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いくつかのユーグレナによる硝酸態窒素の利用

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Kato, S [1] and Ooshima, K.[2]: On utilization of nitrate as the nitrogen source by some *Euglena* spp. Jpn. J. Phycol. (Sorui) 50: 79-82.

Utilization of nitrate as the nitrogen source was studied using axenic cultures of *Euglena gracilis*, *E. granulata* and *E. polymorpha*. While *E. gracilis* could not utilize nitrate as reported previously, both *E. granulata* and *E. polymorpha* could utilize nitrate for their growth as evidenced by the effects on cell multiplication and consumption of nitrogen from the nitrate medium. This result indicates that both nitrate and ammonium are good nitrogen sources for some *Euglena* species.

Key Index Words: *axenic culture*, Euglena, E. gracilis, E. granulata, E. polymorpha, *nitrate*, *nitrogen source*

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Cramer and Myers（1952）は *E. gracilis* Klebs var. *bacillaris* Pringsheim の無菌培養株を用いた実験を行い，ユーグレナは窒素源として硝酸態窒素を利用できないことを明らかにした。その後，Huzisige and Satoh（1960）や Oda *et al.*（1979）も *E. gracilis* var. *bacillaris* を用いた実験結果から，ユーグレナは窒素源としてアンモニア態窒素は利用できるが硝酸態窒素は利用できないことを報告した。これらの報告に基づいて、現在ではユーグレナは硝酸態窒素を利用できないとされている（北岡 1986）。

今回，*E. gracilis* Klebs, *E. granulata* (Klebs) Lemmermann および *E. polymorpha* Dangeard の無菌培養株を用いて，これら3種のユーグレナが硝酸態窒素を利用できるかどうかを調べた。その結果，*E. gracilis* は従来報告されてきたように硝酸態窒素は利用できなかったが，*E. granulata* および *E. polymorpha* の両種はアンモニア態窒素だけでなく，硝酸態窒素も利用できることが判明したので，ここに報告する。

材料と方法

実験には *E. gracilis* として E-1533株（千葉県山武郡成東町の溝，1998年2月18日採集），*E. granulata* として E-1657株（神奈川県横浜市緑区寺家町の大池，2000年5月26日採集），*E. polymorpha* として E-1327株（沖縄県南大東町の溜池，1991年7月19日採集）の無菌培養株をそれぞれ用いた。無菌培養株は，それぞれの採集地点から得られた試料からピペット洗浄法で1個体を単離し，AF-6培地（加藤 1982）で温度20℃，照度4,000 lux，12-12 hrs の明暗周期で継代培養されているものである。

硝酸態窒素及びアンモニウム態窒素の利用について調べるため，それぞれ3種の対数増殖期にある継代培養物をnitrogen-free 培地で数回洗浄したのち，*E. gracilis* については3,000細胞 /m*l*, *E. granulata* および *E. polymorpha* については300細胞 /m*l* になるように nitrogen-free 培地，nitrate 培地および ammonium 培地（Tab. 1）に接種し，1週間後および2週間後にその細胞数を血球計算盤を用いて測定した。また，nitrate培地で培養したものについては同時に培地中の硝酸態窒素の濃度を分光光度計（島津製作所UV-1200）を用いて測定した。なお，これらの実験は温度，照度，明暗周期などの条件をすべて継代培養と同じ条件下で行い，それぞれの測定時にはB-III及びB-IV培地（Ichimura and Watanabe 1977）を用いて無菌テストを行った。

結果

nitrogen-free培地，nitrate培地および ammonium 培地におけるユーグレナの3種の細胞数の変化を Figs 1-3 に示す。*E. gracilis* は ammonium 培地では，接種時の3,000細胞 /m*l* から1週間後には59,000細胞 /m*l*，2週間後には133,000細胞 /m*l* とよく増殖した。これに対して，nitrate 培地では1週間後には4,600細胞 /m*l*，2週間後には6,500細胞 /m*l* に過ぎず，ほぼ nitrogen-free 培地での1週間後の3,300細胞 /m*l*，2週間後の5,200細胞 /m*l* と似た増殖を示した。また，ammonium 培地では，2週間後の細胞さえも貯蔵物質のパラミロン粒は少なく，葉緑体はよく観察できた（Fig. 4c）。nitrate 培地のものでは nitrogen-free培地のものと同様に1週間後にもかなり多くのパラミロン粒が認められ，2週間後の細胞では多数のパラミロ

Table 1 Compositions of three culture media

Components	nitrogen-free medium	nitrate medium	ammonium medium
$NaNO_3$	—	121 mg	—
$(NH_4)_2SO_4$	—	—	95 mg
$MgSO_4 \cdot 7H_2O$	30 mg	←	←
KH_2PO_4	10 mg	←	←
K_2HPO_4	5 mg	←	←
$CaCl_2 \cdot 2H_2O$	10 mg	←	←
Fe-citrate	1 mg	←	←
Citric acid	1 mg	←	←
Trace metals [1)]	3 m*l*	←	←
Biotin	2 μg	←	←
Thiamine HCl	10 μg	←	←
Vitamin B_6	1 μg	←	←
Vitamin B_{12}	1 μg	←	←
Distilled water	997 m*l*	←	←
pH	6.6	←	←

[1)] 1m*l* of the trace metals solution contains; 0.2mg $FeCl_3 \cdot 6H_2O$, 0.08mg $MnCl_3 \cdot 4H_2O$, 0.001mg $ZnSO_4 \cdot 7H_2O$, 0.0004 mg $CoCl_2 \cdot 6H_2O$, 0.0008mg Na_2MoO_4, 1.5mg Na_2-EDTA

ン粒のために，葉緑体はほとんど観察できなくなっていた (Figs 4a, b)。

E. granulata は，ammonium 培地では接種時の 300 細胞 /m*l* から 1 週間後には 2,900 細胞 /m*l* に，2 週間後には 5,500 細胞 /m*l* になった。nitrate 培地では 1 週間後には 2,300 細胞 /m*l*，2 週間後には 4,400 細胞 /m*l* まで増殖した。これに対して，nitrogen-free 培地では接種時の 300 細胞 /m*l* から 1 週間後には 900 細胞 /m*l* に，2 週間後には 1,200 細胞 /m*l* にまで増殖したが，ammonium 培地および nitrate 培地と比べて細胞数は少なかった。また，nitrogen-free 培地では 1 週間後の細胞がすでにかなり多くのパラミロン粒を含みり，2 週間後の細胞は多数のパラミロン粒で満たされていた（Fig. 5a)。しかし ammonium 培地および nitrate 培地では，2 週間後の細胞にもパラミロン粒は少なかった（Figs 5b, c)。*E. polymorpha* は，ammonium 培地では接種時の 300 細胞 /m*l* から 1 週間後には 2,800 細胞 /m*l* に，2 週間後には 5,900 細胞 /m*l* となった。nitrate 培地では 1 週間後には 2,400 細胞 /m*l* に，2 週間後には 7,000 細胞 /m*l* になり，ammonium 培地とほぼ同様の増殖を示した。これに対して，nitrogen-free 培地では 1 週間後には 1200 細胞 /m*l* まで増殖したが，2 週間後には 700 細胞 /m*l* にまで減少してしまった。そしてその 2 週間後の細胞にはかなりのパラミロン粒が認められた (Fig. 6a)。しかし ammonium 培地および nitrate 培地では 2 週間後の細胞でもパラミロン粒は少なかった (Figs 6b, c)。

次に接種から 1 週間後および 2 週間後における nitrate 培地中の硝酸態窒素の濃度を Fig. 7 に示す。接種時に 19.9 mg N/*l* であった硝酸態窒素は，*E. gracilis* では 1 週間後に 19.7 mg N/*l*，2 週間後には 19.8mg/*l* で，ほとんど減少していなかった。それに対して，*E. granulata* では 1 週間後には 18.9 mg N/*l*，2 週間後には 17.7 mg N/*l* となり，*E. polymorpha* では 1 週間後には 19.2 mg N/*l*，2 週間後には 17.8 mg N/*l* と減少していた。

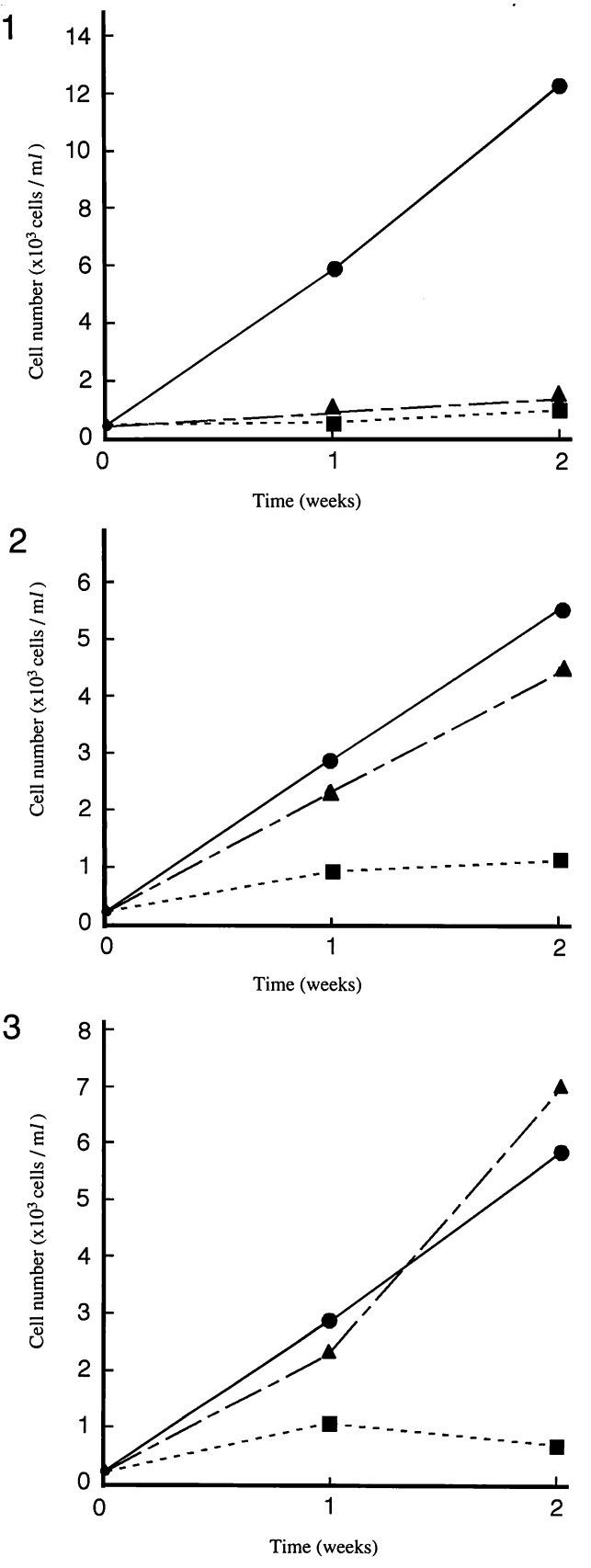
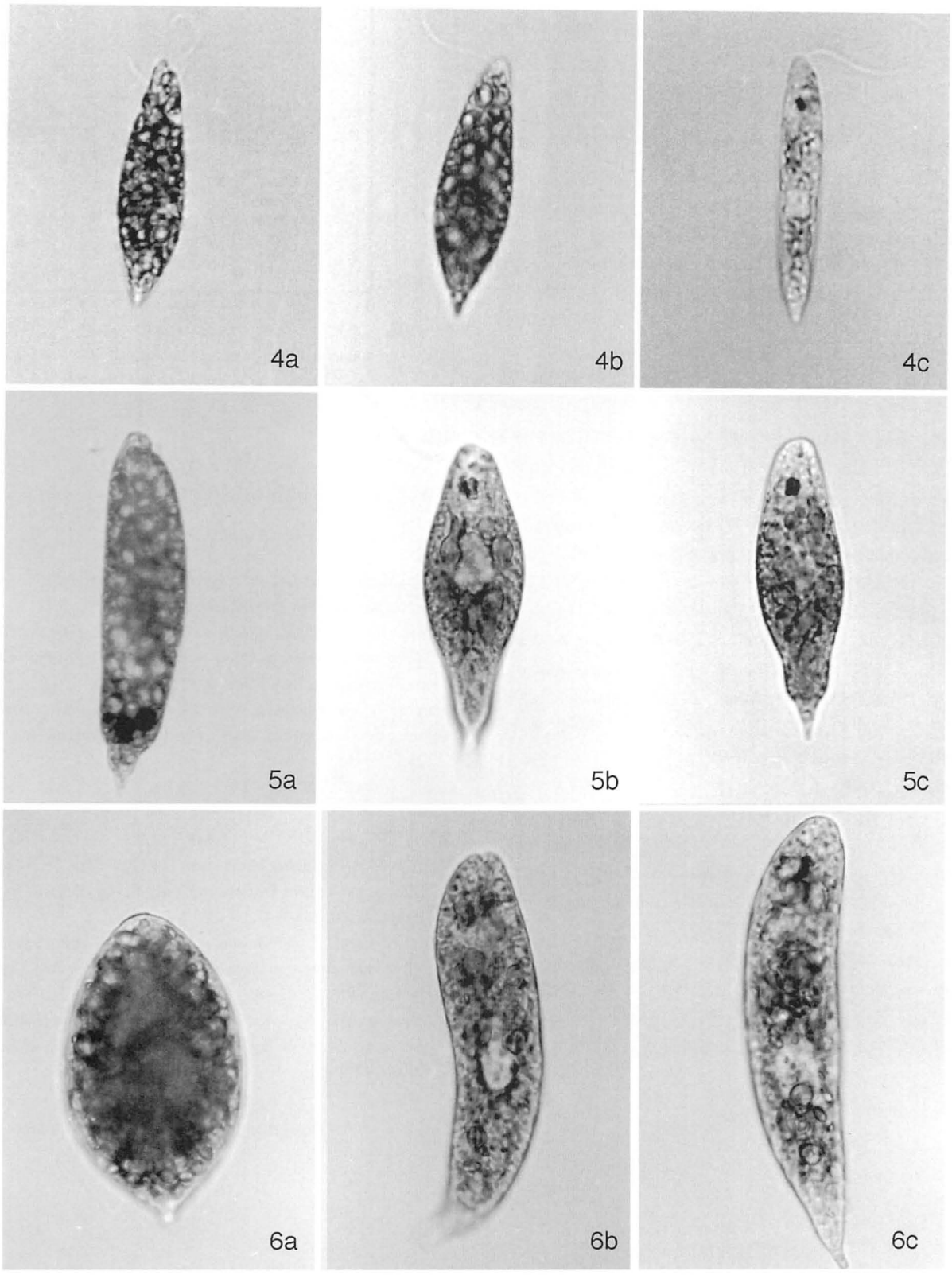


Fig. 1-3. Growth of three species of *Eugena* in three media.
Fig. 1. *E. gracilis*, Fig. 2. *E. granulata*, Fig. 3. *E. polymorpha*
Symbols; ●: ammonium medium, ▲: nitrate medium, ■: nitrogen-free medium



Figs 4-6. Cells of *Euglena* spp. grown in three media.

Fig. 4. *E. gracilis* (x 900); Cell grown in (b) is filled with many paramylon granules the same as in (a).

Fig. 5. *E. granulata* (x 625); Cell grown in (b) has a few paramylon granules the same as in (c).

Fig. 6. *E. polymorpha* (x 625); Cell grown in (b) has a few paramylon granules the same as in (c).

a: nitrogen-free medium, b: nitrate medium, c: ammonium medium

考察

ユーグレナの窒素源に関しては，Pringsheim (1914)が硝酸態窒素をアンモニア態窒素と同じように利用するとしたのに対して，Mainx (1928) は硝酸態窒素よりもアンモニア態窒素の方を利用するとして，両者には見解の相違が見られた。しかし，ユーグレナの無菌培養が困難であったため，どちらの見解が正しいかについては決定されないでいた。その後，Cramer and Myers (1952) は無菌培養株を用いた栄養要求実験から，*E. gracilis* var. *bacillaris* は硝酸態窒素は利用できない報告した。さらに，Huzisige and Satoh (1960) や Oda *et al.* (1978) も同様に *E. gracilis* var. *bacillaris* は硝酸態窒素は利用できないことを確かめた。これらの報告から，ユーグレナは硝酸態窒素を利用できないとされてきた (北岡 1986)。しかし，ユーグレナは100種以上を含む多様な分類群で，富栄養水域ばかりではなく貧栄養水域にまで広く生息しているので，窒素源としてアンモニア態窒素だけでなく硝酸態窒素も利用できる種も存在する可能性は否定できない。そこで今回，既に硝酸態窒素を利用できないとされている *E. gracilis* と，有機物汚染があまり進んでいない湖沼などにも出現する *E. granulata* および *E. polymorpha* について，硝酸態窒素を利用できるかどうかをそれらの無菌培養株を用いて調べた。その結果、実験に用いた *E. gracilis* は従来の報告と同じように硝酸態窒素は利用できなかった。しかし， *E. granulata* および *E. polymorpha* は Figs 2-3に示されているようにnitrate培地でもammonium培地のときと同様に増殖した。さらに Fig. 7 に示されたように増殖するにつれて培地中の硝酸態窒素は減少することから，この両種は硝酸態窒素を利用していることが判明した。

E. gracilis は無菌培養が容易であるために，ユーグレナの生理・生化学的研究に広く用いられ，ユーグレナを代表する種の１つとされているが，自然水域での出現頻度はかなり低く，生活排水が流れ込むような汚染水域にのみ出現する比較的稀な種である。今回，*E. granulata* および *E. polymorpha* の両種のような硝酸態窒素も利用できる種の存在が明らかになったことは，*E. gracilis* がアンモニア態窒素しか利用できないために有機物汚染が激しい場所でしか生息できなくなった種で，有機物汚染があまり進行していない場所に生育しているユーグレナは硝酸態窒素を利用している可能性があることを示唆している。

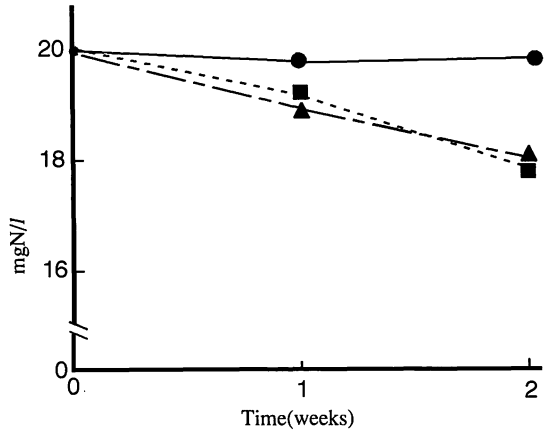


Fig. 7. Consumption of nitrogen in nitrate media by *Euglena* spp. Symbols; ●: *E. gracilis*, ▲: *E. granulata*, ■:*E. polymorpha*

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海外藻類事情

筒井　功：ベトナム海藻事情（2）
キリンサイ類・オゴノリ類の生産と利用

海藻類の利用調査で各地の市場に行くと，たいていチェー（che）を食べることから調査を始める。チェーはベトナムの「ぜんざい」あるいは「あんみつ」とも呼ぶべき甘味食品(1)だが，その具として海藻類が使われることが多いからだ。近年，メコンデルタの多くの町では，キリンサイ類もチェーの具に加わり始めた。

チェーの注文は簡単で，いくつも並べられた容器の中にそれぞれ異なった具が入っているので，自分の好みのものを選ぶだけである（図1）。ベトナム中南部以南の地域で比較的よく食べられているものとして，チェー ダウサン フォーターイ（che dau xanh pho tai）があげられるが，これは甘く煮たダウサン（緑豆）とフォターイ（コンブ）に砕いた氷とココナツミルクなどとを混ぜたものだ。もちろん北の地方や南部でも気温の低い時期などは，氷なしの冷たくないチェーを食べることも多い。

注文を終え椅子に座ると，最初は具の種類や客層などを黙って観察する。もちろんチェーの味も楽しむ。いろいろな話を聞かせてもらうのは，支払いが終わってからのことである。それまでは可能な限りベトナム人になりすましておく。そうしなければ「外国人料金」で請求されることもあり，「本当の値段」がわからなくなってしまうからだ。ベトナムでは定価というものがない。ベトナム人でさえも，よそからきて間がなくその町の物価がわからないときは，相場よりも高い買い物をすることがよくあるという。

チェーの店の形態は様々で，立派なお店を構えているところもあれば，天秤棒で具といくつかの食器，そして小さな椅子を担いで売り歩くような簡単な屋台もある。店や屋台は市場だけに限らず，公園や学校などのまわりに行けば見つけることができるだろう。多くの女性と子供は甘味が好きなので，このような人たちがよく集まる場所を探せばよいのである。

本稿では，チェーとの関わりが深いキリンサイ類とオゴノリ類について，ベトナムでの採取・養殖の現状とその利用を紹介したい。

図1 ビンロンのチェー屋　画面中央の大きなボールに*K. alvarezii*が入っている

キリンサイ類の採取と養殖

ベトナムでは2種のキリンサイ類が流通している。そのひとつはホンヴァン（hong van）と呼ばれるカタメンキリンサイ *Betaphycus gelatinum* (Esper) Doty ex Silva *et al.* で，もうひとつはロンスン(2)（rong sun）という標準和名を持つ *Kappaphycus alvarezii* (Doty) Doty ex Silva in Silva *et al.* である。

カタメンキリンサイのベトナムにおける分布は限られており，主として中南部のファンラン（Phan Rang）（図2）周辺のリーフに生育している。しかしながらその生育量は限られ

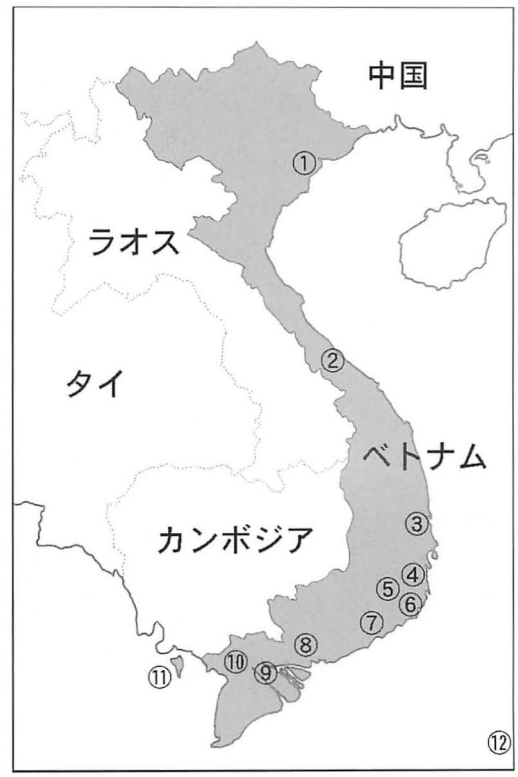

図2 本稿に関係するベトナムの町の位置　①ハイフォン，②フエ，③ソンカウ，④ニャチャン，⑤ダラット，⑥ファンラン，⑦ファンティエット，⑧ホーチミン市，⑨ビンロン，⑩ロンスェン，⑪フークォック島，⑫チュォンサー諸島

ており，沿岸住民によって採取される量も多くはない。このため本種はファンランとニャチャン周辺にしか流通していない。

ベトナムにおける*K. alvarezii*の養殖は，1993年に高知大学で保存されていたフィリピン株のうち500gをベトナムに移植したことから始まる（大野ら 1995）。移植後数年間の経過や生長状況は大野ら（1995）に詳しいのでここでは詳述しない。筆者の知る限りでは，ウシエビ養殖池が*K. alvarezii*の養殖に使用される事例はベトナム以外にない。本種の養殖には適度な海水流動が必要と考えられているからである。しかし水温がおよそ30℃以下の場合は，海水流動があまりなくても十分生長するのだという（Huynh 私信）。ファンランでは雨季の11月から翌年２月頃までの間は気温や水温があまり高くならない。逆に３～10月頃までの乾季には気温や水温はかなり高くなるが，常に強い風が吹いており，ウシエビ養殖池のような閉ざされた場所でも風によって波が立ち常時海水流動がおこっている（図３）。ファンランがこのような状況にあるため，*K. alvarezii*の養殖がウシエビ養殖池でも可能だったのではないだろうか。

ファンランのソンハイ（Son Hai）集落周辺では，現在までに海面及びラグーンでの養殖が定着し，約150戸が漁業などとの兼業で*K. alvarezii*の養殖にたずさわっているという。モノライン方式による養殖が主流で，６月から翌年の２月まではラグーンで養殖し，ラグーン内の水温が高くなりすぎる３～６月頃のみ海面に場所を移す。海面での養殖面積はおよそ8ha，ラグーンでのそれは約12haほどあるという。年間およそ乾燥重量にして，50トンほどが生産される。いっぽうウシエビ養殖池での*K. alvarezii*養殖は，まだ試験段階でほとんど行われていない。本年からは，ベトナムの汽水・海水域養殖を対象としたデンマーク政府による国際協力プログラムが始まり，ウシエビ養殖池での本種の養殖もこのうちのひとつに採用された。これまで，塩濃度の低下する雨季の間はウシエビの養殖ができないため，池はそのまま放置されていた。そこでその期間，空いたウシエビ養殖池で*K. alvarezii*を養殖することにより，ウシエビ養殖池やラグーンの水質改善と収入増を同時にはかるという，より環境に配慮した「持続可能な養殖技術」が検討されることになっている。

次に，現時点で最大の養殖規模を持つフークォック島に話を移す。*K. alvarezii*の養殖が行われているのは，島の東側中央部に位置するハムニン（Ham Ninh）集落地先の遠浅砂浜海岸である（図４）。ここでの養殖は，Institute of Materials Science, Nhatrang Branchのフイン・クアン・ナン（Huynh Quang Nang）氏の研究室と香港のカラゲナン製造会社K社との共同事業として，1999年から始まった。研究室が技術開発を行い，K社が資金面でのサポートをしている。また，ハムニン地先で生産された海藻はK社が買い取ることになっているので，専用養殖場ともいえよう。周辺の漁業者らが養殖を希望した際には，研究所の技師が技術指導をおこない，株の分与を無償で行っている。2001年末の時点で，ハムニン集落の約40戸が漁業との兼業で養殖を行っており，全体で約50haの養殖面積をもつ。また今後養殖に使用できる場所はおよそ300～500ha程と見積もられている。雨季は５～10月頃でファンランと比べて長く，特に雨季の終盤に毎年のようにおこるメコン川の増水により，運河を経て大量の河川水がタイ湾側に流れ込む。この塩濃度の低い水塊が東の季節風によってたまに島へ運ばれてくるときがあり，養殖場付近の表水は塩濃度が約15pptほどまで下がることがあるという。しかしながら，塩濃度の比較的高い層まで養殖水深を低めることで，約10日程度であれば問題がないとのことである。また乾季は11月から翌年４月頃で，この時期には表面水温は約30℃以上に達することが多い。このように年間の塩分や水温の変化が多く，また雨季も長い場所での養殖なので，養殖する水深や藻体をロープにつるす間隔，乾燥方法などについて，これまでに多くの工夫が試みられノウハウが蓄積されてきた。*K. alvarezii*の養殖によって得られる年間収入は，その家庭が養殖している面積にもよるが，およそ円換算にして約11～17万円程(3)で，年間収入の約25～30%を占めるという。退官前の研究公務員の基本給一年分が10万円程(4)であるので，かなりの収

図３ ファンランの試験養殖池に立つHuynh Quang Nang氏　強い風が吹き池の水面が波立っている

図４ フークォック島の*K. alvarezii*養殖場

入をもたらすといってよいだろう。養殖にたずさわっている人のひとりは，簡単な労働で収入が増えるのがうれしいし，何よりも漁業は収入が不安定だが，海藻養殖はある程度安定しているのがよいと語った。これまでに養殖された*K. alvarezii*の一部は，後述するようにメコンデルタの各地に運ばれ食用とされ始めた。また，ベトナムではこれまでキリンサイ類をカラゲナン原藻として輸出した実績はないが，2002年末には100トンほどの原藻が香港に向け輸出される見通しである。

上述の場所以外では，チュォンサー（Truong Sa）諸島に駐留する兵士への野菜代用品として*K. alvarezii*を利用するため，1999年から養殖試験が行われている。またファンティエット（Phan Thiet）の沖に浮かぶフークイ（Phu Quy）島やビンディン（Binh Dinh）省クイニョン（Qui Nhon），フーイェン省ソンカウ（Song Cau），ニャチャンの北方約150kmに位置するカインホア省ヴァンニン（Van Ninh）などでも養殖が開始されるなど，この一年間でにわかに養殖地が拡がってきた。これらの新しい養殖地のいくつかは，フークォック島でK社が行ったのと同じように，ベトナムと中国のカラゲナン製造会社との共同開発事業である。ここ数年間のうちで，中国系カラゲナン製造会社数社がベトナムとのキリンサイ養殖の合同プロジェクトの立ち上げを打診してきているという。これまでの主要輸入先であったインドネシアやフィリピンの生産地周辺での情勢不安，またベトナムからの輸送コストの方が安価なためであるという。日本企業はどうかと関係者らに尋ねたところ，商社を通じて買い付ける話あるいは値段の折衝だけで，共に新たなキリンサイ養殖地を開発する姿勢はないようだと語る。各国・各社それぞれにビジネスのやり方があるのだろうが，フークォック島でのK社とベトナム人技術者，地先の漁民らの信頼関係を見ていると，中国系の人々は商売がうまいと素人ながらに感じる。

1993年以前にベトナム国内で*K. alvarezii*の養殖に向けた動きが全くなかったわけではではない。1980年代半ば，フィリピンで行われた水産関連のトレーニングコースに*K. alvarezii*養殖も含まれており，ベトナム政府の水産幹部が一名参加したが，ほとんど現場や専門から離れた人だったという。今ではかなり減ってきたとはいうが，共産党員など政治的に「血筋のよい人物」あるいはその知人などが，本来行くべき専門家などを差しおいて海外への研修や会議に参加することなどはベトナムでは珍しいことではない。結局，当時はベトナム国内でオゴノリ類が注目を浴びていたこともあり，研修後キリンサイの養殖は試みられることはなく，その人はもう公務員を辞め，ベトナム中部地方でウシエビ養殖をしていると聞いた。

オゴノリ類の採取と養殖

ベトナム沿岸には15種のオゴノリ類が生育するとされている（Huynh 1998）。またOhno *et al.*（1999）は，ベトナム新参種などを含む9種のオゴノリ類について，その形態や生態，採集地などを報告した。これらのオゴノリ類のうち，多くの種が沿岸住民などによる採取の対象となっており，自宅で消費されたり地元ならびに周辺の町の市場などで販売されたりしている。ベトナム中南部に位置するニャチャンを例にとると，ニャチャンでは10種弱のオゴノリ類が生育しているが，そのうち市場価値が高くさかんに採取されているのは，リュウキュウオゴノリ *Gracilaria eucheumoides* Harvey とシマテングサ *Gelidiella acerosa* (Forsskal) Feldmann et Hamelの2種である。前者は比較的海水流動の大きい海岸の潮下帯に生育し，生育量の周年変化はあまり大きくない。男性の素潜りなどによって採取されるが，10月頃から翌年2月頃の間は北東季節風の影響により波浪が高く潜れないので，採取されることは滅多にない。これに対し後者は礁原の潮間帯に生育し，干潮時に女性や子供たちによって採取されることが多い。年間を通じて採取されるが，季節的変化が比較的はっきりしており，2～6月頃に生育量が増え多く採取される。採取後はどちらの種も淡水で洗浄し不純物を取り除いたのち，数日かけて海岸や自宅の庭などで天日で乾燥させる（図5）。

ベトナムではオゴノリ類の養殖も行われている。養殖対象種は地方で異なり，北部のハイフォン（Hai Phong）周辺では *G. vermiculophylla* (Ohmi) Papenfuss[5]が，中部のフエ（Hue）周辺では *G. tenuistipitata* var. *liui* Zhang et Xia が，中南部のソンカウ周辺では *G. heteroclada* Zhang et Xia がそれぞれ養殖されている。養殖状況などは各地で異なるが，紙面の都合上ここではフーイェン（Phu Yen）省ソンカウのオゴノリ養殖をとりあげる。ソンカウ集落一帯にはウシエビ養殖池とオゴノリ類の養殖池が混在している。かつてはマングローブ林だったという。オゴノリ養殖池の大きさは様々であるが，水深はどれも約50～70cm程で底質は泥である。池の周辺には幅1mほどの水路があり，高潮時に海水が流入し，低潮時には池の表水が流出する仕組みになっている。オゴノリ類はこのような池に散布され，底に沈んだ状態で生長する。乾季である2～9月頃の間は，一ヶ月に一回ほどの頻度で採取し，その際に採取しなかった他のオゴノリ類に付着したジュズモ類やイバラノリ類などの雑藻を取り除く。それ以外の手入れはほと

図5 ニャチャンの漁村で乾燥中の *Gelidiella acerosa*

んど行わない（図６）。採取後はオゴノリ類を池の畦や自宅周辺で乾燥させ，乾燥した藻体がある程度の量まとまった段階で，寒天製造工場のあるハイフォンへ向けて出荷する。雨季である10月から翌年１月頃の間は，池の塩分が低下し藻体が育たないため採取は行わなず，そのまま藻体を池に放置しておく。池の規模によりまちまちであるが，乾燥重量にして２～３トン，日本円に換算しておよそ８～９万円程の年間収入を得る家もある。

ところでベトナムで本格的にオゴノリ類の養殖が行われるようになったのは，北部で1970年頃，中部および南部で1980年頃である（Huynh 1998）。1986年の刷新（ドイモイ）政策以降，国策としてオゴノリ類の養殖が発展した。前報で述べたとおり，ナン氏の研究室の設立当初の目的はオゴノリ類の養殖拡大のための技術開発であり，研究室設立の時期が上記とほぼ一致する。ところが1990年代に入ると，それまで原藻の主要輸出先であったソ連が崩壊するとともに，中国もベトナムからの原藻輸入をやめるようなった。またベトナム産オゴノリ類の品質はあまり高くなく，日本などの西側諸国への原藻輸出も散発的で終わった。いっぽう高くない品質のオゴノリ類から寒天を製造するため，ベトナムでは寒天製造過程で多くの化学薬品を使うという。このため製品にその薬品が残留し(6)，寒天としての輸出も伸ばすことができなかった。さらに1990年代からは，主として西側諸国へ輸出するためのウシエビ養殖が盛んになった。エビから得られる収入がオゴノリの養殖とは比較にならないほど高額であったため，オゴノリ養殖池はウシエビ養殖池へと転用されるようになり，次第にオゴノリ類の養殖は衰退の傾向を見せている。現在ではオゴノリ類は自国向けのみに用途をせばめ，後述するように養殖オゴノリ類は寒天工業の原藻として，また天然オゴノリ類は食用として利用されるのみである。

キリンサイ類・オゴノリ類の利用

a. キリンサイ類

ベトナムではカラゲナンの工業的製造は行われていない。しかしながら，つい先日ベトナム・香港のカラゲナン製造企業(7)・フィリピンのＭ社との間で，ベトナム国内に共同でカラゲナン製造工場を設立することに合意が得られたという。

フークォック島での大量養殖が成功した後，ここ一年半ぐらいの間に，メコンデルタ西北部一帯では，乾燥した *K. alvarezii* を水でもどしたものが，チェーの具として盛んに利用されはじめた（図７）。藻体そのものには味付けなどはしないため味はほとんどないが，緑豆を甘く煮たものなどと共に食べると，その強い甘みを押さえてくれるし，またコリコリとした食感を楽しむことができる。チェーを頼んだ際の具の種類や数によって値段は異なるが，上述の緑豆とコンブのチェーに本種を加えてもらっても，円換算にして20円弱である。

これに対し中南部の町ダラット（Da Lat）などでは，ファンラン産のものが５年以上前から普通に出回っており，他の野菜類と共にサラダとして利用されることが多いという。市場の乾物屋などで乾物が売られるのはメコンデルタ各地と同様であるが，水でもどしたものが八百屋で販売されている（図８）。

b. オゴノリ類

乾燥オゴノリ類は，煮溶かした液にライム汁を少々加え濾過液を冷却凝固させたスーソア（xu xoa）というトコロテン

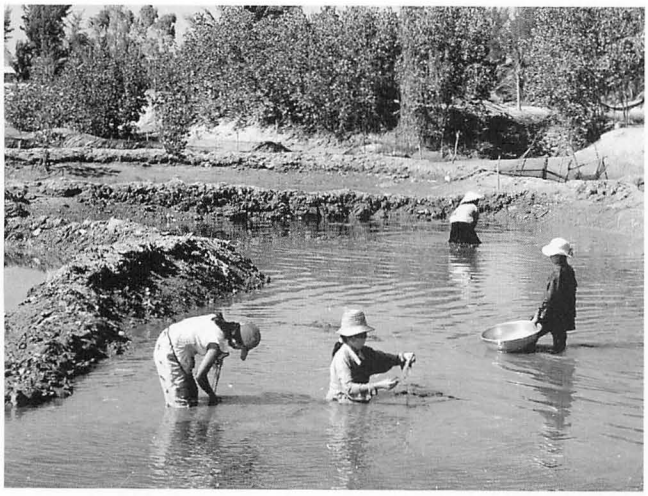

図６ ソンカウの *Gracilaria heteroclada* 養殖池で雑藻をとる家族

図７ ロンスェンのチェー材料屋　手前中央のボールには *K. alvarezii* が，手前右のボールにはドンスォンがそれぞれ入っている

図8 ダラットの八百屋で売られている *K. alvarezii*

様食品の原料として利用されている（図9）。甘く味付けたココナツミルクあるいは生姜とライム汁，砂糖を混ぜた甘い液をかけて食べる場合が多い。シマテングサを使ったものの品質がよいとされている。日本のトコロテンよりもやや硬く透明感は少ないが，香りは日本のものにちかい。去熱効果があるので暑い時期に好んで食べるという。スーソアはチェーの店などではたいてい扱われており，茶わん一杯が円に換算して4～9円ほどである。ところで海岸から離れた都市や町などでは，乾燥オゴノリ類が市場で販売されていないことが多い。このような町でもやはりチェーの店があり「トコロテン」が売られているが，これらは粉末寒天や糸寒天から作られたものでドンスォン（dong suong）と呼び，乾燥オゴノリ類から作られたスーソアとは呼び名の点で区別されていることが多い。ニャチャンなどの海岸部の町でこれらのことについて話してみると，スーソアのほうが磯臭い(8)けれど，柔らかくて安全なのだという。上述したように，ベトナムの寒天には製造過程で使用した薬品が残留しているからであろう。本当なのかどうか，また本当であればどのくらいの量残留しているのか，機会があれば化学分析を行ってみたいと考えている。

リュウキュウオゴノリはチェーラウカウ（che rau cau）の原料として用いられる。乾燥したリュウキュウオゴノリを水で一晩もどした後煮て，柔らかくなった段階ですりおろした生姜・ライム汁・砂糖を煮汁の中に入れ，そのままオゴノリごと冷却凝固させる。スーソアと比べるとかたく，やや粘りがある。中南部地方のチェーの店などで扱われており，茶わん一杯円換算にして20円弱である。

図9 スーソア（左）と，そのかけ汁

このほかオゴノリ類は，他の野菜類と共にサラダに利用されることもある。生あるいは乾燥したものを水でもどし利用する。

おわりに

ベトナムのキリンサイ類とオゴノリ類について，その生産と利用の概要を述べた。

ベトナムにおいて1986年の刷新政策以降，国策としてオゴノリ類の養殖が発展した。しかしながら現在では，ベトナム国内の社会状況の変化，オゴノリ類の世界市場，また西側諸国でウシエビ消費の拡大などの中で，その養殖規模はかなり減少している。現在筆者は関連の一次資料を収集することに努めているが，まだ十分とはいえない。今後も資料収集に努め，ベトナムのオゴノリ養殖がどのように発展し，またどのような状況・要因の中で次第に衰退傾向へと変化していったのか，詳しく解き明かしてゆきたいと考えている。

その一方で，筆者がベトナムに滞在するこの数年間のうちに，*K. alvarezii*の大量生産（養殖）技術ができあがり，養殖地があちこちに拡がろうとしている。また数年のうちに原藻輸出という第2段階およびカラゲナンの自国生産という第3段階への第一歩が踏み出されるだろう。一般庶民のレベルでは，2年前にメコンデルタを訪ねた際にはキリンサイは販売されていなかったところが，昨年あたりからあちこちの市場で乾燥キリンサイが販売されるようになり，庶民の味ともいえるチェーの具として盛んに利用されるようになった。キリンサイ類に関して激動とも言えるまさにその時期に，筆者自身がベトナムに滞在し観察させてもらえるというのは，非常に幸運に恵まれていたのだと感じる。

今後，ベトナムのオゴノリ類とキリンサイ類に関連する事柄は，どのように変化してゆくのだろうか。これからもできるだけ長くその動向を見守りたいと考えている。

本稿の執筆にあたり，高知大学海洋生物教育研究センターの大野正夫教授，ならびにInstitute of Materials Science, Nhatrang Branchの Huynh Quang Nang 副所長および Tran Kha 研究員にご教示いただいたので，ここに感謝の意を表します。

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脚注

(1) 北部では茶を意味することもある。

(2) 標準越名はロンスンだが, 一般的には海藻類全般を意味するジョンビエン（rong：藻, bien：海）と呼ばれている場合が多い。前回述べた「ホンダワラ類を利用した清涼飲料」やその原料であるホンダワラ類もやはりジョンビエンとふつう呼ばれている。たとえば,「ホンダワラ類を利用した清涼飲料」の屋台ではジョンビエンといえば飲み物を指すが, 市場の乾物屋では, ジョンビエンは清涼飲料にするものか（ホンダワラ類), チェーにするほうか（キリンサイ類）聞かれることが多くまぎらわしい。なおオゴノリ類はラウカウと呼ばれ, ジョンビエンとは言わない。

(3) 2002年5月の時点で1円＝約115ドンである。

(4) もちろんこれだけでは一家は養ってゆけない。

(5) ベトナムの研究者らの間では、これまで *G. asiatica* Zhang et Xia とされてきたが、吉田（1998）にしたがう。

(6) 情報提供者の立場が悪化する可能性があるので, 現時点では薬品名などについて詳しく報告できない。

(7) 企業名は不明だが, フークォック島で養殖を行っている香港のカラゲナン製造会社とは別の企業とのことである。

(8) ベトナムの人々の中には, おそらく日本人の多くが芳香と感じるであろうアマノリ類の香りやコンブ佃煮の香りなどに対して, 拒否反応を示す人が少なからずいる。また「ホンダワラ類を利用した清涼飲料」を作る際などにも, におい消しあるいは芳香をつけるため菊花などを少量混ぜる工夫をしている屋台もある。なおベトナム語では, 海藻類の磯臭さに対しも魚介類の生臭さに対してもTanhという単語を用い, 両者のにおいを特に区別することがない。

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シリーズ
藻場の景観模式図

寺脇利信[1]・新井章吾[2]：10. 新潟県佐渡島・真野湾二見地先

はじめに

本シリーズでは，岩礁域の藻場について，北海道，本州の日本海岸および太平洋岸，九州，さらに瀬戸内海の各地先での景観模式図を掲載してきた。また，前回から，自然の生育基盤のみならず，人工の生育基盤での藻場の景観も加えた（寺脇・新井 2002）。今回からさらに範囲を広げ，砂泥域の基盤に生育する海草類の藻場についても，景観模式図の報告を続けてゆく。

筆者らは，岩礁域において海藻類がつくる藻場のみならず，砂泥域において海草類がつくる藻場についても，深い関心を抱いている。今回は，冬季の季節風浪が厳しい日本海に浮かぶ佐渡島沿岸の中でも，静穏な環境条件のため，海草類の植生も豊富な真野湾北岸の二見地先の藻場を観察する機会を得た。本シリーズ第1回の富山県氷見市宇波地先（寺脇・新井 1999）の場合と同じ日本海沿岸に三たび戻り，改めて今回を，海草・藻類を含めた藻場の景観模式図の初回と位置づけたい。

10. 新潟県佐渡島・真野湾二見地先

現地の概要と方法

本州の日本海沿岸の北部の新潟県沖に浮かぶ佐渡島では，基本的に，冬季の北西からの季節風浪によって大きな影響を受ける。しかし，佐渡島沿岸においても，南西方向に開けた真野湾の北岸に位置する二見地先（図1）では，湾口部の城ヶ鼻・二股岩地先から約2km湾奥側へ入り込んだ内湾部であるため，冬季の北西からの季節風浪の影響が小さい。二見地先において物理的な底質攪乱が生じる場合は，夏から秋季の台風で発達したうねりが南西から押し寄せる時期に限られる。以上のことなどから，二見地先では，砂泥が堆積しやすい環境条件となり，浅海域まで砂泥底が広がっている。ただし，二見地先の砂泥海底では，全体的な表面観では砂泥であるが，泥岩の平坦な岩盤に砂泥が薄く被覆した状態が広く，その上に砂岩の巨礫が点在している。一方，泥岩の凹部に砂泥が堆積した場合のみならず，砂礫の集積域が砂泥に被覆されて砂礫基盤となっている場合も多い。砂に薄く被覆された泥岩の岩盤および砂岩の巨礫では大型海藻・ホンダワラ類が優占し，一方，砂礫および砂泥基盤では大型海草・アマモ類が優占している（図2）。

1992年6月8日に，SCUBA潜水により，真野湾北岸の二見地先において，水深2mの海底10m×20mの範囲を観察した。岩盤および巨礫で特に目立った群落を形成していたヨレ

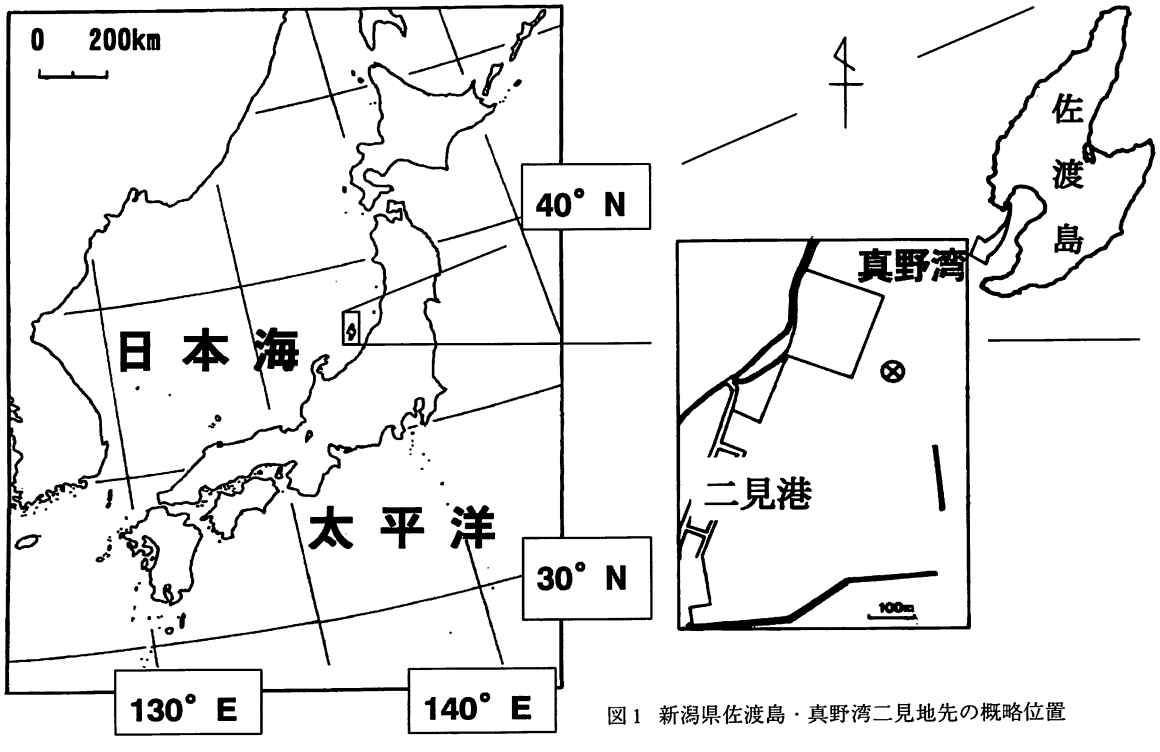

図1 新潟県佐渡島・真野湾二見地先の概略位置

図2　新潟県佐渡島・真野湾二見地先における藻場（上：ホンダワラ類中心，下：スゲアマモ（左）とウスイロモク（右）中心）

モク *Sargassum siliquastrum*, マメタワラ *S. piluliferum* およびウスイロモク *S. pallidum*, 一方, 砂礫基盤で特に目立った群落を形成していたスゲアマモ *Zostera caespitosa* および砂泥基盤のアマモ *Zostera marina* の優占域の中心部0.5m×0.5mを測点とした。それぞれの測点において，基盤の硬度（中山式土壌硬度計標準型による支持強度；図3），砂層厚，優占する海草・藻類の被度，および草・藻体の基盤への固着力（バネばかりによる引張強度；図4）を測定した。

図3　土壌硬度計を基盤に垂直に押しつけ、反発力（支持強度）で基盤硬度の測定（砂泥基盤の例）

結果

新潟県佐渡島・真野湾二見地先における藻場の景観模式図を図5に示す。

ヨレモク群落：水深2.0mの砂泥上に点在する，硬度200kg/cm^{2}の砂岩の巨礫（長径60-150cm）の砂面からの比高0.4mでは，被度100%で優占し，藻体の固着力は10kg以上であった。

マメタワラ群落：水深2.2mの砂泥上に点在する，硬度200kg/cm^{2}の砂岩の巨礫でも，砂面からの比高0.2mでは，被度100%で優占し，藻体の固着力は10kg以上であった。

ウスイロモク群落：水深2.4mの，硬度10kg/cm^{2}の泥岩の岩盤で，砂層厚1cm未満で砂泥に被覆された場所では，被度50%で優占し，藻体の固着力は2kgであった。

スゲアマモ群落：水深2.4mの，砂層厚1cm未満で砂泥に被覆された，硬度1kg/cm^{2}の砂礫基盤では，被度95%で優占し，草体の固着力は2kgであった。

アマモ群落：水深2.4mの，砂層厚9cmで砂泥に被覆された，硬度1kg/cm^{2}未満の砂泥基盤では，被度40%で優占し，草体の固着力は1kgであった。

その他の主要種：カタツルモ *Chorda rigida*，コアマモ *Z. japonica* およびウミヒルモ *Halophila ovalis* が，ウスイロモクと類似した条件の場所に点生していた。

まとめ

1992年6月8日に，新潟県佐渡島・真野湾の二見地先の水深2mでは，硬度200kg/cm^{2}の砂岩で比高0.4mにヨレモクが，比高0.2mにマメタワラが，硬度10kg/cm^{2}の泥岩でウスイロモクが，砂礫基盤で海草のスゲアマモが，砂泥基盤でアマモが優占していた。

注目点

二見地先の水深2mでは，硬度200kg/cm^{2}の砂岩で比高0.4mと相対的に高い場所にヨレモクが10kg以上の固着力で，比高0.2mと相対的に低い場所にマメタワラが10kg以上の固着力で，硬度10kg/cm^{2}の泥岩でウスイロモクが2kgの固着力で優占していた。ヨレモクおよびマメタワラはホンダワラ類の中

図4　海草・藻体の基部にくくりつけたロープをバネばかりで45度方向へ引っ張り、引張強度で基盤への固着力の測定（フシスジモク幼体の例）

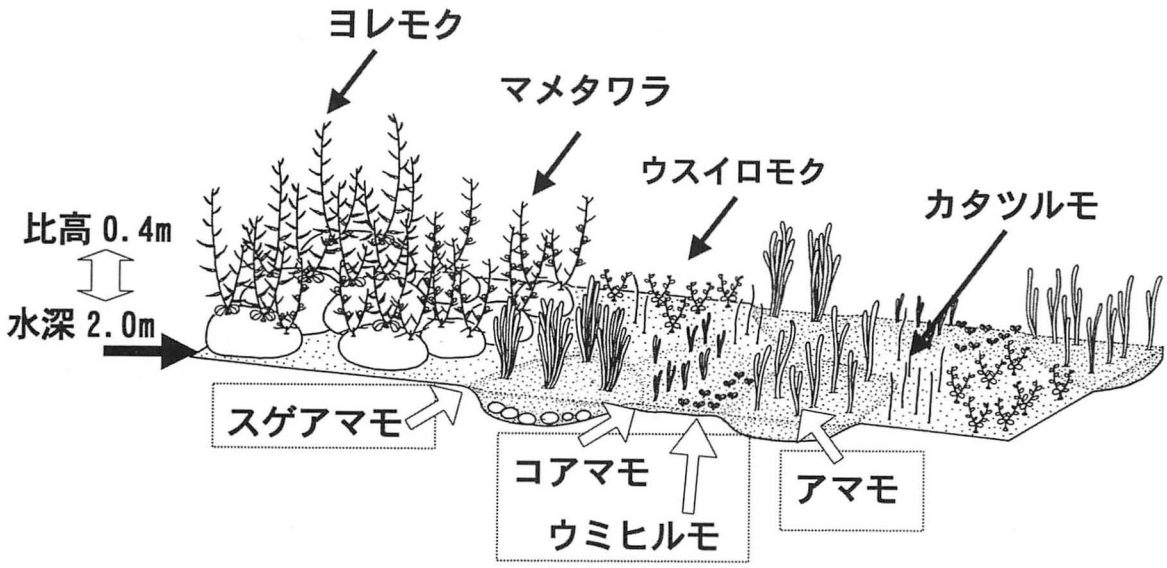

図5 新潟県佐渡島・真野湾二見地先の藻場の景観模式図（1992年6月）

でも比較的安定した環境に生育し（今野 1984），一方，ウスイロモクは砂の被覆の影響が強くより不安定な環境に生育する（新井ら 1990,1996）。これらのことから，水深がほぼ等しい岩礁域において，基盤の硬度がホンダワラ類の固着力に影響を及ぼし，優占するホンダワラ類の種類が変化する可能性が示唆された。

二見地先の水深2mでは，砂礫基盤で海草のスゲアマモが，砂泥基盤でアマモが優占していた。スゲアマモが優占する砂礫基盤では，砂層厚が薄く，礫を含み，硬度が1kg/cm^{2}で，海草体の固着力が2kgと大きかった。一方，アマモが優占する砂泥基盤では，砂層厚が9cmと厚く，硬度1kg/cm^{2}未満であり，海草体の固着力が1kgと小さかった。スゲアマモは，主に北海道，本州，朝鮮半島および中国の日本海沿岸に分布し，匍匐茎を有しないため株全体が叢生することで，他のアマモ属3種と区別された(Miki 1932)。しかし，スゲアマモの生態に関する知見は，アマモに比べてきわめて少ない。Miki(1933)によると，相対的に，アマモが湾最奥部の静穏な泥質基盤に主に分布するのに対して，スゲアマモはやや波あたりの強い湾口部の砂質基盤に主に分布する。スゲアマモの根茎は，他のアマモ属3種と異なり，節間が短い上に1～35mmまで変化するため，密に分枝し，密着して塊状となる（大森・相生 1998）。これらのことなどから，水深がほぼ等しい砂泥域において，基盤の硬度は，ホンダワラ類の場合と同様に，アマモ類の固着力にも影響を及ぼし，優占するアマモ類の種類が変化する可能性が示唆された。

さらに，二見地先では，泥岩で優占するホンダワラ類のウスイロモクと砂礫基盤で優占する海草類のスゲアマモとは，固着力が2kg/cm^{2}と同等であった。今後，海草・藻類の局所的な分布の決定に関わる物理的外力要因について，繰り返し測定の伴う実験的な調査により，同じ測定項目を用いての解析が実現することが期待される。

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藻類学最前線

本多 大輔：全真核生物における藻類の位置づけ

1980年代から急速に広まった分子系統学的手法によって，生物の進化の道筋がようやく明らかになってきた。また同時期に広まったPCRの手法によって，少量のサンプルから容易にDNA配列を決定できるようになり，多様な生物を対象にすることが困難ではなくなった。特にリボソームRNA遺伝子は適当な間隔で保存的な配列を含んでいることから，系統的に離れた生物に対しても同じプライマー配列を用いることが可能なこともあって，非常に多くの配列データが蓄積されてきており，その勢いは未だとどまるところを知らない状況である。実際，原核生物では16Sリボソーム RNA遺伝子配列の相同性やGC率が分類基準にも導入され，真核生物でも18Sリボソーム RNA遺伝子の分子系統解析の結果が分類に反映されているなど，その知見は生物全体の系統の隅々までに及んでいると言って過言ではない。しかし，あらためてリボソーム RNA遺伝子の分子系統樹を見てみると，真核生物では界や主要な門に属する生物が単系統群を形成することは示せても，それらの高次分類群の分岐順については明確な結論が導けない状況にある。これは同時期に適応放散したことを樹冠の枝振りに例えて，クラウン生物群[1]と呼んでいることにも現れている。この現象は変化の速い領域での多重置換の蓄積や，タンパク・コード遺伝子と比べて正確なアラインメントが困難といったリボソームRNA遺伝子の解析限界を示すものであると言える。

この問題に対応するため，複数の遺伝子を合わせて解析する手法がとられるようになってきた。中でも以下のタンパク・コード遺伝子は，比較的進化速度が遅く置換が飽和していないことが期待されること，系統ごとの進化速度が極端には異ならないこと，系統ごとに見られる塩基組成の不均一性が少ないことなどの理由から取り扱われることが多い：cytosolic 70-kD heat shock protein (HSP70-cy)，tubulin alpha subunit (a-tubulin)，tubulin beta subunit (b-tubulin)，actin，vacuolar ATPase (V/A-ATPase)，elongation factor-1a (EF-1a)，elongation factor -2 (EF-2)，RNA polymerase II largest subunit (RPB1)。図1はBaldaufら[2]が2000年にScience誌に発表したactin, a-tubulin, b-tubulin，EF-1aに基づく系統樹を改変したものである。それぞれの内部の枝に示された1から18までの番号は，表1のノード番号に対応しており，これら18個のノードで括られるグループの単系統性は上記の4遺伝子を合わせて解析することで支持された。一方，いくつかのノードは番号が付けられていないが，これは4遺伝子によってあまり支持されなかったことを示している。

この系統樹では真核生物は少なくとも以下の7大系統群から構成されることが示されている。

1）Opisthokonta系統群：微胞子虫類を含む菌界および動物界の生物から構成される大系統群。この呼称は，opistho-が「後部の」，kontaが「糸・鞭毛」を意味し，鞭毛を細胞の後方で運動させる精子，遊走細胞の特徴から名付けられた[3]。

2）Amoebozoa系統群：タマホコリカビ類，ホコリカビ類といったいわゆる動菌類，Amoebaを含む葉状根足虫類，ミトコンドリアを欠くEntamoebaの生物などが含まれる。

3）Plantae系統群：陸上植物類と緑藻類を合わせた緑色植物類に，紅藻類，灰色植物類が加わったグループである。葉緑体が2重の包膜をもつことでまとめられる。光合成色素がクロロフィル *a*+*b* である緑色植物とクロロフィル *a* とフィコビリンタンパクである紅藻，灰色植物では差が見られることや，緑色植物と灰色植物の鞭毛装置には多層構造体が見られるものがあるのに対し，紅藻では鞭毛装置そのものが見られないばかりか，分裂時の中心子さえも現れないなどの類似点，相違点からこれらが近縁かどうかについては従来は決着がついていなかったが，この系統樹ではこれらの単系統性が支持されている。またこのことは，シアノバクテリアと真核生物の共生による葉緑体の成立は1回しかなかったとする考え方[4]と矛盾しない。

4）Heterokonta系統群（＝stramenopile系統群）：卵菌類と褐藻類で比較できるDNA配列データが報告されているに過ぎないが，これらは良くまとまった単系統群であることが示されている。Cavalier-Smith[5]はHeterokonta系統群にクリプト藻類とハプト藻類を加えて，クロミスタ界（Kingdom

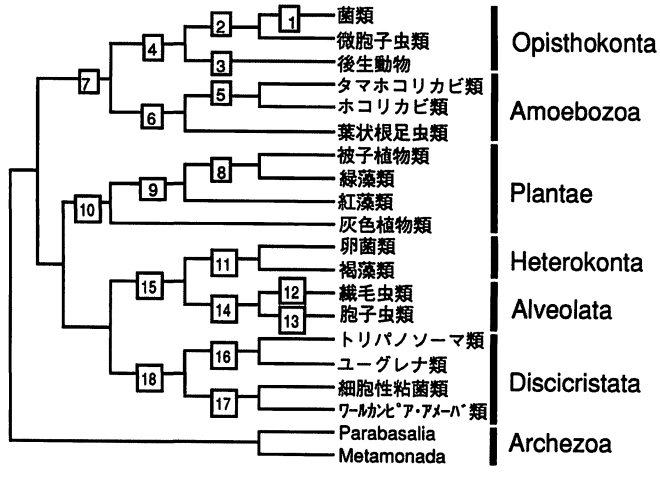

図1　Baldaufら[2]が示した分子系統樹を改変したもの。各ノードの数字は表1に示したノードに対応している。

表1　図1の各ノードの支持・不支持を分子種別に示したもの(Baldaufら[2]の改変)。
a：リボソームRNA遺伝子，b：Baldaufら[2]では解析に使用しなかったタンパク・コード遺伝子，c：Baldaufら[2]で解析を行ったタンパク・コード遺伝子，d：cの4つの遺伝子を合わせて解析したもの。
○：支持についてのブートストラップ確率（BP）が70%以上，△：支持のBPが70%以下，／：不支持のBPが70%以下，×：不支持のBPが70%以上。

	genes											
	a			b				c				d
nodes	SSU rRNA	LSU rRNA	LSU + SSU	EF-2	V/A-ATPase	HSP70-cy	RPB1	actin	α-tubulin	β-tubulin	EF-1α	combined
1	○	○	○	△	○	○	○	○	／	／	○	○
2	×	×	×	△			○		○	○	×	○
3	△	○	○	△	○	○	○	△	○	○	○	○
4	○	／	／	○	／	／	△	○	○	○	○	○
5	×	×	×					△	／	○	○	○
6	○							○				○
7	×	×	×	／		△	／	△	／	／	△	○
8	○		○	○	○	○		○	／	○		○
9	／		／	○	△	×	／	△	／	×	／	○
10	／					△		△	／	×		△
11	○		○					○		○		○
12	○		○	△		○		×	△	／	／	○
13	○	○	○	△		○		○	○	○	○	○
14	○	○	○	○		○		×	△	○	／	○
15	△	／	／	／		○		×	／	△		○
16	○	／	○	○				△	△	×	○	○
17									○			○
18	×							×	△	△	／	○

Chromista）を提唱しており，葉緑体が核の外膜から連続する粗面小胞体の中に位置して計4重の包膜をもつことでまとめられる。しかしクロミスタ界の単系統性については，リボソームRNA遺伝子などの分子系統樹では基本的に支持されないことから疑問視されることが多い。今回の系統樹構築に用いた遺伝子のDNA配列データが得られれば，上記の問題に対する結論が得られるかもしれない。

5）Alveolata系統群：マラリア原虫を含む胞子虫類と繊毛虫類に，この系統樹では登場しないが渦鞭毛藻類が加わった系統群である。細胞膜のすぐ内側にアルベオールと呼ばれる袋状の裏打ち構造があることを共通の形質としてもつ。渦鞭毛藻類が葉緑体をもつことと，胞子虫類にも葉緑体が光合成能を失って矮小化したと思われる35kbの環状DNAを含む包膜構造があることから，Alveolata系統群は分岐した初期にPlantae系統群の生物と真核生物－真核生物間共生が成立していたとする考えがある[6]。

さらにHeterokonta系統群とAlveolata系統群が単系統群を形成することが示唆された。表1のノード15の列に示されているように，それぞれの遺伝子を単独に扱って解析すると両者の単系統性はあまり支持されないが，遺伝子をまとめて解析することにより強く単系統性が支持された。またArisueら[7]は，Heterokonta系統群の初期に分岐したと思われる葉緑体を持たない*Blastocystis hominis*について同様の遺伝子群を解析し，このデータを付加すると，Heterokonta系統群とAlveolata系統群の単系統性がより強く示唆されたとする結果を報告している。Cavalier-Smith[8]はHeterokonta系統群とAlveolata系統群を合わせた大系統群にchromalveolatesという呼称を与えている。

6）Discicristata系統群：ユーグレナ類，トリパノソーマ類，細胞性粘菌類などが含まれる系統群。ミトコンドリアのクリステが平らで基部がくびれることで円盤状となる共通の形質をもち，この呼称の由来にもなっている。図1の系統樹には現れないが，Discicristata系統群の姉妹群であることが期待されるCercozoa類にはクロララクニオン藻類が属する（図2）。

7）Archezoa系統群：ミトコンドリアを欠くMetamonada類，ミトコンドリアの代わりにヒドロゲノソームをもつParabasalia類から構成される系統群で，真核生物の中では最初に分岐する系統樹が得られることが多いので，ここでは外群として位置づけられている。

真核藻類は非常に多様な形態をもち，その色素組成など生理学的な特徴も多様であることから，少なくとも9つの分類群として認識されており，葉緑体を獲得するための細胞内共生が分類群の数だけ何度も起こったことで理解する考え方がある。一方で今回の4遺伝子からの系統樹を基にして真核藻類の系統的分布を考えると，緑色植物／紅藻／灰色植物，chromalveolates，Discicristata / Cercozoa，の大きな3つの系統群としてまとまってくることがわかる。その上で共生のイベントの回数を予測した場合，緑色植物／紅藻／灰色植物の系統の初期に一次共生が成立し，他の2つの系統で二次共生が別個に計2回が起こったと考えることが最節約的な解となる（図2）。

まだまだこのような調査がなされた生物数が少ないこと，複数の遺伝子の配列データを合わせて解析する方法が正しい系統関係を推定できるかどうかにも疑問の余地があることなど，今後のデータ蓄積によってようやく明確になっていくことも多いと思われるが，真核生物全体における藻類の系統学的概念が改めて構築されようとしている時に居合わせているように思う。そしてその新たな概念は真核生物の多様性の理解をもたらすだけでなく，材料としての藻類という観点からも，研究対象とする生物の選択に対し有効に働くと期待できる。

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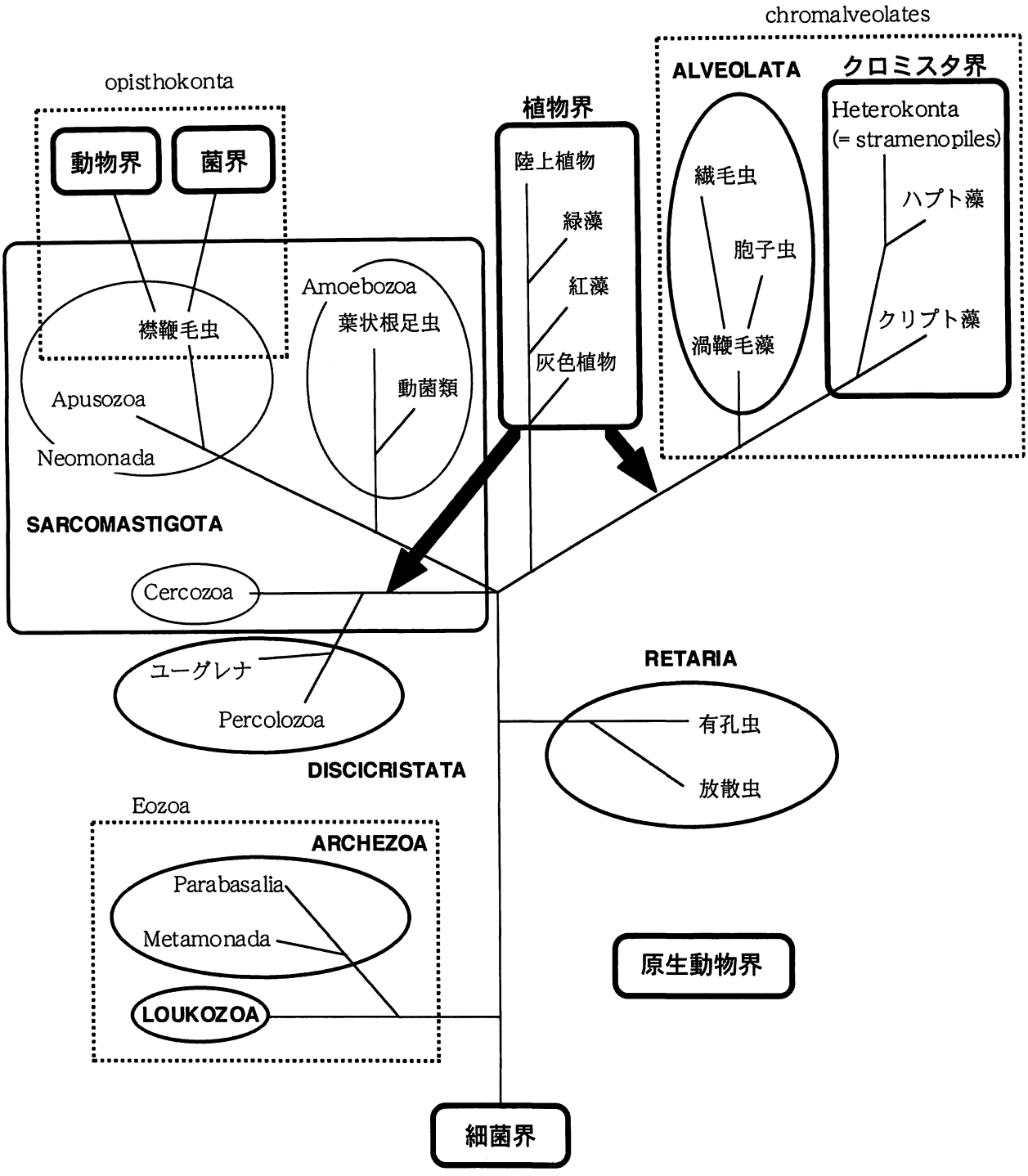

図2　Cavalier-Smith [8]が示した生物全体の系統関係に関するスキームを改変したもの。原生動物の多系統性，界や門の範疇を越えた大系統群としてopisthokonataやchromalveolataの存在が表現されている。太い矢印は予想される二次共生による葉緑体の獲得を示している。すべてを大文字のアルファベットで示した分類名は，Cavalier-Smith [8]が提唱する原生動物界内に属する6つの下界（infrakingdom）である。

pp. 339-353.
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吉田 忠生：太平洋の有用海藻分類学ワークショップ
Workshop on Taxonomy of Economic Seaweeds

海藻が分類学以外のさまざまな分野で関心を持たれるようになってきて，対象としている海藻の種類を明確にする必要に迫られることが多くなった。国により，研究者によって異なった名前が用いられれば，それから得られる知見を利用するのが困難である。このような観点から，各国の研究者が集まって名前の統一を図る試みがハワイ大学の Abbott 教授と California Sea Grant College Systemの Sullivan 所長の協力によって実現した。

はじめ Workshop on Taxonomy of Economic Seaweeds として発足した集まりは，環太平洋諸国の研究者がそれぞれ関係の標本を持ち寄り，それをいっしょに観察したり議論したりしてそれぞれの種についての知識を深め，各国で違った名前で呼ばれているものがあれば統一に努力するという，これまでにない画期的な企画である。

第1回目はグアム大学の Roy Tsuda（以下敬称略）のお世話で，University of Guam の Marine Laboratory において 1984 年 6 月 15 日から 20 日にわたって開催された。日本からはオゴノリ属が専門の山本弘敏（北大水産学部），ホンダワラ属のグループとして吉田忠生（北大理学部）が招待され，参加することになった。オゴノリグループは山本のほかに I.A. Abbott, J.N. Norris, R.T. Tsuda, 夏邦美（Xia Bangmei），S. Fredericq, 江永綿（Young Meng Chiang）がメンバーになった。ホンダワラグループは曾呈奎（C.K. Tseng），江永綿と吉田の 3 人で構成された。ほかにテングサグループとして B. Santelices, J.G. Stewart，キリンサイグループは M.S. Doty と J.N. Norris であった。グループは決まっていても，ときどき他のグループの議論に加わることもあり，これも有意義なことであった。

図 1 第 1 回ワークショップ。グアム大学 Marine Laboratory にて。
前列左より Fredericq, Tsuda, 江 , Stewart, 山本
後列左より Santelices, Garique, 夏 , Abbott, 曾 , 吉田 , Doty, Norris

あいだに 1 日の休みを置いて 1 週間，毎日持ち寄った標本を前にして議論をするのは初めての経験であり，さらに慣れない英語でのやりとりは非常な苦労だったし，有益でもあった。これは山本も同様で，お互いに強烈な印象を持ち，夜には山本と二人で日本酒を酌み交わすこともあった。

最終日には全員が集まって報告会をして，各グループの成果を発表し，お互いの理解を深めた。

ホンダワラ属については，吉田が Bactrophycus 亜属のモノグラフを纏めた直後ということもあり，この亜属の種について中国・台湾の種類との異同を論じた。ほかのグループの結果と共に報告書として纏められ，Taxonomy of Economic Seaweeds, with reference to some Pacific and Caribbean species として 1985 年 9 月に出版された。

第 2 回目は曾呈奎がホストとなり，中国科学院海洋研究所（青島）が開催場所となった。1986 年 9 月で，このときは日本からはホンダワラグループに吉田のみが参加した。青島までは直接行けないようで，9 月 18 日に札幌を出発して成田空港で国際線に乗り，北京で一泊して夜行列車で 21 日午後にやっと青島に着いた。

22 日に開始されたワークショップは，ホンダワラグループに曾呈奎，陸保仁，Roy Tsuda, W.H. Magruder と吉田が加わり，オゴノリグループは Abbott, 夏邦美, 張俊甫（Zhang Junfu）からなり，テングサグループは Santelices, 張俊甫, 夏恩湛（Xia Enzhan），ソゾグループは Karla McDermid と張俊甫というメンバーだった。

ホンダワラ属については，第 1 回で日本を中心としたアジアに分布する Bactrophycus 亜属を片付けたことになっている

図 2 第 2 回ワークショップ。中国科学院海洋研究所（青島）にて。
前列左より 張 , Tsuda, 曾 , Abbott, Sullivan, 吉田
後列左より 陸 , McDermid, Magruder 一人おいて , 夏

ので，亜熱帯から熱帯に広く分布しているSargassum亜属に取りかからなければならない。台湾の江永綿が中国にこられないのが残念である。ハワイの標本，グアムの標本を見ていくと，太平洋中央部の地域で種類数が少ないことがはっきりする。中国からは多数の種類が記載され，お互いの関係を明らかにするまでには行かない。各地域の種類を詳しく記載することとして，吉田は日本と台湾から記録されているものを検討した。

開催が9月で，採集にはいい時期ではなかったけれども，青島の近くで観察したフシスジモクは日本のものとかなり異なっている印象だった。それでも*Sargassum pallidum*の名前で呼ぶことはできないと中国側に納得してもらうのは大変だった。

3回目は1989年8月に開催され，Vancouverで行われたInternational Phycological Congress (Vancouver) の直前に期間が設定された。スポンサーのひとつのCalifornia Sea Grant College Systemがある San Diegoで，Scripps Institution of Oceanographyが会場となった。

テングサグループはR.A. Melo, R.E. Norris, B. Santelices, J.A. Stewartで，オゴノリグループにはI.A. Abbott, Nguyen Huu Dinh, J.N. Norris, 夏邦美，張俊甫が参加した。ホンダワラグループは鯵坂哲朗，江永綿，J.A. Kilar, 李仁圭（In Kyu Lee），曾呈奎，陸保仁，G. Trono Jr., 吉田が加わってもっとも人数が多くなった。

第2回目からSargassum亜属を扱うようになって吉田だけでは対応しきれないので，熱帯のホンダワラ類にくわしい鯵坂（京都大学）に参加してもらった。韓国にはSargassum亜属の種は分布しない。そこで李仁圭には別に韓国の種について纏めてもらうことにした。Kilarは大西洋の種類を扱っており，Smithsonian Institutionの多数の標本も参考にして，大西洋と太平洋に共通種があるかどうかを検討した。よく似た種はあっても同じ種とは認められない，共通種はないという結論になった。中国とPhilippinesは種類が多く，それらの分布範囲もはっきりしないので，別々に報告をしてもらうことになった。

閉会の集まりで次は札幌という声が出た。

第4回のワークショップはなんとなく札幌で開催することを引き受けざるを得ない状況になってしまった。参加者の交通費は大部分アメリカ側で負担するとはいえ，滞在費などの経費を受け持つのに苦労した。北大の杉野目記念会に支援をお願いしたり，オプザーバーの参加費を頂いたりして充当し，さまざまな雑事については教室の諸君にも多大の迷惑をかけることになってしまった。こうして1991年7月に北海道大学理学部での開催にこぎつけた。

テングサグループに李海福（Lee Hae Bok），宮田昌彦（千葉県立中央博物館），B. Santelicesが加わり，オゴノリグループはI.A. Abbott, 大野正夫（高知大学），Anong Chirapart, Alan Critchley, Phang Siew Moi, K. Lewmanomont, 夏邦美，山本弘敏で組織され，オキツノリ科グループに増田道夫（北海道大学），R.E. Norris, 張俊甫，夏邦美が参加した。

ホンダワラグループとして曾呈奎，陸保仁，江永綿，G. Trono Jr.のほか日本から鯵坂，野呂忠秀（鹿児島大学），吉田のメンバーとなった。野呂には在外研究のあいだにオーストラリアの種類を勉強してもらっていた。今回も中国とPhilippineの種類については別に多くの新種の記載がなされた。日本グループは分岐した葉をもつ種群，平たい枝をもつ種群という共通の特徴を持っている種類について調べた。

アメリカの大学では学生寮を利用することができる。日本では無理な相談で，学内にあるクラーク会館のほか市内のホテルも使用するなど宿舎についても問題があった。また，これまでの集まりの間には主催者の自宅に参加者全員を招待するのが習慣になっているけれども，主催した吉田にとっては不可能なので，市内の和風レストランでの夕食にした。エクスカーションとしては小樽市忍路の臨海実験所にいって日本の海藻を観察してもらう機会を作った。いろいろな困難があったけれども，何とか役目を果たすことが出来たと思っている。

第5回のワークショップはAbbottのいるUniversity of Hawaii at Manoaが会場になった。1993年6月である。

グループのメンバーはいつも多少の入れ替わりがある。テングサグループは李海福，宮田昌彦，Santelicesとなり，オゴノリグループにはAbbott, 馬場将輔（海洋生物環境研究所），李仁圭，K. Lewmanomont, J.N. Norris, Phang Siew Moi, G.R. South, 夏邦美，山本弘敏，張俊甫が加わり，ホンダワラグループは曾呈奎，陸保仁，鯵坂哲朗，野呂忠秀，吉田，Phang Siew Moi, Nguyen Huu Dinh, Nang Hynh Quangで構成され，N. Phillipsは論文のみで参加した。曾呈奎らは中国の種だけに関心を持っていて，他国の種類には意見を述べない。われわれはできるところから種の形態的な変異や分布についていくつかの種を取り上げて詳しく記述することに努めた。

6回目の会場はKuala LumpurのUniversiti Malaysiaで1995年7月に開催された。テングサ属についてはSantelices一人の参加で，オゴノリグループのAbbott, A.J.K. Millar, 大野正夫，夏邦美，山本弘敏のほか，イバラノリグループが新しく加わり江永綿，増田道夫，山岸幸正（北海道大学），K. Lewmanomont, 夏邦美で組織された。ホンダワラグループも鯵坂哲朗，Put O. Ang, Jr., 陸保仁，Hyunh Quang Nang, Nguyen Huu Dinh, Phamg Siew Moi, 吉田というようになった。Vietnamの種類を中心に，Malaysiaで発見された新種のホンダワラ類などについて報告を纏めた。

2年ごとに定期的に実施されるようになったワークショップの第7回は1997年5月にThailandのPhuket島にあるMarine Biological Centerが会場になった。

テングサ属は前回同様Santelicesだけ，オゴノリグループにはAnong Chirapart, Millar, 大野正夫，山本弘敏は欠席で寺田竜太（北海道大学），G.S. Gerungが参加した。新しいイソノ

ハナ *Halymenia* グループは川口栄男（九州大学），K. Lewmanomont, 夏邦美, Abbottで組織され，ホンダワラグループは前回のメンバー鯵坂哲朗，Put O. Ang, Jr., 陸保仁，Hyunh Quang Nang, Nguyen Huu Dinh, Phamg Siew Moi, 吉田のほかに曾呈奎，野呂忠秀とD. Rodriguezが加わった。中国，Malaysia, Vietnamのそれぞれの地域についての知見を報告した。メキシコ太平洋岸からのホンダワラ属の標本も調べて，太平洋のアメリカ側とアジア側でも共通種がないという印象を持った。

ワークショップの詳細については寺田：藻類45:137-138, 1997が記録している。

第8回はVietnamのNhatrangにあるInstitute of Oceanographyで1999年4月に行われた。この研究所には以前Pham Hoang Hoが在職し，Feldmann, Dawsonや田中剛が研究に訪れたことがある。

ここでもテングサ（Santelices, 増田道夫，嶌田智），オゴノリ・キリンサイ（Abbott, 夏邦美, Pham Huu Tri, Lewmanomont, A. Chirapart, 寺田竜太，J. Fisher），イソノハナ（川口栄男，K. McDermid, Abbott）とホンダワラ（曾呈奎，陸保仁，鯵坂哲朗，野呂忠秀，Nguyen Huu Dai, 吉田）の4グループでそれぞれ合議しながら標本の観察を行った。

ホンダワラグループでは，Pham Hoang Hoのコレクションを検討することから始まり，Daiの著書についても元となった標本を調べて，ヴェトナムのホンダワラの種類をはっきりさせるのに重点を置いた。エクスカーションの際に採集した材料などを持ちかえってDNA抽出をして，分子系統の立場からホンダワラ属の系統を考えられるようになった。

このワークショップの経過は嶌田：藻類47:153-154, 1999に詳しい。報告書は2002年9月発行予定である。

9回目を数えるワークショップは事情により開催が遅れ，2002年5月にハワイ島にあるUniversity of Hawaii at HiloのMarine Science Buildingで実施された。今回はWorkshop on Taxonomy and Diversity of Economic Seaweeds in the Pacific Basinという名称になっていた。

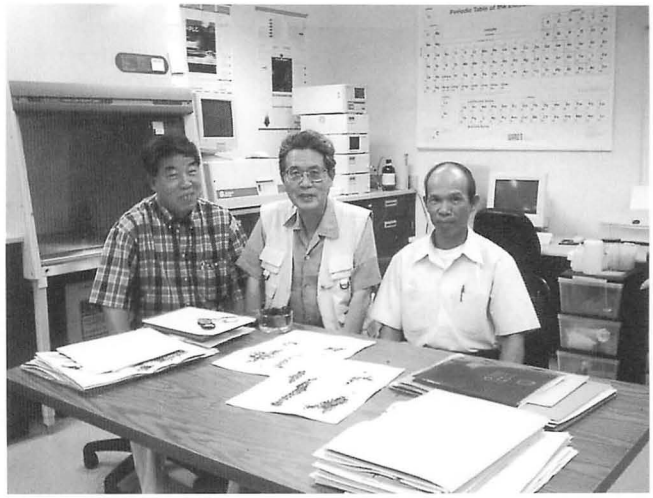

図4 第9回ワークショップ。ハワイ大学ヒロ校にて。
左より 陸，吉田，Dai

オゴノリグループは寺田，Abbott, R. South, P. Skelton, Lewmanomont, Chirapart, 夏邦美，ムカデノリ科グループに川口，Millar, J. Fisher, Skelton, J. Norris, 夏邦美，イワズタグループにL. Hodgson, Pham Huu Tri, C. Puttock, Lewmanomont，アミジグサグループはR. Tsuda, Abbott, Norris，テングサグループはSantelices, R. Okano, B. Stuercke，ソゾグループには南基完（Nam Ki Wan), K. McDermid, Millar，ミルグループにP.C. Silva, C. Puttock, Lewmanomont, J. Huisman，ホンダワラグループは陸保仁，Nguyen Huu Dai，吉田であった。

ホンダワラ属については，中国から4新種を記載することとし，ヴェトナムからは前回の検討で同定の誤りを指摘されていた2種を記述しようとしていた。吉田はヤツマタモク群の再検討を試みるというように，別の報告を書く事にした。

今回はじめて参加したP.C. Silvaは分類と命名についての講義や，長年研究しているミル属の話しも有益だった。Huismanによるオーストラリアの海藻のスライドショーもあった。

研究室での活動とは別に毎朝のように近くの海岸に出かけて，海藻の生態を観察した。波の荒いところにはサイミが群落を作り，穏やかなところにはフシクレノリが多量に生育し

図3 第8回ワークショップ。ベトナムにて。ホンダワラグループ。
左2人目より Dai, 野呂，曾，吉田，陸，鯵坂

図5　第9回ワークショップ開催地（ハワイ大学ヒロ校キャンパス）

ていた。市場ではサラダとしてミル，“なます”などの加工品にオゴノリ属のいくつかの種がOgoという名前で使われているのを見た。

ハワイ島Konaの近郊にある海洋深層水施設のちかくでオゴノリ属数種のタンク養殖をしている人が生の材料や製品を披露してくれた。

エクスカーションで訪れたPunalu'u Black Sand BeachやHawaii Volvanoes National Parkも楽しかったし，降雨林の中にあるKarla McDermidの自宅にも驚かされた。

19年間に9回開催されたワークショップは参加者の顔ぶれもさまざまであったし，国別でいえばアメリカ，日本，韓国，中国，台湾，ヴェトナム，タイ，マレーシア，オーストラリア，南アフリカ，フィージー，チリー，メキシコと太平洋を囲む多くの国から集まった。9回のすべてに出席できたのはConvenerのAbbottのほかSantelices, 夏邦美，吉田だった。

幾つかの国の研究者が標本を持ち寄ってそれについての意見を述べ合い，顕微鏡観察をしながら議論する，これまでにない集まりで，各分類群について多くの成果をうることができた。つぎつぎに新しい研究者も加わり，少数ながら若い世代にも刺激を与えることが出来たと信じている。日本から参加した若い世代が各グループで主導的な役割を果たしていたのも心強い限りであった。

各回ごとに報告書Taxonomy of Economic Seaweedsが California Sea Grant College System (University of California, 9500 Gilman Drive, La Jolla, CA 92093-0232) から出版されている。価格は1冊10 USドルで，上記のところから入手できる。

（818-0103 太宰府市朱雀6-13-13）

学会・シンポジウム情報

2002　秋季藻類シンポジウムのご案内

「新しい海藻由来の製品の科学的検討」

海藻は，伝統食品として日本人の健康に大きく関わっており，その効果が科学的に解明して価値が再認識されている。さらに，海藻の抽出成分を素材にした健康・化粧製品が注目されており，海藻多糖類の寒天，カラギナン，アルギン酸の利用も多様な分野にわたっている。しかし，私達は身近な製品が海藻由来のものであることを知らない。

そこで，最新の海藻由来の素材・製品を科学的に紹介して，その効能を知り，海藻が２１世紀の資源として利用の可能性を，一般の方々にも理解できるように講演される。進んでご参加，ご聴講下さい。

日　時：平成１４年１２月６日（金曜日）
　　午後１時より５時，シンポジウム終了後懇親会
場　所：ロイヤル・パークホテル
　　（東京・日本橋，地下鉄半蔵門線，水天宮駅隣接）

参加費：シンポジウム無料（講演集 1,000 円）
　　懇親会：8,000 円

共　催：日本藻類学会，マリンバイオテクノロジー学会，日本海藻協会

参加・問い合わせ先：
日本海藻協会事務局：大野正夫
（高知大学海洋生物教育研究センター）
TEL& FAX : 088-856-0462　E-mail: mohno@cc.kochi-u.ac.jp

会場準備のために，シンポジウムと懇親会参加者は，10 月末日までに上記の事務局へで御連絡下さい

2002 年度「藻類談話会」のお知らせ

「藻類談話会」は藻類を研究材料とする幅広い分野の研究者の集まりで，西日本を中心に講演会や研究交流を行っています。今年度は以下の講演を企画しています。ふるってご参加くださいますようご案内申し上げます。

日　時：2002 年 11 月 9 日（土）13：00-17：00
場　所：京都大学農学部総合館 W-524 号室
　〒606-8502　京都市左京区北白川追分町

講演予定（敬称略）
坂東忠司（京都教育大・教育）：
　　ホウネンエビと共に生きる藻類
大城　香（福井県立大・生物資源）：
　　ラン藻の窒素固定
左子芳彦（京都大院・農）：
　　有毒渦鞭毛藻の遺伝子診断法の開発
伊藤裕之（神戸市水道局・水質試験所）：
　　浄水処理障害の原因となる藻類について
吉田吾郎（水産総合研究センター瀬戸内海区水研）：
　　広島湾におけるホンダワラ類の生態研究と藻場造成

参加費：500 円（通信費など）

談話会終了後，北部キャンパス内生協 2 階「ほくと」で懇親会を行います。会費：一般 3,500 円，学生 1,500 円）。

談話会および懇親会の参加希望者は 10 月 28 日(月）までに電子メールかファックスで下記の宛先へお申し込みください（当日参加も可）。また最新情報は下記ホームページに適宜，掲示しますのでご覧ください。

＊会場への道順
市バス「百万遍」下車徒歩約 5 分，または「農学部前」下車すぐ。京阪出町柳駅から徒歩約 15 分。

参加申込・問合先
〒606-8501 京都市左京区吉田二本松町
京都大学総合人間学部自然環境学科　幡野 恭子
TEL : 075-753-6854　FAX : 075-753-6864
e-mail : hatano@bio.h.kyoto-u.ac.jp
2002 年度藻類談話会ホームページ：
http://biotech1.nikkeibp.co.jp/100HP/ より「バイオ・基礎医学関係者の皆のホームページ」→「学会・研究会」の項目をお選び下さい。

会員のページ

アマモ場についてのビデオ紹介と頒布のお知らせ
—海の草原 ～ アマモ場回復のために—

浅海域の砂泥底に拡がるアマモ場は稚仔魚の隠れ家や餌場として重要で，沿岸漁業においてはその存在が漁獲量に直接の影響を与えることもある。しかしながら，日本各地のアマモ場は沿岸域の埋め立てや汚染によって次々に失われてきた。このビデオは，アマモ場の回復のために立ち上げられ成功をおさめたプロジェクトについての記録である。

この研究プロジェクトでは，やみくもにいろいろな復元技術を試してみるのではなく，まずはアマモ場が存続できない要因が何であるのかについての解明に取りかかり，その後にその制限要因をいかに緩和するかについての対策を講じている。「アマモ場の再生を行う」というただひとつの共通の目的のもとに，生物学者のみならず海岸工学者や漁業者等がグループを作り，共同で研究調査に関わったということも特筆に値する。

ビデオの企画者は社団法人マリノフォーラム21の浅海域緑化技術開発グループ（幹事：森田健二氏）である。アマモ場回復のモデル地区となったのは，以前から地元漁協組合員によるアマモ場復元の試みが続けられていた岡山県日生町で，平成9年から平成13年に調査と試験が行われた。

アマモ場消失に働く複合要因についての検討に基づいて，プロジェクトのスタートとともに3つの課題設定が行われた。まず，平成10年には，アマモ場の光環境についての調査が行われ，純生産量が光環境の評価に役立つことが明らかとなった。次の年には，水理環境についての調査が行われ，波や流れの強度変化が気象条件や海底地形と併せて解析され，アマモの生育可能な波高の限界値が求められた。続いて底質環境についての調査が行われた。地下茎を有するアマモの支持基盤としての調査から，好適な底質粒度が求められた。これらの調査結果を踏まえて，復元を要する海域において何が制限要因になっているのかを見極めたうえで，それぞれの場所に最適な制限要因緩和策が講じられていった。それによって無駄の少ない効率的な浅海緑化を行うことができたのである。

新しい播種技術についても紹介されている。土嚢を用いたマット法，コロイダルシリカによる播種，粘土利用の移植法が紹介され，復元すべき海底の状況によっての使い分けが行われている。このビデオにより，アマモ場再生技術の最前線に触れることができる。また，プロジェクトの立ち上げから課題設定，調査と復元作業に至る流れから，異分野間にまたがる基礎調査を野外での応用技術に結びつけていく作業過程の良き手本を見ることができる。藻類学会の会員の方々のみならず，藻場造成に関わる研究機関や企業，環境問題についての研究者，海洋土木工学の研究者，群集研究などを行う海洋生物学者などの方々にも是非手元に置いていただきたいビデオである。

ラストシーン近くで流れる瀬戸内海区水産研究所の寺脇利信氏による次のコメントは，藻場の回復技術というものを今後どのように理解して人々が使っていくべきかについての示唆を与えている。「アマモ場回復のために現地の海底で行われる技術についてはこのように着実に進歩してきています。しかし，これらの技術の効果を飛躍的に高めるためには，汚れのないきれいな水の海であることが最も大切なことになります。アマモ場回復という問題は私達の普段の生活と深く関わっているのです。」

このビデオの他に，磯やけ問題についての啓蒙ビデオおよびガラモ場植生の回復についての実地試験についてのビデオも頒布中である（いずれもVHS）。

1.「海の草原～アマモ場回復のために」（20分）
2.「磯やけって何？～長崎県野母崎町を訪ねて」（20分）
3.「豊かな暮らしのために～自然の藻場にならう階段藻礁」（23分）

ビデオ入手方法

申し込み（FAXに限る）：FAX 03-5202-6066「毎日EVRシステム内　藻類絵はがきの会ビデオ係」
申込書記入事項：希望ビデオのタイトルと本数・氏名（振込者名）・住所・電話番号・FAX番号
料金（税・送料700円込）はいずれも，1本3,360円，2本5,985円，3本8,610円
振込先：三和銀行東京営業部　当座預金口座005902
（株）毎日イーヴィアール・システム
振込手数料は申込者負担。振込確認次第ビデオを発送。
問合先：毎日EVRシステム　佐藤建男 Tel 03-5202-6060

（青木優和　筑波大学生物科学系下田臨海実験センター）

Phycological Research

英文誌 49巻4号掲載論文和文要旨

Bayer, M. M.・Droop, S. J. M.・Mann, D. G.：藻類研究，特に微細藻研究に関するデジタル顕微鏡について

Micha M. Bayer, Stephen J. M. Droop and David G. Mann: Digital microscopy in phycological research, with special reference to microalgae

デジタル画像技術は生物学的研究のあらゆる分野で徐々に組み入れられているが，各専門分野の科学者を対象にした情報源は明らかに不足している。標本の形態計測あるいは視覚解析，分類学的データベース，および従来の顕微鏡写真に代わるデジタル写真の発行など，藻類学にデジタル画像技術を取り入れる価値はある。ここでは一般的なデジタル画像技術と，特に微細藻研究分野における可能性について紹介する。また，初心者が高解像度のデジタル画像ライブラリーを構築するための本質的な画像撮影や最適化について数多くの技術を紹介する。デジタルカメラを使う際に必要な解像度について説明し，顕微鏡の解像度と比較する。デジタル画像技術の利点について議論し，従来のハロゲン化銀技術と対比する。(Royal Botanic Garden Edinburgh, UK)

辻村茂男[1]・石川可奈子[2]・塚田創[2]：琵琶湖と余呉湖に出現したラン藻 *Aphanizomenon flos-aquae* の増殖に及ぼす温度の影響

Shigeo Tsujimura, Kanako Ishikawa and Hajime Tsukada: Effect of temperature on growth of the cyanobacterium *Aphanizomenon flos-aquae* in Lake Biwa and Lake Yogo

アオコを形成するラン藻 *Aphanizomenon flos-aquae* Ralfs ex Bornet et Flahault（ラン藻綱ネンジュモ目）が琵琶湖と余呉湖で1999年に初めて出現した。その形態特徴を野外試料を用いて記述した。琵琶湖と余呉湖に出現する他のアオコ形成ラン藻類 *Microcystis* や *Anabaena* と違って，*A. flos-aquae* は夏期に少なく冬季に増殖する傾向があり，この種が好低温性あるいは耐低温性であることが示唆された。増殖に及ぼす温度の影響を調べるため，琵琶湖より分離した無菌培養株を用いて培養実験を行った。この培養株は23-29℃で最適温度を持ち8℃で増殖可能であった。5℃においても低光条件では少なくとも25日の生存が確認できた。これらの結果は，晩秋から冬におけるアオコ形成が可能であることを示すものであった。しかし，なぜ *Microcystis* と *Anabaena* のブルームが消滅した直後の秋でなく，水温が約10℃まで低下する12月になって *Aphanizomenon* のアオコが生じたかはまだ不明である。([1]琵琶湖研，[2]京大院・農)

小亀一弘[1]，上井進也[1]，川口栄男[2]：*Asteronema rhodochortonoides*（褐藻綱）の太平洋における初記録

Kazuhiro Kogame, Shinya Uwai and Shigeo Kawaguchi: First record of *Asteronema rhodochortonoides* (Phaeophyceae) for the Pacific Ocean

南日本の福岡県津屋崎（33°48'N, 130°27'E）で採集した材料において，ウミトラノオ（*Sargassum thunbergii*）に付着していた小さな分枝する糸状の褐藻の形態学的観察を行った。この藻は，中央に複数のピレノイドが集まった星状に配置する葉緑体をもち，頂端生長的で，細胞糸は細く，直立糸に頂生または側生する楕円形または幅広の楕円形の複子嚢をもつことから，*Asteronema rhodochortonoides* (Børgesen) Müller et Parodi と同定した。日本の材料とカナリー諸島の *A. rhodochortonoides* との核小サブユニット rDNA の部分塩基配列の比較では，2または3塩基の違いしか見られなかった。これは，太平洋での初報告として日本の藻をこの種に当てた我々の同定を支持している。室内培養において，複子嚢から放出された遊走細胞は，野外で採集した植物体と同様の形態をもつ植物体に発達した。我々の培養において，このサイクルは単子嚢を形成せずに繰り返した。([1]北大院・理，[2]九大院・農)

West, J. A.[1]・Hommersand, M.[2]・Zuccarello, G. C.[3]：培養条件下における *Bostrychia pinnata* の形態と生殖

John A. West, Max Hommersand and G. C. Zuccarello: Morphology and reproduction of *Bostrychia pinnata* (Rhodomelaceae, Ceramiales) in laboratory culture

Bostrychia pinnata の栄養藻体株は単藻培養下においてしばしば四分胞子嚢を形成し，正常な四分胞子を放出した。ほとんどの四分胞子は成熟前に正常に分枝したが，なかには分枝しない幼体（<1mm）上にプロカルプを形成し，その後に不動精子嚢を形成する胞子もあった。正常に分枝した配偶体（>2mm）のほとんどは初期には雌で，古く大きくなると（>5mm）両性化した。単性の雄性配偶体は培養下ではみられなかった。果胞子体は成長が遅く（40-70日），不稔か果胞子嚢の数が減少することがあった（天然では40-50個であるのに対し培養藻体で10-15個）。果胞子は四分胞子よりも発芽が遅く，四分胞子体は成熟するまでに6ヶ月を要した。イトグサ型生活史は9-12ヶ月で完結した。ひげ状付着器（peripherohaptera）は培養藻体では観察されなかった。フロリダ，グアテマラ，ペルーの培養株は有性生殖器官を形成しなかったが，下部の枝を定期的に脱離することによって栄養繁殖を行った。何年にもわたる培養の結果，ほとんどの培養株が自家不和合になった。雌性配偶体では，1つもしくは2つのプロカルプが各セグメントに形成され，ほとんどが4細胞性で，3細胞性もわずかにみられた。3—7細胞の不稔細胞群もプロカルプに1つずつ観察された。受精後，造果器内の複相核は2回分裂し，キャッピング構造と受精毛を切り離し，助細胞に隣接する連結構造を形成した。通常の成熟した嚢果では不稔細胞群は残存し，中央の空洞に粘液を分泌した。成

熟した果皮は4細胞層（1層の主軸フィラメントと3層の皮層細胞）で構成されていた。偽嚢果は普通にみられ，果胞子嚢を作らず，伸長した不稔細胞を含み，部分的に発達した果皮によって覆われていた。造果器には通常4つの核がみられることから，発達に失敗して偽嚢果になったものと思われる。（[1]Univ. Melbourne, Australia, [2]Univ. North Carolina, USA, [3]Univ. New South Wales, Australia）

Yang, Y.[1]・ Gao, K.[1,2]・Xia, J.[2]：*Chlamydomonas reinhardtii* の成長と光合成に対する大気中のCO_2濃度倍加による効果

Ying Yang, Kunshan Gao and Jianrong Xia: Effects of doubled atmospheric CO_2 concentration on the growth and photosynthesis of *Chlamydomonas reinhardtii* (Volvocales, Chlorophyceae)

淡水産微細藻 *Chlamydomonas reinhardtii* Dangeard を350ppmvおよび700ppmvのCO_2濃度下で培養し，成長と光合成に対するCO_2濃度倍加の影響を測定した。成長速度，光合成効率，最大純光合成率および光飽和点は，2つのCO_2濃度条件で有意差はみられなかった。どちらのCO_2濃度条件下で培養した細胞も，高光量子束密度条件にさらすと，光阻害のために光依存O_2放出率と光化学効果（F_v/F_m）が減少した。しかし，高CO_2濃度下で培養した細胞は光阻害効果がより弱く，初期の回復過程中の弱光もしくは暗黒下においてより良い回復効率を示した。（[1] Shantou Univ., China, [2] Chinese Academy of Sciences, China）

Necchi, O. Jr.・Zucchi, M. R.：温度，光強度，pHおよび日長条件に対する淡水産紅藻の光合成活性

Orlando Necchi Jr. and Marcelo Ribeiro Zucchi: Photosynthetic performance of freshwater Rhodophyta in response to temperature, irradiance, pH and diurnal rhythm

淡水産紅藻8種15集団の天然および培養藻体において，温度，光強度，pH／無機炭素および日長に対する純光合成速度の反応を解析した。光合成速度は明瓶暗瓶法を使って酸素濃度により決定した。光合成-光強度曲線から導かれたパラメーターにより，解析した全ての淡水産紅藻が低い光強度に対して適応していることが示され，これらの藻類が低照度条件下で生育しやすいことが確かめられた。ある程度の光合成阻害（ß=-0.33-0.01mgO_2 g^{-1} DW h^{-1}(µmol photons m^{-2} s^{-1})$^{-1}$）は解析した全ての種および集団でみられたが，光補償点（lc）は全ての藻体で極めて低かった（≦2 µmol photons m^{-2} s^{-1}）。飽和点も全ての藻体で低かった（lk = 6-54 µmol photons photons m^{-2} s^{-1}; ls = 20-170 µmol photons photons m^{-2} s^{-1}）。純光合成速度と暗呼吸速度は温度変化に相関を示した。純光合成に対する至適温度は種や集団によって異なっていたため，最も高い光合成活性は別々の温度（10，15，20もしくは25 °C）で観察された。暗呼吸速度は温度とともに上昇する傾向があり，20-25°Cで最も高い値を示した。1種を除き，pH8.5もしくはpH6.5で最も高い光合成活性を示し，重炭酸塩のような無機炭素に対して高い親和性をもつか，あるいは重炭酸塩やフリーの二酸化炭素をわずかに利用することが示唆された。調査した藻体すべてにおいて，日長の変化によって普遍的な光合成活性パターンがみられ，ある程度の変異はあったが2つのはっきりしたピークが観察された。はじめのより高いピークは午前中（7-11時）にあり，2番目のより低いピークは午後（14-18時）にみられた。*Batrachospermum* 1集団において，シャントランシア期の藻体とその配偶体の光合成活性を比較したところ，配偶体においておよそ2倍の活性を示し，これは以前に報告された値よりかなり低い結果となった。シャントランシア期の生理学的役割についてさらに解析が必要である。（São Paulo State Univ.）

Ghoshal, D.・Goyal, A.：単細胞緑藻の溶存無機炭素摂取における酸素阻害

Durba Ghoshal and Arun Goyal: Oxygen inhibition of dissolved inorganic carbon uptake in unicellular green algae.

単細胞緑藻は無機炭素を細胞や葉緑体に濃縮するために，DICポンプと呼ばれる溶存無機炭素（DIC）濃縮機構を持っている。DICポンプ活性は通常，DIC依存の光合成による酸素発生速度が最大値の半分となるようなpHでのCO_2＋HCO_3^-濃度と等しい$K_{0.5}$(DIC)か，あるいは制限された量の$NaH^{14}CO_3$を用いたシリコンオイル遠心法を用いて15-60秒で細胞内に蓄積されるDIC量により測定される。溶存酸素は，単細胞緑藻 *Chlamydomonas reinhardtii* Dangeard 株137および細胞壁のない海産藻類 *Dunaliella tertiolecta* Butcher において，DIC摂取を阻害もしくは減少させる。これらの細胞は，アッセイ培地内の酸素量がわずかあるいは全くない場合に，最も高いDIC量を濃縮した。これらの結果は，DICポンプアッセイ前にO_2とDIC量を入念にモニターしなければならないことを示唆している。（Univ. Minnesota Duluth, USA）

英文誌 50巻1号掲載論文和文要旨

Paula, E. D.[1]・Pereira, R. T. L.[2]・Ohno, M.[3]：ブラジル・サンパウロ州の暖海域に移植されたカラギナン藻*Kappaphycus alvarezii*の成長速度

Edison D. Paula[1], Ricardo Toledo Lima Pereira[2] and Masao Ohno[3]: Growth rates of the carrageenophyte *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) introduced in subtropical waters of São Paulo State, Brazil

ブラジルのサンパウロ州のウバツバ湾（232°6.9', 45°0.3'）において1995年10月から1996年10月まで，*Kappaphycus alvarezii* (Doty) Doty ex P. Silvaの枝20本（それぞれ約3 g）を海面に張られたロープに結び移植した。この海域の月平均水温は20.3～28.5℃（Min.17.0℃, Max.31.0℃）であった。移植した藻体は2ヶ月後に採取し，再び100～150 gずつに分けて養殖を行った。日間成長率は各月ごとの測定から換算した。室内で培養された藻体を海中養殖に供すると，全ての季節で良好な生育を示した。移植直後の日間成長率は6.5～10.7%であり，その後の海中養殖の個体の日間成長率（4.5～8.2%）よりも高い値を示した。後者の値は，他の養殖海域で報告されている本種の日間成長率の範囲内であった。季節的な成長率の変動は水温の変動と関連していた。これらの結果は，ウバツバ湾において事業レベルの浮動方式の*K. alvarezii*養殖が可能であることを示している。([1] Univ. São Paulo, Brazil, [2] APTA da Secretaria de Agricultura e Abastecimento do São Paulo, Brazil, [3]高知大）

福田　覚[1]・大塚周二[1]・北出幸広[2]・渡邊俊樹[3]・嵯峨直恆[1,2]：スサビノリ（紅色植物門，ウシケノリ目）の延長因子(EF-1 α)をコードするcDNAの単離，同定と発現解析

Satoru Fukuda, Shuuji Ootsuka, Yukihiro Kitade, Toshiki Watanabe and Naotsune Saga: Isolation, characterization and expression of a cDNA encoding an elongation factor-1 α from *Porphyra yezoensis* (Bangiales, Rhodophyta)

海産紅藻スサビノリのポリペプチド延長因子(EF-1 α)をコードするcDNAの単離，同定そして発現解析について報告する。cDNAクローンは，葉状の配偶体由来のcDNAライブラリーから単離し，その塩基配列を解析した。このクローンは，既知のEF-1 αと類似性を示す449個のアミノ酸からなるタンパク質の読み取り枠を含んでいた。アミノ酸配列は，アマノリ属の一種*Porphyra purpurea*の糸状の胞子体で特異的に発現するタイプのEF-1 α (*tef-s*)よりも，糸状の胞子体と葉状の配偶体の両世代で発現するタイプのEF-1 α (*tef-c*)に対してより高い類似性を示した。ノーザン解析の結果，本研究で単離したEF-1 αは葉状の配偶体と糸状の胞子体の両世代で発現するタイプであることが明らかになった。([1]東海大院・海洋，[2]東海大・先端技術センター，[3]東大・海洋研）

Freshwater, D. W.・Thomas, D. T.・Bailey, J. C.：紅藻*Gelidium crinale*のグルタミン合成酵素IIをコードするcDNAの特徴

D. Wilson Freshwater, David T. Thomas and J. Craig Bailey: Characterization of a cDNA encoding glutamine synthetase II from *Gelidium crinale* (Rhodophyta)

逆転写酵素PCRと5'-および3'-RACE法を用いて，紅藻*Gelidium crinale* (Turner) Gaillonのグルタミン合成酵素（GS）をコードするcDNAを解析した。1231bpのGS cDNA転写産物の塩基配列を解析したところ，5'および3'末端に翻訳されない部位があり，推定タンパク質コード領域には352のアミノ酸ポリペプチドがコードされていた。GS塩基配列を他の生物と比較したところ，*G. crinale*のcDNAはtype-II GSをコードしており，N末の色素体シグナル配列を欠くことから，細胞質のイソ酵素と考えられる。GSIIアミノ酸配列の系統解析により，細胞質と色素体のイソ酵素が真核生物の進化の間に何度も生じたことが指示された。(Univ. North Carolina, USA）

Bañares, E.・Altamirano, M.・Figueroa, F. L.・Flores-Moya, A.：南イベリア半島の無節石灰藻3種の発芽体の成長における紫外線照射の影響

Elena Bañares, María Altamirano, Félix L. Figueroa and Antonio Flores-Moya: Influence of UV radiation on growth of sporelings of three non-geniculate coralline red algae from Southern Iberian Peninsula

Melobesia membranacea (Esper) Lamouroux，*Lithophyllum incrustans* Philippiおよび*Mesophyllum lichenoides* (Ellis) Lemoineの発芽体の成長における紫外線照射(UVR)の効果を調べるために，光合成活性を示す照射（PAR）のみ，およびPARとUVRを組み合わせた異なる条件下で藻体を培養した。自然条件下では，3種が生育している場所での光環境は実質的に異なっている。*M. membranacea*および*L. incrustans*は潮間帯の日向および漸深帯の上部に生育しているが，*M. membranacea*は潮間帯の日陰になった割れ目（照射量は日向の10%未満）において他の海藻に付着して生育している。これら無節石灰藻の発芽体の相対成長速度（RGR）はUVRによって変化した。3種において，成長速度に及ぼす有害なUVR効果の範囲は照射量の対数と同様の増加パターンを示し，所定の作用スペクトルに対して同じ様な勾配で減衰する事からも推察された。日向に生育している2種の無節石灰藻では，生物学的に効果のある照射量対数に対してRGRが同じような中断・減衰パターンを示したことから，この2種がPAR+UVR下での成長阻害で同じ様な特性を有することがわかった。(Univ. Málaga, Spain）

河地正伸[1]・井上勲[2]・本多大輔[2]・O'Kelly, C. J.[3]・Bailey, J. C.[3]・Bidigare, R. R.[4]・Andersen, R. A.[3]：オメガ3脂肪酸含有量の高い独立栄養性ストラメノパイル類の新綱ピングイオ

藻綱

Masanobu Kawachi, Isao Inouye, Daiske Honda, Charles J. O'Kelly, J. Craig Bailey, Robert R. Bidigare and Robert A. Andersen: The Pinguiophyceae classis nova, a new class of photosynthetic stramenopiles whose members produce large amounts of omega-3 fatty acids

独立栄養性ストラメノパイル類の新綱を記載した。本綱は5つの属，*Glossomastix*, *Phaeomonas*, *Pinguiochrysis*（タイプ属），*Pinguiococcus*そして*Polypodochrysis*を含む。これらの種はいずれも高含有の高度不飽和脂肪酸(PUFA)，中でも特に20:5 (n-3)(EPA, エイコサペンタエン酸)を多く含むことで特徴づけられる。これらの脂肪酸に認められる特徴に基づいて，綱の名称の語根としてラテン語名"Pingue"（= fat, grease）を用いた。核コードの18S rRNA遺伝子および葉緑体コードのrbcL遺伝子の配列データの解析から，本藻群は他のいかなる藻綱にも含まれることなく，独立した単系統群を形成することが示された。形態的には全て単細胞性の微細藻であり，ピコサイズの種から，40 μmを越えるものまで存在する。各々の種は葉緑体包膜とガードルラメラをもつ典型的な葉緑体を1ないし2個有する。葉緑体の中のピレノイドは埋没型から突出型のものまで多様だが，全ての属において膜の陥入が見られた。*Phaeomonas*は2本鞭毛の運動性の細胞を有し，前方に伸びる鞭毛はマスチゴネマ（管状小毛）で覆われている。他の2属（*Glossomastix*と*Polypodochrysis*）は，マスチゴネマのないスムーズな1本鞭毛の遊走子を生じる。この鞭毛は明らかに成熟した鞭毛であり，従来の独立栄養性ストラメノパイルでは知られていない特質である。ピングイオ藻の主要なカロチノイド色素はフコキサンチン，ヴィオラキサンチン，ゼアキサンチンそしてb-カロテンであり，更にクロロフィル*a*とクロロフィル*c*関連色素が存在する。こうした特徴は新綱ピングイオ藻綱を藻類のユニークなグループとして認知させるものである。([1]海洋バイオ研釜石・[2]筑波大・[3]Bigelow Lab. Ocean Sciences・[4]Univ. Hawaii)

河地正伸・熱海美香・池本尚人・宮地重遠：太平洋から分離した新しいピコプランクトンの新属新種*Pinguiochrysis pyriformis*（ピングイオ藻綱）

Masanobu Kawachi, Mika Atsumi, Hisato Ikemoto and Shigetoh Miyachi: *Pinguiochrysis pyriformis* gen. *et* sp. nov. (Pinguiophyceae), a new picoplanktonic alga isolated from the Pacific Ocean

新属新種の*Pinguiochrysis pyriformis*は褐色で不動の裸細胞の海産ピコプランクトンである。1991年に西太平洋の熱帯域において採取した表層サンプルから本種の培養株を確立した。*P. pyriformis*の典型的な細胞は独特な西洋梨の形で，1個の卵形の葉緑体をもつ。この2つの特徴により本種は他のピコ植物プランクトン種から区別されるが，細胞の形状は常に西洋梨型を保っているわけではなく，容易に丸く変形する。葉緑体やミトコンドリアの微細構造は，本種が独立栄養性ストラメノパイルに所属することを示した。*P. pyriformis*の微細構造上の他の独特な特徴として，(i) ピレノイド内に入り込んでチューブ状の陥入構造を形成する葉緑体膜，(ii) いくつかの細胞で認められた3層以上のチラコイドラメラ，(iii) 鞭毛基部や中心体の欠如，そして(iv) 鱗片やその他の細胞外被構造を欠くといった特徴が見出された。形態の特徴に基づいて，このピコプランクトン種を新種として記載し，他で報告した分子系統解析および生化学的結果に基づいてピングイオ藻綱に所属させた。(海洋バイオ研釜石)

Andersen, R. A.・Potter, D.・Bailey, J. C.：新属新種の海産微細藻*Pinguiococcus pyrenoidosus* gen. et sp. nov.（ピングイオ藻綱）

Robert A. Andersen, Daniel Potter and J. Craig Bailey: *Pinguiococcus pyrenoidosus* gen. et sp. nov. (Pinguiophyceae), a new marine coccoid alga

水産養殖場の水槽から採集した球形の海産藻類を培養株(CCMP1144)として維持し，光学顕微鏡および電子顕微鏡を用いて観察した。若く増殖速度の速い細胞はほとんどが丸く，直径は約46 ㎛だった。古い細胞はしばしば突起や偽足状の伸張構造を形成し，そのためにアメーバのようにみえるが，アメーバ状の動きは観察されず，偽足状の伸長部は活発な動きを示さなかった。葉緑体は一つで，独立栄養性ストラメノパイルにみられる典型的な構造をしていた。大きな突出型ピレノイドは光学顕微鏡でも容易に観察された。微細構造的には，ピレノイドの顆粒部は貫入した葉緑体膜によって区切られていた。ミトコンドリアはしばしばピレノイドに隣接していたが，隣接していないものもあり，時にミトコンドリアが突出したピレノイドを覆うようにキャップ構造を形成していた。ゴルジ体は（断面を見たときに）核に向かって湾曲していた。網状につながった管状膜の周縁ネットワークは細胞膜直下に位置していた。2つの中心小体は核膜に隣接していた。脂質状で電子透過性の液胞がみられた。本研究およびこれまでに発表されたデータ（エイコサペンタエン酸，18S rRNAおよび*rbc*L遺伝子の大部分）に基づき，本藻を*Pinguiococcus pyrenoidosus* gen. et sp. nov. として記載した。(Bigelow Lab. Ocean Sciences, USA)

Okelly, C. J.:南オーストラリアで採集された球状でコロニーを形成する黄金色藻の新属新種*Glossomastix chrysoplasta* n. gen., n. sp.（ピングイオ藻綱）について

Charles J. Okelly: *Glossomastix chrysoplasta* n. gen., n. sp. (Pinguiophyceae), a new coccoidal, colony-forming golden alga from southern Australia

オーストラリアのビクトリア州モーニングトン半島ソレントバックビーチの砂岩瓦礫から単離培養した*Glossomastix chrysoplasta* gen. et sp. nov. を記載する。本藻は細胞壁を持たない球状の栄養細胞を形成し，これらの細胞は粘液質のコロニーとして集合体を形成し，二分裂によって継続的に増殖する。色素体はガードルラメラと部分的に埋没したピレノイドを有し，細胞質がピレノイド内を貫通している。遊走子は単一の鞭毛で殆ど遊泳せず，主な運動は細い舌状の偽足による

ものである。鞭毛はマスチゴネマ，鞭毛膨潤部および自家蛍光を欠き，他の黄金色藻の後鞭毛と同等である。前鞭毛は存在せず，鞭毛基部としても目立たないものである。鞭毛装置は2つの鞭毛基部，3つの微小管性鞭毛根およびライゾプラストからなっている。後鞭毛（古い方）の鞭毛基部は基底板の根本側にヘリックス構造をもつ。*Glossomastix chrysoplasta*は塩基配列や生化学的データに基づいてピングイオ藻綱に置かれており，この綱の他の仲間とはいくつかの微細構造の特徴を同じくする。特に*Polypodochrsis teissieri*は類似した遊走子を形成するが，多くの点で他のピングイオ藻綱とは異なってもいる。*Glossomastix chrysoplasta*はピングイオ藻の中で（ただし伸長した細胞を除く）平均的に最も大きな細胞を形成し，コロニーを形成する唯一の種類である。（Bigelow Lab. Ocean Sciences, USA）

本多大輔・井上勲：海産独立栄養性ストラメノパイル生物*Phaeomonas parva* gen. *et* sp. nov.（ピングイオ藻綱）の鞭毛装置構造を含む微細構造と分類

Daiske Honda and Isao Inouye: Ultrastructure and taxonomy of a marine photosynthetic stramenopile *Phaeomonas parva* gen. *et* sp. nov. (Pinguiophyceae) with emphasis on the flagellar apparatus architecture

カロリン諸島近傍の外洋水から採取された海産独立栄養性ストラメノパイル生物を新属新種*Phaeomonas parva*として記載した。細胞は細胞外被を持たず球形から卵形である。この藻類は明期では細胞の側面から生じる2本の鞭毛によって遊泳し，暗期では鞭毛を細胞内に取り込み球形となる。長い方の前鞭毛（成熟していないNo. 2鞭毛）は，3部構成の管状マスチゴネマを有している。短い方の後鞭毛（成熟したNo. 1鞭毛）は，平滑で基部に自家蛍光物質を含んでいる。明るい褐色の葉緑体はカップ型で，細胞の後方を占めている。葉緑体のカップの内側はピレノイドによって埋められている。鞭毛装置構造にはいくつかの点で通常とは異なる特徴が見られた。鞭毛移行部には2枚の基底板と2回転のプロキシマル・ヘリックスがあり，この特徴は*P. parva*がペラゴ藻綱，ディクティオカ藻綱と分類群不明の独立栄養性ストラメノパイル生物*Sulcochrysis biplastida*と近縁であることを示唆している。R3とR4鞭毛根はループを形成しており，これは補食性の黄金色藻類のものと類似している。しかし，*Phaeomonas*は補食能を持たないこと，R3ルートの微小管の本数が異なること，餌を取り込む口部装置となるR3ルートの隙間が形成されないことから，この類似は外面的なものであると考えられる。また，*Phaeomonas*は「バイパッシング・ルート」をもっていることが明らかになった。この構造は褐藻綱，*Giraudyopsis stellifera*（クリソメリス藻綱），*Ankylochrysis lutea*（ペラゴ藻綱と思われる）のみに見られるものである。以上のように*P. parva*の分類学的位置については微細構造の特徴からだけでは特定できない。しかし分子系統学的および生化学的解析は，*P. parva*とストラメノパイル生物4属（*Glossomastix*，*Pinguiochrysis*，*Piguiococcus*，*Polypodochrysis*）の近縁性を強く支持した。これらの藻類は形態学的に区別されるが，高度不飽和脂肪酸，特にエイコサペンタエン酸の含有率は他の藻類では見られないほど高いことで共通していた。そこでこのストラメノパイル生物の新たな系統群を新綱ピングイオ藻綱（Pinguiophyceae）として分類した。*P. parva*はこの新綱に属する唯一の2鞭毛遊泳性の藻類である。（筑波大）

河地正伸[1]・Noel, M.-H.[2]・Andersen, R. A.[3]：海産の「黄金色藻」*Polypodochrysis teissieri*（ピングイオ藻綱）に関する再調査

Masanobu Kawachi, Mary-Helene Noel and Robert A. Andersen: Re-examination of the marine "chrysophyte" *Polypodochrysis teissieri* (Pinguiophyceae)

特徴的なロリカをもつ海産黄金色藻*Polypodochrysis teissieri* Magneについて，光学顕微鏡と電子顕微鏡を用いて再調査した。光学顕微鏡による観察結果は，基本的にMagneの記載と一致していたが，彼の論文で記載された不動胞子は実は独特な1本鞭毛性の遊走子であることが判明した。葉緑体やミトコンドリアの一般的な微細構造は典型的な独立栄養性ストラメノパイルのものであった。埋没型のピレノイドは葉緑体の中央に位置し，葉緑体膜の陥入が認められた。2個のゴルジ体は常に核に近接し，栄養細胞では互いに直角，遊走子では平行に配置していた。ロリカの超薄切片像は，珪藻殻のような珪酸質の構造に類似したが，EDX分析の結果では，珪素は極僅かであった。裸の遊走子はマスチゴネマのない単一の鞭毛をもち，鞭毛膨潤部や眼点を欠いていた。遊走子は基物表面上で滑走する動きを見せた。二つの異なる細胞質突出（偽足）が遊走子で観察された。舌状偽足の一つは，鞭毛の基部から生じ，いつも鞭毛に沿って存在した。もう一つの偽足は細胞が運動方向を変えるときにのみ形成された。鞭毛装置構造の調査から，鞭毛の鞭毛移行領域に単一の基底板が存在し，その鞭毛基部に近接して第二の鞭毛基部が存在すること，3つの微小管性鞭毛根と一組の細胞骨格系微小管そして第二の鞭毛基部と殻をつなぐ痕跡的なライゾプラストの存在が示された。*Polypodochrysis*の遊走子に見られる多くの特徴は，*Glossomastix chrysoplasta*（ピングイオ藻綱）の遊走子と類似しており，*Polypodochrysis*と*Glossomastix*で見られる単一の鞭毛が相同であることを示唆した。この単一の鞭毛はストラメノパイル類における短い方の後鞭毛として一般に認識されている成熟鞭毛に相当するようであり，ストラメノパイル類におけるユニークな鞭毛構成を示すものである。こうした形態上の特徴および別論文で報告した他の知見（18S rRNAやrbcL遺伝子と生化学的特徴）に基づき，*Polypodochrysis*を黄金色藻綱から新綱ピングイオ藻綱に移行することにした。

（[1]国環境研・[2]筑波大・[3]Bigelow Lab. Ocean Sciences, USA）

学 会 録 事

平成14年度日本藻類学会の運営方法のその後について

先の学会録事（「藻類」49巻3号）で3月中に持ち回り評議員会を開催し，昨年度の学会活動報告と今年度の予定等を7月の総会に代わって，評議員会で仮の承認をいただくことになっておりました。残念ながら事務処理が大幅に遅れ，ここにその報告ができず，ご迷惑をおかけしておりますこと深謝いたします。

日本分類学連合設立について

去る1月12日・13日，国立科学博物館分館にて同連合設立総会とその記念シンポジウムが開催されました。総会は日本爬虫両棲類学会会長松井正文氏を議長に選出し，規約，役員（会長に加藤雅啓氏（東大）が就任），事務局の設置，活動計画，分担金などを審議し，正式に設立・発足しました。これらの詳しい内容は同連合のホームページ（http://www.bunrui.info)に，事務局へは tomokuni@kahaku.go.jp にアクセスしてください。本学会を含めて19，参加予定学会1が紹介，登録されました。総会に引き続き，記念のシンポジウム（3セッション）と特別講演（荒俣宏氏：分類学の先駆者は偉かった・・・が，しかし）が両日にわたって行われました。本学会として学会長と日本分類学会連合設立準備委員会委員の川井浩史氏が参加しました。

植物分類学関連学会連絡会について

去る3月17日，国立科学博物館分館にて頭書の連絡会が開催されました。藻類学会からは原慶明会長が出席しました。日本植物分類学会代表の綿野泰行氏の進行で，1）連絡会合同名簿発行の報告と校正のあり方，2）日本植物学会時の合同シンポジウム企画（タイトルは「集める・貯める・使う：生物多様性情報の体系化と活用」，日本蘚苔類学会の神田啓史氏が纏め，藻類分野の話題提供の協力依頼があった)。3）連絡会の今後，4）植物分類学国際会議（IAPT2004)への取り組みが議論され，また合同名簿作成の会計報告が行われました。

次期会長・評議員選挙について

現在の会長および評議員は本年12月31日で任期満了となります。今年度から評議員会による会長候補者推薦の方法が改正され，場合によっては時間を要すること（「藻類」49巻2号学会録事，持ち回り評議員会報告参照)，7月にAlgae2002（JSP総会）が開催される変則的な年間予定であること，加えて，英文誌，和文誌それぞれの編集長の交代（現会長と次期会長の合議が必要）があることなど学会運営が錯綜しております。したがいまして，次期会長および評議員選挙を例年より時期を早めて挙行する予定です。選挙日程の詳細については追って連絡いたします。

訂正のご案内

50巻1号に掲載の新山優子著「江津湖の藻類」の図の説明に以下の訂正があります。

正　図2　江津湖周辺の様子

誤　図2　図2　神水（くわみず）駐車場付近。湧水池のひとつ。季節によって出現種が異なるが，アミミドロ，*Tribonema*，珪藻類，*Scenedesmus* などが多数出現した場所。

編集後記

いつになく（いつものことか...）どたばたと編集作業をしてしまった。ディスプレイとプリンターを買い換えて新たな気分だったが，ついそちらの使い心地が気になって，校正等に身が入らない。今号は記事が少ない分たっぷりとalgae2002講演英文要旨が詰め込まれているので堪能できますよ。

（ＪＴ）

筑波での学会要旨も大量に割り付け，和文誌なのか英文誌なのか分からないような雑誌になりましたが，見栄えのする号になった気がします。

（ＴＮ）。

賛助会員

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世界の淡水産紅藻

熊野　茂 著

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清澄な水域に生息している淡水産紅藻は，環境汚染に極めて敏感であるため，地球的規模での水の汚染の危険を人類に知らせる有効な指標としての役割を担っている．しかし水質の汚染に伴い残念ながら淡水産紅藻種のいくつかの種は既に絶滅し，また多くの種の絶滅が危惧されている．本書は淡水産紅藻という分類群の現時点での研究成果をまとめたものであり，世界で認められている淡水産紅藻の大部分の分類群を，種，変種のランクまで収録する．

淡水藻類入門

淡水藻類の形質・種類・観察と研究

山岸 高旺　編著

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「日本淡水藻図鑑」の編者である著者がまとめる，初心者・入門者のための書．多種多様な藻類群を，平易な言葉で誰にも分かるよう，丁寧に解説する．Ⅰ編，Ⅱ編で形質と分類の概説を行い，Ⅲ編では各分野の専門家による具体的事例20編をあげ，実際にどのように観察・研究を進めたらよいかを理解できるように構成する．

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新日本海藻誌 — 日本産海藻類総覧 —

吉田 忠生 著

B5判・総頁1248頁・本体価格46000円

本書は古典的になった岡村金太郎の歴史的大著「日本海藻誌」(1936) を全面的に書き直したものである．「日本海藻誌」刊行以後の約60年間の研究の進歩を要約し，1997年までの知見を盛り込んで，日本産として報告のある海藻（緑藻，褐藻，紅藻）約1400種について，形態的な特徴を現代の言葉で記載する．植物学・水産学の専門家のみならず，広く関係各方面に必携の書．

近刊　**小林珪藻図鑑**

小林　弘
南雲　保・出井雅彦・真山茂樹・長田敬五　著

藻類の生活史集成

堀　輝三　編

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陸上植物の起源

—緑藻から緑色植物へ—

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堀　輝三　共訳

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最初に海で生まれた現生植物の祖先は，どのような進化をたどって陸上に進出したのか——．分子生物学，生化学，発生学，形態学などの成果にもとづく探求の書．

日本淡水藻図鑑

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千原光雄　編著

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藻類の今を見渡し，理解するための最適の書．斯界の第一人者により，藻学および周辺領域の膨大な知識の蓄積が整理され，新しい研究成果も取り入れられている．藻学を学ぶ方，またこの分野に興味のある方の新たなスタンダード．

日本の赤潮生物

—写真と解説—

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原生生物の世界

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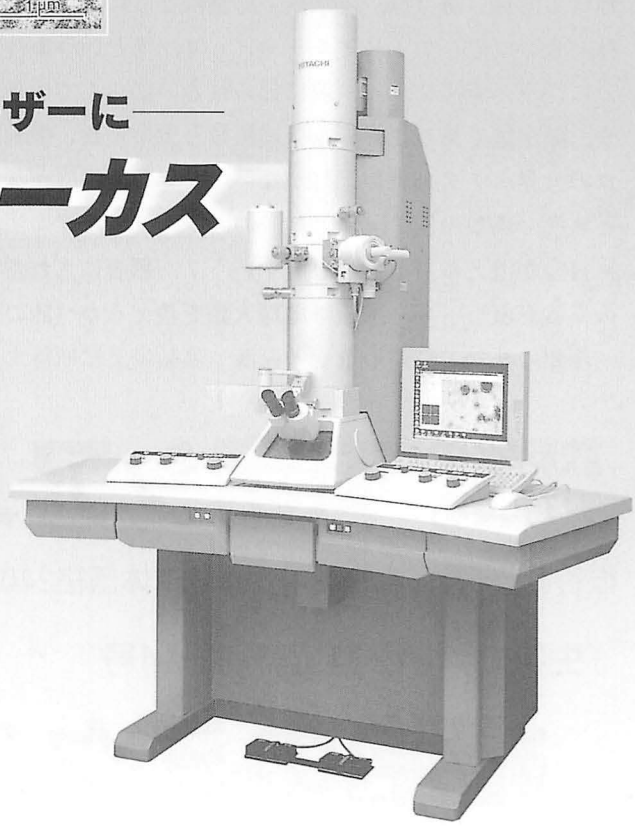

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裳華房の生物系書籍

ポピュラー・サイエンス 240

人も環境も藻類から

石川依久子 著　四六判 210頁 本体価格1600円＋税　ISBN4—7853—8740—8

生物進化の原点，生き物に満ちあふれる地球環境をつくった立て役者．けれど，いかにも地味な存在であるためか，「藻類」の認知度はあまり高まる様子がありません．そうかと思えば赤潮やアオコで一方的な悪者とみなされたり．本書では，さまざまな角度から藻類の真の姿を浮き彫りにします．

〈主要目次〉 1．エネルギー編（藻類は飛行機を飛ばす／太陽エネルギーを「カロリー」に／藻類が作るクリーンエネルギー／石油に代わる天然ガス）　2．地球生命編（クロロフィルがすべての原点／藻類は酸素を排泄した／鉄の文明は藻類から／らん藻という生き物／藻類から陸上植物へ）　3．地球生態編（海は海，陸は陸／海藻はどこに／微細藻類はどこにも／海藻で飾られた日本／海の大森林／サンゴ礁／アイスアルジー）　4．海という条件編（色が消える世界／重力のない世界／月の引力に従う／潮流に乗る／波に揺られる／膨らんで生きる／カルシウムを信号に／カルシウムを着る）　5．顕微鏡で見る世界編（微細藻類と大型藻類／微細藻類は数万種もいる／共生による多様化／ミクロの世界・ナノの世界／目がないのに見る／細胞が泳ぐ／動物か植物か）　6．生殖の原点編（有性と無性／藻類の親子／卵と精子の起源／メスを探す法／体を分断して子どもを作る／多産多死の戦略／自分が消える／遺伝子を消す）　7．悪者にされた藻類編（赤潮／水の華，アオコ／港湾汚染／毒のある海藻）　8．藻類は地球人類を救えるか（陸の森林に代わる／藻場をはぐくむ／海藻を食べる／藻類の薬効／藻類の遺伝子資源／藻類産業に期待する／藻類と共に宇宙へ）

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彼谷邦光 著　A５判 160頁 本体価格2400円＋税　ISBN4-7853-5835-1

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Joint Conference of Japanese Society of Phycology and 3rd Asian Pacific Phycological Forum

26th annual and 50th anniversary

Algae 2002

Tsukuba, Japan

19–24 July 2002

ABSTRACTS

July 19 (Fri) — 24 (Wed), 20[illegible]

National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 1
National Institute for Environmental Studies (NIES)

Host organization

Japanese Society of Phycology (JSP)
Asian Pacific Phycological Association (APPA)
National Institute of Advanced Industrial Science and Technology (AIST)
National Institute for Environmental Studies (NIES)

Cosponsorship

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Local Organizing Committee of the Algae2002
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Congress Secretariat for Algae2002

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http://www.biol.tsukuba.ac.jp/~algae/algae02/

Algae 2002: Agenda

	Events	Time	Auditorium A	Auditorium B	Lobby
	Registration	10:00-17:00			
19 July (Fri)	Extramural Class for Citizens	10:00-12:00			Lectures and Seaweed Specimen Preparation Practical Class (language: Japanese)
	Lunch	12:00-13:00			
	JSP 50th Anniversary Symposium for Public.	13:00-16:00	JSP 50th Anniversary Symposium for Public. (language: Japanese)		
	Get Together	17:00-18:00			Get Together (snacks and drink)
20 July (Sat)	Registration	9:00-17:00			
	Opening Ceremony	10:30-11:30	Algae 2002: Opening Ceremony		
	Lunch	11:30-12:30			
	JSP 50th Anniversary Special Lectures	12:30-14:30	JSP 50th Anniversary Special Lectures Prof. Y. Aruga and Prof. I. K. Lee		
	Award Ceremony	14:30-15:00	Award Ceremony (Japanese)		
	Poster Session	15:00-16:00			Poster Session <1> Poster presentation by high school students Presenters of *odd-numbered posters* are requested to explain their works.
	JSP Business Meeting	16:00-17:00	JSP Business Meeting (Japanese)		
	Welcome and JSP 50th Anniversary Party	17:30-20:00	at the University of Tsukuba (Transport by coaches)		
21 July (Sun)	Plenary Lecture	8:00-9:00	PL1: J. D. Pickett-Heaps and T. Spurck		
	Symposia	9:00-11:00	S1: Mangrove Ecosystem	S2: Contribution of Genetics and Genomics to Phycological Research	
	Lunch	11:00-12:00			

	Events	Time	Auditorium A	Auditorium B	Lobby
21 July (Sun)	Symposia	12:00-14:00	S3: Structuring Algal Assemblages: Biotic and Abiotic Interferences	S4: The biology of Thraustochytrids and Labyrinthulids	
	Symposia	14:00-16:00	S5: Algal Reproduction and Development	S6: Coralline Blooms and Loss of Kelp Beds	
	Coffe break	16:00-16:30			
	Students Competition	16:30-18:30	Students Competition <C1-C8>	Students Competition<C14-C21>	
22 July (Mon)	Plenary Lecture	8:00-9:00	PL2: Y. Shizuri and Y. Matsuo		
	Symposia	9:00-11:00	S7: Algal Physiology	S8: Mariculture	
	Lunch	11:00-12:00			
	Symposia	12:00-14:00	S9: Cell dynamics	S10: Some Aspects of Bangiales Biology	
	Symposia	14:00-16:00	S11: Systematics of Macroalgae	S12: Algal Roles in theIntegrated Aquaculture System	
	Coffe Break	16:00-16:30			
	Poster Session	16:30-17:30			Poster Session <2> Presenters of *even-numbered posters* are requested to explain their works.
	Students Competition	17:30-18:45	Students Competition <C22-C26>	Students Competition <C9-C13>	
23 July (Tue)	Satellite Symposium	9:00-16:00	CULTURE COLLECTION AND ENVIRONMENTAL RESEARCHES at National Institute for Environmental Studies (NIES) (Transport by coaches)		
	Excursion: Oarai Beach Excursion: Mt. Tsukuba & Botanical Garden	7:45-15:30 13:00-16:30			
	Banquet	17:30-20:00	Algae 2002 Banquet: Dinning Room at AIST		
24 July (Wed)	PlenaryLecture	9:00-10:00	PL3: M. J. Wynne		
	Symposia	10:00-12:00	S13: Phylogeography	S14: Harmful Algae	
	Symposia	13:30-15:30	S15: Systematics of Microalgae	S16: The Frontiers of MarimoResearch	
	APPA Business Meeting	15:30-16:30	APPA Business Meeting		
	Closing Ceremony	16:30-17:00	Closing Ceremony		

PLENARY LECTURES

PL-1 STRATEGIES FOR SURVIVAL: LIFE AT THE BOTTOM OF THE FOOD CHAIN

Pickett-Heaps, J D*; Spurck, T: *University of Melbourne, AUSTRALIA*

PL-2

1: NATURAL PRODUCTS FROM MARINE BACTERIA AND MARINE ORGANISMS

2: ALGAL MORPHOGENESIS INDUCED BY MARINE BACTERIA

Shizuri, Y; Matsuo, Y

Marine Biotechnology Institute (MBI), JAPAN

PL-3

THE STATUS OF OUR UNDERSTANDING OF THE BENTHIC MARINE ALGAL FLORA OF THE SULTANATE OF OMAN AND THE BROADER BIOGEOGRAPHICAL IMPLICATIONS

Wynne, M J

University of Michigan, USA

SYMPOSIA

SYMPOSIUM 1: *MANGROVE ECOSYSTEM*

Conveners: Jiro TANAKA (*Tokyo University of Fisheries, JAPAN*), John WEST (*University of Melbourne, AUSTRALIA*)

S1-1: DIFFERENCES IN MOLECULAR PHYLOGENY AND REPRODUCTION BETWEEN *BOSTRYCHIA MORITZIANA* POPULATIONS FROM THE WESTCOAST AND EASTCOAST OF NEW CALEDONIA

West, J* (1); Zuccarello, G (2); Loiseaux-de Goër, S (3)

(1) University of Melbourne, AUSTRALIA; (2) Marine Biological Association, U.K; (3) 11 rue des Moguerou, 29680 Roscoff, FRANCE

S1-2: SPECIATION AND PHYLOGEOGRAPHY OF MANGROVE RED ALGA *CALOGLOSSA*

Kamiya, M: *Kobe University Research Center for Inland Seas, JAPAN*

S1-3: SPECIES DIVERSITY OF UNICELLULAR RED ALGAE FROM MANGROVES

Yokoyama, A*; Hara, Y: *Yamagata University, JAPAN*

S1-4: INSIGHTS INTO ALGAL SPECIES: THE EVOLUTIONARY HISTORY OF *BOSTRYCHIA*

Zuccarello, G C* (1); West, J A (2); Sandercock, B (2); King, R J (3)

(1) Marine Biological Association, U. K.; (2) University of Melbourne, AUSTRALIA; (3) University of New South Wales, AUSTRALIA

S1-5: CLADOPHORACEAN PLANTS GROWING IN AND AROUND MANGROVE FORESTS IN JAPAN

Miyaji, K* (1) and Takaso, T (2) : *(1) Toho University, JAPAN; (2) University of Ryukyu, JAPAN*

SYMPOSIUM 2: *CONTRIBUTION OF GENETICS AND GENOMICS TO PHYCOLOGICAL RESEARCH*

Conveners: Naotsune SAGA (*Hokkaido University, JAPAN*), Siew Moi PHANG (*University of Malaya, MALAYSIA*)

S2-1: FUNCTIONAL GENOMICS IN CYANOBACTERIA

Ohmori, M*; Yoshimura, H; Okamoto, S; Katoh, H; Fujisawa, T: *University of Tokyo, JAPAN*

S2-2: GENETIC APPROACHES TOWARDS THE STUDY OF MALAYSIAN *GRACILARIA*

Phang, S-M: *University of Malaya, MALAYSIA*

S2-3: GENETIC ENGINEERING TOWARDS CONSTRUCTION OF A MULTIPLE-FUNCTIONAL KELP BIOREACTOR

Qin, S*; Tseng, C: *Chinese Academy of Sciences, CHINA*

S2-4: *PORPHYRA YEZOENSIS* AS A MODEL PLANT FOR GENOME SCIENCES

Kitade, Y: Hokkaido University, JAPAN

S2-5: APPLICATION OF GENOMICS APPROACHES TO GENETICS IN ALGAE: EST ANALYSIS IN *PORPHYRA YEZOENSIS*

Asamizu, E (1); Nakajima M (2); Nakamura, Y (1); Nikaido, I (2,3); Kitade, Y (2,4); Saga, N (2,4); Tabata, S* (1)

(1) Kazusa DNA Research Institute, JAPAN; (2) Tokai University, JAPAN; (3) RIKEN Yokohama Institute, JAPAN; (4) Hokkaido University, JAPAN

SYMPOSIUM 3: *STRUCTURING ALGAL ASSEMBLAGES: BIOTIC AND ABIOTIC INTERFERENCES*

Conveners: Put ANG (*The Chinese University of Hong Kong, CHINA*)
Gary KENDRICK (*University of Western Australia, AUSTRALIA*)

S3-1: DISEASE AS A BIOLOGICAL FACTOR IN ALGAL GROWTH AND PRODUCTIVITY
Ganzon-Fortes, E T*; Butardo, V; Lluisma, A O; Quitoriano, M L; Montaño, M N E; Mendoza, W
University of the Philippines, PHILIPPINES

S3-2: PRODUCTION ECOLOGY OF *ECKLONIA CAVA*, VARIATIONS IN PRODUCTIVITY AND PHOTOSYNTHATE ACCUMULATION OVER LIFE HISTORY STAGES
Maegawa, M: *Mie University, JAPAN*

S3-3: STOCHASTICITY OF ALGAL RECRUITMENT IN PING CHAU, HONG KONG
Kong, S L; Ang, P O, Jr.* : *The Chinese University of Hong Kong, CHINA*

S3-4: THE ROLE OF DISTURBANCE ON MAINTAINING DIVERSITY IN MACROALGAE
Kendrick, G A: *University of Western Australia, AUSTRALIA*

SYMPOSIUM 4: *THE BIOLOGY OF THRAUSTOCHYTRIDS AND LABYRINTHULIDS*

Conveners: Toro NAKAHARA (*National Institute of Advanced Industrial Science and Technology, JAPAN*)
Daiske HONDA (*Konan University, JAPAN*)

S4-1: PHYLOGENY AND TAXONOMY OF THE LABYRINTHULIDS AND THRAUSTOCHYTRIDS
Honda, D: *Konan University, JAPAN*

S4-2: DEGRADATION OF FALLEN MANGROVE LEAVES AND LABYRINTHULOMYCOTA
Nakahara, T*; Yokochi, T: *National Institute of Advanced Industrial Science and Technology, JAPAN*

S4-3: ECOPHYSIOLOGY OF MANGROVE THRAUSTOCHYTRIDS
Fan, K W*; Jones, E B G; Vrijmoed, L L P: *City University of Hong Kong, CHINA*

S4-4: UTILIZATION OF THRAUSTOCHYTRIDS FOR FISHERY FEED
Hayashi, M: *Miyazaki University, JAPAN*

SYMPOSIUM 5:*ALGAL REPRODUCTION AND DEVELOPMENT*

Conveners: Gwang Hoon KIM (*Konju University, KOREA*), Taizo MOTOMURA (*Hokkaido University, JAPAN*)

S5-1: PYRENOID FORMATION ASSOCIATED WITH THE CELL CYCLE IN THE BROWN ALGA, *SCYTOSIPHON LOMENTARIA* (SCYTOSIPHONALES, PHAEOPHYCEAE)
Nagasato, C* (1); Yoshikawa, S (1); Kawai, H (2); Motomura, T (1)
(1) Hokkaido University, JAPAN; (2) Kobe University Research Center for Inland Seas, JAPAN

S5-2: CELLULAR AND MOLECULAR STUDIES ON REPRODUCTION AND SEX DETERMINATION IN *AGLAOTHAMNION OOSUMIENSE* ITONO (RHODOPHYTA)
Chah, O-K* (1); Lee, I K (2); Kim, G H (1) : *(1) Kongju National University, KOREA; (2) Korean Institute of Biodiversity, KOREA*

S5-3: SEXUAL INCOMPATIBILITY IN RED ALGAE
Wilson, S M*; West, J A; Pickett-Heaps, J D: *University of Melbourne, AUSTRALIA*

S5-4: MORPHOGENESIS IN GIANT ALGAL CELLS
Mine, I*; Okuda, K : *Kochi University, JAPAN*

SYMPOSIUM 6: *CORALLINE BLOOMS AND LOSS OF KELP BEDS*

Conveners: Daisuke FUJITA (*Toyama Pref. Fis.Res.Inst., JAPAN*), Jeong Ha KIM (*Sungkyunkwan University, KOREA*)

S6-1: THE CURRENT STATUS OF BARREN GROUND PHENOMENA ON THE COASTS OF JAPAN AND KOREA
Fujita, D (1); Kim J.H (2): *(1) Prefecture of Toyama, JAPAN; (2) Sungkyunkwan University KOREA*

S6-2: EFFECTS OF FISH GRAZING ON TEMPERATE KELP FORESTS IN SOUTHERN JAPAN
Arai, S: *Marine Algae Research Co., Ltd., JAPAN*

S6-3: VEGETATIONAL CHARACTERISTICS OF BARREN GROUNDS ON THE EASTERN COAST OF KOREA
Kim, H G: *Kangnung National University, KOREA*

S6-4: NUTRIENTS AND SNAIL GRAZING COULD RECOVER THE ALGAL VEGETATION ON COBBLES COLLECTED FROM A BARREN GROUND IN SOUTHWESTERN HOKKAIDO
Fujita, D: *Toyama Prefectural Government, JAPAN*

S6-5: THE ROLE OF CRUSTOSE CORALLINE ALGAE IN THE ESTABLISHMENT OF MACROALGAE AND IN ALGAL SUCCESSION
kim, J H*; Lee, H A; Park, S W: *Sungkyunkwan University, KOREA*

SYMPOSIUM 7: *ALGAL PHYSIOLOGY*

Conveners: Antonietta QUIGG (*Rutgers University, USA*), Hiroyuki SEKIMOTO (*University of Tokyo, JAPAN*)

S7-1: GAMETE RECOGNITION IN A RED ALGA *AGLAOTHAMNION OOSUMIENSE*: CYTOCHEMICAL AND BIOCHEMICAL APPROACHES
Kim, G H: *Kongju National University, KOREA*

S7-2: GENE EXPRESSION INDUCED BY NITROGEN STARVATION AND SUBSEQUENT GAMETIC DIFFERENTIATION IN *CHLAMYDOMONAS REINHARDTII*
Matsuda, Y: *Kobe University, JAPAN*

S7-3: SEXUAL REPRODUCTION OF *CLOSTERIUM PERACEROSUM-STRIGOSUM-LITTORALE* COMPLEX: PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR BIOLOGICAL APPROACHES
Sekimoto, H* (1, 2); Tsuchikane, Y (3), Akatsuka, S (3), Fukumoto, R (3), Fujii, T (3)
(1) University of Tokyo, JAPAN; (2) PRESTO, JAPAN; (3) Toyo University, JAPAN

S7-4: CO_2 CONCENTRATING MECHANISMS IN ALGAE: THEIR ROLE AND REGULATION BY ENVIRONMENTAL FACTORS
Beardall, J: *Monash University, AUSTRALIA*

S7-5: MOLECULAR ANALYSES OF CELL RESPONSES TO CHANGES IN CO_2 LEVEL AND LIGHT: CARBON CONCENTRATING MECHANISM AND ITS REGULATORY PATHWAYS
Fukuzawa, H: *Kyoto University, JAPAN*

SYMPOSIUM 8: *MARICULTURE*

Conveners: Masao OHNO (*Kochi University, JAPAN*)
Alan T. CRITCHLEY (*Degussa Texturant Systems France SAS, Research Centre, FRANCE*)

S8-1: MARICULTURE OF SEAWEEDS WITH DEEP SEAWATER IN JAPAN
Ohno, M* (1); Hiraoka, M (2) : *(1) Kochi University, JAPAN; (2) Kochi Prefectural Deep Seawater Laboratory, JAPAN*

S8-2: INTRODUCTION ON ADVANCED AND NEWLY DEVELOPED SEAWEED CULTIVATION IN KOREA
Sohn, C H: *Pukyong National University, KOREA*

S8-3: ADVANCEMENT OF *PORPHYRA* CULTIVATION IN CHINA
Yan, X-H: *Shanghai Fisheries University, CHINA*

S8-4: THE SYNERGY OF SEAWEED HYDROCOLLOID (CARRAGEENAN AND ALGINATE) PRODUCTION AND APPLICATION: AN INDUSTRIAL EXAMPLE
Critchley, A T: *Degussa Texturant Systems France SAS, FRANCE*

SYMPOSIUM 9: *CELL DYNAMICS*

Conveners: Yoshiaki HARA (*Yamagata University, JAPAN*), JohnWest (*University of Melbourne, AUSTRALIA*), Christopher S. LOBBAN (*University of Guam, USA*)

S9-1:THE CELL BIOLOGY OF DIATOMS RECORDED BY VIDEOMICROSCOPY
Pickett-Heaps, J D*; Spurck, T
University of Melbourne, AUSTRALIA

S9-2: FUNCTION OF CENTRIOLES ON THE DEVELOPMENT OF *FUCUS* ZYGOTES
Motomura, T: *Hokkaido University, JAPAN*

S9-3: ORIGIN, ROLE AND DYNAMICS OF APICOPLAST
Matsuzaki, M*; Kita, K; Kuroiwa, T: *University of Tokyo, JAPAN*

S9-4: THE CILIATE *MARISTENTOR DINOFERUS* AND ITS ZOOXANTHELLAE, *SYMBIODINIUM*
Hara, Y* (1); Kajikawa, M (1); Yokoyama, A (1); Lobban, C (2): *(1) Yamagata University, JAPAN; (2) University of Guam, USA*

SYMPOSIUM 10: *SOME ASPECTS OF BANGIALES BIOLOGY*

Conveners: C. H. SOHN (*Pukyong National University, KOREA*), M. NOTOYA (*Tokyo University of Fisheries, JAPAN*)

S10-1: MORPHOLOGICAL CHARACTERISTICS AND PHYSIOLOGICAL RESPONSES OF *PORPHYRA SUBORBICULATA* KJELLMAN (BANGIALES, RHODOPHYTA) FROM FIVE LOCALITIES
Monotilla, W D*; Notoya, M.: *Tokyo University of Fisheries, JAPAN*

S10-2: GEOGRAPHICAL DISTRIBUTIONS AND THE PHYSIOLOGICAL CHARACTERISTICS OF *PORPHYRA* SPP. (BANGIALES, RHODOPHYTA) ALONG THE COASTS OF KOREA

Kim, N-G: *Gyeongsang National University, KOREA*

S10-3: CELL DIFFERENTIATION FROM THE BLADE TISSUE OF *PORPHYRA VIETNAMENSIS* TANAKA ET HO (BANGIALES, RHODOPHYTA) FROM THAILAND

Ruangchuay, R* (1); Notoya, M (2): *(1) Prince of Songkla University, THAILAND; (2) Tokyo University of Fisheries, JAPAN*

S10-4: LIFE MODES AND SPECIES OF THE GENUS *BANGIA* (RHODOPHYTA) IN JAPAN

Iijima, N* (1); Notoya, M (2): *(1) Marutomo Co. Ltd., JAPAN; (2) Tokyo University of Fisheries, JAPAN*

S10-5: MORPHOLOGY OF THREE SPECIES OF *BANGIA* (BANGIACEAE, RHODOPHYTA) IN JAPAN

Kikuchi, N* (1); Miyata, M (1); Notoya, M (2): *(1) Natural History Museum and Institute, JAPAN; (2) Tokyo University of Fisheries, JAPAN*

SYMPOSIUM 11: *SYSTEMATICS OF MACROALGAE*

Convener: Sung Min BOO (*Chungnam National University, KOREA*), Max HOMMERSAND (*University of North Carolina, USA*)

S11-1: TAXONOMY AND PHYLOGENY OF THE BROWN ALGAL ORDER SCYTOSIPHONALES

Kogame, K: *Hokkaido University, JAPAN*

S11-2: THE ORDER LAMINARIALES (PHAEOPHYCEAE) FROM THE FAR EASTERN SEAS OF RUSSIA

Klotchkova, N G: *Kamchatka Research Institute of Fishery and Oceanography, RUSSIA*

S11-3: SIMPLICITY AND COMPLEXITY - *BANGIA* IN NEW ZEALAND

Broom, J E* (1); Nelson, W A (2); Farr, T J (2); Jones, W A (1)

(1) University of Otago, NEW ZEALAND; (2) NIWA (National Institute for Water and Atmospheric Research), NEW ZEALAND

S11-4: SYSTEMATICS OF THE CERAMIACEOUS RED TRIBE CERAMIEAE BASED ON MORPHOLOGY AND DNAS

Boo, S M*; Cho, T O : *Chungnam National University, KOREA*

S11-5: CLASSIFICATION OF THE RED ALGAL FAMILY CERAMIACEAE AT THE SUBFAMILY AND TRIBAL LEVELS

Hommersand, M H* (1); Fredericq, S (2); Freshwater, D W (3): *(1) University of North Carolina at Chapel Hill, USA; (2) University of Louisiana at Lafayette, USA; (3) University of North Carolina at Wilmington, USA*

SYMPOSIUM 12: *ALGAL ROLES IN THE INTEGRATED AQUACULTURE SYSTEM*

Conveners: Ik Kyo CHUNG (*Pusan National University, KOREA*), Charles YARISH (*University of Connecticut, USA*)

S12-1: ECOSYSTEM MODELING: A TOOL TO UNDERSTAND THE INTERACTIONS BETWEEN EXTRACTIVE AND FED AQUACULTURE

Yarish, C* (1); Rawson, Jr. M V (2); Chopin, T (3); Wang, D R (4); Chen, C (4); Carmona, R (1,5); Chen, C (6); Wang, L (4); Ji, R (6); Sullivan, J (6)

(1) University of Connecticut, USA; (2) Georgia Sea Grant College Program, USA; (3) University of New Brunswick, CANADA; (4) Marine and Fishery Dept. of Hainan Province, P. R. CHINA; (5) Universidade do Algarve, PORTUGAL; (6) The University of Georgia, USA

S12-2: APPLICATION OF MARINE ANIMAL EFFLUENTS FOR CULTIVATION OF *GRACILARIA* AND *CAULERPA* IN THAILAND

Chirapart, A : Kasetsart University, THAILAND

S12-3: COMPARISON OF THE BIOREMEDIATION POTENTIAL OF *PORPHYRA* SPP.

Kraemer, G-P (1); Yarish, C (2); Carmona, R *(2,3)

(1) State University of New York, USA; (2) University of Connecticut, USA; (3) Universidade do Algarve, PORTUGAL

S12-4: APPLICATION OF *PORPHYRA* CULTIVATION IN FISH FARM EFFLUENT TREATMENT.

Chung, I K* (1); Kang, Y H (1); Lee, J A (2): *(1) Pusan National University, KOREA; (2) Inje University, KOREA*

SYMPOSIUM 13: *PHYLOGEOGRAPHY*

Conveners: Wendy NELSON (*National Institute for Water and Atmosphere Research, NEW ZEALAND*)
Hiroshi KAWAI (*Kobe University Research Center for Inland Seas, JAPAN*)

S13-1: THE SOUTH WEST PACIFIC RIM: PRELIMINARY BIOGEOGRAPHIC ANALYSES OF EAST AUSTRALIAN MARINE MACROALGAE

Phillips, J A : *The University of Queensland, AUSTRALIA*

S13-2: A COMPLEX PHYLOGEOGRAPHIC STORY - THE CHALLENGE OF *PORPHYRA/BANGIA*

Nelson, W A *(1); Broom, J E (2); Farr, T J (1); Jones, W A (2)

(1) NIWA (National Institute for Water and Atmospheric Research), NEW ZEALAND; (2) University of Otago, NEW ZEALAND

S13-3: ARCTIC OCEAN—BRIDGE OR BARRIER? REFLECTIONS ON THE BIOGEOGRAPHY OF SEAWEEDS IN COLD NORTHERN WATERS

Lindstrom, S C: *University of British Columbia, CANADA*

S13-4: PHYLOGEOGRAPHY OF SOME CIRCUM-ARCTIC SPECIES OF LAMINARIALES AND CHORDARIALES (PHAEOPHYCEAE)
Kawai H *(1); Kim S-H, Sasaki, H (2).
(1) Kobe University Research Center for Inland Seas, JAPAN; (2) Graduate School of Science and Technology, Kobe University, JAPAN

SYMPOSIUM 14: *HARMFUL ALGAE*

Conveners: Ichiro IMAI (*Kyoto University, JAPAN*), Chang Hoon KIM (*Pukyong National University, KOREA*)

S14-1: BLOOM DYNAMICS OF TOXIC DINOFLAGELLATE *ALEXANDRIUM TAMARENSE* IN JAPANESE COASTAL WATERS.
Itakura, S*; Yamaguchi, M: *National Research Institute of Fisheries and Environment of Inland Sea, JAPAN*

S14-2: OCCURRENCE OF HABS AND BIOGEOGRAPHY OF PSP TOXIGENIC DINOFLAGELLATES IN KOREA
Kim, C-H* (1); Kim, Y-S (1); Kim, K-Y (1); Park, T-G (2): *(1) Pukyong National University, KOREA; (2) University of Tasmania, AUSTRALIA*

S14-3: RED TIDE OCCURRENCES AND PREVENTION STRATEGIES IN JAPAN
Imai, I: *Kyoto University, JAPAN*

S14-4: MARINE HARMFUL MICROALGAE OF SOUTHERN PACIFIC WITH SPECIAL EMPHASIS ON BLOOM-FORMING SPECIES IN AUSTRALASIA
Chang, F H: *National Institute of Water & Atmospheric Research Ltd., NEW ZEALAND*

SYMPOSIUM 15: *SYSTEMATICS OF MICROALGAE*

Conveners: Ken-ichiro ISHIDA (*Kanazawa University, Kanazawa, Ishikawa, JAPAN*)
Woongghi SHIN (*Rutgers University, USA*)

S15-1: ENDOSYMBIOTIC LATERAL TRANSFER OF THE PLASTID: A DRIVING FORCE OF ALGAL DIVERSITY
Ishida, K: *Kanazawa University, JAPAN*

S15-2: EVOLUTION OF GREEN ALGAE: A REVIEW BASED ON MOLECULAR PHYLOGENETIC ANALYSES
Friedl, T*; Hepperle, D: *University of Goettingen, GERMANY*

S15-3: PLASTID ORIGIN(S) AND PHYLOGENETIC RELATIONSHIPS OF HETEROKONT ALGAE
Andersen, R A: *Bigelow Laboratory for Ocean Sciences, USA*

S15-4: THE IMPACT OF NOVEL SEQUENCES FROM FRESHWATER AND BENTHIC MARINE DINOFLAGELLATES ON THE PHYLOGENETICS OF DINOFLAGELLATES
Horiguchi, T; Takano, Y: *Hokkaido University, JAPAN*

SYMPOSIUM 16: *THE FRONTIERS OF MARIMO RESEARCH*

Conveners: Isamu WAKANA (*Akan Town Board of Education, JAPAN*), Arni EINARSSON (*Myvatn Research Station, ICELAND*)

S16-1: NEW VIEWS OF PHYLOGENETIC RELATIONSHIP BETWEEN MARIMO (*AEGAGROPILA LINNAEI*) AND SOME SPECIES OF CLADOPHORALES
Hanyuda, T*; Ueda, K : *Kanazawa University, JAPAN*

S16-2: ECOLOGICAL FREATURES OF FRESHWATER GREEN ALGA MARIMO *AEGAGROPILA LINNAEI* IN THE BALTIC SEA AREA
Reitalu, T* (1); Wakana, I (2): *(1) University of Tartu, Estonia; (2) Akan Town Board of Education, JAPAN*

S16-3: MARIMO AEGAGROPILA LINNAEI IN ICELAND
Einarsson, A* (1); Wakana, I (2): *(1) Myvatn Research Station, ICELAND; (2) Lake Akan Eco-Museum Center, JAPAN*

S16-4: WATER QUALITY AS MACRO- AND MICRO-ENVIRONMENTAL FACTORS IN THE MARIMO HABITATS.
Honoki, H: *Toyama Science Museum, JAPAN*

S16-5: COMPENSATION DEPTH OF MARIMO AGGREGATIONS AND ITS ANNUAL FLUCTUATIONS AT LAKE AKAN
Wakana, I *(1); Nagao, M (2): *(1) Akan Town Board of Education, JAPAN; (2) Hokkaido University, JAPAN*

SATELLITE SYMPOSIUM (at NIES): *CULTURE COLLECTION AND ENVIRONMENTAL RESEARCHES*

Conveners: Fumie KASAI (*National Institute for Environmental Studies, JAPAN*)
Kunimitsu KAYA (*National Institute for Environmental Studies, JAPAN*)
Aparat MAHAKHANT (*Thailand Institute of Scientific and Technological Research, THAILAND*)
Susan I. BLACKBURN (*CSIRO Microalgae Research Centre, AUSTRALIA*)

SS-1: NEW FACILITIES: BIOLOGICAL RESOURCE COLLECTION AND ENVIRONMENTAL RESEARCHES
Watanabe, M M: *National Institute for Environmental Studies, JAPAN*

SS-2: ALGAE: 3.5 BILLION YEARS EVOLUTION AND DIVERSIFICATION.

Isao Inouye: *University of Tsukuba, JAPAN*

SS-3: SITUATION OF TOXIC CYANOBACTERIAL BLOOMS IN THAILAND

Mahakhant, A* (1); Khaiprapai, P (1); Tungtananuwat, M (1); Polchai, J (1); Keovara, L (2); Sano, T (3); Kaya, K (3); Arunpairojana, A (1)

(1) Thailand Institute of Scientific and Technological Research, THAILAND; (2) Chiang Mai Provincial Water work Authority, THAILAND; (3) National Institute for Environmental Studies (NIES), JAPAN

SS-4: EVALUATION OF TOXIC CYANOBACTERIA BLOOMS IN LAKE DIANCHI, TAIHU AND CHAOHU

Song, L R; Liu, Y D; Lei, L M; Shen, Q; Zhu, Y Z: *Institute of Hydrobiology, The Chinese Academy of Sciences, CHINA*

SS-5: THE CSIRO COLLECTION OF LIVING MICROALGAE: AN AUSTRALIAN PERSPECTIVE ON MICROALGAL BIODIVERSITY AND APPLICATIONS.

Blackburn, S I (1); Frampton, D (1); Gallori, S (4); Mansour, M P (1); Nichols, P N (1); Parker, N S (1); Robert, S (1); Volkman, J K (1); Bolch, C J S (2); Negri, A (3); Tredici, M R (4)

(1) CSIRO Marine Research, AUSTRALIA; (2) University of Tasmania, AUSTRALIA; (3) Australian Institute of Marine Science, AUSTRALIA; (4) Universita' degli Studi di Firenze, ITALY

SS-6: DETECTION OF SINGLE CELLS OF BLOOM-FORMING TOXIC *MICROCYSTIS* (CYANOBACTERIA) USING IMMUNOLOGICAL PROBES

Hiroishi, S*; Yuki, Y; Yoshida, T: *Fukui Prefectural University, JAPAN*

SS-7: BIOACTIVE COMPOUNDS OF FRESHWATER CYANOBACTERA

Kaya, K: *National Institute for Environmental Studies, JAPAN*

SS-8: PROVASOLI-GUILLARD NATIONAL CENTER FOR CULTURE OF MARINE PHYTOPLANKTON: PAST, PRESENT AND FUTURE

Andersen, R A: *Bigelow Laboratory for Ocean Sciences, USA*

SS-9: CRYOPRESERVATION AND THE PROBLEM OF FREEZE-RECALCITRANCE, IN ALGAL CULTURE COLLECTIONS

Day, J G: *Culture Collection of Algae and Protozoa (CCAP), UK*

SS-10: EXPLORING EVOLUTIONARY RELATIONSHIPS OF MICROALGAE: MOLECULAR PHYLOGENETIC ANALYSES AND CULTURE COLLECTIONS

Friedl, T*; Hepperle, D; Müller, J; Behnke, A; Lorenz, M: *University of Goettingen, GERMANY*

STUDENTS COMPETITION

C1: A NOVEL REPRODUCTIVE ORGAN AS THE PROPAGULE OF *CODIUM EDULE* (BRYOPSIDALES, CHLOROPHYTA)

Chang, J-S*; Dai, C-F: *National Taiwan University, TAIWAN*

C2: GENETIC AND MORPHOLOGICAL VARIATION AMONG POPULATIONS OF *SARGASSUM HEMIPHYLLUM* (PHAEOPHYTA)

Cheang, C C*; Chu, K H; Ang, P O: *The Chinese University of Hong Kong, CHINA*

C3: MORPHOLOGICAL DIFFERENCES IN THE SIX SPECIES OF UNARMORED DINOFLAGELLATES *COCHLODINIUM, GYMNODINIUM* AND *GYRODINIUM*

Cho, S Y*; Han, M S: *Hanyang University, KOREA*

C4: PHYLOGENY OF *LAMINARIA* (PHAEOPHYCEAE) BASED ON ITS AND RUBISCO SPACER REGION

Cho, G Y* (1); Yoon, H S (1); Klochkova, N (2); Yarish, C (3); Yotsukura, N (4); Kain (Jones), J M (5); Druehl, L D (6); Boo, S M (1)

(1) Chungnam National University, KOREA; (2) Kamchatka Institute of Fishery and Oceanography, RUSSIA; (3) University of Connecticut, USA; (4) Hokkaido University, JAPAN; (5) University of Liverpool, UK; (6) Bamfield Marine Station, CANADA

C5: A NEW CRUSTOSE RED ALGA *PEYSSONNELIA PLURICRUSTIS* AND A FIRST REPORT OF *P. HARVEYANA* (GIGARTINALES, RHODOPHYTA) FROM JAPAN

Kato, A *; Masuda, M: *Hokkaido University, JAPAN*

C6: MOLECULAR DIFFERENTIATION OF *GRACILARIA SALICORNIA* AND CLOSELY RELATED SPECIES

Lim, P-E*; Phang, S-M; Thong, K-W: *University of Malaya, MALAYSIA*

C7: THE ULTRASTRUCTURE AND 18S RDNA ANALYSIS OF A NEW JAKOBID FLAGELLATE

Moriya, M*; Nakayama, T; Inouye, I: *University of Tsukuba, JAPAN*

C8: TAXONOMIC STUDY OF THE TETRASPORALEAN GENUS *ASTEROCOCCUS* (CHLOROPHYTA) BASED ON COMPARATIVE MORPHOLOGY AND MOLECULAR PHYLOGENETIC ANALYSIS

Nakazawa, A*; Nozaki, H: *University of Tokyo, JAPAN*

C9: LIGHT AND ELECTRON MICROSCOPY AND 18S RDNA ANALYSIS ON AN UNDESCRIBED EUKARYOTE INHABITING INTERTIDAL SANDY FLATS

Okamoto, N*; Matsunaga, S; Nakayama, T; Inouye, I: *University of Tsukuba, JAPAN*

C10: REORIENTATION OF THE FLAGELLAR ROOT SYSTEM DURING MITOSIS OF A UNICELLULAR GREEN ALGA *MESOSTIGMA* (PRASINOPHYCEAE)

Sakaushi, S* (1); Watanabe, S (2); Miyamura, S (1); Hori, T (1): *(1) University of Tsukuba, JAPAN; (2) RIKEN Cell Bank, JAPAN*

C11: FURTHER TAXONOMIC STUDY OF *NITELLA* (CHARALES, CHAROPHYCEAE) FROM EAST ASIA, BASED ON SEM OOSPORE MORPHOLOGY AND MOLECULAR PHYLOGENETICS

Sakayama, H* (1); Hara, Y (2); Nozaki, H (1): *(1) University of Tokyo, JAPAN; (2) Yamagata University, JAPAN*

C12: PRESENCE OF *rbc*L IN COLORLESS HETEROKONT ALGAE

Sekiguchi, H*; Moriya, M; Nakayama, T; Inouye, I: *University of Tsukuba, JAPAN*

C13: MOLECULAR PHYLOGENY AND ULTRASTRUCTURE OF A NEW SPECIES OF THE HAPTOPHYTA WITH UNIQUE SILICEOUS SCALES FROM SHIRIBESHI SEAMOUNT

Yoshida, M* (1); Noel, M-H (1); Kawachi, M (2); Inouye, I (1): *(1) University of Tsukuba, JAPAN; (2) National Institute for Environmental Studies, JAPAN*

C14: MULTIFUNCTION OF A SEX PHEROMONE IN *CLOSTERIUM-PERACEROSUM-STRIGOSUM-LITTORALE* COMPLEX

Akatsuka, S* (1); Fukumoto, R (1); Sekimoto, H (2, 3); Fujii, T (1): *(1) Toyo University, JAPAN; (2) University of Tokyo, JAPAN; (3) PRESTO, JAPAN*

C15: INFLUENCE OF DIFFERENT FACTORS ON CARRAGEENAN FROM RED ALGA *TICHOCARPUS CRINITUS* (TICHOCARPACEAE) OF RUSSIAN PACIFIC COAST

Barabanova, A O*; Yermak, I M; Nagorskay, V P; Reyunov, A V; Solov'eva, T F: *Pacific Institute of Bioorganic Chemistry, RUSSIA*

C16: ECOLOGICAL CHARACTERISTICS OF ATTACHED DIATOMS AND ENCRUSTING CORALLINE ALGAE IN THE ALGAL WHITENNING AREAS OF JEJU COAST, KOREA

Choa, J-H*; Lee, J-B : *Cheju National University, KOREA*

C17: CYANOBACTERIAL SIDEROPHORES

Itou, Y*; Okada, S; Murakami, M: *University of Tokyo, JAPAN*

C18: CYTOCHEMICAL AND ULTRASTRUCTURAL STUDIES ON PROTOPLAST FORMATION FROM DISINTEGRATED CELLS OF A MARINE GREEN ALGA *CHAETOMORPHA AEREA* (CHLOROPHYTA)

Klochkova, T A* (1); West, J A (2); Kim, G H (1): *(1) Kongju National University, KOREA; (2) University of Melbourne, AUSTRALIA*

C19: TRYING TO CULTIVATE THE TOXIC DINOFLAGELLATES *DINOPHYSIS* SPP.

Nishitani, G* (1); Yamaguchi, M (2); Miyamura, K (3); Imai, I (1): *(1) Kyoto University, JAPAN; (2) National Research Institute of Fisheries and Environment of Inland Sea, JAPAN; (3) Oita Institute of Marine and Fisheries Science, JAPAN*

C20: SEASONAL VARIATIONS IN THE POPULATIONS OF *CHONDRUS OCELLATUS* AND *C. YENDOI* FROM OKIRAI BAY, NORTHERN JAPAN

Pe-Montebon, M J* (1); Nanba, N (2); Ogawa, H (2); Ota, A (2)

(1) University of the Philippines, PHILIPPINES; (2) Kitasato University, JAPAN

C21: PHOTOSYNTHESIS AND RESPIRATION OF *LAMINARIA JAPONICA* FROM HIGASHIDOURI, AOMORI PREFECTURE, NORTHERN JAPAN

Suzuki, M* (1); Nanba, N (1); Ogawa, H (1); Honda, M (2); Kamimura, R (3); Okamura, T (4)

(1) Kitasato University, JAPAN; (2) Central Research Institute of Electric Power Industry, JAPAN; (3) Tokyo Electric Power Environmental Engineering Co., Inc., JAPAN; (4) The Tokyo Electric Power Co., Inc., JAPAN

C22: CHARACTERIZATION OF TWO SEX PHEROMONES IN *CLOSTERIUM*

Tsuchikane, Y* (1); Fukumoto, R (1); Fujii, T (1); Sekimoto, H (2, 3)

(1) Toyo University, JAPAN; (2) University of Tokyo, JAPAN; (3) PRESTO, JAPAN

C23: THE INFLUENCE OF HERBIVOROUS SNAILS ON GREEN TIDES

Yang, S Y*; Kim, J H: *Sungkyunkwan University, KOREA*

C24: NUCLEAR HISTONE PROTEINS OF GAMETES IN BROWN ALGAE

Yoshikawa, S * (1); Nagasato, C (1); Makino, Y (2); Murakami, A (3); Kawai, H (3); Ichimura, T (1); Motomura, T (1)

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Handa, S* (1); Nakahara, M (2); Tsubota, H (2); Deguchi, H (2); Nakano, T (3)

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Yumoto, K* (1); Kawachi, M (1); Nakayama, T (2); Kasai, F (1); Watanabe, M M (1)
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Science and Technology, JAPAN; (3) Tokushima Agriculture, Forestry and Fisheries Technology Center, JAPAN; (4) Kochi University, JAPAN

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Iima, M* (1); Hanyuda, T (2); Yoshizaki, M (3); Ebata, H (4): *(1) Nagasaki University, JAPAN; (2) Kanazawa University, JAPAN; (3) Toho University, JAPAN; (4) Fuyo Ocean Development & Engineering Co. Ltd., JAPAN*

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Klochkova, N G: *Kamchatka Research Institute of Fishery and Oceanography, RUSSIA*

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Traichaiyaporn, S* (1); Chainapong, T (1); Tularak, P (1); Dolprasit, S (2); Waraegsiri, B (3)

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Yano, T* (1); Kamiya, M (2); Arai, S (3); Kawai, H (2)

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Yoshizaki, M: *Toho University, JAPAN*

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Junshum, P *; Trichaiyaporn, S; Promya, J; Sirirattanawarabgkul, W; Tularak, P; Chainapong, T; Suwattanakupt, S
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Kim, Y-S*; Kim, C-H: *Pukyong National University, KOREA*

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Kuntanawat, P* (1); Peerapornpisal, Y (1); Bhumiratana, A (2): *(1) Chiangmai University, THAILAND; (2) Mahidol University, THAILAND*

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Lim, W-A* (1); Jung, C-S (1); Lee, C-K (1); Lee, S-G (1); Kim, H-G (1); Chung, I-K (2)
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Panuvanitchakorn, N*; Peerapornpisal, Y: *Chiang Mai University, THAILAND*

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Kobayashi, Y*; Torii, A; Kato, M: *Ochanomizu University, JAPAN*

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Yoshimi, K *; Dan, A : *Fisheries Research Institute, Tokushima Agriculture, Forestry and Fisheries Technology Center, JAPAN*

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Hanawa, Y*; Iwamoto, K; Shiraiwa, Y: *University of Tsukuba, JAPAN*

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P159: MOLECULAR EVIDENCE FOR PLASTID ROBBERY IN *DINOPHYSIS*, A DINOFLAGELLATE CAUSING DIARRHETIC SHELLFISH POISONING

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Day, J G* (1); Benson, E E (2); Natanson, L (2); Bremner, D (2); Santos, L (3); Santos, F (3); Friedl, T (4); Lorenz, M (4); Lukesova, A (5); Elster, J (6); Lukavsky, J (6); Herdman, M (7); Rippka, R (7); Hedoin, H (8); Hall, T (8)

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Erata, M* (1); Mori, F (1); Yumoto, K (1); Kawachi, M (2); Kasai, F (2); Watanabe, M M (2)

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Fukuda, H* (1); Kawashima, S (2); Yokohama, Y (3); Noda, M (4); Okuda, H (1); Ishihara, K (1); Imuro, A (5)

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Ohmura, Y* (1); Kawachi, M (2); Sekiguchi, H (2); Takeshita, S (3); Kasai, F (2): *(1) Domestic Research Fellow, JAPAN Society for the Promotion of Science, JAPAN; (2) National Institute for Environmental Studies, JAPAN; (3) Hiroshima University, JAPAN*

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Phang, S-M * (1); Chu, W-L (2) : *(1) University of Malaya, MALAYSIA; (2) International Medical University, MALAYSIA*

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Suda, S*; Atsumi, M; Takishita, K; Kurano, N: *Marine Biotechnology Institute, JAPAN*

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Tanabe, Y*(1); Kaya, K(1); Watanabe, MM(1): *(1) National Institute for Environmental Studies, JAPAN*

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Yamashita, N*; Matsuda, T; Shimizu, Y; Matsui, S: *Kyoto University, JAPAN*

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Yan, X-H* (1); Zhu, W-R (1); Aruga, Y (2): *(1) Shanghai Fisheries University, CHINA; (2) Tokyo University of Agriculture, JAPAN*

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Wang, G*; Hu, S; Tseng, CK: *Chinese Academy of Sciences, CHINA*

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Satoh, D; Fujiwara, Y; Satoh, T: H*okkaido Sapporo Kiyota High School, JAPAN*

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Ogata, A(1); Moriya, Y(1); Takahashi, S(2); Members of Natural Science Club(1)

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PL-1

STRATEGIES FOR SURVIVAL: LIFE AT THE BOTTOM OF THE FOOD CHAIN

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Just as in the world we are familiar with, the inhabitants of the microscopic world can be divided into two basic groups of organisms distinguished by their mode of mutrition: those that are primary producers (photosynthetic algae) and those which are predators, feeding on other cells. The microscopic realm is just as much a dangerous jungle as the macroscopic world we are familiar with. Rapacious single-celled organisms have devised all sorts of strategies to catch and eat their prey, while the prey organisms have evolved many different mechanisms for escaping predators and surviving.

Using videomicroscopy coupled with a variety of optical techniques, we provide a glimpse into this jungle and illustrate some of the techniques these tiny organisms employ to catch their prey. Some single cells such as *Noctiluca* and *Peranema* are particularly relentless predators. Flagella play an central role in both prey capture and avoidance in different species. For example, many organisms use their flagellar systems create water currents to bring their prey close enough for capture and ingestion, while the ability of *Chlamydomonas* to shed its flagella now appears an efficient mechanism for escaping capture.

PL-2

1: NATURAL PRODUCTS FROM MARINE BACTERIA AND MARINE ORGANISMS

2: ALGAL MORPHOGENESIS INDUCED BY MARINE BACTERIA

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MBI has been identified hundreds of biologically active compounds from marine organisms and microorganism. In the first part, we will report the results and further efforts to develop as commercial products. In the second part, we will present our recent results on the chemical component isolated from one of epiphytic marine bacterium, which strongly induce algal morophogenesis. A part of this work was performed as the Industrial Science and Technology Frontier Program supported by New Energy and Industrial Technology Development Organization.

PL-3

THE STATUS OF OUR UNDERSTANDING OF THE BENTHIC MARINE ALGAL FLORA OF THE SULTANATE OF OMAN AND THE BROADER BIOGEOGRAPHICAL IMPLICATIONS

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The northern Arabian Sea represents one of the last remaining "little explored" regions in terms of its benthic marine algae. Collections have been made during the past three years, mostly from the southern (Dhofer) region of the Sultanate of Oman. During the summertime monsoon winds blow surface water away from the Arabian shoreline, and this surface water is replaced by nutrient-rich water coming from a deep, northward flow. Water is supplied from much greater depths than is usual in other coastal upwelling system. Water temperatures drop to less than 20 °C during the monsoon, even to 16-17 °C or colder, in contrast to a typical water temperature range of 24-28 °C o in the non-monsoon season. The monsoon also brings increased nutrients and increased wave action. For the stretch of coastline between Salalah and Sadh, the impact of the monsoon is most pronounced because of the relatively narrow extent of the continental shelf. So despite their location in tropical latitudes, the southwestern coastline and offshore islands of the Sultanate of Oman reveal a benthic marine algal flora made up of typical tropical and subtropical components along with species more typical of cold-temperate regions, reflecting the complicated oceanographic conditions of the monsoon and non-monsoon seasons.

Some of the species we are finding in Oman represent the first reports from the entire Indian Ocean. An interesting floristic connection that has emerged is between the northern Arabian Sea and Japan and environs, some species found in Oman previously known only from eastern Asia. Other taxa were previously known from the Mediterranean Sea and Atlantic Europe. Other connections are with southern Africa and even with Brazil (Jolyna).

Some new genera (*Pseudogrinnellia*, *Stirnia*) and new species (in *Champia*, *Plocamium Dipterocladia, Turbinaria*, and *Cryptopleura*) have been described. Some species characteristic of the region impacted by the monsoon.

S1-1
DIFFERENCES IN MOLECULAR PHYLOGENY AND REPRODUCTION BETWEEN *BOSTRYCHIA MORITZIANA* POPULATIONS FROM THE WESTCOAST AND EASTCOAST OF NEW CALEDONIA

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Based on sampling in 1998 and 2001 we have shown that 15 isolates of *Bostrychia moritziana* from western New Caledonia are **all asexual**. Most (Koumac, Plage de Poé, Plage de Foué, Nera River and Conception) are of haplotype AU which is characteristic of all sexual and asexual isolates from Australia, New Zealand and South Africa. Two isolates from Plage de Foué are of two distinct haplotypes NC1 and NC2. By contrast the 9 isolates from mangroves around the nickel ore port of Kouaoua in eastern NC are all probably sexual and from a different series of haplotypes, One isolate (4158) is linked to a clade that includes 3751 (Palmerston, NT, Australia) and 3453 (Sulawesi, Indonesia). The other eight isolates are linked with a clade including 4119 (Florida USA), 4069 (Saudi Arabia) and 4109 (Malaysia). All these isolates from other regions are **sexual**. The Kouaoua isolates have formed viable tetrasporangia and spores but have not yet produced mature gametophytes. The New Caledonia isolates are linked with widely divergent phylogenetic clades from diverse geographic regions and it appears that the genetic diversity of *B. moritziana* from New Caledonia is much greater than in Australia. Australia has a much longer coastline over 14,000 km extending over such a diverse climatological and geographical (10-43° S) range whereas New Caledonia has a coastline of about 900 km ranging from 20-23° S. These results have important implications in understanding the biodiversity of mangrove algae. If a goal of managing biodiversity is the maintenance of genetic diversity within a species, a simple morphological analysis of biodiversity would not suffice, as all isolates of *B. moritziana* from New Caledonia are morphologically indistinguishable. These results also indicate that the biogeography of mangrove algae can be complex, as found on either side of New Caledonia have different phylogenetic histories.

S1-2
SPECIATION AND PHYLOGEOGRAPHY OF MANGROVE RED ALGA *CALOGLOSSA*

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Algal biodiversity in mangrove ecosystems has been uncovered in this decade, and it becomes possible to argue speciation processes and dispersal patterns of mangrove algae. *Caloglossa leprieurii* (Delesseriaceae, Ceramiales) is one of the most abundant and widespread red algae in mangrove ecosystem. This species includes two morphological types based on the number of cell rows at the blade node, single cell row type and multiple cell rows type. The single cell row type is restricted around Indo-Pacific coasts, and the crossability tests revealed five mating groups in this type, three of them from Australia. In contrast, the multiple cell rows type is distributed worldwide and also involves five mating groups, but many of them showed partial reproductive reactions with each other. In spite of the more limited distribution, the maximum genetic distance within the single cell row type is one and a half times as much as that within the multiple cell rows type on the basis of both Rubisco spacer and lsu rDNA sequences, so the latter may have dispersed worldwide for a relatively short period in recent times. Because the evolutionary relationship within the single cell row type is similar to that within *C. postiae*, which is also limited to the Indo-Pacific region, there may be certain physical barriers to affect their dispersal patterns around this region. The reproductive compatibility of *C. leprieurii* correlates with genetic distance rather than geographical distance: sympatric mating groups were completely incompatible and genetically distant (4.1-7.7% in the Rubisco spacer); allopatric mating groups demonstrated partial reproductive reactions and much lower genetic distance (0.4-2.4%). This fits with a view that genetic divergence and reproductive isolation were established allopatrically, in which case, the allopatric speciation theory can be applied to this mangrove alga.

S1-3

SPECIES DIVERSITY OF UNICELLULAR RED ALGAE FROM MANGROVES

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The type localities of unicellular red algae are quite variable, however, almost all of them are cosmopolitan. For the last two decades of our mangrove research projects, we found eight species and several undescribed taxa, including in five genera such as *Porphyridum*, *Rhodella*, *Dixoniella*, *Rhodosorus* and *Rhodospora*-like were found and isolated from field samples in mangrove forests and adjacent areas of Japan, Hong Kong, Thailand, Indonesia, Australia and Mexico. Their habitats in surface soil of mangrove forests are exposed by freshwater and salt water twice a day. Depending on turbulence by tidal conditions, brackish water with various salinities also covers them. Consequently, unicellular reds can survive, when they are tolerant to salinity changes and desiccation.

From the results of molecular phylogenetic analyses of the Porphyridiales using 18SrRNA, *psb*A, *rbc*L genes, it revealed that they were not monophyletic and divided into four groups, *Cyanidium*-, *Rhodosorus*-, *Porphyridium*- and *Rhodella*-groups. The latter three groups include species that inhabit mangroves. Each group includes a few freshwater or terrestrial members. *Rhodosorus marinus* and *R. magnei* in the second group have never been collected from mangrove environments, although they are distributed on the surface sands of seashores or on other algae near mangroves. They showed the marine type of growth pattern under various salinity conditions in culture. Based on these findings, the nature and phylogeny of unicellular red algae from mangroves will be discussed.

S1-4

INSIGHTS INTO ALGAL SPECIES: THE EVOLUTIONARY HISTORY OF *BOSTRYCHIA*

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Mangrove ecosystems contain a unique compliment of algae of which *Bostrychia* spp. are an important component. Molecular methodologies have aided in the understanding of the evolution of species within this genus. DNA sequence data from chloroplast and nuclear genes shows that the split into 2 genera (*Bostrychia* and *Stictosiphonia*) is not supported. Many characters that were considered important in species-level description (cortication, monosiphonous branches, number of tier cells) are plastic and can not be used in many cases. The systematics of this genus needs reevaluation. Molecular analyses within morphologically defined species has shown that species can have high levels of genetic variation and can be reproductively isolated. Molecular clock calibration suggests that isolates of *B. calliptera* have a most recent common ancestor and have remained morphologically similar for over 40 million years. Highly diverse lineages are also seen in the *B. radicans*/ *B. moritziana* species complex, with isolates from different lineages unable to cross suggesting that this lineage is composed of multiple cryptic species. Population genetic analysis of *B. moritziana*, using microsatellite loci, reveals that populations even within relatively short geographic distances are genetically isolated. This genetic isolation can lead to local adaptations and reproductive isolation which could be one explanation for the large number of distinct genealogical lineages within *B. moritziana*. Though populations seem to be genetically isolated over short time frames, the geographically wide-spread nature of these genealogical lineages is probably due to rare long-distance dispersal events over much longer time scales. The history of this well studied red algal genus reveals a complex and fascinating systematic, evolutionary and population genetic history.

S1-5
CLADOPHORACEAN PLANTS GROWING IN AND AROUND MANGROVE FORESTS IN JAPAN

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Two species of green algae, *Rhizoclonium kerneri* Stockmayer and *R. africanum* Kützing, belonging to the Cladophoraceae have been reported to be associated with mangrove forests in Japan. We have carried out taxonomical, morphological and ecological studies of the cladophoracean plants native to the mangrove forests extending from Kiire, Kagoshima, to Iriomote Island, Okinawa, Japan, and found three species of *Rhizoclonium* and three species of *Chaetomorpha*, none of which have been described or reported previously, in addition to *Chaetomorpha linum* and one *Rhizoclonium* species. Of the four species of *Rhizoclonium*, three belong to the *Rhizoclonium tortuosum* complex and have very similar morphology, with slender uniseriate filaments and rare intercalary rhizoids containing several nuclei per cell, except the chloroplasts and pyrenoids. One species has lacy chloroplasts with polypyramidal pyrenoids, another has stellate chloroplasts with bilenticular pyrenoids, and the third has spongy chloroplasts with bilenticular pyrenoids. The other *Rhizoclonium* species, which also inhabits the estuaries of many Japanese rivers, has many nuclei and spongy chloroplasts with polypyramidal pyrenoids. The four species of *Chaetomorpha* include *Chaetomorpha linum* Kützing and three unidentified species. One species has slightly broad filaments with polypyramidal pyrenoids and polarity. We can detect a basal rhizoid, and an acute distal portion in most of the filaments. Another species has twisted filaments with cells of uniform diameter throughout, numerous elliptical chloroplasts and bilenticular pyrenoids in culture, and the third species has slender filaments with cells about 35 μm in diameter, stellate chloroplasts, polypyramidal pyrenoids and up to 20 nuclei per cell.

Among the eight species, four (the first of the *R. tortuosum* complex and the three species of *Chaetomorpha*) grow only epiphytically on the bark of aerial mangrove roots and stems. The other four species grow on mud, fallen logs, and riverbanks inside and outside the mangrove forests.

S2-1
FUNCTIONAL GENOMICS IN CYANOBACTERIA

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Sequencing of a genome of a unicellular cyanobacterium *Synechocystis* sp. PCC 6803 has been completed in 1996. By utilizing the sequence information, many studies concerning the structure, function and expression of *Synechocystis* genes have been carried out up to present. Consequently, a large quantity of knowledge on the functions of 3 thousand genes in the 3.57 Mb genome has been accumulated. Very recently, Kaneko et al. reported the complete sequence of the entire genome of a filamentous cyanobacterium *Anabaena* sp. strain PCC 7120. This strain has been used extensively for genetic studies of nitrogen fixation and of differentiation of cells during the heterocyst formation, thus, combined with the information of the entire genome structure, provides an excellent system to study common and unique features of genes among cyanobacteria. We have isolated twelve genes for Cya from various cyanobacteria. Those proteins are classified into 6 groups by their deduced amino acid sequences. The Cya catalytic domains were C-terminal, whereas various putative input domains were N-terminal in all Cya proteins. Four Cya proteins had GAF domain(s) and the four GAF domains of CyaB1 and CyaB2 were predicted to bind cGMP. A photoreceptor having phosphorylation capability and which might control the activity of CyaC via the phosphotransfer reaction has been predicted. For the mutant analysis, we first surveyed approximately 150 amino acid residues that are recognized as chromophore binding GAF domain in *Anabaena* genome, and determined the 23 chromophore binding GAF domains in the 15 ORFs. And then disrupted these ORFs by the insertion of antibiotic restriction cassettes. We found that the *all2699* disruptant did not respond to far-red light. The ORF *all2699* named *aphC* encodes a protein with 920 amino acids. The results obtained in the present experiment suggest that the AphC controls the activity of the CyaC.

S2-2

GENETIC APPROACHES TOWARDS THE STUDY OF MALAYSIAN *GRACILARIA*

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More than ten species of *Gracilaria* are found in Malaysia. Of these, *Gracilaria changii* (Xia et Abbott) Abbott, Zhang et Xia (1963) is one of the most widely distributed agarophytic seaweeds in Malaysia. It contains large amounts of high quality agar and agarose, and this seaweed is now the focus of our research. The Algae Laboratory at the University of Malaya is a newcomer to the area of Seaweed Genetics and Molecular Biology. Our early interests have been on the use of molecular techniques for species identification and the understanding of phylogenetic and evolutionary relationships amongst economically important seaweeds in Malaysia, focusing on *Gracilaria* and *Sargassum*. RAPD (random amplified polymorphic DNA) followed by sequencing of selected genes like the rubisco large subunit gene is used. We are presently involved in a national project on "Genomic approaches to seaweed genes and natural product discovery". This research focuses on the "application of expression profiling and proteomics in natural product discovery" and the "cultivation and strain improvement of the seaweed". Our laboratory is involved in the construction of a cDNA library for *Gracilaria changii* which may lead to identification of important genes through microarray approach. We are in the process of developing a genetic transformation and gene expression system for *Gracilaria changii*. This paper will discuss our results on the use of RAPD and gene sequencing in species differentiation, establishment of RNA extraction techniques and experience in genetic transformations in *Gracilaria changii*.

S2-3

GENETIC ENGINEERING TOWARDS CONSTRUCTION OF A MULTIPLE-FUNCTIONAL KELP BIOREACTOR

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Large-scale seaweed cultivation is performed mainly in Asia-Pacific region and the annual production of kelp (*Laminaria japonica*) in China has reached 3.10 million tons fresh weight (about 0.52 million tons of dry materials). Kelp shows a great ability to absorb C, N, P and other elements to produce biomass. The matured kelp plants are harvested to leave marine environment and used as health food and a source of iodine, mannitol and alginate. Since 1993 the research target of us is to make kelp a multiple-functional bioreactor by applying genetic engineering effecting in the following aspects:

1. Fixation of CO_2 and release of O_2;
2. Removal of heavy metal pollutants;
3. Removal of eutrophicated elements such as N and P;
4. Production of high-value products such as oral vaccines.

The establishment of methodology of genetic engineering has been carried out on the basis of plant genetic engineering and seaweed life cycle. The project mainly includes translocation of an effective direct-gene-transfer method, selection of effective promoter-report gene cassette, determination of the most appropriate host cell and investigation of selectable markers in kelp.

The result of introduction of the human hepatitis B surface antigen (HBsAg) gene into kelp showed a promising prospect of kelp bioreactor in transforming environmental unwelcome N and P into valuable peptide. Safety cultivation of transgenic kelp is also discussed in this presentation.

S2-4
PORPHYRA YEZOENSIS AS A MODEL PLANT FOR GENOME SCIENCES

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The members of Rhodophyta (red algae) have never got to land, and almost of them have adapted themselves to marine environment and accomplished their unique evolution. A marine red alga *Porphyra* includes several economically valuable species *P. yezoensis*, *P. tenera*, etc. They are the most valuable marine crops in the world. *Porphyra* has a dimorphic life cycle which consists of a leafy gametophytic generation and a filamentous sporophytic one and some species of *Porphyra* have an asexual cycle consisting of repetition of leafy gametophytes through monospores. These two generations sometimes seem to be different species even though they have the same genome. The morphogenetic modes of them are different, and this may be explained by different gene expression. *Porphyra* has recently been recognized as a model plant for basic and applied studies in marine sciences. There are several advantages to study the genetics and genomics. For example, the establishment of several pure lines, the small genome size (estimated to be $2.6x10^8$ bp consisting of 3 chromosomes) and the short generation time (a few months) are suitable for genetic analyses. Linkage analyses, including both classical and molecular marker (AFLP, microsatellite DNA) studies are in progress. Moreover, a large scale expressed sequence tags (ESTs) analysis of *P. yezoensis* has recently been performed. Now, we are performing the following subjects in order to make *P. yezoensis* a sophisticated model organism: cryo-preservation, establishment of mutant cell lines, development of host-vector system for transformation, *Porphyra* genome project which includes genetic map, EST, cDNA macro/micro array, etc. The present status of infrastructure arrangements for advanced research on *P. yezoensis* which is a key to open the door toward the new phycology world for macroalgae will be introduced, featuring the activities of our research group, in this presentation.

S2-5
APPLICATION OF GENOMICS APPROACHES TO GENETICS IN ALGAE: EST ANALYSIS IN *PORPHYRA YEZOENSIS*

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With an objective to understand the whole genetic system in red algae, we initiated a large scale expressed sequence tag (EST) analysis of *Porphyra yezoensis*. Normalized and size-selected cDNA libraries were generated from both gametophytic and sporophytic stages of *P. yezoensis* Ueda (strain TU-1), and single-pass sequencing was performed from the 5'-end of each cDNA clone. As of April, 2002, 10,154 and 10,625 5'-end ESTs were established from gametophytic and sporophytic stages, respectively. These EST sequences were clustered into 4896 non-redundant groups. Database search of the 4896 non-redundant ESTs by BLAST algorithm showed that approximately 40 % have similarity to those of registered genes from various organisms including higher plants, mammals, yeasts, and cyanobacteria, while remaining 60 % are novel. Among the 4896 non-redundant ESTs, 1055 (21.5%) groups were classified as ESTs that commonly occurred in both stages, whereas relatively large number of groups appeared only in sporophyte and gametophyte EST population, 1797 (36.7%) and 2044 (41.8%) groups, respectively. The 1797 and 2044 groups appeared only in sporophyte and gametophyte EST population may include genes that underlie structural and functional differences between the two developmental stages.

The sequence data of individual ESTs are available at the web site http://www.kazusa.or.jp/en/plant/porphyra/EST/.

S3-1

DISEASE AS A BIOLOGICAL FACTOR IN ALGAL GROWTH AND PRODUCTIVITY

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The effect of "ice-ice" disease (thallus whitening and softening) on the growth and productivity of the green morpho-type of *Kappaphycus striatum,* "sacol" variety farmed in Batangas, Luzon Is., Philippines was studied. The occurrence of the disease was determined as well as the extent to which each cultivar was infected in both the floating and fixed bottom monoline methods of farming. The growth rates of the cultivars were also monitored, including the photosynthesis-irradiance responses of the healthy and diseased cultivars. Results showed that when there is high occurrence of the disease, the degree of infection was also high. Productivity of the uninfected part of the diseased thallus decreased by 40-50%, while that of the infected part decreased by 80%. Their photosynthetic efficiency was reduced by more than 70% (as indicated by decreased alpha values and increased compensation points). Consequently, growth rates of the diseased cultivars were lower than those of the healthy cultivars, yet such decrease may not always be highly significant. However, if diseased plants/cultivars were not harvested at an earlier time, the eventual softening of the thallus would lead to breakage, resulting in enormous losses in biomass production due to losses of thalli in the field.

S3-2

PRODUCTION ECOLOGY OF *ECKLONIA CAVA*, VARIATIONS IN PRODUCTIVITY AND PHOTOSYNTHATE ACCUMULATION OVER LIFE HISTORY STAGES

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Ecklonia cava have a wide distribution around the southern coast of Japan. This species make a dense population called "Marine Forest" in subtidal zone from 3 to 20 m in depth. It is known that *Ecklonia* marine forests show a high productivity compared with land forests. *E. cava* grows up rapidly from winter to early summer, and hardly grows up from summer to autumn during mature seasons of this species. Photosynthetic activities of *E. cava* were measured by many researchers under various temperature or light conditions with viewpoints of the base of growth analysis. E. cava performed high photosynthetic activity all the year around, even in summer and autumn during low grow rate seasons of this species, although respiratory rates were little higher than the other seasons because of high water temperature. Then, we propose a new hypothesis. From winter to early summer during the rapid growth seasons, photosynthates are promptly used for developing the leafy blades. From summer to autumn during low growth rate seasons, photosynthates are accumulated and used for maturation, producing a huge amount of zoospores. The leafy blades of *E. cava* have roles as not only the photosynthetic organs but also reproductive organs. So, *E, cava* should develop a lot of leafy blades. Several kinds of photosynthates are accumulated in the leafy blades of *E. cava*, such as mannitol, alginic acid, laminaran and so on. We paid attention to the laminaran. *E. cava* accumulated the laminaran only in the mature seasons from summer to autumn. At other seasons laminaran was not detected in all organs of *E. cava*. In addition, laminaran was contained only in the leafy blades immediately before maturity, and not contained in fully matured leafy blades with sori. It was thought that mannitol played an important role as an energy source of the maturity of *E. cava*.

S3-3
STOCHASTICITY OF ALGAL RECRUITMENT IN PING CHAU, HONG KONG

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Algal recruitment in A Ma Wan, Ping Chau, Hong Kong was evaluated from 1997 to 1999 by the seasonal assessment of the presence of algal spores in the water column, biweekly and monthly provision of artificial substrata, and artificial clearing of natural substrata. Two types of clearing plots were created: one with all the algal thalli removed (chiseled), and one with the top few mm of the substrata removed (hammered). New clearing plots were created monthly, and the succession of recruits on these plots was monitored in the subsequent months. Algal spores were found to be present in the water column at all times. Algal recruits were observed on artificial substrata throughout the study period but with significant temporal variation in their recruitment pattern. Significant variations in the species composition and percentage cover of algal recruits were also observed in the clearing plots, indicating that the pattern of algal recruitment between seasons and between years did not follow any predictable pattern. However, all variations in the recruitment pattern were wiped out by the summer die off of most algae. This suggested that not withstanding any inter-annual variation in the recruitment pattern, an over-riding physical disturbance in summer was far more important in setting back the successional sequence of algal recruitment in Ping Chau.

S3-4
THE ROLE OF DISTURBANCE ON MAINTAINING DIVERSITY IN MACROALGAE

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Temperate Western Australia (WA) is home with one of the most speciose macroalgal assemblages in the world. Locations along the WA coastline have high local species diversity with high spatial variability at the smallest scale of sampling, replicate quadrats within locations. Results from previous research (Kendrick et al. 1999) have demonstrated that assemblage structure at local scales, between replicate quadrats, was influenced by the presence and density of the kelp, *Ecklonia radiata.*

In this presentation, I first describe species diversity in WA subtidal macroalgal assemblages along 1200 km of temperate coastline. I then detail recent research into how the kelp canopy influences both the physical environment and the resulting species-poor macroalgal assemblage. I then demonstrate the importance of disturbance in maintaining species diversity in macroalgal assemblages. This is shown though two experiments. The first experiment tests the effect of clearing size on persistence of alternative macroalgal states to kelp dominated. This experiment manipulates clearances between 0 and 200 m^2, which is the range in size of naturally occurring clearings within *Ecklonia radiata* kelp beds. The second experiment details the diversity of alternative states in >300m^2 clearances during recolonisation by macroalgae.

I then propose a conceptual model that links patch creation in *Ecklonia radiata* kelp beds and maintenance of the patch in an alternative state, to the species diversity and small-scale species turnover of contemporary temperate Western Australian macroalgal assemblages.

S4-1

PHYLOGENY AND TAXONOMY OF THE LABYRINTHULIDS AND THRAUSTOCHYTRIDS

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Labyrinthulids and thraustochytrids are marine, biflagellate protists. Initial classifications of the thraustochytrid in the Oomycetes and labyrinthulids in the Fungi, Protozoa and algae were based on their different superficial morphologies. The subphylum Labyrinthista (phylum Labyrinthulomycota, phylum Labyrinthomorpha) is composed of these groups based on the following common unique characters: sagenegenetosome, an ectoplasmic net and a cell wall composed of non-cellulosic scales. This subphylum is classified in the phylum Sagenista, infrakingdom Heterokonta (nearly equal to stramenopiles), kingdom Chromista based on the tripartite tubular flagellar hairs on the locomotive anterior flagellum of the zoospore. The labyrinthulids *sensu stricto* and thraustochytrids are respectively classified as the families Labyrinthulaceae and Thraustochytriaceae in the single order Labyrinthulales and class Labyrinthulea. The genera in the thraustochytrids are characterized by the patterns of life history and light microscopic morphologies. However, the taxonomic system of thraustochytrids has problems because of several species sharing two or three generic diagnostic characters. The molecular phylogenetic work also suggests that thraustochytrid genera do not form monophyletic groups. Moreover, these organisms are separated, based on signature sequences and unique inserted sequences, into two major groups, which were named the labyrinthulid phylogenetic group (LPG) and the thraustochytrid phylogenetic group (TPG). These groupings are in disagreement with the current taxonomic system and many conventional taxonomic characters. Thus, the currently-used taxonomic criteria need serious reconsideration.

S4-2

DEGRADATION OF FALLEN MANGROVE LEAVES AND LABYRINTHULOMYCOTA

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Fallen mangrove leaves are the main organic source for the mangrove ecosystem, from which various Labyrinthulomycota have been isolated. Docosahexaenoic acid (DHA) and other polyunsaturated fatty acids (PUFA) produced by Labyrinthulomycota are essential nutrient for some marine faunae. We investigated the relationship between degradation of the fallen leaves and the family of the isolated Labyrinthulomycota including Thraustochytrid (Thra) and Labyrinthulid (Laby).

Fallen leaves of *Bruguiera gymnorrhiza* and *Rhizophora stylosa* (two typical mangroves) at various extent of degradation were collected from the Fukido River in Ishigaki Island, Okinawa, Japan in April, June, September 1999, January, April and June 2000. Thraustochytrids were isolated using a pine pollen baiting method and Laby by our method using the plate of *Psychrobacter phenylpyruvicus*. Isolated strains were cultured in liquid or solid medium and the fatty acid profiles of the isolates and leaves were analyzed by GLC. The color of fallen leaves varied from greenish yellow to blackish brown according to the extent of degradation. Using the ratio of α-linolenic acid/palmitic acid in the leaves, the degradation degree of the leaves from D1 to D4 was determined.

Average degradation degree of the leaf sample resulted in higher level in September than the other months. Though no effect of two type of mangrove leaves on Thra/Laby occurrences was observed, Thra/Laby occurrences were higher in June when the seawater temperature was higher than the other months. Effect of degradation degree on the microbial occurrence was different between Thra and Laby, that is, Thra was observed almost at the same rate from D1 to D4 but Laby was observed more often in the more degraded leaves. This result indicated that Thra and Laby might serve differently in the course of degradation of mangrove. The isolated Thra was categorized into three types using PUFA profile. In June when Thra occurrence was high, occurrence of lower-DHA-content-Thra was increased.

S4-3
ECOPHYSIOLOGY OF MANGROVE THRAUSTOCHYTRIDS

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This study unravels the life history of thraustochytrids and their successful survival in the mangrove habitat through a series of laboratory and field experiments. Selected thraustochytrid isolates were used to evaluate the physiological conditions for growth. Laboratory studies indicate mangrove thraustochytrids can grow and reproduce well within a wide range of temperature (15-30°C) and salinity (0-30‰) levels at a pH range of 4 to 9 with optima between 4 and 7. These results indicate that mangrove thraustochytrids are able to tolerate the fluctuating physical parameters in their immediate environment, an essential attribute to survival in the mangroves.

Colonization of mangrove litter, especially submerged leaves, by thraustochytrids relies on the success of their reproductive units to secure nutritional and substrate resources rapidly. Although zoospore motility has been investigated in a numbers of Öomycetes, thraustochytrid zoospore motility, in terms of speed and tactic response, was investigated in detail for the first time. Curvilinear velocity (VCL) and straight line velocity (VSL) of zoospores of selected thraustochytrid species were measured. They were motile over a wide range of salinities (0-100‰) and remained active up to 120 min. with VCL and VSL ranging from 109.3 and 77.7 μm/s respectively for *S. mangrovei* KF6 in distilled water. Zoospore chemotaxis of thraustochytrids was examined by using a capillary root model. Most leaf extracts of mangrove plants were shown to induce moderate chemotactic responses in zoospores of *S. mangrovei* KF6 and *Ulkenia* sp. KF13. Thraustochytrid zoospores are likely to rely on the presence of these compounds as an environmental cue to locate the surface of a mangrove leaf for colonization.

Schizochytrium mangrovei was the most abundant colonizer of decaying leaves of *Kandelia candel*. Seasonal variations of thraustochytrid abundance was observed, with an overall higher number of cells / g of detritus in winter/spring season than in summer/fall season. The observed results may be attributed to the difference in salinity regimes due to the dry vs wet subtropical winter and summer climate in Hong Kong. The nutrient status of the decomposing leaves may be another contributing factor.

The DHA yield in *S. mangrovei* strains were exceptionally high as compared with temperate strains, with production from 747.7 to 2778.9 mg/L after 52 h fermentation at 25°C. These findings indicate that mangrove thraustochytrids might be important producers of polyunsaturated fatty acids (PUFAs) and thus an important natural supplier of PUFAs in the food chain of the mangrove ecosystem.

S4-4
UTILIZATION OF THRAUSTOCHYTRIDS FOR FISHERY FEED

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For mass production of marine fish larvae, zooplanktons such as rotifer and *Artemia* have been used as primary feed for fish larvae in hatcheries. The importance of docosahexaenoic acid (DHA) as essential fatty acid for marine fish is widely recognized. Prior to the feeding of rotifer and *Artemia* to fish larvae, these zooplanktons are nutritionally fortified by feeding of the commercial DHA-enriched algae, such as *Euglena* and *Chlorella.* But these commercial algae are enriched with DHA by uptake of exogenous DHA that is prepared from fish oil. A novel feed for the zooplanktons that can produce more DHA in the cells is required.

Twelve strains of highly DHA-accumulated thraustochytrids were isolated from coastal sea water in Japan. The lipid contents in the cells and the DHA contents in the total fatty acid of these isolates were 13.7 - 23.0% and 21.5 - 55.4%, respectively. For the utilization of these isolates as a feed for nutritional enrichment of rotifer and *Artemia*, dispersibility of the cells in the water was evaluated. The cells of the several isolates were agglutinated in sea water or tap water, and brought high mortality of rotifers and *Artemia* during their nutritional enrichment. Strain KY-1, however, showed high DHA content and good dispersibility in the water. Moreover, the DHA in KY-1 cells was rapidly transferred into the rotifers and *Artemia* fed on KY-1 cells during their nutritional enrichment. These results showed that the strain KY-1 would be a novel and profitable feed for nutritional enrichment of rotifer and *Artemia.*

S5-1

PYRENOID FORMATION ASSOCIATED WITH THE CELL CYCLE IN THE BROWN ALGA, *SCYTOSIPHON LOMENTARIA* (SCYTOSIPHONALES, PHAEOPHYCEAE)

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Pyrenoids are proteinaceous structures in chloroplasts observed in many eukaryotic algae and hornworts. It has been confirmed that the main component of the pyrenoids in all algae is ribulose-1, 5-bisphosphate carboxylase/oxygenase (RUBISCO), a key enzyme involved in carbon dioxide fixation. RUBISCO consists of eight large and eight small subunits, and in brown algal cells, both the large (*rbc*L) and small (*rbc*S) subunit of RUBISCO is chloroplast-coded. At present, it is unclear why and how RUBISCO proteins gather locally in the pyrenoid.

Scytosiphon cell characteristically has only one chloroplast with a prominent protruding pyrenoid though the life cycle. In this study, we examined how the number of pyrenoid in cell is maintained, and when new pyrenoid appears within chloroplast during the cell cycle. We prepared a polyclonal antibody against the rbcL extracted from *Scytosiphon,* and observed the behavior of pyrenoid though the first and second cell cycle of zygotes which developed synchronously, by immunofluorescence and electron microscopy. Just fertilized zygotes had two chloroplasts derived from male and female gametes. At the first cell division, each of the chloroplasts was distributed into the daughter cells, respectively. From observations by immunofluorescence and electron microscopy, it became clear that the new pyrenoid was formed during mitosis. At this time, the old pyrenoid remained, and several masses which were detected by anti-rbcL antibody, appeared at some regions of chloroplast stroma. Under electron microscopy, these were observed as electron dense material. Eventually, they gathered in one place and chloroplast had two protruding pyrenoids. It was also suggested that the new pyrenoid formation would be depended on the cell cycle rather than the light and dark cycle in culture as the result of experiments using some cell cycle inhibitors.

S5-2

CELLULAR AND MOLECULAR STUDIES ON REPRODUCTION AND SEX DETERMINATION IN *AGLAOTHAMNION OOSUMIENSE* ITONO (RHODOPHYTA)

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Sex determination and development of reproductive structures in *Aglaothamnion oosumiense* were examined by cytogenetic and molecular studies. A chromosome studies on male and female reproductive cells indicated that the sexuality of this species might be determined by a sex chromosome. Chromosome counts in female and male gametophyte gave 37 and 36, respectively. Sex ratio of gametophytes was 1:1. Both male-derived bisexual plants were observed. They were different in morphology, position of carpogonial branches and chromosome number. Some male developed parasporangia in addition. Chromosome number of the paraspore germlings was the same as male plants. A novel method combining element of suppression subtractive hybridization (SSH) with high throughput differential screening permited an efficient and rapid cloning of rarely transcribed differentially expressed genes. Potential of the method is demonstrated by the isolation of 212 subtractive clones that were differentially expressed in male and female plant. About 18 out of them were confirmed by reverse northern. Two genes, AOMS-1 and AOMS-2, which showed male specific expression were analyzed. These genes seem to be involved in differentiation of male reproductive structures.

S5-3

SEXUAL INCOMPATIBILITY IN RED ALGAE

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The events of fertilisation in outcrosses of several red algae have been carefully followed with time-lapse videomicroscopy. **1. *Bostrychia mortiziana* x *Murrayella periclados***.Time-lapse videomicroscopy reveals that these species closely resemble each other in events of fertilisation but they do not interbreed. Under laboratory conditions, crossing does not yield viable carposporophytes. At what precise stage during fertilisation does a spermatium from one species and the carpogonium from another fail? Spermatia of *B. moritziana* successfully bind to the trichogynes of *M. periclados*. These spermatia divide and the fertilisation pore forms but the migrating male nuclei "plug" the pore and are unable to enter the trichogyne cytoplasm. By contrast, spermatia of *M. periclados* adhere to *B. moritziana* trichogynes but nuclear division does not occur and fertilisation proceeds no further. **2. *B. moritziana* x *B. simpliciuscula***. Spermatia of *B. moritziana* readily adhere to trichogynes of *B. simpliciuscula*. The spermatial nucleus divides but usually a fertilisation pore does not form. However, when sexual plasmogamy occurs the migrating male nucleus does not fully enter the trichogyne cytoplasm but remains at the pore junction. Spermatia of *B. simpliciuscula* bind to trichogynes of *B. moritziana* but generally the spermatial nucleus does not divide. Occasionally spermatial mitosis occurs but no further events of fertilisation are observed during this crossing experiment. Incompatibility is expressed at different stages in the various hybridization experiments described here. Other interspecific (*B. moritziana* x *B. radicans*) and intraspecific (*B. moritziana* x *B. m.*, *B. radicans* x *B. r.*) crosses have also been tested. These investigations help to understand the mechanisms that maintain genetic isolation between species and populations of red algae.

S5-4

MORPHOGENESIS IN GIANT ALGAL CELLS

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There are algae that consist of cells reaching scores of millimeters in size, which include the genera *Bryopsis*, *Valonia*, *Acetabularia*, and *Vaucheria*. These algae have relatively simple organizations, but the constituent cells undergo a variety of fundamental plant morphogenesis, such as growth, development, and differentiation. In addition, the cells are so giant that they are easy to deal with for experiments. Therefore, the giant algal cells have been used as some model systems to study plant morphogenesis. Plant morphogenesis encompasses changes in cytoskeletal elements, cell wall structures, and gene expression. Immunofluorescence microscopy has contributed to understanding functions of cytoskeletal elements in some green coencytes. It has been suggested that microtubules and actin filaments are involved in organelle movement, localization and direction of tip growth, nuclear divisions, and wound-healing reactions. Spatial and temporal differentiation of cells is also genetically regulated. It is the case that the transportation and localization of messenger RNA are required for morphogenesis in *Acetabularia*. In tip growth, the polar deposition and loosening of cell wall occur coordinately to keep cell shape. As an approach to clarify mechanisms controlling tip growth, cell wall extensibility in xanthophycean alga *Vaucheria* has been examined.

S6-1

THE CURRENT STATUS OF BARREN GROUND PHENOMENA ON THE COASTS OF JAPAN AND KOREA

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Deterioration and/or disappearance of seaweed beds have been paid more attention than before in the eastern Asia as well as the other areas in the world. In Japan, the phenomena are called "Isoyake". "Isoyake" was a dialect of Izu Peninsula, referring to the episodicdecrease of *Gelidium* and/or *Ecklonia*, which in turn causes the reduction of abalone and prawn. After the academic use by Yendo (1902),the word has covered various types of decrease of seaweed in all over the country. Some of such phenomena seem to be caused by oceanographic events, while the others seem to be generated by anthropological reasons. In Korea, barren ground phenomenon coupled withblooms of crustose coralline algae has been first documented scientifically in 1982 and became a major environmental problem in coastal ecosystem. Korean people called 'getnogum' or 'bekhwa' phenomena indicating the disappearance of macroalgal thallus and turning into white colored barren ground after the death of crustose corallines. Regarding the causes of the barren ground phenomenon,most people believe that there is no single factor for it, rather it could be one of the following processes: (1) Being grazed by herbivores (mainly sea urchins or fish), (2) Deteriorating in high water temperature/low nutrient conditions or turbid waters, (3) Being detached from substrata by high waves during storms, (4) Failing in community reproduction by decreased water motion and/or mud accumulation. We will introduce barren ground phenomenon in this talk, and present some of the results of on-going researches and discuss on possible causes of barren ground phenomenon in both countries in this symposium section.

S6-2

EFFECTS OF FISH GRAZING ON TEMPERATE KELP FORESTS IN SOUTHERN JAPAN

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The high water temperature and lack of nutrition salt by approach on the coast of the Kuroshio has been considered to be the cause by which the temperate kelp forests disappear on a large scale in a short term. On the other hand, it was reported that the macro brown algae transplanted to the Isoyake areas disappeared by the grazing of the herbivorous fishes and the algae grew up in the cage. The observations by SCUBA diving and the laboratory experiments in recent years suggest that the overgrazing by the herbivorous fishes, such as *Signus fuscescens* and *Calotomus japonicus*, is the cause of Isoyake. It is pointed out that especially the grazing of *Signus fuscescens* affects the disappearance of the kelp forests. It is reported that the consumption of the algae by *Signus fuscescenses* decreases as water temperature falls from 20 degrees. If mean water temperature in winter is 18 degrees or more, it is thought that *Signus fuscescens* is possible to winter in the areas where the kelp forests develop. It is guessed that the young fishes are produced from the wintered *Signus fuscescens* is survived without restriction by the quantity of food, and the disappearance of the kelp forests actualizes in the autumn when the young fishes grow. The water temperature in winter of 1972-1973, 1975 years and 1989, 1991-2001 years was higher than 18.1 degrees of the mean in the Miyazaki Pref. central part. It is surmised that there was a similar tendency in the whole area of the coast in the southern Japan. According to the documents and the catching investigations, many of Isoyake in the southern Japan have occurred in the first half of 1970's and 1990's. It is mostly in agreement in the period when winter water temperature is high.

S6-3

VEGETATIONAL CHARACTERISTICS OF BARREN GROUNDS ON THE EASTERN COAST OF KOREA

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This study investigated the influence of environmental factors on the spontaneous occurrence barren ground including the changing variation of seaweed vegetation in the eastern coast of Korea. Four different coastal areas were studied(Galnam, Gyoungpo, Youngok and Jeodo area located in DMZ) between 1994~2001. Barren ground expansion was measured by the seaweed biomass and the frequency of occurrency of crustose coralline algae. Barren ground occurred in the south coast of Gangwon in 1997~1998 but did not appear in the north coast area until 1998~1999. Algal standing crops on the Gyoungpo area were 3~4kg/m^2 in 1994; however, fleshy algae had mostly disappeared in 1997. The frequency of the crustose coralline algae in Galnam located in the southern part of Gangwon coastal area was very high as over 50%. The Jeodo area located near the Demilitarized Zone(DMZ, latitude 38°) showed the lowest frequency of crustose coralline algae. These areas were less contaminated than any other areas. The barren ground phenomenon occurred in the lower part of the littoral zone at Jeodo, while it happened in the upper part of the sublittoral zone at Galnam. The character of the area where the barren ground occurred that the crustose coralline algae were growing the beyond the pier. This indicated that the population abundance of the crustose coralline algae was affected by water currents caused by the geographical configuration of the coast area.

S6-4

NUTRIENTS AND SNAIL GRAZING COULD RECOVER THE ALGAL VEGETATION ON COBBLES COLLECTED FROM A BARREN GROUND IN SOUTHWESTERN HOKKAIDO

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Coralline-covered cobbles collected from a barren ground in Southwestern Hokkaido were exposed to herbivores in aquariums (35 l) using flowing seawater from 1994 to 1995 (exps. I-IV) and from 2000 to 2002 (exps. V-VII). In exps. I-III, a few cobbles were exposed to three species of herbivores (5 sea urchins: *Strongylocentrotus nudus*; 10 snails :*Chlorostoma turbinatum* or *Ompharius rusticus*, or 5 sea stars: *Asterina acutinifera*) using flowing ambient (nutrient-poor) seawater (5-24°C) of the barren ground. The cobbles in sea-star aquariums and control (no animals) were covered with diatoms and filamentous algae, while those in other aquariums were kept clean. Sea urchin and snail grazed corallines heavily and slightly, respectively (resulted in maturity of corallines in the latter); sea star induced multi-layer shedding of corallines. In exp III, *Laminaria* never appeared in all of the aquariums although its zoospores had been released. In exp. IV, in which the cobbles were exposed to 0, 1, 3 or 5 sea urchins, diatoms covered only in the absence of sea urchins. Exps. V-VII were conducted in two aquariums (absence or presence of snail) using deep-sea (nutrient-rich, clean) water of Toyama Bay kept around 11°C all through the year. Cobbles were kept clean only in the presence of snail; macroalgae (up to 20 species including *Laminaria*) grew on various types of refuges in spite of relatively high water temperature. In addition, some cobbles turned brown because encrusting brown algae overwhelmed the corallines. In exp. VI, cobbles (*Laminaria* zoospores released) were heavily covered with the kelp in the presence of snail. Extensive cover of diatoms disturbed coralline algal recruitments as well as growth of fleshy algae. These results suggest that nutrients and moderate grazing are needed to recover the algal vegetation in the barren ground.

S6-5

THE ROLE OF CRUSTOSE CORALLINE ALGAE IN THE ESTABLISHMENT OF MACROALGAE AND IN ALGAL SUCCESSION

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To investigate the potential role of crustose corallines and herbivores in the establishment of macroalgae at the early successional stage, we conducted a field experiment using cages with settlement blocks in a tide pool where crustose corallines dominated. A separate laboratory experiment was also carried out to examine the effect of coralline algae on algal spore settlement using coralline crust-covered rocks featuring four experimental conditions: live corallines, dried corallines, EtOH-extracted corallines, bare rocks. Sloughing rate of crustose corallines was indirectly evaluated by counting conceptacles under three different temperature levels. Algal settlement was facilitated in the absence of herbivores from the earlier stage of succession, and the pattern was continuously maintained until the end of experiment. As a substrate for algal settlement, coralline crust-covered rocks showed the higher values in number of species and total biomass of macroalgae than bare rocks. This field observation was largely supported by the results of the laboratory experiment. In that, number of germinating spores on the coralline crust-covered rocks (i.e., live, dried, extracted) was greater than that on the bare rocks with some variations depending on algal species. Number of conceptacles was greater at 25°C than at 20 and 15°C, indicating that reproduction and sloughing were enhanced at the higher temperature. In general, herbivores inhibited the settlement of frondose macroalgae during succession. This, in turn, caused corallines, the potential competitor of frondose algae, to increase at the end. Facilitation rather than inhibition in algal establishment by coralline crusts implies that natural recovery from existing barren grounds can be possible if certain conditions (e.g., herbivory, temperature, etc.) are given.

S7-1

GAMETE RECOGNITION IN A RED ALGA *AGLAOTHAMNION OOSUMIENSE*: CYTOCHEMICAL AND BIOCHEMICAL APPROACHES

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In red algae, fertilization begins with gamete-gamete contact between the trichogyne cell wall of the female carpogonium and spermatial coverings. The gamete binding is highly selective suggesting the presence of recognition factors along their surfaces. In the previous studies, we have reported that spermatial binding to trichogynes of the red alga, *Aglaothamnion oosumiense*, is mediated by a lectin-carbohydrate complementary system, Spermatial binding to trichogynes was inhibited by pre-incubation of trichogynes with N-acetyl-D-galactosamine and D-glucose and hence lectins specific to these sugars were expected to present on the surfaces of trichogyne cell wall. FITC-conjugated lectin showed the presence of the complementary carbohydrates on the spermatial surfaces. We have isolated a new lectin from female trichogynes of *Aglaothamnion oosumiense* by the use of agarose bound N-acetyl-D-galactosamine affinity chromatography and named it as rhodobindin. Rhodobindin agglutinated human erythrocytes as well as spermatia of *Aglaothamnion oosumiense*. The agglutinating activity of this lectin was inhibited by N-acetyl-D-galactosamine and N-acetyl-D-glucosamine. SDS-PAGE results showed that this lectin might be monomeric. The molecular weight was determined as 21,876 dalton by matrix-assisted laser desorption ionization (MALDI) mass-spectrometry. N-terminal amino acid sequence of the lectin was analyzed and revealed to have no identity with those of known proteins. On the other hand, male recognition glycoprotein was isolated and purified from released spermatia using SBA affinity chromatography. This glycoprotein had a N-acetyl-D-galactosamine residue and blocked gamete binding very effectively. Degenerative primers for both proteins were designed based on the amino acid sequences and the genes encoding both proteins were analyzed. Purification and characterization of male and female gamete recognition molecules from *Aglaothamnion oosumiense* supported our previous cytochemical studies.

S7-2

GENE EXPRESSION INDUCED BY NITROGEN STARVATION AND SUBSEQUENT GAMETIC DIFFERENTIATION IN *CHLAMYDOMONAS REINHARDTII*

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The unicellular green alga *Chlamydomonas reinhardtii* is an excellent model system for studies on gametogenesis and mating. In this organism, vegetative cells of the mating-types plus and minus differentiate independently into gametes when deprived of nitrogen (N), especially ammonium ions. Light and a specific period in the G1 phase of the cell cycle are also required for sexual differentiation. When N is depleted, the vegetative cells undergo two critical programs: They set up an adaptation program such that they adjust to nitrogen starvation, and they also express a gamete program which permits them to recognize, agglutinate and fuse with gametes of opposite mating-type.

We searched genes from 10,368 different EST clones, whose expression is markedly induced during nitrogen starvation and gametogenesis (*nsg*). The synchronized vegetative cells in the early G1 phase were harvested, resuspended in either +N or –N medium, and incubated in the light for 8 h until they had become fully competent gametes under –N conditions. Macroarray analyses between two targets isolated from +N and –N cell mixtures were able to isolate over 100 *nsg* genes, and about 2/3 of them were confirmed by Northern blot analyses. The kinetics of RNA transcription of *nsg* genes under –N conditions were analyzed, and they were ordered with respect to the timing of their expression.

S7-3

SEXUAL REPRODUCTION OF *CLOSTERIUM PERACEROSUM-STRIGOSUM-LITTORALE* COMPLEX: PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR BIOLOGICAL APPROACHES

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The conjugation process of *Closterium peracerosum-strigosum-littorale* complex (*C. pslc*) consists of sexual cell division (SCD), which is responsible for differentiation to gametangial cells, pairing, release and fusion of gametic protoplasts and formation of zygotes. Two glycoproteinaceous sex pheromones (PR-IP and PR-IP Inducer) involved for the progress of sexual reproduction have been well-characterized. The PR-IP Inducer was known to induce the release of PR-IP from mating-type plus (mt^+) cells, whereas PR-IP induces the release of gametic protoplasts from mating-type minus (mt^-) cells, during latter steps of the sexual reproduction.

Recently, four additional pheromonal acitivities were detected. These were two SCD-inducing pheromones (SCD-IP) and two Insoluble-Polysaccharide Secretion Inducing Pheromones (IPS).

The SCD-IP-minus was released from mt^- cells and induced the SCD of mt^+ cells and vice versa. Partially purified SCD-IP-minus showed quite similar characters to a PR-IP Inducer. Actually, the activity of SCD-IP-minus was declined by immunoprecipitation using anti-PR-IP Inducer antiserum and recombinant PR-IP Inducer produced in yeast could induce not only releasing of PR-IP but also SCD of mt^+ cells.

On the other hand, IPS-plus was detected as a pheromone that was released from mt^+ cells and induced the secretion of uronic acid-rich insoluble polysaccharides (IP) from mt^- cells. Similar to the case of PR-IP Inducer, purified PR-IP was shown to induce the IP secretion from mt^- cells. In addition, it could also promote the sexual cell division of mt^- cells. It was proposed that PR-IP could function in three steps of the sexual reproduction; IP secretion for forming the cell assembly as IPS-plus, the sexual cell division for forming gametangial cells as SCD-IP-plus, and the protoplast-release for forming zygotes as PR-IP.

From these results, possible mode of sexual reproduction of *C. pslc* was discussed.

S7-4

CO_2 CONCENTRATING MECHANISMS IN ALGAE: THEIR ROLE AND REGULATION BY ENVIRONMENTAL FACTORS

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Most algae possess the ability to actively transport inorganic carbon as HCO_3^- and/or CO_2, and to accumulate CO_2 at the active site of RuBISCO. Various physiological and ecological roles have been attributed to these CO_2 concentrating mechanisms (CCMs) and these will be discussed. CCMs are regulated by a number of environmental factors. Some of these environmental factors, such as photon flux, can influence the instantaneous activity of a CCM without necessarily affecting the capacity of the cell to transport inorganic carbon; others regulate the expression of genes associated with CCMs and photorespiration. This paper will consider the complex interactions between environmental factors in controlling CCM activity. In particular the consequences of global climate change (elevated CO_2 and UVB radiation) for the performance of algae with and without CCMs will be discussed.

S7-5

MOLECULAR ANALYSES OF CELL RESPONSES TO CHANGES IN CO_2 LEVEL AND LIGHT: CARBON CONCENTRATING MECHANISM AND ITS REGULATORY PATHWAYS

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Aquatic photosynthetic organisms can acclimate to environmental stresses such as low CO_2-supply, high-light by inducing a set of genes for a carbon-concentrating mechanism (CCM) through putative signal transduction pathways. Expression of several CCM-related genes, *Cah1* coding for a periplasmic carbonic anhydrase (CA), *Mca* for a mitochondrial CA and *Ccp1* for a chloroplast envelope protein LIP-36, is induced by lowering CO_2 in a green alga, *Chlamydomonas reinhardtii.* This acclimation to CO_2-limiting conditions suggests the existence of sensory mechanisms by which cells perceive the shortage of CO_2 and pathways by which the signal is transduced into the specific gene regulation. We have cloned a nuclear regulatory gene, *Ccm1*, which complements the mutation in the pleiotropic high-CO_2 requiring mutant C16 (Fukuzawa 2001). *Ccm1* is constitutively expressed both in high- and low-CO_2 conditions. His-54 in the zinc-finger motif of CCM1 is essential to its regulatory function. To isolate target genes which are controlled by CCM1, we have developed cDNA macroarray containing 10,368 EST (Fukuzawa *et al.* 2002). RNA samples from WT cells cultured in high-CO_2 or low-CO_2 and also from the low-CO_2-acclimated C16 were labeled with ^{32}P-dCTP and hybridized with cDNA array membranes. Fifty nine low-CO_2 inducible EST clones were not induced in the C16 mutant, suggesting that these are regulated by CCM1. Time-dependent expression patterns of EST clones enabled us to detect low-CO_2 inducible (Lci) genes containing those for photorespiration or ion-transporters. Relationship between CO_2-limiting stress and photorespiration or high-light stress will be discussed as well as CO_2-signal transduction pathways deduced from array analyses of regulatory mutants.

Fukuzawa, H. *et al.*: Proc. Natl. Acad. Sci. USA.98: 5347-5352 (2001)

S8-1

MARICULTURE OF SEAWEEDS WITH DEEP SEAWATER IN JAPAN

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The east coasts of Muroto in Kochi faces to the Pacific Ocean, and has a steep sea floor. Thus, the deep seawater can be obtained only 2 km off the coast from 320 m of depth. The characteristic of deep seawater are to be colder water temperature of 12°C, greater quantities of nitrogen, phosphates and silica. The seawater temperature in the tank suppied deep seawater, ranged among 15 and 23°C by the effects of air temperatures. The materials of useful seaweed, *Enteromorpha prolifera,* edible green seaweed, *Scytosiphon lomentaria*, edible brown seaweed, *Porphyra dentata, Eucheuma serra* and *Meristotheca papulosa,* edible red seaweeds, grew well in the outdoor tank. The growth rates of *E. prolifera* were significantly greater than that of other species. Their daily growth rates attained to 60% in April and 50 % in October. Their daily growth rates showed 30~46 % in other months. *E. prolifera* fronds cultured in deep seawater become the best grade as commercial products. The daily growth rates of *P. dentata* were 39% in January. The quality of their fronds was also good grade as commercial products.

The daily growth rates of *S.lomentaria* ranged among 25 and 30 % in winter season. The average daily growth rates for *E. serra* showed 2.0~2.5 %. The average daily growth rates for *M.papulosa* also showed 1.5~2.0%. These growth rates of them are similar to the results of their cultivation in the sea. The daily growth rates for *E. serra* attained more than 3.0 %, when their plants were cultured under temperature control condition of deep seawater.

S8-2

INTRODUCTION ON ADVANCED AND NEWLY DEVELOPED SEAWEED CULTIVATION IN KOREA

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Porphyra cultivation in Korea came to a turning point in 1990's during which the exposure method advanced and marketing system in management was changed. The new exposure method by floating buoys that attached one side of the floating net makes to change the exposure system. Using the small boat that attached the stainless steel roller at the one side of the boat, floating *Porphyra* nets can be easily and very quickly turn upside down, then the time is less than 2 min. per 100 m of net. Otherwise, some cultural methods such as exposure time, net size and establishment of net were modified. Another important change is separation of farmer and processor in cultural management system.

Recently, according to increasing demand of nutrient health foods, species diversification of seaweed cultivation is needed to extend the opportunities for developing new markets. For this reason, the two species, *Capsosiphon fulvescens* and *Costaria costata*, were successfully developed as new cultivation species in Korea.

Capsosiphon fulvescens is a filamentous green algae growing on the high tidal level than *Porphyra.* It is also traditionally utilized as food in the form of boiled soup with oyster in western part of the south coast. Artificial seed production was experimented by the means of gametes liberation. Also pilot and large scale of cultivation techniques are established for mass culture of this alga. The retail price is about $6.2/kg as fresh condition in this year.

Costaria costata, brown algae, started in cultivation at the eastern coast of Korea. Now, the alga transplanted to the culture grounds in southern coast, successfully. The cultivation technique is very similar to *Undaria* or *Laminaria* because of its taxonomical similarity. The price is also good as any other brown algae in domestic market.

S8-3

ADVANCEMENT OF *PORPHYRA* CULTIVATION IN CHINA

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In China, two species of *Porphyra*, *P. haitanensis* and *P. yezoensis* are cultivated and many progresses of *Porphyra* cultivation have been mad in the resent years. In 2000, China produced some 44000 t dry weight of *Porphyra* by cultivation from about 20300 hectares of farms. The southern species, *P. haitanensis* being mainly cultivated in two provinces of Zhejian and Fujian, accounts for about 90% of this production. The production averages about 2.6 and 0.6 t dry weight per hectare for *P. haitanensis* and *P. yezoensis*, respectively. In the last five years, there were several big technical progresses in *Porphyra* cultivation of China, one was applying of cold storage of nursery nets and of the improved varieties in *P. yezoensis* cultivation; and another one was development of half-floating cultivation technique instead of using the traditional full-floating cultivation technique in *P. haitanensis* cultivation. These new techniques significantly increased the cultivation areas, production and qualities of *Porphyra* in China.

S8-4

THE SYNERGY OF SEAWEED HYDROCOLLOID (CARRAGEENAN AND ALGINATE) PRODUCTION AND APPLICATION: AN INDUSTRIAL EXAMPLE

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This presentation outlines the use of seaweed raw materials for the production of carrageenan and alginate by Degussa Texturant Systems SAS France (DTS). The company is positioned as a technical solutions provider to commercial users of a wide range of industrial hydrocolloids. In addition to a carrageenan production plant, Baupte, France also houses an Application Services Centre (ASC) where new applications and formulations used in commercial applications are developed. DTS produces an very wide range of hydrocolloids and a range of examples for the applications of these will be presented (e.g. fruit, dairy, meat, etc. and nonfood applications, see also www.texturantsystems.com). The synergy of production and commercial application of seaweed hydrocolloids is extremely important and provides solutions directly to commercial customers. Innovation, new products and rapid responses to market demands are strategically valuable in expanding the use of seaweed hydrocolloids, which in turn increases the demand for raw materials. New opportunities for use of seaweed hydrocolloids will be discussed.

Traditionally, wild stocks of French *Chondrus crispus* and *Laminaria digitata* have been harvested and used by DTS for the production of carrageenan and alginate respectively. The present strategy with regard to access to raw materials will be discussed. DTS is not involved in seaweed cultivation, but increasing demand for raw materials is met by purchasing globally from farmed crops. Seaweed farming is a sustainable coastal enterprise, with few negative environmental impacts. It provides much needed opportunities for employment and economic development in emerging economies. In addition, important sources of raw material are obtained from wild stocks in Chile (e.g. *Gigartina, Iridaea*, etc). A list of taxonomic names and centres of production will be presented.

S9-1

THE CELL BIOLOGY OF DIATOMS RECORDED BY VIDEOMICROSCOPY

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In 1896, the great german microscopist Robert Lauterborn published a superb treatise on diatoms (when he was is his early twenties!). His drawings through the microscope are remarkable: in them, we can clearly identify golgi bodies, microtubules, centrosomes and other organelles. He was one of the first biologists to watch chromosome movement in living mitotic cells.

Inspired by this work, we have devoted years to watching these beautiful cells under the microscope. Using a wide variety of centric and pennate diatoms and recording in real time or in timelapse, we have recorded many aspects of the biology of living cells. I will present a video that will show: the interphase cytoskeleton; mitosis and cell division; morphogenesis including chloroplast and nuclear movements before and after mitosis; secretion of the valve and girdle bands; secretion of silica spines; secretion of mucilage and holdfasts; the mechanism of gliding movement in centric and pennates; diurnal movement of chloroplasts; and sexual reproduction.

S9-2

FUNCTION OF CENTRIOLES ON THE DEVELOPMENT OF *FUCUS* ZYGOTES

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The oogamous brown algae, *Fucus* and *Pelvetia*, has been used for investigating various aspects of developmental biology, particularly the establishment of polarity during rhizoid formation. We have examined fertilization, zygote development, and parthenogenesis of unfertilized eggs in *Fucus distichus* by immunofluorescence and electron microscopy, especially focusing on the behavior of centrioles and the spindle formation (Motomura 1994, 1995, Bisgrove *et al.* 1997, Nagasato *et al.* 1999, 2000). From these studies, we have clarified the following points, 1) eggs originally do not have centrioles, and sperm introduces them into zygotes after plasmogamy, 2) this pair of centrioles duplicates after karyogamy, and centriolar bipolar mitotic spindle is formed, 3) when karyogamy is inhibited by colchicine, egg chromosomes become condensed after DNA synthesis, but sperm chromosomes do prematurely, 4) when karyogamy is inhibited, acentriolar mono or bipolar spindle is formed in egg nucleus, 5) when multinucleate egg is fertilized, supplementary mitotic poles are formed adding to two centriolar mitotic poles, and 6) gamma-tubulin is not detected in these acentriolar mitotic poles. Recently, we have developed a new non-destructive technique using mineral oil. By this method, we can clearly follow mitosis and cytokinesis in vivo by flattening just fertilized zygotes under DIC microscopy. Moreover, we can prevent karyogamy of egg and sperm nuclei without colchicine treatment, and make cytoplasmic fragments containing only egg and sperm nucleus after plasmogamy. It became clear that each centrosome defines territories off daughter cells during cytokinesis. In cytoplasmic fragment containing only egg nucleus, it entered into mitotic period, but the chromosomes remained condensed, not dispersed. Centrosome might be related the spindle checkpoint. We will also introduce these topics using video microscopy.

S9-3

ORIGIN, ROLE AND DYNAMICS OF APICOPLAST

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Apicoplast, a non-photosynthetic plastid found in protozoan phylum Apicomplexa (McFadden *et al.* 1996), is essential for apicomplexan parasites, e.g. *Plasmodium falciparum*, a notorious causative agent of malaria. This plastid has close sister relationship to 'red-lineage' secondary plastids of heterokonts and dinoflagellates, which are originated from a single red alga (Fast *et al.* 2001). While the function and what is essential are still an open question, it could be said that apicomplexan parasites are algae adapted for parasitism and that apicoplasts are plastids for parasitism.

While morphological dynamics of this organelle has been known in early observation using electron microscopy, the whole picture of apicoplast division mechanism is quite unclear. The only thing known is the association with the centrosomes (Striepen *et al.* 2000). Generally, the plastid division is caused by contractile structure designated the plastid-dividing ring (PD ring; Mita *et al.* 1986, Hashimoto 1986, Miyagishima *et al.* 2001). As PD rings have been also observed in heterokonts, it is natural to suppose that apicoplast are also divided using PD rings.

In this session, we will talk about the background and current topics of origin, role, and dynamics of apicoplast, and introduce our research on *P. falciparum* and *Toxoplasma gondii*. Apicoplasts had high copy number of DNA suggesting strong activity of the organelles. A homolog of HU, a major DNA binding protein of bacterial nucleoid, was found in the apicoplast nucleoid (Matsuzaki *et al.* 2002). Several structures with potential involvement in apicoplast division were observed (Matsuzaki *et al.* 2001): 1) The "cap" structures are possibly a linker to the centrosomes or a remnant of cytoplasmic segregation factors. 2) The "ring-like" structure is a potential PD ring that will generate the division force. 3) The "granular" structure is hypothesized as a microbody.

S9-4

THE CILIATE *MARISTENTOR DINOFERUS* AND ITS ZOOXANTHELLAE, *SYMBIODINIUM*

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Maristentor dinoferus was recently described a new genus and species from coral reefs on Guam. It has since been found in Saipan (Northern Mariana Islands) and Palau and may be much more widespread. It forms black clusters, visible to the naked eye, especially when they are on the seaweed *Padina* and other light-colored backgrounds. When fully extended, this sessile ciliate is trumpet-shaped, up to 1 mm tall and 300 μm wide across the cap. It resembles the famous freshwater protozoan *Stentor* but has important morphological differences, which required a new genus. The ciliate is host to 500–800 symbiotic algae (zooxanthellae), which, on the basis of electron microscopy and molecular biology, are *Symbiodinium*. The symbiosis has some intriguing features. It is interesting as a comparative organism for the coral symbiosis because while the algal partner occurs in coral and many other invertebrates on coral reefs, the host is not in the animal kingdom (currently classified in Alveolatae, together with the dinoflagellates).

Identifcation of *Symbiodinium* species is difficult. Classical taxonomy of thecate dinoflagellates is based on detailed anatomy of the thecal plates (the "shell") and several free-living species have been described. In symbiosis, however, *Symbiodinium* lacks thecal plates, and taxonomic identification is based on molecular studies. Strains of *Symbiodinium* from a wide range of reef invertebrates have been grouped into four clades on the basis of rbcL sequences and isozyme analyses. Initial identification of the strain from *Maristentor* had shown that it fit with clade C. We compared the strain from new samples from Guam with strains from invertebrates collected in Palau and found only 1 substitution (in 1300 bp) compared to a clade C strain from the clam *Fragum unedo*. However, in other samples of *F. unedo* from Palau we found *Symbiodinium* clade A. To better understand the host-symbiont relationship in *M. dinoferus*, we have to analyze allopatric and sympatric collections of *Symbiodinium*. We have collected samples from Saipan and Palau and expect to present the results of our analyses at Algae 2002.

S10-1
MORPHOLOGICAL CHARACTERISTICS AND PHYSIOLOGICAL RESPONSES OF *PORPHYRA SUBORBICULATA* KJELLMAN (BANGIALES, RHODOPHYTA) FROM FIVE LOCALITIES

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The influence of temperature and photoperiod on blade growth, morphology and sporulation of five local strains of *Porphyra suborbiculata* from Ilocos Norte, Philippines; Shangwei, China; Kagoshima, Japan; Enoshima, Japan and Tong Yong, South Korea were investigated under two photoperiod (14L:10D and 10L:14D) regimes at different temperature (5, 10,15, 20, 25, and 30°C) conditions in laboratory. The blades of Philippines and China strains grow at 15-30°C with optimum growth at 20-25°C under both photoperiods. The Kagoshima, Enoshima and Korean taxa tolerate a temperature range of 10-25°C under both photoperiods and grow best in 10-15°C at short photoperiod. Cell production and development of the Kagoshima, Enoshima and Korean specimens appear parallel to the blade surface under long daylength resulting to round to ovoid blades while linear cell arrangement produced linear-lanceolate to reniform blades under short day conditions. Archeospore liberation is observed earlier in higher temperature for all strains regardless of photoperiod. Sexual reproduction is prevalent within the optimum growth conditions of all strains under both photoperiods. The appearance of 2-3 celled marginal denticulations is generally earlier at higher temperature and long day conditions with slight difference in morphology (4-5 cells) for the Chinese strain at 25°C. Thallus growth of both Philippines and Chinese strains is indicative of tropical water conditions while the Kagoshima, Enoshima and Korean specimens are representative of warm temperate environments. *Porphyra suborbiculata* exhibited wide temperature tolerance that could imply species adaptability to varying environmental conditions. This also suggests that during winter months in the northern hemisphere, warm continental waters could possibly reach the northern coasts of Philippines and southern China provinces where day-length remains within 11-12 hours. It can be speculated that under natural conditions, a relative small photoperiod difference of 1 hour between regions is effective for blade growth and conchosporangia production for tropical strains.

S10-2
GEOGRAPHICAL DISTRIBUTIONS AND THE PHYSIOLOGICAL CHARACTERISTICS OF *PORPHYRA* SPP. (BANGIALES, RHODOPHYTA) ALONG THE COASTS OF KOREA

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Distribution of thirteen species of *Porphyra* along the coasts of Korea was studied. The influence of temperature, light intensity and day length for growth and maturation of blade and conchocelis of the *Porphyra* spp. were observed in culture. The distribution pattern of the *Porphyra* spp. along the coasts of Korea was divided into three groups. First group is distributed along the northeastern coasts, classified as Liman Current species: *P. katadae, P. koreana, P. okamurae* and *P. pseudolinearis*. The second group, the Kuroshio Current species; *P. dentata, P. ishigecola, P. kuniedae, P. lacerata, P. seriata, P. suborbiculata, P. suborbiculata* f. *latiforia and P. tenera* is prevalent along the western and southern coasts. *Porphyra yezoens*is that can be found all over the coasts of Korea occupy the third group. Conchocelis of all species grew at a wide temperature range. Maturation of conchosporangia of *P. dentata, P. katadae, P. pseudolinearis* and *P. seriata* was observed at 5-30°C with an optimal range between 10-25°C. Conchocellis maturation of archeospore liberating species of *P. ishigecola, P. suborbiculata* and *P. lacerata* had a very narrow temperature range of 20-25°C. However, first maturation of conchosporangial branches of most species was observed at 20-25°C. Big blades of *P. koreana, P. kuniedae* and *P. pseudolinearis* grew at lower temperatures of 5-10°C in culture while other species grew at higher temperatures of 10-15°C. First maturation of blade in any species was observed at 15-20°C. From these results, it is considered that the distribution of species depends on temperature adaptation of the blade phase.

S10-3

CELL DIFFERENTIATION FROM THE BLADE TISSUE OF *PORPHYRA VIETNAMENSIS* TANAKA ET HO (BANGIALES, RHODOPHYTA) FROM THAILAND

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A convenient seedling technique for *Porphyra* cultivation and the ability for the cell differentiation on the excised blade tissues of *Porphyra vietnamensis* from Thailand were examined. Young blades of *P. vietnamensis*, 2.3 cm in length and 0.5 cm in width, obtained from laboratory culture were used. Small disks of excised tissues (ca. 1mm diameter) from different parts of blades were cultured at various temperatures (20, 25 and 30°C) under the photoperiod of 14L:10D, 80 μmol $m^{-2}s^{-1}$ photon flux density at a salinity of 25 ‰ in order to study the ability of cell differentiation, the excised tissues from blades cultured at 20, 25 and 30°C; 14L:10D and 80 μmol $m^{-2}s^{-1}$ at a salinity of 25 ‰ were again cultured at 30°C under both photoperiods (14L:10D and 10L:14D) at 80 μmol $m^{-2}s^{-1}$ under different salinities (10, 15, 20, 25, 30, 35 and 40 ‰). It was found that the disk tissues obtained from the upper part of the blade produced regenerated tissues and differentiated into archeospores and male gametes. While those obtained from the lowest part of the blade differentiated to rhizoids. This was found under each temperature of the culture. Temperature and salinity affected on the number of archeospore liberation from the excised blade tissues were significant (P <0.01) whilst photoperiod did not. The amount of archeospores release from excised tissues obtained from the middle part of the blade under 30°C, 14L:10D, 80 μmol $m^{-2}s^{-1}$ and salinity of 25‰ was shown to be the highest. At various salinities, cultured tissues under 30 ‰ produced a significant quantity of archeospores (ca. 2,802 spores). For the seedling technique, high liberation rate of archeospores from blade tissues of *P. vietnamensis* was shown within eight days under 30°C, 30- 35 ‰,14L:10D and 10L: 10D at 80 μmol $m^{-2}s^{-1}$.

S10-4

LIFE MODES AND SPECIES OF THE GENUS *BANGIA* (RHODOPHYTA) IN JAPAN

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Bangia spp. were collected from 24 sites of river and marine coasts in Japan. A detailed life history and morphological differences of these plants have been investigated under laboratory conditions. As a result, three species of *Bangia* in Japan were recognized. Likewise, three different types of plant habitat are known in Japan, *viz.* marine epilithic, marine epiphyte and freshwater epilithic. Plants from the rocks of marine coasts, sexual and asexual types of plant were identified. The life history of the sexual type of plant has macroscopic phase of dioecious gametophyte with asexual sub-cycles of archeospores and apogamic germlings from each gametophyte and microscopic phase of conchocelis. The asexual marine type was determined only by archeospores production. The life history of marine plant epiphytic on the *Gloiopeltis furcata* was observed as dioecious gametophyte producing archeospores and conchocelis phase. The life history of the freshwater plant from the Amahata river is similar to the marine asexual plants producing only archeospores. The length and width of macroscopic plant was clearly different between sexual marine epilithic and epiphytic plant.

Previously, we have reported the results of analysis on the SSU rDNA sequences of these plants. The sexual and asexual marine plants were quite closely related, however, the asexual plants of marine and freshwater origin were very different. We concluded that the three species of *Bangia* in Japan were recognized as sexual and asexual marine plant *B. fuscopurpurea*, freshwater type *B. atropurpurea* and *B. gloiopeltidicola* epiphytic on *Gloiopeltis furcata.*

S10-5

MORPHOLOGY OF THREE SPECIES OF *BANGIA* (BANGIACEAE, RHODOPHYTA) IN JAPAN

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Reexamination of taxonomy of genus *Bangia* (Bangiaceae, Rhodophyta) from Japan in morphology was investigated. The materials were collected from Tateyama and Katsuura, Chiba and Fukaura, Aomori in sexual thalli of *Bangia fuscopurpurea* (Dillwyn) Lyngbye, Haneda, Tokyo and Enosjima, Kanagawa in asexual thalli of *B. fuscopurpurea*; a branch of Amehatagawa-River, Hayakawa, Yamanashi in *B. atropurpurea* (Roth) C. Agaradh and Choshi, Chiba in *B. gloiopeltidicola* Tanaka. The authors investigated several characteristics, such as length and diameter of ereect thalli, cell size, number and size of cell with rhizoidal filaments and formation of male and female reproductive organs. As a result, the difference of number of the cell with rhizoidal filaments, as 19 cells in *B. fuscopurpurea,* 32 cells in *B. atropurpurea* and 5 cells in *B. gloiopeltidicola,* is most important criteria to discrete three species of *Bangia*. On *B. atropurpurea*, formation of male and female reproductive organ was not observed. The length of erect thalli of *B. gloiopeltidicola,* 1.5cm in length, was shoter than the thalli of *B. fuscopurpurea* and *B. atropurpurea.* Rhizoids of *B. gloiopeltidicola* penetrated into host seaweed *Gloiopeltis furcata*, but rhizoids of *B. fuscopurpurea* did not penetrate when archeospores attached to surface of *G. furcata*. Morphology of sexual and asexual thalli of *B. fuscopurpurea* were same. As a result of this study, there are three species of *Bangia* from Japan in morphology and SSU rRNA gene sequences.

S11-1

TAXONOMY AND PHYLOGENY OF THE BROWN ALGAL ORDER SCYTOSIPHONALES

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The order Scytosiphonales has been characterized by having a single chloroplast with a large pyrenoid and a heteromorphic life history alternating a large gametophyte and a small sporophyte. This order now includes two families and nine genera. Taxonomy of this order has been based on the external morphology and structure of gametophytic thalli. However, some generic criteria has been pointed out to be unclear or confused by some authors. Although morphology of sporophytic thalli is varied showing filamentous thalli, discs or confluent crusts in each species, it has not been applied to the genus or family-level taxonomy. A molecular phylogenetic study of this order was carried out using DNA sequences of *rbc*L, partial *rbc*S and partial large subunit (LSU) nrDNA. The results suggested doubts about autonomy of the Chnoosporaceae and monophyly of the genera *Colpomenia*, *Scytosiphon* and *Petalonia*. Morphological characters of the sporophytes, such as thallus structure and presence or absence of plurilocular zoidangia, were congruent with the molecular phylogeny. They are likely to be more important as taxonomic criteria at the generic or family level in the Scytosiphonales than morphology of the erect gametophytes. Other molecular studies suggested that the genera *Myelophycus* and *Melanosiphon* (Dictyosiphonales) are the members of the Scytosiphonales although they were reported to show isomorphic life histories. Molecular phylogenetic studies have supported the Ectocarpales *sensu lato* which includes the Scytosiphonales, and the reduction of the Scytosiphonales to a familial rank is widely accepted at present. On these circumstances, taxonomical revisions of the Scytosiphonales are needed with more morphological and molecular data.

S11-2

THE ORDER LAMINARIALES (PHAEOPHYCEAE) FROM THE FAR EASTERN SEAS OF RUSSIA

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According to the previous taxonomic revision of the order Laminariales from the Russian Far Eastern seas (RFES), 37 species were distributed in this area (Petrov, 1975). The present study presents the new taxonomic revision of Laminariales from RFES. Presently, the laminareacean flora of RFES appears to be counted as 44 species and 23 genera. Also, 2 new species belonging to new genera were established, and 1 genus and 2 species were found in this region for the first time. The number of some taxa was reconsidered. As suggested, 11 genera include only one species, and 7 genera are endemics for RFES. 10 genera are distributed along the coasts of the Bering Sea and Southeastern Kamchatka. Floras of the continental coast of the Sea of Ochotsk, Kurile Islands and Sea of Japan include 8, 15 and 8 genera, respectively. The analysis of kelp distribution along 40 different areas of the Russian Far Eastern coast showed that the most abundant and specific laminareacean flora is present on the Kurile Islands, especially on Simushir Island (11 genera and 22 species), Iturup Island (10 genera and 24 species), and Kunashir Island (9 genera and 24 species). In other areas, only 5-15 species and 4-10 genera are found. Many species (e. g. *Tauya, Costularia, Undariella* etc.) have very narrow distribution and inhibit places with very specific hydrological conditions. During the past two centuries, distribution of some species had changed significantly. Currently, penetration of the American and Japanese species into the Russian algal flora is observed. The comparative morphological analysis and florogenetic investigations of the members of Laminariales showed 5 centres of species diversity in the northern Pacific hemisphere. The laminareacean flora of RFES was formed under the influence of 3 centers. Possible ways of the historical development of this order were also suggested.

S11-3

SIMPLICITY AND COMPLEXITY - *BANGIA* IN NEW ZEALAND

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Members of the genus *Bangia* are filamentous red algae which are distributed world wide in both marine and freshwater environments. Molecular studies have shown that *Bangia* and *Porphyra* are not only closely related, but are paraphyletic with respect to one another. *Bangia* has a very simple morphology, with either uniseriate or multiseriate filaments. This haploid phase alternates with a filamentous conchocelis phase analogous to that of *Porphyra*. We have sampled *Bangia* populations from locations around New Zealand, and assessed both morphological and molecular data. Our results show that there are at many more lineages of *Bangia* present in New Zealand than previously suspected. We currently distinguish 14 groups using nuclear SSU sequence data. Phylogenetic analysis shows that these fall into several distinct monophyletic groups. Many of these SSU entities appear to be seasonal, and we have observed very rapid turnover of populations as well as ecological partitioning at sites Some entities are very restricted in distribution, while others are widespread throughout New Zealand. At a number of geographically separated sites, two or more entities are found growing coincidentally. We suggest that these entities are members of well separated phylogenetic lineages which are exploiting the environment in subtly different ways, leading to a complex spatial and temporal distribution pattern.

S11-4

SYSTEMATICS OF THE CERAMIACEOUS RED TRIBE CERAMIEAE BASED ON MORPHOLOGY AND DNAS

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The ceramiaceous tribe Ceramieae is one of the most familiar red algae because the tribe has a well-known large, cosmopolitan genus *Ceramium,* which is also the type of the order Ceramiales. Despite its long history of taxonomic studies, monophyletic categories at all ranks in the Ceramieae and their phylogenetic relationships have been less demonstrated. We studied the vegetative, reproductive, and developmental morphology of *Campylaephora, Carpoblepharis, Centroceras, Ceramium, Corallophilla, Herpochondria, Microcladia,* and *Reinboldiella* from our collections and loaned specimens of established herbaria. Lectotypes of *Ceramium californicum, C. gardneri,* and *C. pacificum* were designated from original material. A new candidate in *Ceramium,* found on the East coast of Korea, was distinct in being slightly twisted thallus with strongly inrolled apices, a single row of spines, and gland cells. Although male and female structures were very similar to each other within the Ceramieae, tetrasporangial and vegetative features were not consistent. Nucleotide sequences of 18S rDNA in nucleus and *rbc*L and *psb*A gene in plastid were determined from samples of the Ceramieae and other ceramiaceous algae. Results of separate and concatenated molecular data are compared with morphological data in establishing a more natural classification system of the minute, variable and beautiful tribe.

S11-5

CLASSIFICATION OF THE RED ALGAL FAMILY CERAMIACEAE AT THE SUBFAMILY AND TRIBAL LEVELS

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The Ceramiaceae is one of the largest, most readily recognized families of red algae with vegetative and reproductive structures having been illustrated for most genera and many species. Despite the wealth of published information,, the classification of the Ceramiaceae at the subfamily and tribal levels remain problematic. Our investigations with Wittmann's hematoxylin stain indicate that early stages in the development of the fruiting body (carposporophyte) from the fertilized egg cell are highly conserved. We hypothesize three major developmental lines, equivalent to subfamilies, that are characterized by the manner in which a derivative of the fertilization nucleus is transferred to the auxiliary cell: (1) through a tube-like protrusion or homologous connecting cell emanating from the carpogonium that typically attaches near the base of the auxiliary cell followed by the division of the auxiliary cell into a gonimoblast initial and a foot cell containing a diploid and a haploid nucleus; (2) through a tube-like protrusion that deposits a diploid nucleus that remains in the primary gonimoblast cell while a foot cell is cut off that contains only the haploid nucleus; (3) through one to three connecting cells that contain highly condensed nuclei. In this third case, nuclear transfer is effected by an auxiliary cell that expands and either fuses directly with one of the connecting cells or sends out a protuberance that initiates the fusion. In this case the haploid nucleus is cut off basally in a foot cell or laterally in a "disposal cell". DNA sequence analyses are used to test the robustness of these hypotheses. Initial molecular phylogenies based on separate and combined analyses of data from plastid-encoded *rbc*L and 16S rDNA, and nuclear-encoded 18S rDNA and 26S rDNA genes lend support to the proposed classification. The position of approximately thirty presently recognized tribes will be evaluated in the light of this classification.

S12-1

ECOSYSTEM MODELING: A TOOL TO UNDERSTAND THE INTERACTIONS BETWEEN EXTRACTIVE AND FED AQUACULTURE

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One of the most difficult tasks resource managers face is understanding the carrying capacity of coastal waters for aquaculture. Aquaculture, like many other human activities, can threaten coastal waters. Aquaculture producing shrimp and finfish depends on supplemental feeding and can contribute to eutrophication. A second type, involving shellfish and seaweeds, extracts plankton and nutrients from surrounding waters, and can have a significant positive impact on moderately eutrophic waters. Ecosystem modeling offers a 3-dimensional physical, chemical and biological simulation that can help understand and predict the impacts of aquaculture on coastal embayments. Such a model is being explored for Xincun Bay (22 km^2), which is located in southeastern Hainan Province, China. Aquaculture in Xincun Bay includes about 6500 fish pens, 100 ha of shrimp ponds, pearl oyster culture rafts and *Kappaphycus alvarezii* culture that produces 2,000 mt (Oct.-May). The surrounding area has ~15,000 people and Xincun City is a major offshore fishing and tourist center. The annual nitrogen and phosphorus removal capacity of *Kappaphycus* in Xincun Bay may be as much as 53.8 and 3.7 mt, respectively, during the 1999-2000 growing season. We are developing a prototype model that may hold the promise for showing the importance of integrating seaweed culture activities in the maintenance and health of coastal embayments.

S12-2

APPLICATION OF MARINE ANIMAL EFFLUENTS FOR CULTIVATION OF *GRACILARIA* AND *CAULERPA* IN THAILAND

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In Thailand, several cultivation techniques have been previously used for the mass production of the red algae *Gracilaria*, these including long line, fish net or cage, tank and pond culture systems. Most development of *Gracilaria* cultivation mainly employed the long line method in tanks and ponds. However, during the past few years, cultivation of *Gracilaria fisheri* and *G. tenuistipitata*, and the green algae, *Caulerpa lentillifera*, was done in outdoor tanks and ponds using shrimp and fish effluents. The N-enriched effluents have result in increased growth rate for *Gracilaria* and *Caulerpa* species. Under pond culture conditions, growth rates of *Gracilaria* generally increased during the rainy months. Maximum growth rate of 3.1 ± 1.1 $\%d^{-1}$ and 2.7 ± 1.8 $\%d^{-1}$ obtained from *G. fisheri* and *G. tenuistipitata*, respectively, during the cold months. Total production of *G. fisheri* (1000 g wet wt) and *G. tenuistipitata* (961 g wet wt) increased to 2-3 times of their initial weights (250-500 g wet wt) during the best growing season. Integrated cultivation of *G. fisheri* with sailfin molly was also studied under a semi-enclosed culture system, which obtained the highest growth rate of 12.8 ± 1.6 $\%day^{-1}$. Coincidentally, the growth of *Caulerpa lentillifera* in outdoor tanks using N-enriched effluents from fish culture was better than those cultured in non-enriched seawater. The maximum growth rate was 7.8 ± 0.9 $\%day^{-1}$. Seaweed cultivation using N-enriched effluents from shrimp and fish farms may promote further mass production for marine animal feed as well as for agar industry of the country.

S12-3
COMPARISON OF THE BIOREMEDIATION POTENTIAL OF *PORPHYRA* SPP.

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Many aquaculture industries generate a nutrient-rich waste stream that can lead to eutrophication of coastal waters. To address this pressing environmental issue, the bioremediation potential of several *Porphyra* spp. and strains was assessed using short- and long-term experiments. Short-term measurements were conducted over ca. 20 min in 30 mL tubes at 5-15 °C at high (10 g L^{-1}) stocking density. Long-term experiments were run over 30 d periods during which tissue was cultured in 1-L flasks at 15°C at lower stocking densities (0.4 –0.8 g FW L^{-1}). At three- to seven-day intervals growth rate, nutrient uptake and tissue accumulation, and phycobiliprotein concentration were determined as functions of nitrogen (N) concentration (25-150 μM and 1450 μM) and N source (nitrate vs. ammonium). Growth and tissue N reached maximal levels between 150-300 μM inorganic N. Maximum growth rates ranged from 13-18% d^{-1}, although induction of monospore production reduced average growth rates. No evidence of ammonium toxicity (reductions in growth rate or maximum photosynthetic rate) was observed. Under experimental conditions, *Porphyra* spp. removed 90-100% of N within 3-4 days at N concentrations up to 150 μM.. However, *Porphyra* spp. were less efficient in removing inorganic phosphorus (25-82% removal). Larger scale experiments (1 m^3) are planned. Overall, *Porphyra* appears to be an excellent choice for bioremediation of moderately eutrophic effluents, with the added benefit that tissue may be harvested for sale.

S12-4
APPLICATION OF *PORPHYRA* CULTIVATION IN FISH FARM EFFLUENT TREATMENT

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The seaweed biofilter/production system has been developed to reduce the environmental impact of marine fish farm effluent in the coastal ecosystem as a part of integrated polyculture practice. Several known seaweed cultivars and other possible field species have been considered as biofilter organisms based on their species-specific physiological properties such as nutrient uptake kinetics, growth potential, required environmental factors, and their economic feasibility.

Porphyra (Gim in Korean) has been used as a nutrient scrubber for bioremediation and oxygen producer with very high commercial value. It is an excellent candidate and shows efficient nutrient extraction properties. The tissue nitrogen contents of *Porphyra* were up to 5.7% (5.4±0.26%) of dry wt and showed highest value among seaweeds tested. *Sargassum* spp., *Enteromorpha* spp., and *Undaria* spp. showed 4.7%, 4.6%, and 4.2% of tissue nitrogen contents respectively where the ambient total nitrogen contents in seawater were 6.28-16.14 μM in winter season of the study area.

Rates of ammonium uptake were maintained at around 2.5 μmoles g dw^{-1} min^{-1} under high ammonium concentrations. A simple STELLA model predicts the production of biomass and removal of ammonium in the seaweed biofilter/production system. The simulation experiments with known physiological properties have been conducted to verify the system's behavior and scale-up possibility in maintaining water quality, reducing the flow-through rate of seawater, and reducing the nutrient load in effluents.

S13-1

THE SOUTH WEST PACIFIC RIM: PRELIMINARY BIOGEOGRAPHIC ANALYSES OF EAST AUSTRALIAN MARINE MACROALGAE

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The east coast of mainland Australia which trends north-south from tropical Cape York (11° 54'S 142° 15'E) to cool temperate Wilsons Promontory (39°00'S; 146°22'E) provides numerous habitats for macroalgae in the many bays and estuaries, in the Great Barrier Reef system and on the extensive wave-exposed coast south of the Great Barrier Reef. The majority of macroalgal species recorded from the Australian east coast are restricted to sections of the coastline, with relatively few species ranging along the entire east coast. Strictly tropical species generally have a southern distribution limit around 19 to 23°S. Macroalgal species with a tropical to warm temperate distribution pattern differ markedly as to the southward limit of their range. Warm temperate species also occur on the east coast. Relatively few cool temperate species which either occur on the Australian south coast or on the west and south coasts also range onto the east coast, with the northern distribution limit on this coastline varying with the species. Future collecting effort will provide the fine resolution required to determine more accurately the latitudinal extent of macroalgal species and the areas of macroalgal species richness and endemism on the Australian east coast.

S13-2

A COMPLEX PHYLOGEOGRAPHIC STORY - THE CHALLENGE OF *PORPHYRA/BANGIA*

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The genera *Porphyra* and *Bangia* have an ancient history and are distributed worldwide with more than 140 species described. 18S rDNA sequence analyses have enabled us to examine relationships within *Porphyra* and *Bangia* at a number of scales - within the New Zealand archipelago, between New Zealand and other portions of the southern hemisphere, within the Pacific (both northern and southern hemisphere) and the wider world. Although there are more than 50 distinct entities within the New Zealand region that belong to these genera, it is our conclusion that in order to understand the phylogenetic relationships of the New Zealand taxa one has to include data from throughout the geographical range of this widespread and ancient group. Traditional morphological characters previously used to separate sub-genera of *Porphyra* have in recent years been abandoned as initial molecular analyses suggested that some of their shared features were in fact homoplastic rather than reflecting a shared common ancestry. Using a fuller phylogeographic analysis than has been possible previously, we have been able to re-evaluate morphological/anatomical characters and identify those which are phylogenetically informative.

S13-3

ARCTIC OCEAN—BRIDGE OR BARRIER? REFLECTIONS ON THE BIOGEOGRAPHY OF SEAWEEDS IN COLD NORTHERN WATERS

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The Arctic Ocean affords a marine connection between the North Pacific and North Atlantic Oceans. However, this connection is mostly covered with ice and is largely a depositional environment, offering little suitable substrate for seaweeds. Limited collecting in this remote environment has revealed a paucity of species, and controvery has surrounded the affinities of these species. Various research fields have provided different perspectives on what that connection means. Paleontological studies have revealed that the Arctic provided a conduit for Pacific species to migrate into the North Atlantic as well as Atlantic species to migrate into the North Pacific. The former tracks are thought to have occurred post-Panamanian Isthmus closure (ca 3.5 mya) whereas the latter are thought to have occurred prior to that geological event. Studies of extant species, using molecular tools, provide some support for these scenarios. However, other patterns are emerging. These include examples of what appear to be relatively recent dispersal through the Arctic and the recognition of a significant number of Arctic endemics.

S13-4

PHYLOGEOGRAPHY OF SOME CIRCUM-ARCTIC SPECIES OF LAMINARIALES AND CHORDARIALES (PHAEOPHYCEAE)

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Regarding the phylogeny of Laminariales and its related taxa, recent molecular phylogenetic analyses using Rubisco gene and ribosomal DNA sequences revealed that *Akkesiphycus* (Akkesiphycaceae), *Pseudochorda* (Pseudochordaceae), form a monophyletic clade with so-called advanced kelps (Alariaceae/Laminariaceae/Lessoniaceae) as well as *Chorda* (Chordaceaee), and are considered to be ancestral taxa of kelps. These results support the notion that Laminariales originated in the northwestern Pacific Ocean and diverged expanding their distributions along the Pacific and Atlantic Ocean. In contrast, *Halosiphon tomentosum* (Halosiphonaceae) and Phyllariaceae, which used to be included in Laminariales, were shown to be rather distant from them but grouped with Tilopteridales. They are considered to have evolved and diverged principally in the Atlantic, although their geographical origin is still uncertain.

Morphological and molecular phylogenetic analyses of some circumpolar taxa such as *Chorda filum* (Laminariales), *Chordaria flagelliformis* and (Chordariales) revealed the following aspects: 1) the genus *Chorda* included at least four independent taxa: *Chorda filum* s.s. (Atlantic), *C. rigida* (NW Pacific), *C. hakodatensis* (sp. nov., NW Pacific), and *C. asiatica* (sp. nov., NW Pacific). Greater genetic diversity within each taxon in the Pacific as well as the occurrence of more taxa in the region support the Pacific origin of the genus; 2) *Chordaria flagelliformis* f. *chordaeformis* was shown to be independent from *C. flagelliformis* at the species level and should be treated as *C. chordaeformis*. In the molecular phylogenetic tree, each species divided into Pacific and Atlantic clades and the Pacific population had greater diversities in *C. chordaeformis*, suggesting its Pacific origin. However, such tendency was not seen in *C. flagelliformis*.

S14-1

BLOOM DYNAMICS OF TOXIC DINOFLAGELLATE *ALEXANDRIUM TAMARENSE* IN JAPANESE COASTAL WATERS.

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In Japanese coastal waters until the 1980s, the toxic dinoflagellate *Alexandrium tamarense* (Lebour) Balech had been distributed mostly over the northern part of the Pacific coast, such as the Hokkaido and Tohoku regions. In recent years, however, *A. tamarense* seems to have become a conspicuous species in south-west Japan, including Hiroshima Bay. In order to know the relationship between the occurrence of *A. tamarense* bloom and the local hydrographic conditions, we have conducted long-term observations on the resting cysts and vegetative cells of *A. tamarense* at one fixed station in northern Hiroshima Bay from April 1994. The resting cysts of *A. tamarense* have a temperature "window" (ca. 10 to 15°C) for germination, which controls the seasonal change in germination ability. High germination success rates (> 50 %) of the resting cysts were observed between December and April each year. From June to November, germination success rates were considerably lower (0 to 40 %). Vegetative cells of *A. tamarense* were detected each year within the period from January to June. Observed annual maximum cell densities of *A. tamarense* reached to 10^3 - 10^4 cells/L, and mostly peaked at 5m depth layer of the station in April or May. Within the bloom period, water temperatures ranged from 10.2 °C to 20.2 °C and thermal stratification was gradually developed around April. Thus, the temperature "window" of cysts was almost consistent with the temperature range of bloom period. These results indicate that the germination characteristics of *A. tamarense* resting cysts are well adapted to the ambient water temperature rhythm in temperate shallow coastal environments, allowing *A. tamarense* to seed vegetative cell populations for the recurrent spring bloom.

S14-2

OCCURRENCE OF HABS AND BIOGEOGRAPHY OF PSP TOXIGENIC DINOFLAGELLATES IN KOREA

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Harmful algal blooms occur so frequently regardless of seasons and coasts and extend from the southern coast to the other coasts of Korea. Especially, the summer outbreak of harmful algal blooms, mainly consisting of dinoflagellate *Cochlodinium*, and the spring outbreak, accompanied by paralytic shellfish poisoning, have caused fatal damage to the cultural production of fish and shellfish. Toxins found in shellfish from winter through spring have raised some problems with fishery food sanitation as well as coastal culture. Above all, the occurrence of PSP has spread in the southern coast and the ban on harvesting and selling shellfish such as mussels has been imposed ever since 1993. After then, toxification has become more severe and yearly culture ground closing in spring were continued. Based on the monitoring data on the outbreaks of PSP, it was suggested that *Alexandrium* spp. caused the regional shellfish toxification in the early spring season. But *Gymnodinium catenatum* was newly found in the Korean coasts and its toxin production was confirmed. Thirty strains of *G. catenatum* were divided into three groups as a result of cluster analysis based on toxin composition. The Yellow Sea and the South Sea were identified to have different populations with different toxin composition. By the result of sequence analysis of LSU rDNA D1-D2 region, all *Alexandrium tamarense/catenella* isolates established from various parts of Korean coasts were divided into two ribotypes, North American and temperate Asian. Toxigenic *A. tamarense/catenella* populations were distributed in all three major coasts of Korea without clear geographical isolation. The high genetic homogeneity between nearshore and offshore populations indicated a frequent genetic exchange between the two areas.

S14-3
RED TIDE OCCURRENCES AND PREVENTION STRATEGIES IN JAPAN

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The incidents of noxious red tides had dramatically increased in frequency and scale in Japanese coastal waters, especially in the Seto Inland Sea, along with serious eutrophication in 1960s and 1970s. The maximum incident 299/yr was recorded in 1976. The most important red tide organisms causing huge fishery damages by fish-kill were *Chattonella antiqua*, *C. marina* and *Heterosigma akashiwo* (Raphidophyceae), and *Gymnodinium* (*Karenia*) *mikimotoi* (Dinophyceae). Average economic loss has been estimated to be about 1 billion yen per year thereafter in the Seto Inland Sea. The level of nutrient salts had been lowered and red tide incidents had consequently decreased thereafter and reached about 100/yr in late 1980s, but this level of red tides has been kept so far with the level of nutrients supporting red tides. In 1990s, however, new red tide dinoflagellate, *Heterocapsa circularisquama* appeared and has killed bivalves of both nature and culture recurrently. Currently, useful tools are urgently needed to reduce the impacts (mass mortalities of fish and bivalves) of red tides. Algicidal bacteria and viruses play an important role in terminating red tides in coastal sea. Those bacteria and viruses have been detected abundantly at and just after the end of red tides and actually isolated from seawater. On the surface of macroalgae such as *Ulva* sp. and *Gelidium* sp., algicidal bacteria were found to be attached numerously. Algicidal bacteria were also much abundant in seawater at seaweed beds in a coastal area. It is hence proposed that co-culturing of finfish with macroalgae is a new and gentle prevention strategy for the occurrences of noxious red tides in enclosed and small-scale embayments by virtue of continuous release of many algicidal bacteria from the surface of macroalgae. Algicidal viruses are also hopeful tools by virtue of specificity to host algae and extremely high reproduction rates.

S14-4
MARINE HARMFUL MICROALGAE OF SOUTHERN PACIFIC WITH SPECIAL EMPHASIS ON BLOOM-FORMING SPECIES IN AUSTRALASIA

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A great variety of harmful algal blooms (HAB) have been recorded in the Southern Pacific. Some of them from to time present health and economic problems to the region. This review focuses on the harmful species that have been detected south of equator (Oceania) and in the southwest region (Australasia). In Oceania, on-going problems are mainly ciguatera related seafood poisoning (CSP) linking to a group of epiphytic/benthic dinoflagellates (e.g., *Gambierdiscus*, *Ostreopsis*, *Coolia*, and *Prorocentrum*). Papua New Guinea appears to be the only country in the region that has also experienced PSP (paralytic shellfish poisoning) associated with *Pyrodinium bahamense*. There have been, however, a greater variety of harmful species reported in Australasia than in Oceania. On top of the common toxic/potentially harmful epiphytic/benthic dinoflagellates found in the warm, northeastern tropical/subtropical waters of Australasia, there have been a great variety of other harmful species reported in the region. In both Australia and New Zealand several members of *Alexandrium* and one *Gymnodinium* species (*G. catenatum)* have been associated with PSP. *Prorocentrum lima* and several *Dinophysis* spp. have been linked to DSP (diarrhetic shellfish poison) detected in shellfish, and *Pseudonitschia* spp. to ASP (amnesic shellfish poison). Recently in New Zealand *Protoceratium reticulatum* has been confirmed as a yessotoxin-producer. On the northeastern coast of New Zealand, *Karenia* cf. *brevis* was implicated as the causative organism for the first ever cases of human respiratory illness and NSP (neurotoxic shellfish poisoning) outside the Atlantic coast of the USA. In both Australia and New Zealand, several species of microflagellates (e.g., *Chattonella marina*, *Heterosigma akashiwo*, *Prymnesium calathiferum*) and dinoflagellates (e.g., *Karenia mikimotoi*, *Karenia brevisulcata*, *Karenia* sp., *Amphidinium carterae*) have been linked to marine life kills. In New Zealand the newly described marine life killer *K. brevisulcata*, has also been associated with outbreaks of human respiratory illnesses.

S15-1

ENDOSYMBIOTIC LATERAL TRANSFER OF THE PLASTID: A DRIVING FORCE OF ALGAL DIVERSITY

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Recent advances in electron microscopy, molecular phylogenetics, and genomics have proved that the endosymbiotic lateral transfer of the plastid has been a major driving force of the diversification of algal cells. All the evidence currently available indicate that all plastids most likely originated from a single cyanobacterium-like endosymbiont that was engulfed by a non-photosynthetic eukaryotic ancestor. This process is called primary endosymbiosis. The resulting first photosynthetic eukaryote subsequently diversified into three major algal lineages: the glaucophytes, the rhodophytes, and the chlorophytes, all of which have plastids with two envelope membranes. Several (at least three) secondary endosymbioses, where a non-photosynthetic eukaryote engulfed a photosynthetic eukaryote (one of those that originated from the primary endosymbiosis), took place independently in different protistan lineages. They gave rise to other algal groups: the euglenophytes, the chlorarachniophytes, the dinoflagellates, the cryptophytes, the haptophytes, and the heterokonts, whose plastids are surrounded by three or four membranes. These findings have led us to realize that the protistan evolutionary tree is reticulate, and we are no longer able to talk about algae without considering their non-photosynthetic relatives.

Based on this new evolutionary picture, several protistan lineages, such as Euglenozoa, Cercozoa, stramenopiles, and alveolates have been recognized. Two algal groups, the euglenophytes and the chlorarachniophytes, are now recognized as photosynthetic euglenozoans and cercozoans, respectively. The stramenopiles includes the heterokont algae and their non-photosynthetic relatives that have tripartite tubular flagellar hairs. The alveolates are composed of the ciliates, the dinoflagellates, and the apicomplexans. They share a single ultrastructural character: the presence of cortical alveoli, thin membraneous sacks beneath the plasma membrane. A broader lineage, the chromalveolates, which includes the alveolates and the chromists (the stramenopiles, the haptophytes, and the cryptophytes), has also been proposed and is supported by a phylogenetic analysis of glyceraldehyde-3-phosphate dehydrogenase and the presence of chlorophyll c in its photosynthetic members.

S15-2

EVOLUTION OF GREEN ALGAE: A REVIEW BASED ON MOLECULAR PHYLOGENETIC ANALYSES

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Recent phylogenetic analyses of 18S rRNA gene sequences had important implications for both, the phylogenetic relationships of green algae and the assessment of their biodiversity. The monophyletic origin of two distinct lineages of green algae, the Chlorophyta and the Streptophyta, is supported. While the Chlorophyta comprises the main diversity of green algae, the Streptophyta also contains the higher land plants with the charophytes as its closest living sister group. Present molecular data support the monophyletic origin of three main evolutionary lineages within the Chlorophyta, i.e. a weakly supported sister-group relationship of the two classes Chlorophyceae and Trebouxiophyceae, and the Ulvophyceae. While the Trebouxiophyceae contains mainly terrestrial coccal green algae and lichen symbionts, the Chlorophyceae is characterized by mainly flagellates and colony-forming green algae. In contrast to traditional taxonomic concepts, molecular phylogenies are congruent with multiple origins of the coccal growth form and lichen symbioses as well as the independent loss of flagellated stages. The Ulvophyceae exhibits a broad range of morphologies from unicellular freshwater to tissue-forming marine algae. Basal to the almost simultaneous radiation of these three classes is a heterogenous assemblage of lineages representing scaled flagellates, the prasinophytes with *Mesostigma* even most ancestral within the Streptophyta. Examples from subaerial green algae revealed an unexpected diversity despite almost no morphological differences were found. *Chlorella*-like algae from a small area of a green subaerial biofilm on urban buildings were found to belong to at least five distinct lineages and significant genetic distances were among morphologically uniform strains of the same species of the soil alga *Dictyochloropsis*. Molecular methods are essential for an adequate assessment of the biodiversity of terrestrial microalgae. Secondary structure elements of the ITS-1,2 regions may contain 'signature sequences' for the unequivocal identification and definition of species of coccal green algae.

S15-3

PLASTID ORIGIN(S) AND PHYLOGENETIC RELATIONSHIPS OF HETEROKONT ALGAE

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The origin(s) and evolutionary relationships of the heterokont algae (= chromophytes = stramenochromes) were unknown prior to the advent of electron microscopic studies. Approximately ten years of electron microscopic study revealed several unifying characters, including (a) chloroplast lamellae composed of three thylakoids, with a single girdle lamella usually present, (b) mitochondria with tubular cristae, and (c) tripartite flagellar hairs on one flagellum. At about the same time, modern biochemical studies revealed the presence of a small molecular weight carbohydrate storage product (laminaran/chrysolaminaran) as well as chlorophyll *c*-type molecules and more detailed information about carotenoids. From these studies, it was postulated that heterokont algae formed a monophyletic group that arose following a secondary endosymbiosis event. Nevertheless, the nonphotosynthetic ancestors and the relationships among classes remained unclear. Molecular sequence data, especially 18S rRNA, suggested that Oömycete fungi (and relatives) and other heterotrophic stramenopiles were the nonphotosynthetic ancestors of the heterokont algae. The 18S rRNA and *rbc*L gene sequences established some evolutionary relationships among the heterokont algae, but incongruences were also evident. Within the past year, evidence has appeared that challenges the monophyly of the heterokont algae, and more rigorous combined data analysis suggests the incongruences are less serious. Concomitant with electron microscopic, biochemical and molecular results, a number of heterokont algal classes were described (i.e., Dictyochophyceae, Eustigmatophyceae, Pelagophyceae, Pinguiophyceae, Phaeothamniophyceae, and Synurophyceae) and the class Prymnesiophyceae was removed from the heterokont algae. The historical and current data for origin(s) and evolutionary relationships will be discussed.

S15-4

THE IMPACT OF NOVEL SEQUENCES FROM FRESHWATER AND BENTHIC MARINE DINOFLAGELLATES ON THE PHYLOGENETICS OF DINOFLAGELLATES

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Recent molecular phylogenetic analyses revealed that the dinoflagellates are phylogenetically closely related to two major protistan groups, i.e. the ciliates and the apicomplexans. This rather unexpected grouping of organisms is now fully recognized and this assemblage is called the Alveolates. The phylogenetic relationships within the dinoflagellates have also been studied by many researchers. Here, we present our recent data on marine benthic dinoflagellates, especially those of the genus *Amphidinium* and the freshwater representatives.

Phylogenetic affinities of benthic members of the genus *Amphidinium* were investigated based on SSU rRNA gene sequences. The species studied include *Amphidinium operculatum* (type species), *A. carterae*, *A. belauense*, *A.* cf. *elegans*, *A. britannicum*, *A. testudo* and two undescribed species, *Amphidinium* sp. (ONN-7) and *Amphidinium* sp. (MAE-18). The phylogenetic analyses showed that: 1) *A. operculatum*, *A. carterae*, *A. belauense* and *A.* cf. *elegans* formed a monophyletic clade (the true *Amphidinium*-clade) separate from the GPP (Gymnodiniales-Peridiniales-Prorocentrales)-complex and formed a sister group to the Gonyaulacales-clade. 2) *Amphidinium testudo* and *Amphidinium* sp. (MAE-18) formed a monophyletic clade within the GPP-complex, and 3) *Amphidinium* sp. (ONN-7) also appeared separately in the GPP-complex.

As to the freshwater dinoflagellates, a total of 37 strains, representing 34 species have been analyzed. The resulting phylogenetic trees revealed that the genera *Gymnodinium*, *Woloszynskia*, *Peridinium* and *Peridiniopsis* were polyphyletic. While *Gymnodiniium palustre*, *G. acidotum* and *G. uberrimum* formed a distinct clade with a type species of the genus, *Gymnodinium helveticum* was resolved as a distinct lineage and this was also supported by morphological data. Some of the species assigned to *Amphidinium*, *Gymnodinium* or *Katodinium* were shown to possess *Woloszynskia* type of thecal plates and they were clustered with some of the *Woloszynskia* species in the molecular trees.

In addition to our own data, recent knowledge on the dinoflagellate phylogeny will be reviewed briefly.

S16-1

NEW VIEWS OF PHYLOGENETIC RELATIONSHIP BETWEEN MARIMO (*AEGAGROPILA LINNAEI*) AND SOME SPECIES OF CLADOPHORALES

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The phylogenetic position of a freshwater green alga, 'Marimo' (*Aegagropila linnaei*, Cladophorales, Ulvophyceae), and the relationships between Marimo, *Cladophora* species, and some other cladophoralean genera were investigated using nuclear 18S rRNA gene sequences. This alga has usually been called *Cladophora aegagropila* or *Cladophora sauteri*, and has long been well known as its beautiful globe-like aggregation and a special natural monument in Japan. Based on morphology, it was formerly classified into the section *Aegagropila* or into the subgenus *Aegagropila*, together with several marine species of the genus *Cladophora*. This classification was not supported by the present phylogenetic analysis in which two very distinct Cladophorales clades were recognized. Marimo groups together in a well-supported clade with Tateyama-Marimo (*Cladophora* sp.), *Pithophora* sp., *Chaetomorpha okamurae*, *Basicladia* sp., *Arnoldiella conchophila*, two *Wittrockiella* species (*W. lyallii* and *W. paradoxa*), and three *Cladophora* species (*C. conchopheria*, *C. kosterae*, and *C. horii*), and other many *Cladophora* species were contained in another clade. Most of families and genera in the Cladophorales were polyphyletic showing that taxonomic characters used for these taxa do not reflect the phylogenetic relationships. Marimo and its closely related species share some ultrastructural and biochemical characteristics like pyrenoid structure and carotenoid. These characteristics might be useful as the taxonomic characters for Marimo and its closely related species. Freshwater species, included in the analysis, were located in two distantly related lineages indicating that adaptation from a marine to a freshwater habitat has happened at least twice independently in the Cladophorales.

S16-2

ECOLOGICAL FREATURES OF FRESHWATER GREEN ALGA MARIMO *AEGAGROPILA LINNAEI* IN THE BALTIC SEA AREA

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Aegagropila linnaei (*Cladophora aegagropila*) is present in number of lakes around the Baltic Sea, in addition it is abundant in the Gulf of Bothnia – northernmost part of the Baltic Sea. Its distribution to southern Baltic is delimited by salinity. *Aegagropila linnaei* is characteristic for clean or weakly eutrophicated lakes with low or average concentrations of mineral and organic substances. Sometimes it can also be found in relatively eutrophicated lakes and sea areas. In most of its' distribution area in the vicinity of the Baltic sea Aegagropila linnaei is loose-lying or attached and does not form balls. The balls are only formed in shallow lakes exposed to moderate winds.

S16-3

MARIMO AEGAGROPILA LINNAEI IN ICELAND

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The Marimo, *Aegagropila linnaei* Kutzing, has been found in 4 lakes in Iceland. Three of the lakes are in the area of active volcanism, associated with the rifting of the mid-Atlantic Ridge. Two of the lakes have been thoroughly investigated. Lake Thingvallavatn is deep (>100 m) and oligotrophic and the species forms an epilithic community at 4-10 m depth. The photosynthetic properties of this community have been investigated by Jonsson (1992). Lake Myvatn is shallow (< 5 m) and eutrophic and *A. linnaei* forms at least three types of community based on growth forms: (1) epilithic in the rocky lava littoral; (2) free-floating filaments forming a carpet together with *Cladophora glomerata* on top of the soft sediment and (3) true lake ball colonies, also in the soft sediment. The lake balls form densely packed colonies in well-defined patches in 3 areas in Lake Myvatn. Aerial photographs suggest that their extent has been reduced by about 60 % since they were discovered in 1978.

The cell walls of *A. linnaei* are very durable and are preserved in the sediment for a long time. A palaeolimnological records shows a log-linear increase with time over the last 2000 years, suggesting a limitation by light. Superimposed on this trend are large long-term fluctuations (centuries) that are negatively correlated with cyanobacterial pigments which in turn seem to be related to regional volcanic activity (Einarsson et al. 1993). Cyanobacterial blooms may reduce the production of *A. linnaei* through shading.

The lake ball colonies in Lake Akan and Lake Myvatn rank among the strangest plant communities on Earth. Their existence depends on the special adaptation of the species combined with the dynamic interaction of wind-induced currents, light regime, lake morphology, bottom substrate and sedimentation.

Einarsson, A. , H. Oskarsson & H. Haflidason 1993. Stratigraphy of fossil pigments and *Cladophora* and its relationship with tephra deposition in Lake Myvatn, Iceland. Journal of Paleolimnology 8: 15-26.

Jonsson, G.S. 1992. Photosynthesis and production of epilithic algal communities in Thingvallavatn. Oikos 64. 222-240.

S16-4

WATER QUALITY AS MACRO- AND MICRO-ENVIRONMENTAL FACTORS IN THE MARIMO HABITATS

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The Marimo *Aegagropila linnaei* is growing in about 20 lakes of the 480 lakes in Japan. To reveal the restriction factor of distribution of the Marimo, examinations of water qualities of lakes, in which the Marimo lives, were carried out. As a result, no tendencies of chemical components for distribution of the Marimo had been observed in these lakes. However, it had been found that there were springs in the Marimo populations at lake bottom. Then, water quality of spring water in the Marimo populations in several lakes were analyzed, and following characters of water qualities had been found.

1) Chemical compositions of water in Marimo populations in Lake Sai, Lake Shirarutoro, Lake Akan and Lake Mivatn were different from those of surface water of lakes. 2) High concentrations of nitrate ions were found in water in the Marimo populations in Lake Akan, and concentrations of nitrate ions were higher in water in the lake deposit under the Marimo populations than that in the Marimo populations. 3) From results of the investigations at Lake Mivatn, no oxygen was found in the surface layers of lake deposit. 4) It were found in Lake Akan and Lake Shirarutoro that there were water with high values of electric conductivity in Marimo populations.

From these results, following assumptions were discussed for the role, as environment factor, of lake bottom spring to form Marimo populations. 1) Sunlight environment: Depositions of fine particles (silts and dead microorganisms) became smaller due to spring flow. 2) Temperature environment: Spring flow keep seasonal changes of water temperature in Marimo populations mild. 3) Marimo populations keep nitrate ions, ammonium ions and phosphate ions as nutrient salt in the spring flow to oneself. 4) Lake bottom environments: Though internal of lake deposits are anaerobic, oxygen in spring flow keep Marimo populations at lake deposits aerobic. 5) Constituents for Marimo growth: Fossil sea-salt and hot spring supplied to Marimo populations constituents for accelerations of Marimo growth.

S16-5

COMPENSATION DEPTH OF MARIMO AGGREGATIONS AND ITS ANNUAL FLUCTUATIONS AT LAKE AKAN

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The marimo *Aegagropila linnaei* is a species of freshwater green algae that is widely distributed in the Northern Hemisphere. Although this alga is well known as forming globular aggregations, large aggregations exceeding 10cm in diameter occur restrictively in a few lakes of the world. On the other hand, a latest experiment investigating the light-photosynthesis relationship has revealed that the light compensation point of aggregation goes up along with diameter of aggregation becoming large. This finding strongly suggests that maximum diameter of aggregation is restricted by light condition. In the present study, to know the cause of the rare existence of Marimo aggregations, relationship between size of the aggregation and light condition was investigated in a Marimo habitat of Lake Akan, Hokkaido, Japan.

In the field, about 47,000 Marimo aggregations were collected from 440 study sites covered an area of 0.2 km^2, and were measured each dimension as well as fresh-weight. The photosynthetic photon flux density and water temperature in the habitat was observed for 7 years. Then, these data were applied to a regression expression regarding to the light-temperature-photosynthesis relationship obtained by the experiment, and annual fluctuations of compensation depths of the Marimo aggregations with various sizes at various water depths were computed. As a result, compensation depths of large aggregations with diameter of 10cm or more did not exceed 2-2.5m in most years theoretically. However, in the natural habitat, such large aggregations were distributed from 1.6 to 3.5 m in depth. Fresh-weights per a unit volume of the aggregations distributed in deeper places were only halves of the ones in shallower places. Although the light condition influences maximum sizes of the aggregations basically, in the deeper places, it seems that the aggregations reduce their light compensation points by lowering the algal density participating in the respiration and develop into larger aggregations.

SS-1

NEW FACILITIES: BIOLOGICAL RESOURCE COLLECTION AND ENVIRONMENTAL RESEARCHES

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Microbial Culture Collection at the National Institute for Environmental Studies (NIES-Collection) was founded in 1983 as the first collection center of environmental microorganisms. Since then, among various kinds of microorganisms associated with environmental issues, microalgae have been collected and preserved the most actively. The total numbers of strains of algae available for distribution are now 850. The regular activity can be summarized as follows; collection of field samples, establishment of culture strains, long term preservation of culture strains and taxonomic identification of culture strains.

In order to enhance the environmental phycology and develop appropriate *ex situ* methodologies for biodiversity conservation, new research facilities was added to the NIES-Collection Building. The additions include facilities for cryopreservation of living somatic or germ cells and tissues, or DNA of animal, plant and microbial species and genotypes under threat of extinction and erosion, for identification and classification of environmental microbes, for evaluation of functions and genotypes, and for biodiversity databases. In the new facilities, development of culture methods, morphological and physiological characterization of culture strains, phylogenetic analyses, construction of their image database and development of preservation methods are more promoted. These activities will contribute to the rapid growth of environmental phycology. Also a number of algal taxa which are in endangered state will be cultured and maintained. For example, since among 74 species and varieties of Charales have been described so far in Japan, 5 taxa were extinct, one was extinct in the wild, 24 were in critically endangered state during the last 30 years, various taxa of Charales have been actively collected, cultured and maintained in the NIES-Collection during the last 10 years. These are conserved as critically endangered algal species in the new facilities.

SS-2

ALGAE: 3.5 BILLION YEARS EVOLUTION AND DIVERSIFICATION

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This presentation aims to review what the algae are and how they should be understood in view of recent studies of the tree of life and the coevolution of the biosphere and geosphere. Algae is an assemblage of organisms defined by elimination, that is, algae comprise all the oxyphotosynthetic organisms excluding the land plants and cover from prokaryotic cyanobacteria (blue green algae) to the green algae that gave rise to the land plants. The photosynthesis that accompanies oxygen evolution was originated by cyanobacteria and first appeared perhaps 3-3.5 billion years ago. This could be called the biggest evolutionary event in the history of life, because, without it, the world of aerobic eukaryotes must have never appeared. Until the advent of eukaryotic autotrophs, cyanobacteria were only the source of molecular oxygen accumulated in the atmosphere (for ca. 2 billion years). The first eukaryotic autotroph acquired the chloroplast by engulfing a cyanobacterium (primary endosymbiosis), and it is now generally accepted that the descendants of the first eukaryotic alga are the glaucophytes, rhodophytes and the chlorophytes. These three algal lineages could be called "true plants." There are several more algal lineages that are unlike the true plants in terms of cell ultrastructure. Where are they from? How did they acquire the chloroplasts? Comparative ultrastructure and molecular phylogeny have answered these questions. It is well accepted that the lineages of six algal phyla transformed from heterotrophic protists to "plants" secondarily by engulfing the true plants. Such events (secondary endosymbioses) definitely occurred more than once. Fossil records suggest that these new algal lineages appeared on earth after the mass extinction in the Permian/Triassic boundary (P/T boundary) that was much later than the occurrence of true plants (250 Ma). Principal primary producers in the ocean should have changed drastically across the P/T boundary from cyanobacteria/green algae to chlorophyll *a* and *c* containing members (heterokont algae, dinoflagellates and haptophytes). This situation of the ocean dominated by these algal lineages has been kept up to the present day earth.

SS-3

SITUATION OF TOXIC CYANOBACTERIAL BLOOMS IN THAILAND

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The situation of toxic cyanobacterial blooms in Thailand was investigated in small storage ponds or rivers in all regions of Thailand. Besides, monitoring program of the problems in 5 aquatic ecosystems was set in Mae Kuang Dam (Chiang Mai Province) and Kwan Phayao (Phayao Province), Northern Thailand (in collaboration with Region Office 9 Chiang Mai, The Provincial Water Work Authority (PWA) ; Lam Takhong Dam, North-eastern Thailand (in collaboration with Nakhon Ratchasima Municipality and Environmental Health Center Region 5, Department of Health, Minister of Public Health) ; Bang Phra Reservior (Chon Buri Province) and Kaeng Krachan Dam (Phetchaburi Province), Central Thailand. It seemed that during the past two years the situation of toxic cyanobacterial blooms in Thailand was not so serious. The cyanobacterial blooms taxa in colony in each aquatic ecosystems were *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii*. Most of the time, the cyanobacterial cell number was in the range between 10^2-10^3 cells or filaments/ml. Concentrations of microcystins in raw water and tap water did not exceed WHO Guideline. While frequently found microcystin variants were MYCST-RR, MYCST-YR and MYCST-LR, respectively. More details of the situation would be presented.

SS-4

EVALUATION OF TOXIC CYANOBACTERIA BLOOMS IN LAKE DIANCHI, TAIHU AND CHAOHU

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Lake Dianchi, Taihu and Chaohu have been increasingly eutrophicated over the past two decades. The Lakes are the major shallow lakes along the Yangtze river region in China, with water surface areas 300km^2, 2338km^2 and 780km^2, respectively. Featured by the frequent outbreak of the cyanobacteria bloom in these lakes, the problem has gained nation-wide attention due to their crucial role in dringking water supply, among others. We have found and identified the major blooming species in the Lakes. Their abundance and biomass change and toxicity variations over the time has been assessed at the same time. Of the major blooms we have tested 80% of them were toxic ones and great majority of bloom species is *Microcystis*. With *Anabaena* , *Oscillatoria* and *Aphanizomenon* bloom occasionally occurred. Based on the cultured species, the toxin profiles, physiological features and phylogenic relationship of the toxic *Microcystis* has also studied in detail. As water blooms are still occurring and causing increasing troubles in drinking water quality and recreation functions, etc., more and more attentions have been focused on the way of controlling and diminishing blooms as well as the long-term assesment of effect of toxic bloom on the ecosystem. The present report will introduce some of the major action in dealing with the bloom problem in the Lakes.

SS-5

THE CSIRO COLLECTION OF LIVING MICROALGAE: AN AUSTRALIAN PERSPECTIVE ON MICROALGAL BIODIVERSITY AND APPLICATIONS

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Microalgae are known for their global distribution at the morphological species level. We have investigated biodiversity of Australian populations of microalgae and compared them globally using strains isolated and maintained as part of the CSIRO Collection of Living Microalgae. The Collection of over 750 strains has been mostly sourced from Australian waters, tropical to polar, and has representatives of all marine microalgal classes and some freshwater classes. We have found that chemical, molecular and physiological diversity within the one species can be strain and population specific, for example tropical versus temperate, and coastal versus oceanic, as well as on larger regional scales: Australian waters versus non-Australian. There is less within-population diversity for prokaryotic cyanobacteria compared with eukaryotic microalgae.

Unique regional biodiversity and strain specificity has implications for applications of microalgae, for example the search for novel bioactive compounds and use of microalgae in human health, agrifood and aquaculture. A particular focus at CSIRO Marine Research is the study of lipids including the omega-3 polyunsaturated fatty acids (PUFA). Microalgae are primary producers of PUFA that are essential for the health of both humans and aquaculture animals. While mixtures of PUFA are available commercially as fish oils, microalgae offer the potential for production of specific PUFA or products containing PUFA. The capacity to cultivate microalgae to produce high biomass gives a "clean, green" biotechnology option against the backdrop of concern about marine bioprospecting. However bioprocessing of microalgae is not a trivial issue. Using laboratory scale photobioreactors we have grown novel Australian strains of the algal classes Dinophyceae, Bacillariophyceae and Cryptophyceae and demonstrated both high biomass and bioactive compound production using different culture and technology conditions at laboratory scale.

In other studies we have examined strain and species differences in toxin producing dinoflagellates, aligning this with the potential to produce renewable high biomass for industry applications.

An alternative to obtaining benefit from microalgal products is using microalgae as a resource of novel genes. Our early discovery of a gene for a novel Δ-6 desaturase from microalgae opens the pathway for development of crop plants with the beneficial long chain PUFA usually only found in the marine food chain.

Our research demonstrates the importance of maintaining and studying different strains of microalgae in culture collections, and exploring the potential of biodiversity of microalgae for industry applications.

SS-6

DETECTION OF SINGLE CELLS OF BLOOM-FORMING TOXIC *MICROCYSTIS* (CYANOBACTERIA) USING IMMUNOLOGICAL PROBES

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Occurrence of water blooms caused by the cyanobacterial genus *Microcystis* in eutrophic lakes, ponds and reservoirs is an increasing worldwide environmental problem. For the prediction of the blooms, a rapid and accurate quantification method of *Microcystis* is needed. While their population size in early spring and winter seasons must be an important factor in the outbreak of the blooms it was extremely difficult to detect single cells or incomplete colonies of this microorganism by the conventional identification methods based on morphology.

Therefore we have tried to develop a new immunological method for the identification of all *Microcystis* including detection of morphologically atypical *Microcystis*. We developed a polyclonal antibody PM-2 and a monoclonal antibody MN-1 reactive with the cells of *Microcystis*. Specificity of these antibodies was examined by an indirect fluorescent antibody technique (IFA) using fluorescence microscopy. It was found that PM-2 was reactive with all the strains of *Microcystis* except for three strains of *M. novacekii*, and MN-1 was reactive with all three strains of *M. novacekii*. Using both antibodies, all the strains of the genus *Microcystis* could be detected. In an experiment examining the ability of these antibodies to recognize *Microcystis* cells in various physiological conditions, this organism was harvested after it was cultured at various growth stages, or after cultured in various nutrient limited media or at various light intensities. All the samples were examined by IFA and flow cytometric analysis. It was found that more than 90% of the *Microcystis* cells could be detected in every culture conditions although FITC intensities of the cells changed depend on the conditions. This technique was then applied to environmental waters. Ten to five hundreds cells/ml of *Microcystis* were detected in Mikata lake, Fukui prefecture, Japan from April, 2000 to February, 2001. In particular *Microcystis* cells collected from the lake in cold seasons were mainly composed of the single cells, which could not be detected by the observation using conventional optical microscopc.

Thus we conclude that the technique using the antibodies is applicable to the detection and quantification of genus *Microcystis* even if they are composed of single cells or incomplete colonies in environmental waters.

SS-7

BIOACTIVE COMPOUNDS OF FRESHWATER CYANOBACTERA

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Toxic waterblooms of cyanobacteria (blue-green algae) have been found in eutrophic lakes, ponds, and drinking water reservoirs. *Microcystis* has most often been involved in poisoning of animals, but has also been implicated in human health problems. Two types of toxins, hepatotoxins and neurotoxins, have been characterized from toxic cyanobacteria. *Anabaena, Microcystis, Oscillatoria* and *Nostoc* produce microcystins as hepatotoxic cyclic peptides and anatoxins as neurotoxic alkaloids. Cyanobacteria produce not only toxins, but also a large number of compounds with varying bioactivities. Here, we show a new classification of bioactive compounds according to their chemical structures. The biggest class is the peptide-group. This group consists of cyclic peptides and linear peptides. The cyclic peptides further can be divided normal cyclic peptides and depsipeptides. The normal peptide class consists of Adda-containing cyclic peptides, ureido-containing cyclic peptides and oscillatoric acid-containing peptides. Hepatotoxins, microcystins and nodularins, belong to Adda-containg cyclic peptides. The cyclic depsipeptide class can be divided tricyclic peptides and Ahp (3-amino-6-hydroxy- 2-piperidone)-containing depsipeptides. Generally, Ahp-containing cyclic depsipeptides consist of a ringed hydroxy group of threonine and a carboxyl group of hydrophobic L-amino acid such as isolucine and valine. These peptides inhibit the activities of chymotrypsin and/or trypsin.

Alkaloides such as anatoxin-a, anatoxin-a(s) and saxitoxin are neurotoxins. These alkaloides have been isolated from *Anabaena, Aphanizomenon,* and *Oscillatoria, Microcystis* species also produces anatoxin-a. A hepatotoxic alkaloide, cylindrospermopsin has been isolated from *Cylindrospermopsis raciborskii* and *Umezakia natans*, and has an acute (24h) LD_{50} of 2.1 mg kg^{-1} (IP).

A thioic *O*-acid ester-containing sulfolipid (thionsulfolipid) was found from *Synechococcus* sp. isolated from Lake Biwa. This lipid is toxic to fish at the concentration of 20 ppm, and is decomposed in natural conditions. By the decomposition, hydrogensulfide as a typical toxic compound is formed from alkylthioic *S*-acid derived from the lipid.

SS-8

PROVASOLI-GUILLARD NATIONAL CENTER FOR CULTURE OF MARINE PHYTOPLANKTON: PAST, PRESENT AND FUTURE

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The collection was originally formed in 1980 from the private cultures of Luigi Provasoli and Robert R.L. Guillard because many of these marine phytoplankters were not being accepted in other collections. The CCMP was first housed at Woods Hole Oceanographic Institution, but after one year the collection was moved to Bigelow Laboratory for Ocean Sciences. Scientists interested in research or aquaculture generously deposited algal cultures and the collection grew quickly in size. In 1985, on advice from an advisory committee, the mission changed from a "collection" to a "center" to promote active research at the CCMP. In 1992, the U.S. Congress designated the CCMP as a National Center and Facility. Currently, the CCMP holds over 1800 strains, and approximately 700 strains are maintained in a cryopreserved condition. All types of strains are being added, but current emphasis is on harmful algae and oceanic picoplankters. The CCMP website is currently under major revision. Strain records will include a map showing the collection site, digital images will be provided for each strain, strain records will have a direct link to GenBank accessions, and searches will be enhanced to allow users to designate geographic areas on regional or global maps. The CCMP will also host a bulletin board for algal culturing, with discussion threads stored in archival files. As the CCMP moves into the future, several goals have been established. Obviously, the size of the collection and the number of cryopreserved strains are expected to increase. The open oceans remain poorly sampled, and the CCMP will help investigate oceanic biodiversity through collection, isolation and maintenance of new oceanic strains. Bigelow Laboratory is moving to a new building complex within five years, and the CCMP will have new and expanded facilities. Molecular facilities are expected to be an ever-increasing component of the CCMP, and strains used in genome studies may become important, new model systems. Finally, the CCMP may begin accepting strains of marine bacteria, protozoa and viruses, thereby becoming a Center for more than just algae.

SS-9

CRYOPRESERVATION AND THE PROBLEM OF FREEZE-RECALCITRANCE, IN ALGAL CULTURE COLLECTIONS

Day, J G

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Long-term conservation of microbial cultures depends on the removal of water and/or rendering it unavailable for biological activity. In general, in microbial collections, organisms are preserved employing drying, freeze-drying (lyophilization), or cryopreservation techniques. For the Culture Collection of Algae and Protozoa (CCAP) where the holdings are restricted to micro-algae (including the prokaryotic cyanobacteria), small thalloid Rhodophytes and free-living non-pathogenic protozoa the use of long-term preservation methods is problematic and only cryopreservation has been found to be effective as a preservation technique.

Cryopreservation forms a pivotal role in most of the major Algal Culture Collections and at present approximately 30% of the CCAPs' holdings are maintained in a cryopreserved state. This relatively low level is primarily because no appropriate protocols have been developed for the majority of protists and many of the more complex and fragile organisms are considered to be freeze-recalcitrant. Traditionally, the development of algal cryopreservation protocols has been empirical, with most "successfully" cryopreserved strains frozen using simple two-step protocols. These techniques have been effective in preserving a relatively limited taxonomic range of algae and have largely been restricted to morphologically uncomplicated, or small species. On the application of sub-optimal protocols intracellular ice formation commonly occurs and, to date, with the exception of *Chlorella protothecoides*, this is invariably lethal to the effected cells. In addition to cell-damage caused by intracellular ice, the failure of protective mechanisms that ameliorate chill injury, dehydration-induced stresses and injury caused by other freeze-induced stresses such as extracellular crushing and the effects of free-radicals can also result in severe membrane damage, gross disruption of intracellular organelles and cell death.

The CCAP maintains an active program of cryopreservation protocol development and fundamental research on freeze-induced injuries in algae and protozoa. The mid-term objective is to significantly increase the number of strains held in a cryopreserved state.

SS-10

EXPLORING EVOLUTIONARY RELATIONSHIPS OF MICROALGAE: MOLECULAR PHYLOGENETIC ANALYSES AND CULTURE COLLECTIONS

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Algal culture collections are important living resources for exploring phylogenetic relationships of microalgae. The paucity of morphological characters may hide a considerable genetic diversity and, therefore, analyses of DNA sequences from marker molecules are required. For example, rRNA gene sequence analyses in green algae and unicellular cyanobacteria showed the same morphotype to have arisen multiple times in independent evolutionary lineages. In culture collections the same algal species is often represented by several strains. ITS rDNA sequence and AFLP (Amplified Fragment Length Polymorphism) analyses showed that there is a considerable genetic diversity even at the level of strains within a species for *Chlorella vulgaris* and *Trebouxia* spp. These studies underline the importance of culture collections as gene banks of algal biodiversity. Cultures are essential for the utilization of microalgae through biotechnology, health care and environmental industries. To ensure the required high quality the unambiguous identification to class and species levels of the maintained algal strains is important. PCR-RFLP has worked rather effectively in several examples from the green algae to identify strains within a reference system. Cultures are extremely important to document characters at both morphological and genetic levels and to assist in a correct identification of microalgae. rDNA sequences from authentic strains (which are derived from the original culture material used for the description of new species) are particularly important as references for studying microalgal taxonomy. These sequences even allow the identification and detection of algal taxa from environmental DNA samples even without culturing. This will be illustrated by examples from terrestrial and biofilm-forming green and heterokont microalgae.

C1

A NOVEL REPRODUCTIVE ORGAN AS THE PROPAGULE OF *CODIUM EDULE* (BRYOPSIDALES, CHLOROPHYTA)

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A novel reproductive structure, the propagule, was found on the side of the utricles of *Codium edule* collected during the later period of its growing season in southern Taiwan. The propagule is usually fusiform and morphologically similar to a gametangium, with no apparent gamete inside. The propagule shows the ability to produce protuberances on its surface, a characteristic not observed in gametangia. The extended filaments arising from the protuberances of a propagule can develop new utricles terminally after two weeks in cultivation. These evidences indicate that the propagules have the potential to form new thalli and can be regarded as an effective reproductive structure. Microspectrophotometric studies with DAPI showed that relative fluorescent units of nuclei in propagules were the same as those in utricles, and were higher than those in gametangia. It is suggested that the propagule is a transformed gametangia after internal fertilization of anisogametes or a similar diploidization event.

C2

GENETIC AND MORPHOLOGICAL VARIATION AMONG POPULATIONS OF *SARGASSUM HEMIPHYLLUM* (PHAEOPHYTA)

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Populations of the brown alga *Sargassum hemiphyllum* are found along the coasts of northeastern Pacific from Japan to Vietnam. Two subspecies are currently recognized: *S. hemiphyllum hemiphyllum* and *S. hemiphyllum chinense*. *S. hemiphyllum hemiphyllum* is mainly distributed in Japan and Korea, and *S. hemiphyllum chinense*, mainly in China and Vietnam. The genetic and morphological differences among populations of these two subspecies were evaluated to elucidate their evolutionary history. Samples were collected from Japan, Korea, and Fujian, Taiwan, Hong Kong and Zhanjiang in China. Restriction fragment length polymorphism analysis in large-small sub-units spacer of *rbc* gene in chloroplast genome was used to investigate the genetic variation among these populations. A pilot study showed the genetic divergence among populations to be 1.89%. Two informative patterns by restriction enzyme cutting were deduced but only samples of population from Korea (and possibly Japan) could be distinguished from the other populations according to both banding patterns. However, this spacer in *rbc* gene may be too conservative to be an appropriate genetic marker at the population level of *Sargassum hemiphyllum*. For the morphological investigation, four individuals from different populations were measured in terms of several basic parameters e.g. level of branching, length to width ratio of blades and vesicles, blade and vesicle shape. Data were analyzed by Principle Component Analysis (PCA) and those associated with continuous or categorized variables were also analyzed by cluster analysis and Chi-square test respectively. The preliminary results showed that some of the continuous variables like length to width ratio of blades and categorized variables like shape and tip of blades are significantly different among populations. Based on the initial findings, a dispersal pattern of this species from north to south is suggested. The species may have originated in Japan, and differentiated into a different subspecies south of Zhejiang Province in China.

C3

MORPHOLOGICAL DIFFERENCES IN THE SIX SPECIES OF UNARMORED DINOFLAGELLATES *COCHLODINIUM, GYMNODINIUM* AND *GYRODINIUM*

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The classification of unarmored dinoflagellates is difficult due to its morphological variability. To evaluate and re-describe the taxonomic characteristics of unarmored dinoflagellates in Korean waters, we carefully re-examined the morphological characteristics of 18 cultured strains representing 6 species: *Cochlodinium polykrikoides, Gyrodinium impudicum, Gymnodinium aureolum, G. mikimotoi, G. catenatum,* and *G. sanguineum.*

In *C. polykrikoides, Gyr. impudicum* and *G. mikimotoi*, there were not shown typical morphological differences between strains, likely due to an intra-specific relation, while the species *G. catenatum* and *G. sanguineum* were shown distinct differences in morphology between strains. Especially, four strains of *G. catenatum* showed the differences according to origin. For example, in the two domestic strains the ridges were aligned in straight lines over epicone and hypocone, while in the other two foreign strains, the ridges were misaligned. In addition, of five strains in *G. sanguineum*, domestic strains' nuclei were located in the center of cell, compared to the nuclei of the foreign strains were located in the epicone. Interestingly, one of foreign strains had five condensed chloroplasts; one in the head part of epicone, two in the lateral positions and the other two chloroplasts in lobes of hypocone.

In *G. catenatum*, we newly observed the fine striae scattered on the cell surface. In addition, one species which has been recorded as *G. aureolum* was in reality more close to *Karenia digitata,* which has a finger-like sulcus extending to the bottom of apical groove and curving to antapex.

In summary, further studies would be necessary to get a better understanding on the intra-specific and/or inter-specific relations among strains, especially on the molecular phylogeny and the fine structure of unarmored dinoflagellates such as striae, thick-short spines and nipple-like structures in cell surface.

C4

PHYLOGENY OF *LAMINARIA* (PHAEOPHYCEAE) BASED ON ITS AND RUBISCO SPACER REGION

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The genus *Laminaria* is one of the best known edible and cosmetic macroalgae, which inhabits temperate and polar waters in the northern Hemisphere and temperate waters in the southern Hemisphere. As currently recognized, 45 species are reported worldwide and most of species are perennials. Despite its familiarity and long history of biology, *Laminaria* is still controversial in taxonomy. Two infrageneric classification systems have long been used. The first system is to classify the genus into two sections based on the forms of blade: section *Digitatae* has members with a digitate blade and section *Simplices* includes simple blade species. The alternate is to subdivide *Laminaria* into four subgeneras based on the forms of holdfast: subgenus *Laminaria* has members with holdfast of hapteras, *Rhizomaria* with rhizomatous holdfasts, *Solearia* with discoid holdfasts, and *Renfrewia*, being annual, with discoid holdfasts. In order to establish a more natural classification system to reflect phylogenetic relationships, nuclear rDNA ITS and plastid-encoded Rubisco spacer region sequences were determined from 54 samples of 18 species from Canada, England, France, Japan, Kamchatka, Korea, South Africa, and USA. Our analyses of the separate and concatenated sequence data show that *Laminaria* is not monophyletic and rather subdivided into two lineages with strong supports; (1) the *L. saccharina* group including *L. bongardiana, L. dentigera, L. diabolica, L. gurjanovae, L. japonica, L. longicruris, L. longipedalis, L. longissima, L. nigripes* and *L. religiosa*; (2) the *L. digitata* group including *L. hyperborea, L. logipes, L. ochroleuca, L. pallida, L. setchellii* and *L. sinclairii.* This molecular phylogeny agrees well with morphological data that the *L. saccharina* group shares bullation on the blade and the *L. digitata* group has a simple blade without bullation. Our molecular analyses do not support any of previous infrageneric classification systems, but strongly propose to classify *Laminaria* into two different taxonomic categories.

C5

A NEW CRUSTOSE RED ALGA *PEYSSONNELIA PLURICRUSTIS* AND A FIRST REPORT OF *P. HARVEYANA* (GIGARTINALES, RHODOPHYTA) FROM JAPAN

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A new crustose red algal species *Peyssonnelia pluricrustis* is established on the basis of material collected from the Pacific coast of Japan (Shizuoka and Chiba Prefectures). It can be distinguished from related species which have *P. rubra-type* anatomy, closely packed perithallial filaments in a firm matrix, hypothallial filaments arranged in parallel appearance, hypobasal calcification and unicellular rhizoids, by the thallus habit and reproductive features. Several thalli are united to form circular patches up to 15 cm in diameter on bedrock in the lower intertidal to upper subtidal zones. Each thallus consists of a primary crust that is loosely adherent to the substratum and has detached margins and secondary crusts, which develop from the dorsal surface of the primary crust and reach 0.5-2.5 cm in diameter. These crusts are 100-300 μm thick. Gametophytes are monoecious and produce female and male reproductive structures in separate nemathecia. Spermatangia are produced in double chains (*P. harveyana* type) of 10-16-layered fertile filaments. Gonimoblasts develop from the auxiliary cell and bear 1-3 carposporangia per gonimoblast, which are 40-110 μm wide and 40-160 μm high. A tetrasporangium is produced terminally on the perithallial filament or laterally on the basal cell of the paraphysis. Cruciately divided tetrasporangia are 60-95 μm wide and 100-150 μm high.

Peyssonnelia harveyana J. Agardh, originally described from Brest in France, is distributed along the North Atlantic coasts of Europe and the Japan Sea coast of Russia. Our specimens were collected from the Pacific and Japan Sea coasts (Kochi and Toyama Prefectures). The thalli grow on pebbles in the upper subtidal zone. They are 1-5 cm in diameter and are tightly adherent to the substratum except margins. This species is similar to *P. pluricrustis* in reproductive features.

C6

MOLECULAR DIFFERENTIATION OF *GRACILARIA SALICORNIA* AND CLOSELY RELATED SPECIES

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Gracilaria (Gracilariaceae, Rhodophyta) is a commercially important agarophyte that is widely distributed throughout the tropical and temperate waters of the world. In Malaysia, there are several species of *Gracilaria* including *G. salicornia, G. changii, G.edulis, G. firma* and *G. coronopifolia*. Due to the importance of *Gracilaria*, proper classification is important for both exploitation for valuable products and in understanding diversity and evolution of the seaweeds. Traditionally, *Gracilaria* species are classified based on morphological features: branching mode, grade of constriction along thallus, shape of thallus and the reproductive structures. However, due to the limitations of distinct morphological and reproductive characteristics, species delineation of *Gracilaria* is problematic. Confusion in the classification of *Gracilaria* is due to the absence of definitive morphological characters, made more difficult through phenotypic convergence and plasticity. Xia (1986) combined *Gracilaria salicornia, G. crassa* (=*G. canaliculata*), *G. cacalia* and *G. minor* into *Gracilaria salicornia* (C. Agardh (Dawson) Xia), the oldest available name. Uncertainties in the classification can be overcome by using molecular techniques because they measure genetic rather than phenotypic changes. This study reports on the use of molecular techniques, RAPD and sequencing of the rubisco large subunit gene for understanding the taxonomic position of *Gracilaria salicornia* and its closely related species. Results show that *Gracilaria salicornia* is different from *G. crassa* and *G. cacalia*.

C7

THE ULTRASTRUCTURE AND 18S rDNA ANALYSIS OF A NEW JAKOBID FLAGELLATE

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Jakobids are free-living, heterotrophic flagellates characterized by a large ventral groove supported by microtubular flagellar roots. Recent ultrastructural studies have suggested that the jakobids are related to some amitochondriate protists which bear a similar ventral groove such as diplomonads, retortamonads, heterolobosea, *Trimastix* and *Carpediemonas*. These taxa are collectively called as "excavates" and thought to be originated from a primitive common ancestor bearing a ventral groove (excavate hypothesis). It has been shown that mitochondrial genome of a jakobid retains more bacteria-like features than that of any other eukaryotes. Jakobids are exceptional among eukaryotes in terms of mitochondrial cristae, i.e., three different types, flat, tubular and discoid cristae are present within the group. Mitochondrial cristae are accepted as a stable character in higher rank classification of eukaryotes. These mitochondrial features suggest that the jakobids are closely related to the eukaryote which obtained the mitochondrion by endosymbiosis and diversified its type of cristae. The jakobids are possibly the key organisms to solve the mystery of early evolution of the eukaryotes and the origin of mitochondrion.

We studied a jakobid-like flagellate isolated from a freshwater pond in Tsukuba. It possesses two flagella, a large ventral groove and a mitochondrion in common with the jakobids. Its flagellar apparatus is highly similar to that of the jakobids. The flagellate is similar to amitochondriate excavates in possessing two vanes along a posterior flagellum, lacking extrusomes, and a right root supporting the groove, but all these features are different from the jakobids so far described. Therefore, it is appropriate to describe the flagellate as a new genus of the jakobids. 18S rDNA phylogenetic analysis showed that the flagellate was a member of the crown eukaryotes, but relationship to other excavate taxa was not clear.

C8

TAXONOMIC STUDY OF THE TETRASPORALEAN GENUS *ASTEROCOCCUS* (CHLOROPHYTA) BASED ON COMPARATIVE MORPHOLOGY AND MOLECULAR PHYLOGENETIC ANALYSIS

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Asterococcus Scherffel (Tetrasporales, Chlorophyta) is an immotile colonial green alga characterized by having an asteroid chloroplast and swollen gelatinized layers around a cell or colony. Since no taxonomic studies based on comparative morphology of cultured material and molecular phylogenetic analyses have been carried out in *Asterococcus,* species in this genus are ambiguous and variable according to the authors. In the present study, taxonomy of *Asterococcus* was studied on the basis of comparative light microscopy and DNA sequence data from eight strains originating from various localities in the Czech Republic, England, France, Japan and Portugal. The strains could be clearly delineated into three species, *A. superbus* (Cienkowski) Scherffel, *A. korschikoffii* Ettl and *A.* sp., based on differences in structure of the swollen gelatinized layers, presence/ absence of papilla-like protrusion on the anterior end of the cell and *rbc*L gene phylogeny. Although *A. korschikoffii* was synonymized with *A.superbus* by Ettl & Gaertner (1988), the present study clearly demonstrated that *A. korschikoffii* is a distinct species characterized by its dendroid form constructed by the gelatinized layers. *A. superbus* and *A.* sp. exhibited a spheroidal colonial structure; however *A.* sp. can be distinguished clearly from *A. superbus* by the presence of papilla-like protrusion in the anterior end of the cell, and may represent a new species. Sequence data of *rbc*L genes from all of the *Asterococcus* strains and related green algae strongly suggested that this genus formed monophyletic group composed of two large clades which are distinguished by the presence (*A. korschikoffii* and *A.* sp.) or absence (*A. superbus*) of the papilla-like structures. In addition, the dendroid species *A. korschikoffii* was positioned distally, suggesting evolution from spheroidal to dendroid colony might have occurred within the genus.

C9

LIGHT AND ELECTRON MICROSCOPY AND 18S rDNA ANALYSIS ON AN UNDESCRIBED EUKARYOTE INHABITING INTERTIDAL SANDY FLATS

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An undescribed eukaryotic flagellate inhabiting intertidal sandy shore was collected from the coast of Wakayama Prefecture. It has some morphological similarities with the cryptomonads (the Cryptophyta) and the kathablepharids (heterotrophic flagellates of uncertain taxonomic position). These include two flagella emerging from subapical depression and two rows of ejectile structures lying near the depression. However, the organism is different from the cryptomonads and the kathablepharids mainly in those features such as the smooth cell surface, the structure of the flagellar transition and the complex flagellar apparatus. The molecular phylogeny based on 18S rDNA sequence suggested that the organism does not belong to any known taxonomic group of the terminal crown. Thus, it was concluded that the organism is an undescribed flagellate of a novel taxon of the eukaryotes that should be recognized at class or division (phylum) rank.

In addition, this organism holds green algal cells in the cytoplasm as endosymbionts. Most cells collected from nature possessed endosymbionts, but cells lacking them also occurred occasionally. In the laboratory, cells retained the endosymbionts for a certain period (up to 3 weeks) but eventually lost them and became colorless. A nucleus, mitochondria and chloroplasts are present in the symbiotic compartment, though other organelles such as endoplasmic reticulum or Golgi apparatus have not been found. The endosymbiont possesses a red eyespot that is always situated at the apex of the host cell. Inside the chloroplast, eyespot granules forms a sheet oriented perpendicular to the thylakoid membranes, in contrast to that the ordinary green algal eyespot lies parallel. The location of the eyespot and its unique orientation in the chloroplast strongly suggest that there is some genetic control from the host to the green algal symbiont. Symbiotic relationship between the organism and the green alga and its evolutionary implication are discussed.

C10

REORIENTATION OF THE FLAGELLAR ROOT SYSTEM DURING MITOSIS OF A UNICELLULAR GREEN ALGA *MESOSTIGMA* (PRASINOPHYCEAE)

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The flagellar root system is an important cytoskeleton in flagellated algae. This apparatus is correctly distributed into the progeny cells through cell division. However, in a few green algae, behavior of the flagellar root system during mitosis has been carefully analyzed. In this study, behavior of the flagellar root system during mitosis of biflagellated green alga *Mesostigma viride* Lauterborn (Prasinophyceae) was investigated by immunofluorescence and electron microscopy. Interphase cells are slightly ellipsoidal disc-shaped and cell size is approximately 12 μm long x 11 μm wide. Two flagella obliquely arise from the cell center and elongate towards anterior end of the cell. The cell shows the cruciate flagellar root system with four microtubular roots extending from the basal bodies. Two nascent basal bodies appear at the proximal region of the d-roots of respective parental basal bodies before mitosis. Subsequently, four diminutive daughter roots are formed laterally. Flagellar axonemes are reduced at early prophase, so whole mitotic events progress under non-motile condition. Coincidentally with basal apparatus (pair of parental and daughter basal bodies, and their roots) separation at prophase, four parental roots shorten and each basal apparatus clockwise rotates approximately at 45°, thus parental roots orient to the division plane. At prometaphase, parental two s-roots transiently connect each other at their distal region but they shorten less than approximately 2 μm before metaphase. Orientation of the basal apparatus is preserved at anaphase. During telophase, parental and daughter roots elongate and the flagellar axonemes are formed. As a result, the anterior-posterior axes of progeny cells almost perpendicularly shift from that of the parental cell. This study shows that behavior of the flagellar root system during mitosis in the ancestral green alga is similar to that of Chlorophyceae.

C11
FURTHER TAXONOMIC STUDY OF *NITELLA* (CHARALES, CHAROPHYCEAE) FROM EAST ASIA, BASED ON SEM OOSPORE MORPHOLOGY AND MOLECULAR PHYLOGENETICS

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In his world-wide monograph of the Charales, R.D. Wood (1965, *A revision of the Characeae*. J. Cramer, Weinheim) reduced many charalean species originating from east Asia to infraspecific rank based on the similarity of the vegetative morphology. Recently, we examined nine species of *Nitella* including five Japanese or east Asian endemics based on the SEM oospore morphology integrated with *rbcL* gene phylogeny, and demonstrated the efficiency of both approaches to address problems at lower taxonomic levels (Sakayama *et al.* 2002, *Phycologia*: in press). In this study, five additional taxa of *Nitella*, collected from the east Asia, were investigated using these approaches to improve understanding of their taxonomic status. The species delineated were: *N. mirabilis* Nordstedt *ex* J. Groves *sensu* Wood, *N. acuminata* A. Braun *ex* Wallman, *N.* sp. [= *N. pseudoflabellata* A. Braun f. *megacarpa* (J. Groves) R.D. Wood *non N. megacarpa* Allen], *N. gracillima* Allen [= *N. gracilis* (Smith) C. Agardh f. *gracillima* (Allen) R.D. Wood] and *N. axilliformis* Imahori [= *N. translucens* (Persoon) C. Agardh f. *axilliformis* (Imahori) R.D. Wood]. Our SEM observations demonstrated that the oospore morphology of two taxa – *N. gracillima* and *N. axilliformis* – was distinctly different from that of the species (*N. gracilis* and *N. translucens*, respectively) to which Wood (1965) assigned as infraspecific taxa. The present *rbc*L sequence data showed that *N.* sp. was separated phylogenetically from *N. pseudoflabellata*, and *N. axilliformis* from *N. translucens* and *N. axillaris* A. Braun [= *N. translucens* var. *axillaris* (A. Braun) R.D. Wood]. Although three species (*N. pseudoflabellata*, *N. leptodactyla* and *N.* sp.) which Wood (1965) assigned to *N. pseudoflabellata* exhibited similar SEM oospore wall ornamentation, they can be clearly distinguished from each other by differences in vegetative morphology and/or by *rbcL* gene phylogeny.

C12
PRESENCE OF *rbc*L IN COLORLESS HETEROKONT ALGAE

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The algae that have secondarily lost photosynthetic ability are known in various taxa (i.e. Chlorophyta, Cryptophyta, Euglenophyta and heterokont algae). Generally, these algae retain the vestiges of chloroplast called the leucoplast. The leucoplast retains and takes over basic morphological features such as the number of membranes from its ancestor chloroplast. As far as is known, the leucoplast retains chloroplast-encoded genes, even though it is highly reduced. Recently, the complete sequence of the leucoplast genome was determined in *Astasia longa*, a heterotrophic euglenoid closely related with *Euglena gracilis*. Interestingly, *A. longa* retains *rbc*L (the gene encoding the large subunit of RuBisCO) as a sole gene related to photosynthesis. This is the only report of *rbc*L in the heterotrophic algae that lost photosynthetic ability secondarily. Nothing has been reported for the colorless heterokont algae.

Two heterotrophic members of the Dictyochophyceae (heterokont algae, stramenopiles), *Pteridomonas danica* and *Ciliophrys infusionum*, were investigated. An undescribed organelle bounded by four membranes and closely associated with the nucleus was detected in the cell of *P. danica*. The outermost membrane was continuous with the outer nuclear membrane. These features strongly suggested that this organelle was a vestigial chloroplast. The *rbc*L was successfully amplified by polymerase chain reaction (PCR) method from *P. danica* and *C. infusionum*. These sequences were readily and well aligned with those of photosynthetic stramenopiles. Phylogenetic trees of 18S rDNA and *rbc*L were constructed. In all the trees obtained, *P. danica* and *C. infusionum* appeared in two different clades, the Pedinellales clade and the Ciliophryales/ Rhizochromulinales clade, each of which contained photosynthetic members as well as heterotrophic members. The results indicated that the loss of photosynthetic ability occurred independently in *P. danica* and *C. infusionum*. This is the first report of the presence of vestigial chloroplast (leucoplast) in colorless dictyochophytes and the presence of *rbc*L in colorless stramenopiles.

C13

MOLECULAR PHYLOGENY AND ULTRASTRUCTURE OF A NEW SPECIES OF THE HAPTOPHYTA WITH UNIQUE SILICEOUS SCALES FROM SHIRIBESHI SEAMOUNT

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When mineralized, haptophytes produce scales composed of calcium carbonate known as coccoliths. A haptophyte bearing unique siliceous scales was discovered from Shiribeshi seamount, Japan in July 2001. To understand biology and phylogeny of this alga, ultrastructural analyses, observations on the life cycle, X-ray analysis on the scales, and molecular phylogenetic analyses were performed.

The scale is hat-shaped, ellipsoidal in top view, 3-5 μm and 5-7 μm across the axes, and perforated by many small pores. X-ray element analysis has confirmed that the major element of the scales is not calcium but undoubtedly silica. Cells are covered by several layers of siliceous scales, 15-25 μm in diameter including scale cover. Cells also have organic scales of typical haptophytes under the siliceous scale layers. Cells are non-motile but have two short flagella hidden in the scale case, but the haptonema is eminent, one and half the cell length, and extending over the scale case. Intracellular features are of the haptophytes, including the peripheral ER, Golgi cisternae with peculiar dilations and the flagellar apparatus. Siliceous scales are produced in the posterior vacuole that is continuous to the peripheral ER. This is in contrast to that of coccoliths which are formed in Golgi or Golgi-derived vesicles.

Motile cells possessing two long flagella and haptonema occurred in culture. The motile cells lacks siliceous scales but are coated with two types of organic scales that are distinct from that of nonmotile cells. The motile cells are capable of independent growth by binary division.

Phylogenetic position was analyzed by neighbor-joining analysis of sequences of *rbc*L and SSU 18S rDNA. The result indicated that this species is the member of the *Prymnesium/Platychrysis* clade.

Biomineralization is an important link between the life and earth. The discovery of siliceous haptophyte would necessitate reconsideration of evolutionary history of life-earth system.

C14

MULTIFUNCTION OF A SEX PHEROMONE IN *CLOSTERIUM-PERACEROSUM-STRIGOSUM-LITTORALE* COMPLEX

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When mating-type plus (mt^+) and minus (mt^-) cells of the *Closterium peracerosum-strigosum-littorale* complex (*C. pslc*) were mixed together in a nitrogen-depleted mating medium, the secretion of uronic acid-rich insoluble polysaccharides (IP) was remarkably activated and the IP accumulated surrounding the cells. Activation of IP secretion from mt^- cells was observed when mt^- cells were cultured in the medium, in which mt^+ cells had been cultured alone. This means that a pheromone with the ability to activate the IP secretion from mt^- cells is released from mt^+ cells into the medium. We designated this pheromone as IPS-plus (Insoluble-Polysaccharide Secretion Inducing Pheromone which was released from mating type plus cells). Another sex pheromone with the ability to activate the IP secretion from mt^+ cells was also detected in the medium, in which mt^- cells had been cultured, and named IPS-minus.

IPS-plus showed similar properties to a previously reported sex pheromone (PR-IP), which induces the protoplast-release from mt^- cells. Indeed, purified PR-IP was shown to induce the IP secretion from mt^- cells. These results strongly suggested that IPS-plus was the same molecule to PR-IP. In addition to these activities, purified PR-IP was shown to promote the sexual cell division of mt^- cells. Namely, PR-IP (IPS-plus) might accelerate three steps in sexual reproduction of *C. pslc*; IP secretion for forming the cell assembly, the sexual cell division for forming gametangial cells, and the protoplast-release for forming zygotes.

From the dose-response experiments, it was revealed that the concentrations of PR-IP required for the progress of each step became higher with the advance of sexual reproductive processes. The result indicated that PR-IP was not a primer pheromone for triggering all over the sexual reproduction, but a multifunctional pheromone for initiating and/or promoting some separate processes of sexual reproduction in *C. pslc*.

C15

INFLUENCE OF DIFFERENT FACTORS ON CARRAGEENAN FROM RED ALGA *TICHOCARPUS CRINITUS* (TICHOCARPACEAE) OF RUSSIAN PACIFIC COAST

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Variation in polysaccharide yield and composition of the red alga *Tichocarpus crinitus* were studied under differing irradiances (30-35% and 10-15% PAR), when seaweed were presented by non-reproductive sterile form. The highest carrageenan yield observed for samples of *T.crinitus* cultured at 10-15% PAR. Algae accumulated mainly non-gelling type of carrageenan under low irradiance.

The chemical structure and biological activity of systems of carrageenan isolated from cystocarpic and sterile plants were investigated. The total polysaccharide content in plants with cystocarpic of *T.crinitus* was observed in two times more than in the sterile state. According to data obtained by C-NMR and Fourier -Transform spectroscopy the cystocarpic plant of *T.crinitus* produces system of carrageenans composed by fractions in similar amounts of gelling carrageenan with kappa/beta- types and soluble classical pure lambda carrageenan.

It was shown that the chemical structures play a determinant role in the biological activity of these polysaccharides. All types of carreganan extracted from *T.crinitus* showed antivirus activity: in tobacco plant. kappa-type of carrageenan from sterile plants of this seaweed had high activity (89%), while in algae with cystocarpic activity of lambda carrageenan increased.

Our results allow us to hope that carrageenan will be use as plant protectants

C16

ECOLOGICAL CHARACTERISTICS OF ATTACHED DIATOMS AND ENCRUSTING CORALLINE ALGAE IN THE ALGAL WHITENNING AREAS OF JEJU COAST, KOREA

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Biomass accumulation of attached diatoms and covering rates of encrusting coralline algae growing on glass slides were studied in relation to environmental parameters. We carried out this study using scuba diving at 4 subtidal zones of algal whitenning coastal area of Jeju Island from April 2001 to March 2002. Glass slides were routinely submersed at 0.5m (surface layer) and bottom layer and retrieved monthly for microscopic examinations, chlorophyll analyses, cell counts and enumeration of covering rates. Monthly cell counts and daily mean chlorophyll *a* varied from $1.2x10^4$ to $1.2x10^6$ cells cm^{-2} and from 0.11 to 0.29 mg m^{-2} day^{-1} , respectively. The dominant attached diatoms by the attaching type of extracellular polymeric substances (EPS) were comprised of four types; stalks type of *Amphora coffeaeformis* Kützing, apical pads type of *Bacillaria paxillifera* (Müller) Hendey, *Nitzschia longissima* (Brébisson) Ralfs, adhering films type of *Cocconeis pellucida* Grunow, *C. scutellum* Ehrenberg, and *Cylindrotheca closterium* (Ehrenberg) Reimann & Lewin, and tubes type of *Navicula* sp. Monthly covering rates and daily covering areas of encrusting coralline algae growing on glass slides varied from 0.2 to 86.7% and from <0.0 to 0.94 cm^2 day^{-1}, respectively, which were higher in the surface layer than in the bottom layer. We suggested that light, water temperature might be related closely to the attached diatoms and encrusting coralline algae in the study area.

C17

CYANOBACTERIAL SIDEROPHORES

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Iron is the most important transition metal ion for all organisms. It forms versatile electron transfer agents in a variety of important proteins for basic physiological processes. However, under aerobic and the neutral pH condition iron forms highly insoluble ferric hydroxide complex to limit the concentration of biologically available iron. In the last decade it has been elucidated that low levels of iron in ocean limit marine microorganisms including phytoplankton. It is well known that under iron deficient condition a large number of microorganisms biosynthesize and secrete the low-molecular weight chelating compounds, siderophores, and acquire the iron-siderophore complex into cells with their outer membrane specific receptors. Cyanobacteria are the prokaryotic phytoplankton and the representative primary producer in aquatic environment. They perform oxygenic photosynthesis using various iron-containing catalysis, and many species of them can also fix nitrogen by using the iron-rich enzyme, nitrogenase. Therefore, the iron-acquisition of cyanobacteria would have seemed of special interest. However little is known about cyanobacterial siderophore and the molecular mechanism to acquire the trace iron of cyanobacteria.

Then, we screened the ability of siderophore production of cyanobacteria by using CAS assay, which estimated the iron-chelating activity, and found that some cyanobacterial species, mainly diazotrophic species as *Anabaena*, produced strong siderophores. We isolated these cyanobacterial siderophores and succeeded to elucidate structures by using NMR and MS techniques. Anachelins from *Anabaena cylindrica* were novel siderophores and contained some unique units, which suggested that cyanobacteria had might independently evolved their siderophores.

The cyanobacterial siderophores will give us much information to investigate microbial competition to sequester iron in the aquatic environment. And cyanobacteria can fix both carbon and nitrogen from the atmosphere. Therefore their positive secretion of siderophores might be available to construct a new production system of designed organic compounds as peptidic drugs by cyanobacteria.

C18

CYTOCHEMICAL AND ULTRASTRUCTURAL STUDIES ON PROTOPLAST FORMATION FROM DISINTEGRATED CELLS OF A MARINE GREEN ALGA *CHAETOMORPHA AEREA* (CHLOROPHYTA)

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Regeneration of protoplasts from extruded cytoplasm and successive development of aplanospores within the protoplasts is described in the marine green alga *Chaetomorpha aerea* (Dillwyn) Kützing (Cladophorales, Cladophoraceae). Agglutination of cell organelles in seawater seemed to be mediated by a lectin-carbohydrate complementary system. Three carbohydrates, D-galactosamine, D-glucosamine, and a-D-mannose inhibited agglutination of cell organelles. The presence of these sugar moieties on the surface of cell organelles was verified with their complementary fluorescein isothiocyanate lectins. Agglutination assay using human erythrocytes showed the presence of lectins specific for the above sugars in the cytoplasm. Fluorescent probe 1-(4-trimethylammoniumphenyl)-6-phenyl-a, 3,5-hexatriene revealed that the envelope initially surrounding protoplasts was not a lipid-based cell membrane. However, it exhibited some functions of cell membranes including semipermeability and selective transport of materials. Fluorescein diacetate staining showed esterase activity in the protoplasts from the beginning of the regeneration process suggesting that their envelopes were intact. Enzyme digestion revealed that the composition of primary envelope initially involves interactions between polysaccharides and then transforms into a polysaccharide-lipid complex, before a complete plasma membrane develops by 6 h after protoplast formation. Electron microscopic observation showed that new plasma membrane formed by incorporation of original cell membrane into the primary envelope. A new cell wall developed within 24 hours after wounding. When the basic regeneration process was accomplished, protoplasts developed aplanospores or swarmers within 10 days after wounding.

C19

TRYING TO CULTIVATE THE TOXIC DINOFLAGELLATES *DINOPHYSIS* SPP.

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The marine dinoflagellate genus *Dinophysis* includes several species that cause diarrhetic shellfish poisoning (DSP). But the biology of *Dinophysis* is poorly understood due to the unsuccessful attempts at cultivation. Recent findings indicate that some *Dinophysis* species are mixotrophic, i.e. capable of both autotrophic and heterotrophic nutrition. In this study, we tried to laboratory cultures of *Dinophysis acuminata* and *D. caudata* apparently responsible for DSP, feeding with various foods (cyanobacteria, cryptophytes, eukaryotic picoplankton, diatom, etc.). Food organisms were killed by ultrasonic treatment and freezing (-30°C) before addition to *Dinophysis* cultures. The best results were the maximum 57 cells from single cell in 35 days for *D. acuminata* and 28 cells from single cell in 22 days for *D. caudata*. High increases in cell numbers of *D. acuminata* and *D. caudata* were observed in cultures where a small centric diatom and an eukaryotic picoplankton were added, respectively. *Dinophysis* cells could be kept alive at least for 2 months although cells became thinner and thinner, with reduced plasmatic contents. In one case of *D. caudata* cultured with an eukaryotic picoplankton, cells were filled with densely plasmatic contents in spite of after 45 days. In further growth experiments of *D. caudata* in Erlenmeyer flasks, new cells of smaller size appeared. These small cells are originated from *D. caudata* and cannot be considered as a separate species (*D. diegensis*). These results seem to indicate that the addition of food organisms is the first step to cultivate *Dinophysis*.

C2

SEASONAL VARIATIONS IN THE POPULATIONS OF *CHONDRUS OCELLATUS* AND *C. YENDOI* FROM OKIRAI BAY, NORTHERN JAPAN

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Chondrus ocellatus and *C. yendoi* are commonly occurring carrgeenophytes in Japan with the former distributed along the mainland and southern coasts while the latter abundantly found in the Hokkaido area. They are found to co-occur in patchy distributions along the northern coast of Okirai Bay, Northern Honshu forming a belt in the intertidal zone. Biomass, frond density and blade size frequency were obtained monthly while the proportion of life cycle stages (gametophytes to tetrasporophytes) was investigated every other month following the resorcinol-acetal method. Additionally, permanent bases were set up within the *Chondrus* zone and recruitment substrates of polyvinylchloride (pvc) overlain with sand, were deployed monthly. Environmental parameters were similarly taken. Maximum *C. ocellatus* biomass occurred from late summer to early fall coinciding with peaks in fertility of both tetrasporic and cystocarpic thalli as well as maximum temperature. *C. yendoi* biomass, in contrast, did not vary much. However, spikes were observed to occur during the colder seasons and at spring time. From spring to early summer, cystocarpic *C. ocellatus* thalli were not observed and an overall *C. ocellatus* tetrasporophyte dominance was observed from June to October reaching peak fertility in October. Throughout the monitoring period gametophyte dominance was observed for *C. yendoi* and tetrasporophyte dominance for *C. ocellatus*. Between species, *C. ocellatus* dominated both in biomass and frond proportion (except from February to early spring). Recruitment wise, peaks were observed on June, coinciding with the highest proportion of fertile fronds of both spore types of *C yendoi*, and November, when *C. ocellatus* thalli were fertile. No recruits were observed from January to March as fertile fronds of both species were also low in proportion. The dynamic trend of alternating dominances between species and spore types provide an insight as to why these two species coexist.

C21

PHOTOSYNTHESIS AND RESPIRATION OF *LAMINARIA JAPONICA* FROM HIGASHIDOURI, AOMORI PREFECTURE, NORTHERN JAPAN

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Laminaria japonica thalli were collected from Higashidouri, Aomori prefecture, Northern Japan. Thalli grew quickly in winter and halted in summer. Therefore, the gross photosynthetic and respiration rates were compared between summer and winter thalli. Photosynthesis and respiration were monitored using the oxygen evolution technique in a closed system. Photosynthesis-irradiance (P-I) curves were obtained under controlled laboratory conditions with photons irradiance levels ranging from 0-720 μmol m^{-2} s^{-1} and simulated *in situ* temperatures: 7 °C for winter and 18 °C for summer. P-I curves for both seasons were almost same. Light saturation point was not reached within the irradiance range used in this study. The respiration rate of the summer thalli was higher than that of the winter thalli. Hence, the compensation irradiance of the former was also higher as a consequence of the higher temperature used for that season. The highest gross photosynthetic rate was obtained at 720μmol m^{-2} s^{-1}. This irradiance was used to test photosynthetic responses to varying temperatures ranging from 6-26 °C. The same temperature range was used for respiration in the absence of light. Gross photosynthetic rate of the winter thalli was higher than that of the summer thalli within the temperature range used for this study. The optimum temperature for maximum gross photosynthetic rate for both seasons was from 16-21 °C. The respiration rates of both summer and winter thalli exhibited the same trend, wherein rates increased with increasing temperature. In summary, winter thalli have a higher photosynthetic potential, which enable them to grow quickly under low temperatures.

C22

CHARACTERIZATION OF TWO SEX PHEROMONES IN *CLOSTERIUM*

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When mating type plus (mt^+) and minus (mt^-) cells of the *Closterium peracerosum-strigosum-littorale* complex are mixed together in a nitrogen-depleted mating medium, their sexual reproduction process makes progress. The sexual reproduction process consists of following five steps: 1) sexual cell division (SCD) resulting in the formation of gametangial cells, 2) pair formation of sexually competent cells, 3) formation of conjugation papillae, 4) release of gametic protoplasts from paired cells, and 5) fusion of protoplasts to form a zygote. Two novel sex pheromones [protoplast-release-inducing protein (PR-IP) and PR-IP inducer] involved in the latter steps had been characterized biochemically and molecular biologically.

The presence of two additional sex pheromones, sexual cell division-inducing pheromone (SCD-IP)-plus and SCD-IP-minus, involved in the first step, was recently demonstrated. SCD-IP-plus was released from mt^+ cells and induced the SCD of mt^- cells and vice versa.

Partially purified SCD-IP-minus showed quite similar characters to a PR-IP inducer, which induces the release of PR-IP from mt^+ cells. Moreover, the activity of SCD-IP-minus was declined by immunoprecipitation using anti-PR-IP inducer antiserum. These results strongly suggested that SCD-IP-minus must be the same molecule as PR-IP inducer. For further confirmation, recombinant PR-IP inducer produced in yeast cells was prepared and assayed for both biological activities. Both production of PR-IP and induction of SCD were observed after treatment of recombinant pheromone to mt^+ cells. It was strongly indicated that PR-IP inducer (SCD-IP-minus) exerted two different biological activities appropriately for the progress of the sexual reproduction processes.

C23
THE INFLUENCE OF HERBIVOROUS SNAILS ON GREEN TIDES

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This paper describes the temporal and spatial distribution of herbivorous snails and dominant macroalgae in a green tide area on the south coast of Korea, and also showed results of laboratory experiments on feeding preference of the herbivore. The study site, rocky intertidal zone of Songmoon near Namhae Island, was dominated by herbivorous snail, *Littorina brevicula*, *Ulva pertusa*, *Enteromorpha linza* and a turf-forming red alga, *Caulacanthus okamurae*. Vertical and horizontal distribution of these organisms were monitored from April 1998 to December 2001. The abundance of *Ulva* and *Enteromorpha* was inversely proportional to that of *Littorina* during the study period. Blooms of green algae together with lower densities of snails were frequently observed until November 1999. However, as snails began to increase, abundance of green algae remained low with the increase of the turf alga. The results indicated that the herbivores lowed the green algae, a potential food, and indirectly caused to increase the turf alga, known as a space competitor of the greens. Abundance of snails and the green algae showed a similar seasonal pattern, high in winter and spring and low in summer and fall. Their vertical distribution, however, was separated; more snails in the upper zone and more greens in the mid-lower zone. Results of laboratory feeding experiments were supportive to field monitoring, showing a stronger preference to *Ulva* and *Enteromorpha* over the turf alga. This study indicated that the herbivorous snails had a strong influence on macroalgal assemblage in the intertidal zone, and that the snails can be a potential controller of green tides in this habitat.

C24
NUCLEAR HISTONE PROTEINS OF GAMETES IN BROWN ALGAE

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It is well known that basic proteins in sperm nuclei of animals are rearranged into sperm-characteristic proteins, and this induces the conspicuous condensation of the sperm nucleus. In brown algae, nuclear chromatin condensation in male gametes becomes more conspicuous from isogamy to oogamy. However, nuclear basic proteins of gametes in brown algae have never been studied. Nuclear basic proteins (histones) were studied in male and female gametes of isogamous *Colpomenia bullosa* (Saunders) Yamada and *Analipus japonicus* (Harvey) Wynne, and sperm of oogamous *Cystoseira hakodatensis* (Yendo) Fensholt and *Sargassum confusum* C. Agardh by using one – dimensional gel electrophoresis, SDS- and AUT-PAGE, and two – dimensional gel eletrophoresis. Four major core histones and several linker histone H1s were detected by electrophoresis. Each of the core histones and histone H1s was identified by analyzing the amino acid sequences and peptide mappings. Electrophoresis patterns of histones were the same between male and female gametes of isogamous brown algae, and quite similar between *C. bullosa* and *A. japonicus*. But the composition of histone H1s in conspicuously chromatin condensed sperm nuclei of *C. hakodatensis* and *S. confusum* was different from that in the isogamous gametes. Electrophoresis after micrococcal nuclease digestion of chromatin in male and female gamete nuclei of *C. bullosa* and *A. japonicus* and sperm of *C. hokodatensis* showed regularly ladder patterns of DNA fragments (ca. 200bp). This shows that the chromatin of the brown algal gametes takes on the typical nucleosome structures. Therefore, in chromatin condensation of sperm nuclei was not associated with by change of core histones, replacement by other basic proteins or adding sperm specific core histones, and changes of repeating patterns or disappearance of nucleosomes. Histone H1s contained in sperm nucleus of *C. hakodatensis* and *S. confusum* may relate to characteristic chromatin condensation of sperm nuclei.

C25

EFFECTS OF *ULVA* MATS ON THE SOFT BOTTOM FAUNA

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On the south coast of Korea, the occurrence of green macroalgal mats, mainly consisting of species of *Ulva,* has become an increasing problem in coastal ecosystems. Drift *Ulva* thalli were accumulated on the upper shore following green algal blooms, which were mostly fragmented and detached thalli transported from the rocky sublittoral zones. To investigate ecological impacts of green tide, we tested effects of *Ulva* mats (control vs. mat) on benthic fauna as a function of season (winter vs. summer) and duration (2 vs. 4 months) using a three way ANOVA in Namhae Island, Korea. Physicochemical parameters of sediment were also monitored. For community parameters(i.e., number of species, total individuals, diversity index, dominance index) during the summer, although there was no significant difference in number of species between control and mat, diversity index was higher in control than in mat. In contrast, total individuals and dominance index was higher in mat. During the winter, the above patterns in diversity and dominance indice were reversed, indicating the influence of *Ulva* mats was different with season. The duration effect was significant, indicating that a longer persisting time of mats caused the greater impacts on benthic fauna. Responses of different taxa to *Ulva* mats were largely variable. Both polychaetes (e.g., *Ceratonereis*, *Nephyts*) and mollusks (e.g., *Musculus*, *Tapes*) were sensitive to impacts of *Ulva* mat regardless of seasons, whereas more arthropods(e.g., *Ampithoe*, *Caprella*, *Hemigrapsus*) were found in mats. Some genera of mollusks and polychaetes showed different patterns with season. Sediment under *Ulva* mats had five times higher in concentration of H2S and exhibited more reduced conditions. In conclusion, even though some taxa appeared to show different responses, effects of drift *Ulva* mats on the soft bottom community were proportional to the persisting time of mats, and their impacts were more severe in summer, probably due to faster decomposition rate of *Ulva* thalli.

C26

MEASUREMENT OF ALGAL AND CYANOBACTERIAL SPECIES DIVERSITY OF SIX FRESH WATER STREAMS OF SCHIRMACHER OASIS, ANTARCTICA

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Species diversity is the substitute for the biodiversity. The species diversity can be expressed at three levels i.e. alpha (α), beta (β) and gamma (γ) diversity. These three diversity values has been calculated for the algal and cyanobacterial species occurring in the six fresh water streams selected in Schirmacher Oasis, Antarctica during the 11th Indian Scientific Expedition to Antarctica (1991-92). Alpha diversity comprises species richness and evenness in the distribution of individuals among species. The Simpson index 1 , Shannon – Wiener diversity index H' and other two diversity indices N_1 and N_2 were calculated. In Species Richness (SR) Margalef Index (R_1) and Menhinick Index (R_2) were calculated for different streams. Different evenness indices E_1, E_2, E_3, E_4, E_5 were also calculated. For calculation of the beta (β) diversity Whittaker's measure, bw is calculated. The gamma (γ) overall diversity of all the streams can be calculated as the number of species found in the stream. It includes both a and b diversty. The maximum number of species was recorded in the stream SEM (24 taxa) and the minimum number of species was recorded in the stream EGF (12 taxa). Also the species diversity were maximum in stream SEM and minimum in stream EGF.

P1

SPECIATION OF CLOSELY-RELATED SPECIES COMPLEX OF THE GENUS *CLOSTERIUM* (CHAROPHYCEAE, CHLOROPHYTA) VIEWED FROM THEIR MOLECULAR INFORMATION

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The genus *Closterium* contains more than 70 species each comprising varieties. Some sexual populations form zygospores within single clones (homothallism), while some others between clones (heterothallism). There are also asexual populations which form thick-walled resistant spores without conjugation (parthenogenesis). In order to get better understanding of speciation of the genus along with evolutionary patterns of their reproductive types, this study has primarily treated with three closely-related species complex: *C. moniliferum-ehrenbergii*; *C. calosporum* and; *C. peracerosum-strigosum-littrale* (psl). Phylogenetic trees inferred from both nuclear SSU rDNA and chloroplast-encoded *rbc*L generally supported diversification into three different clades in each of those species complex. In the *C. moniliferum-ehrenbergii* species complex, the phylogenetic tree built from SSU rDNA data has suggested that homothallic *C. moniliferum* v. *moniliferum* was ancestral to all other populations of this species complex. ML trees constructed from SSU 1506 group I intron and ITS-2 data has shown that populations of *C. ehrenbergii* were not monophyletic. A characteristic duplication at the tip of helix I of their ITS-2 secondary structure may serve as an important clue for evolutionary derivation of asexual populations from sexual ones. In the *C. calosporum* complex, each of the two species *C. calosporum* and *C. spinosporum* has turned out to be polyphyletic. On helix II of their ITS-2 secondary structure of this species complex, a characteristic motif found in homothallic *C. calosporum* v. *himalayense* was duplicated in heterothallic *C. calosporum* v. *galiciense*. Since these two taxa are most closely related with each other, one explanation for this may be that heterothallic *galiciense* has been derived from a homothallic *himalayense*-like ancestor. In the *C. psl* species complex, unfortunately, neither sequence nor structure characteristic for their each reproductive type was found so far.

P2

PHYLOGENY AND TAXONOMY OF *CHRYSOPHAEUM DISPERSUM* SP. NOV. (PELAGOPHYCEAE, STRAMENOPILES)

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Two identical strains of the macroscopic algae, which formed golden gelatinous colonies, were collected from of Iriomote Is., Okinawa, Japan and Guam Is., U.S.A. Cells were obovate, 30-50 μm long and 15-17 μm wide, and possessed 40-60 yellow-brown chloroplasts and a tube-like invagination at one longitudinal end of each cell. Based on these characters, this alga was identified as the monotypic genus *Chrysophaeum*. However, the following features of this alga do not agree with *Chrysophaeum taylori*. First, cells scattered in gelatinous envelope, whereas *C. taylori* formed a cluster of cells. Second, each cell of *C. taylori* possessed a stalk structure at the opposite end to tube-like invagination, but our strains don't have it. Therefore, this alga should be recognized as a new species, *Chrysophaeum dispersum* Fukaya et Honda. To make clear the higher taxonomical position, we studied its ultrastructual features. It was revealed that *C. dispersum* possessed the following characteristics of the Pelagophyceae (stramenopiles): an electron dense thecate cell covering; a projecting pyrenoid penetrated by membranous invaginations; scattered beads of chloroplast DNA; scale-like structures. Furthermore, the molecular phylogenetic analysis with determined 18S rRNA gene sequences clearly showed that two *C. dispersum* strains located in the clade of the pelagophyte algae, especially formed a monophyletic group with the colonial the Sarcinochrysidalean algae, *Sarcinochrysis marina* and *Pulvinaria* sp., not with picoplanctonic pelagomonadaleans. The molecular phylogenetic relationships was not contradictory to the colonial nature of *Chrysophaeum*. Therefore, we concluded that *C. dispersum* should be classified as a new members of the Sarcinochrysidales in the Pelagophyceae.

P3

A NEW SPECIES OF THE GENUS *STICHOCOCCUS* (CHLOROPHYTA) FROM JAPAN

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We isolated an unusual aerial alga growing on the bark of *Cephalotaxus harringtonia* from Taishaku-kyo Gorge, Japan, which displays a form of "budding"-like asexual reproduction. The morphology of the alga was examined by LM and TEM and by molecular phylogenetic analysis. The alga was confirmed to be a species of the genus *Stichococcus*. During the characteristic "budding"-like reproduction, two autospores of different sizes form. The larger of the two remains within the mother cell wall and expands to fill the empty space, becoming the same size as the vegetative cell. The smaller autospore is extruded. Other morphological features include: unicellular vegetative cells, spherical to subspherical, 6-9 μm in width, 7-9 μm in length. Single layer, smooth surface cell wall, 0.2-0.4 μm in logarithmic phase of growth, up to 2 μm in old culture condition. Chloroplast single, parietal and cup-shaped, without pyrenoid. Single nucleus. Smaller autospore spherical to subspherical, 3-4.5 μm in width, 3-5 μm in length. Several species of *Stichococcus* have been reported living in fresh-water, soil and other aerial conditions. None have displayed this type of reproduction. Furthermore, the absence of pyrenoid differs from other *Stichococcus*. Phylogenetic trees constructed with the sequence data from the 18S rRNA gene showed that the alga is closely related to *Stichococcus bacillaris,* and appeared between the genus *Prasiola* and *Stichococcus*. Because of the phylogenetic divergence of *Stichococcus bacillaris*, here we don't propose the alga as a new genus. The findings suggest that the alga be placed in the genus *Stichococcus*.

P4

MORPHOLOGICAL STUDY OF THE MARINE PLANKTONIC DIATOM *CHAETOCEROS CASTRACANEI* KARSTEN FROM ANTARCTIC WATERS, AND POSSIBLE TAXONOMIC RELATIONSHIPS

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During various phytoplankton surveys (including cruises of the Project Argau) in the north coast of the Antarctic peninsula, the Weddell Sea and the Gerlache Strait, preserved phytoplankton samples were collected on different seasons in 1985, 1986, 1999, 2000 and 2001: net (30 μm mesh) and water pumps were used to obtain phytoplankton, samples being fixed with formalin and lugol's solution, respectively. Several species of diatoms of the genus *Chaetoceros* have been found, identified and studied. A fairly common species is *Chaetoceros castracanei* Karsten, originally described in 1907, which morphology, distribution and taxonomy have not been investigated in detail. The species is present mainly from February to April, and was also recorded in subantarctic waters; it showed relative high abundances in the Weddell Sea (up to 95 238 cells L^{-1}). *Chaetoceros castracanei* forms straight, medium-size to large chains, the terminal and intercalary setae are directed in the same direction (in girdle view), the apertures very reduced, centrally constricted and there are numerous chloroplasts in both cells and setae. Details studied by EM show valves randomly perforated by round poroids, no costae and an excentric rimoportula in every valve of the chain; the setae are circular in cross section at the base, but become four-sided distally, there are perpendicular rows of elongate poroids, spines arranged in spirals along the setae and the tip is very pointed. The intercalary setae are fused together in sibling valves. We have found that the species *Chaetoceros impressus* Jensen *et* Moestrup, recently described from Danish waters, is conspecific with *C. castracanei*, and consequently we propose it as a synonym of the later. Therefore, the known distribution of *Chaetoceros castracanei* may be widen to Antarctic, subantarctic and Danish waters. Other possible taxonomic relationships of the species are also discussed, especially regarding *C. danicus*.

P5
ULTRASTRUCTURE AND PHYLOGENY OF BENTHIC DINOFLAGELLATES RELEASING *AMPHIDINIUM*-LIKE SWARMERS

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Three marine benthic dinoflagellates with a distinct helmet-shaped cell covering were studied. The dinoflagellates investigated in this study have been isolated from bottom sand samples collected in the coral lagoons at Kerama Isl., Miyako Isl. (Okinawa, Japan) and Peleliu Isl. (Palau), respectively. Although they stay non-motile for most of their life cycle they produce swimming cells, whose morphologies resemble that of the genus *Amphidinium*. The aim of our study is to elucidate phylogenetic affinities of these non-motile, coccoid dinoflagelaltes with the distinct cell covering. Because they produce *Amphidinium*-like swarmers, their relationships with members of the genus *Amphidinium*, which are usually regarded to be motile dinoflagellates, were also investigated.

The vegetative cells of Kerama strain possess the cell covering with complex hexagonal surface patterns, while the other two are covered with the cell coverings with smooth surface. The motile cells of Peleliu strain are similar to that of *Amphidinium carterae* viz. with a small triangular epicone, which is mostly embedded in the hypocone. The swarmers of other two strains possess different morphology. Although the epicone is considerably smaller than the hypocone, the hypocone is wide and not embedded in the epicone. The chloroplast of Peleliu strain possesses a single chloroplast with multi-stalked type of pyrenoid. The pyrenoid matrix is penetrated by several thylakoids. This type of pyrenoid/chloroplast has been found in the typical *Amphidinium* species. On the contrary, the chloroplasts of other two species are radially arranged and the pyrenoid is located in the proximal end of each chloroplast profile.

The phylogenetic analyses based on 18S rDNA revealed the followings: 1) The monophyly with Kerama and Miyako strains was supported by high bootstrap values. 2) These two were independent from true *Amphidinium* clade, 3) Peleliu strain came to the sister position with *A. carterae* and it was included in a true *Amphidinium* clade.

P6
MORPHOLOGICAL VARIATIONS OF BODY SCALE AND IMPLICATION FOR TAXONOMY OF *HETEROCAPSA* (DINOPHYCEAE)

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Morphological variations of body scales of the genus *Heterocapsa* (Peridiniales, Dinophyceae) were investigated using transmission electron microscopy. Body scales are minute cell covering structure surrounding whole cell body, and are recognized as one of the useful taxonomic criteria at species level, especially in prasinophytes and haptophytes. Although this structure is also known in dinoflagellates, it has been observed only from three genera, *Heterocapsa*, *Oxyrrhis* and *Lepidodinium* so far.

Three-dimensional body scales were found from all *Heterocapsa* specimens of unialgal culture strains and preserved natural samples. These scales were consisted of the reticulated basal plate more or less triangular or circular in outline, and three-dimensional ornamentations such as uprights, bars and spines. Scales were triradiate symmetry in the plain figure and superficially resemble each other. However, almost all *Heterocapsa* species could be distinguished based on shape and number of those ultrastructures. As a result, seven described species, *H. arctica*, *H. circularisquama*, *H. illdefina*, *H. niei*, *H. pygmaea*, *H. rotundata* and *H. triquetra*, and five undescribed species were confirmed using the scale structure as well as cellular morphology. Those undescribed species were tentatively named as *Heterocapsa* sp. 1 - 6. Each species basically possesses a kind of body scale. However, in *H. rotundata* and *Heterocapsa* sp. 2, finenesses of basal plate reticulation were varied between different strains of same species. Since the basal plates with rough reticulation were observed only from old cultures maintained more than 15 years, moreover those of all natural samples were fine reticulation, the fineness could be supposed to be an artificial variation due to long-termed cultivation. Although almost all *Heterocapsa* species could be identified based on body scale ultrastructure, the scale structures of *H. triquetra* and *Heterocapsa* sp. 5 were rather similar. These two were only species, which could not be distinguished based body scale structure alone.

P7

DIVERSITY OF SUBCELLULAR ORGANIZATION OF *RHODELLA* (RHODOPHYTA), INCLUDING UNDESCRIBED SPECIES, WITH SPECIAL REFERENCE TO THE TAXONOMY AND PHYLOGENY

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Four species of *Rhodella, R. maculata, R. violacea, R. grisea,* and *R. cyanea* have been described so far. However, taxonomic problems exist between them, since the generic and specific criteria are indistinguishable. Evans (1970) established the genus, based on the type species, *R. maculate,* which was characterized by its ultrastructural features. The taxonomic problems arose after descriptions of new species and new combinations were performed without distinctive comparisons with their ultrastructural characteristics. If the diversity of subcellular organization is used as a classification criterion, we can easily distinguish the described and undescribed species. In order to elucidate the taxonomy and phylogeny, selective culture strains of the four described species of *Rhodella* and two closely related genera, as well as three undescribed taxa, were provided for molecular phylogenetic analyses using 18SrDNA and sequences of ITS regions. The result of 18SrDNA analysis showed that *R. cyanea,* which has a pyrenoid penetrated by thylakoids, made a clade with *Dixoniella grisea,* while the other species of *Rhodella* with pyrenoids free from any structures made another clade. In particular, *R. maculata, R. violacea* and two undescribed taxa could not be distinguished from each other. From the result of ITS, however, the latter clade could be divided into three groups; which were temporarily referred to as the maculata-violacea, Gamou-Monbetsu and Nagura-Baja California lineages. At present, it can be inferred that *R. cyanea* should be excluded from *Rhodella* if it is defined by the possession of pyrenoids free from any structures. In addition, each of three lineages may be considered as an independent species in this genus.

P8

ULTRASTRUCTURES OF HETEROCOCCOLITH-BEARING CELL OF *CALYPTROSPHAERA* SP.

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A culture strain of *Calyptrosphaera* sp. was isolated from an enrichment sample of seawater collected at Miyake-jima in November 1999. The cup-shaped holococcolith of this species resembled that of *Calyptrosphaera sphaeroidea*, but some morphological features such as size and shape of organic scales are different. The culture strain was found to produce heterococcolith bearing cells. This heterococcolith bearing cells are usually non-motile, and the coccolith resembles cricolith type of coccolith in *Pleurochrysis*, but the central area filled with calcified crystals makes different appearance. The coccolith is circular to oval shape and 0.5-1.3μm x 0.5-0.8μm in size. The organic scales have concentric fibrous patterns in the both faces and distinct rim, suggesting diploid coccolithophorid stage. General ultrastructural features of the heterococcolith bearing cells are typical of the coccolithophorids. Two chloroplasts are located laterally from nearby the basal bodies to the posterior side, and an immersed type of pyrenoid is observed in the center of chloroplast. A nucleus is located in the center of cell, and its envelope is connected with each chloroplast ER. A Golgi body exists between the basal bodies and the nucleus. A large size of vacuole occupies the posterior region, beneath the nucleus, and tends to extend to the left side of cell, reaching nearly the left basal body. Because of the large size and often containing amorphous substances, this vacuole is one of the most conspicuous components in the cell. The flagellar apparatus consists of right and left basal bodies (R and L), haptonemal base, 4 microtubular roots (R1, R2, R3 and R4), a crystalline-root originated from R1, a connecting band between R and L, cytoplasmic tongue with R1. The components and arrangement of flagellar apparatus in the heterococcolith bearing cells is basically identical with the holococcolith bearing cells and follows the common pattern for the other coccolithophorids so far described, except the absence of crystalline-root associated with R2 which is common in some coccolithophorids.

P9

MOLECULAR PHYLOGENY AND PSP TOXIN PRODUCTIVITY OF *ALEXANDRIUM SATOANUM* YUKI AND FUKUYO (DINOPHYCEAE)

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While isolating dinoflagellates from Jinhae Bay, Korea, we established a clonal culture of *Alexandrium satoanum* Yuki and Fukuyo, hitherto unreported from any locals out of its type locality. In this paper, SSU rDNA and partial LSU rDNA sequences of this species were analyzed, and its PSP toxicity was also surveyed.

The PCR amplification of SSU rDNA and LSU rDNA D1-D3 region of *A. satoanum* yielded one clear fragment of approximately 1,800 base pairs (bp) and 900 bp respectively. The distance values of SSU rDNA and LSU rDNA D1-D3 region of *A. satoanum* with other *Alexandrium* species available in GenBank varied 0.0515 to 0.0886 and 0.2596 to 0.3760, respectively. Each molecular phylogenetic tree was constructed with other dinoflagellates. On the whole, the *Alexandrium* species emerged together on a common branch diverging from the other dinoflagellates. *A. satoanum* was clearly separated from other *Alexandrium* species and the most primitive. According to phylogenetic model of *Alexandrium* based on the detailed morphological features (e.g., the shape of the sp, the 1', the 3' and the sulcal platelets), *A. satoanum* was considered as one of the most ancestral species among the *Alexandrium* species, and our data well supported the hypothesis. To understand the accurate phylogenetic position of *A. satoanum*, the more sequence data of other *Alexandrium* species are required.

In the meanwhile, PSP toxin productivity of *A. satoanum* was investigated using HPLC-FD system, and there were no visible peaks corresponding to any known standard PSP toxins. However, because of its close phylogenetic relationship with other *Gessnerium* species of which species were known to produce icthyotoxic or histopathological substances, further study is required for the toxicity of *A. satoanum* other than PSP toxins.

P10

MORPHOLOGICAL STUDY OF *ISTHMIA MINIMA* HARVEY AND BAILEY (BACILLARIOPHYCEAE)

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A marine epiphytic diatom *Isthmia minima* Harvey and Bailay was collected from Douno-ura, Naruto City, Tokushima Prefecture, Japan. Its morphological features were observed with light and scanning electron microscopes and compared with *I. nervosa*, which is the generitype.

Samples were attached on the thallus of *Caulerpa okamurae* Weber-van Bosse (Chlorophyta) making a colony with mucilage secreted from tip of its valve. The cell of *I. minima* is trapezoidal, and its length of apical axis reached till ca. 160μm. Cingulum consists of one closed-type valvocopula and copula whose margin is undulate. Their areolae are elongated and clearly different from those of valve. The valve has coarse and almost square areolae, which can be easily observed under the light microscope. Some sac-like ingrowth of the cribrum appeared especially near the margin of inner surface of valve, and the rimoportulae are present between cribra. Furthermore, no costae are present inner surface of valve of *I. minima* whereas *I. nervosa* has many costae.

These morphological features of *I. minima* are clearly different from the generitype, *I. nervosa.* It is necessary to reassess whether *I. minima* belong to the genus *Isthmia.*

P11

TAXONOMY AND PHYLOGENY OF *GALDIERIA* (CYANIDIALES, RHODOPHYTA)

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Cyanidian algae inhabit acidic and thermal environments throughout the world. They have been classified into three genera, *Cyanidium*, *Cyanidioschyzon* and *Galdieria*, by cell shape, cell size, number of chloroplasts in a cell and number of endospores in each cell. It is hard, however, to understand these morphologies as the generic and specific characteristics from the field samples whenever we try to do practical identification.

During our survey of cyanidian algae in Japan, they have been collected from 52 hot springs. After establishing their unialgal cultures and re-examining their morphological features to be stable and objectively distinguishable under the culture conditions, we could firstly recognize to exist 18 strains of *Galdieria sulphuraria* and *G. maxima* among them (the remains could be identified as *C. caldarium*.). Using these identified strains, we have previously tried to analyze their phylogeny based on the partial sequences of 18SrDNA genes and obtained the result that these two genera were monophyletic. In particular, it was phylogenetically significant that the genetic variability of *Galdieria* was much larger than that of *Cyanidium*.

In this study, further detailed analysis of the phylogeny of *Galdieria* was carried out including the Russian strains of *G. daedala*, *G. maxima*, *G. partite* and *G. sulphuraria* from Italy and Taiwan. The phylogenetic tree constructed by ML showed that they were divided into two large clades. One was composed by 12 Japanese strains, Italy and Taiwan strains of *G. sulphuraria* and *G. daedala*. Three Japanese and Russian strains of *G. maxima* and *G. partita* made the other clade with three Japanese strains of *G. sulphuraria*. From the result obtained here, it is possible that *G. daedala* and *G. partite* are conspecific with *G. sulphuraria* and *G. maxima*, respectively. Three strains of *G. sulphuraria* in the latter clade are investigated on their karyology.

P12

A NEW MARINE CENTRIC DIATOM *COSCINODISCUS JONESIANUS* VAR. *UNCINATUS* NOV. FROM THE SOUTHERN (TONGYENONG AND YEOJA BAY) COASTAL WATERS OF KOREA

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A study on the structure of the marine centric diatom *Coscinodiscus* species has been carried out for taxonomical purpose during the period from September 1998 to October 2000 in the Tongyeong and Yeoja Bay of the southern coastal waters of Korea. Living materials figured here were collected with 30 μm mesh nets on a cruise of KORDI (Korea Ocean Research & Development Institute) and the field collection of SMU (Sangmyung University).

The present study deals with taxonomic characters of *Coscinodiscus* species on the basis of the cell shape, cell diameter, external tube, labiate processes, strutted processes, occluded processes, areolar patterns, areolar shape, number of areolae per units, areolae type on mantle and central area.

Comparative taxonomic characters of *Coscinodiscus jonesianus* taxa were morphologically analyzed and divided into two groups. The first taxon has no occluded process on the valve surface, the second has a numerous occluded processes. We made a propositions of new infra-taxon of the former group: *Coscinodiscus jonesianus* var. *uncinatus*.

All new variety always showed 3-7 external occluded processes on valve surface, but the occluded processes is a characteristic found only in some *Thalssiosira* species. There is a wide difference of the number of areolae in both valve margin and valve center.

Geographical distribution of *Coscinodiscus jonesianus* and *C. jonesianus* var. *uncinatus* were widely ranged from two areas in the same Autumn, but the latter was more abundance than that of the former. *Coscinodiscus jonesianus* is known as planktonic in marine and ecological characteristics have polyhalobous, meioeyryhaline, but *C. jonesianus* var. *uncinatus* was frequency from 22-5-24.2 °C in water temperature and 22.2-29.0‰ in salinity.

In this paper, the number of occluded processes and positions are discussed with regard to taxonomic significance among the various *Coscinodiscus* species and in relation to some *Thalassiosira* species.

P13

PYRENOID IN *CHLOROMONAS* LINEAGE: CORRELATION BETWEEN MORPHOLOGICAL/ PHYSIOLOGICAL ASPECTS AND *rbc*L GENE SEQUENCES

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Pyrenoids are electron-dense bodies found in the chloroplast stroma of most eukaryotic algae and several species of hornworts (Anthocerotae). The matrix of the pyrenoids consists mainly of Rubisco, a CO_2-fixing enzyme in photosynthesis. It has been suggested that the pyrenoid is an important component of the CO_2-concentrating mechanism (CCM), a mechanism for increasing photosynthetic affinity for CO_2. The green algal genus *Chloromonas* is traditionally distinguished from *Chlamydomonas* by the absence of pyrenoids, but recent molecular phylogenetic studies[1, 2] demonstrated that *Chloromonas* and several species of *Chlamydomonas* constitute a closely related group "*Chloromonas* lineage"[3]. In this lineage, various ultrastructural types of pyrenoids are present and these morphological differences are associated with the degree of inorganic carbon concentration inside the cells which is related to the CCM[2, 3].

In the present study, we compared sequences of the *rbc*L genes of *Chloromonas* lineage with those of 45 other green algae within the CW group of the Chlorophyceae, and found that the proteins encoded by the *rbc*L genes have a much higher level of amino acid substitution in members of *Chloromonas* lineage than they did in other algae. Amino acid residues at the intra-dimer interface of Rubisco protein have an especially higher level of amino acid substitution. This kind of elevated substitution rate was not observed in the deduced proteins encoded by other chloroplast genes that we analyzed: *atp*B and *psa*B. In order to experimentally demonstrate the relationship between morphological/physiological aspects of the pyrenoid and *rbcL* gene sequences, we are now trying to transform the *rbc*L gene of the pyrenoid-containing species to the pyrenoid-lacking species within the *Chloromonas* lineage and observe changing in characteristics related to the pyrenoid within the *rbc*L-recombinant.

[1]Buchheim et al. (1997) J Phycol 33: 286-293., [2]Morita et al. (1997) J Phycol 33: 68-72 , [3]Morita et al. (1998) Planta 204: 269-276

P14

CHRYSOCULTER RHOMBOIDEUS GEN. ET SP. NOV. (PRYMNESIOPHYCEAE, HAPTOPHYTA): A NEW HAPTOPHYTE FROM JAPANESE COASTAL WATER

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We isolated an enigmatic *Chrysochromulina*–like alga from Okumatsushima, northern part of Japan. The cell is spindle- to knife-shaped (10-16 x 3-6 μm), and has two equal flagella and a haptonema (4-10 μm) from the anterior tip. This alga is not an active swimmer, but sometimes attached to the substratum by the anterior side of the cell. Two yellowish chloroplasts including an embedded pyrenoid are positioned asymmetrically. Organic body scales, small elliptical scales and large rhomboidal scales, are the most distinctive feature of this alga. The rhomboidal scale has two projections at both ends, and is closely touched to each other. The transition region of the flagellum includes only one basal plate, which is probably homologous to the distal plate in other prymnesiophyceans. The flagellar apparatus of this alga has basic components found in other prymnesiophyceans. Developed R1 extends toward the posterior tip of the cell with associated ER. Conspicuous fiber connects R1 and the left basal body. This structure has reported only in some coccolithophorids. In traditional taxonomy, this alga is apparently a member of the genus *Chrysochromulina* because flagellate cell possesses a long haptonema. However, recent molecular phylogenetic studies have clearly shown polyphyly of the genus *Chrysochromulina*. Diagnostic characters of *Chrysochromulina* are obviously plesiomorphies of the Prymnesiophyceae. Phylogenetic analysis using 18S rDNA and *rbc*L sequences indicated that the alga reported here is not closely related to any other prymnesiophyceans including *Chrysochromulina*. Some results suggested that this alga is situated at the basal position of the coccolithophorids. Based on these results, we propose *Chrysoculter rhomboideus* gen. et sp. nov. for this unique haptophyte.

P15

CLASSIFICATION OF *ANABAENA CIRCINALIS* LIKE SPECIES IN JAPAN

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Anabaena species with characteristics of *Anabaena circinalis* were collected from several Japanese lakes. Through detailed observation and comparison of their morphological characteristics, such as sizes and forms of vegetative cells, heterocytes, akinetes and coiles, etc., three different types were recognized. There is no description about width and distance of trichome coils of *Anabaena circinalis* in the original diagnosis. Komarek (1958) describes the morphological characteristics of *Anabaena circinalis* in detail including the width and the distance of the coils based on Rabenhorest's Alg. exs. no. 209. Among three types of *Anabaena circinalis* like species in Japan, only one type corresponds to the description and to the drawing by Komarek. But other two types are different in several characteristics, especially morphology and site of akinetes.

P16

EUGLENOPHYTA IN MAE SA STEAM, DOI SUTHEP-PUI NATIONAL PARK, CHIANGMAI, THAILAND

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The study of biodiversity of Euglenophyta in Mae Sa steam, Doi Suthep-Pui Nation park, Chiang Mai Province, Thailand were carried out for three years from April 1997 to March 1999. Ten genera and twenty species of living organisms were found, there were *Astasia acus*, *A. Klebsi*; *Chilomonas paramecium* ; *Cryptoglena pigra* ; *Entosiphon sulcatum* ; *Euglena acus*, *Euglena* sp.2, *Euglena* sp.3, *Euglena* sp.4, *Eunotia* sp.; *Phacus longicauda*, *P. pisciformis*, *Phacus* sp.; *Peranema trichophorum*; *Strombomonas* sp.; *Trachelomonas* sp.1, *Trechelomonas* sp.2 and *Trachelomonas* sp.3. The domoinant species was *Trachelomonas volvocina* Ehrenberg, which could be used as an indicator for the quality of water.

P17
COCCOLITHOPHORIDS DIVERSITY AT MIYAKE-JIMA SHORELINE (JAPAN)

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Miayke-jima is an island located 100 km south from Tokyo, which is often in the meander of the warm marine current, Black Stream (= Kuroshio Current). The diversity of the coccolithophorids was investigated using water samples taken from several different ports of the island in November 1999 when the meander of Kuroshio Current was just washing the island. For scanning electron microscope (SEM) observations, coccolithophorids were collected by filtering at least 40 liters of seawater samples. Large particles such as zooplanktons were removed from the sample with plankton nets (100 μm and subsequently 5 μm mesh size), then the sample was filtered using polycarbonate filter (pore size: 0.2 μm).

In order to culture the coccolithophorids, seawater was concentrated in the way same as above (but not filtered completely). Twelve culture strains of 5 genera (*Emiliania*, *Gephyrocapsa*, *Calcidiscus*, *Oolithotus* and *Calyptrosphaera*) were established. These strains are maintained in NIES Microbial Culture Collection. SEM observations revealed a much high diversity of the coccolithophorids. Nearly 40 species have been recorded. Electron micrographs of all the species recorded from filter samples will be presented with information about their dominance, abundance and regular habitat (pelagic, coastal, Pacific, etc.). From this study, it appears that remote islands under the influence of marine currents are excellent sampling sites to investigate open sea species. It does not require any ships and vessels (low cost). It makes scheduling of sampling easier and makes even regular and repetitive sampling possible. In addition, works on the land immediately after the sampling facilitate isolation process and make establishment of cultures effective. More sampling and studies from such islands should be encouraged.

Coccolithophorids blooms have been recorded several times in the path of the Kuroshio Current. Therefore, the background diversity of the coccolithophorids in the Kuroshio Current and the local condition at the bloom sites would be indispensable information for understanding bloom formation. Changes in the coccolithophorids diversity and their morphology are expected to be indicators of the environmental conditions needed to cause blooms. The examinations of the diversity and morphological features of some selected species are therefore important for the investigation of coccolithophorid blooms. Regular sampling at the shoreline of island, such as Miyake-jima, could be a way to achieve it.

P18
FURTHER STUDY OF THE CHLOROPLAST MULTIGENE PHYLOGENY OF THE COLONIAL VOLVOCALES (CHLOROPHYCEAE)

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The genus *Volvox* is characterized by having a >500-celled colony with small somatic cells and exhibiting oogamous sexual reproduction. Since the anisogamous genus *Pleodorina* has 64- or 128-celled vegetative colony with differentiation small somatic cells, it represents the closest ancestral form of *Volvox* in the traditional volvocine hypothesis. Recent molecular phylogenetic studies showed that *Volvox* and *Pleodorina* are polyphyletic. Furthermore, the very recent molecular phylogenetic study using 6021 base pairs from the concatenated chloroplast genes resolved that three species of *Volvox*, *Pleodorina californica* and *P. japonica* constitute a robust clade in which the two *Pleodorina* species are positioned distally (Nozaki et al. 2000, Mol. Phylog. Evol.). Therefore, it was suggested that these two species of *Pleodorina* might have evolved from a *Volvox*-like alga by the decrease in colony cell number and size. However, number of species of the genus *Volvox* was very limited in the multigene phylogeny by Nozaki et al. (2000).

In this study, 6021 base pairs of five chloroplast genes from 10 strains representing seven *Volvox* species were added to the previous data matrix (Nozaki et al. 2000). The sequence data resolved a large monophyletic group comprising five advanced genera of the Volvocaceae (*Yamagishiella*, *Platydorina*, *Eudorina*, *Pleodorina* and *Volvox*), which was subdivided into two clades, one (clade A) containing *Volvox* sect. *Volvox* and the anisogamous genus *Platydorina*, and the other (clade B) composed of three other sections of *Volvox*, *Eudorina*, *Pleodorina* and the isogamous genus *Yamagishiella* positioned basally. Therefore, evolution of anisogamy from isogamy might have occurred twice within the Volvocaceae. In addition, based on the present molecular phylogenetic analysis, species of *Volvox* within the clade B represented three monophyletic groups, which were separated from the two *Pleodorina* lineages resolved. Thus, reverse evolution from a *Volvox*-like alga to *Pleodorina* suggested previously (Nozaki et al. 2000) appears unlikely.

P19

MORPHOLOGICAL CHARACTERISTICS OF THE GENUS *XANTHONEMA* (XANTHOPHYCEAE)

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The genus *Xanthonema* (Xanthophyceae) is one of the most taxonomically difficult genera of xanthophycean algae. Reasons of difficulty of identification are their narrow filament widths (ca 3-5 mm) and simple cell shapes. In *Xanthonema*, cell shape and dimensions, chloroplast number, presence or absence of holdfast, and presence or absence of stigma in zoospores have been considered to be taxonomic criteria. In the present study, we report ranges of variation of these characters in seven species of *Xanthonema* using culture strains and evaluate these taxonomic characters.

Strains were maintained on 1.5 % agar slants of BBM medium under standard conditions: ca. 700-1000 lux on a 12: 12 h light: dark cycle at 15 °C. After one week to one month of incubation, morphological characteristics were assessed at x 1000 or x1250 magnification using light microscope.

Cell shape and dimensions of studied species except *X. montanum* mostly agreed with original descriptions. However, chloroplast number in each cell was different from original descriptions in *X. exile, X. debile, X. hormidioides* and *X. montanum.* Holdfasts at the end of filaments were observed in *X, sessile, X. hormidioides* and *X.* sp. 1. Shape of holdfasts was different in these three species. X.sp. 1 isolated from Antarctic soils was not identical to any species previously described. Vegetative morphology of *X.*sp.1 was close to *X. exile*, but differed from it by making long filaments and having a needle-shaped holdfast at the end of filament.

We will present key to species of *Xanthonema* based on our observation of morphological characteristics.

P20

TAXONOMIC REVISION OF MAJOR SPECIES OF THE GENUS *MICROCYSTIS* (CYANOBACTERIA = BLUE-GREEN ALGAE) UNDER THE RULES OF THE BACTERIOLOGICAL CODE

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The validity of the species classification of the cyanobacterium *Microcystis* have been discussed in a number of papers. It was primarily cell size, colonial form, and sheath characteristics that were used as the taxonomic criteria for *Microcystis* species. However, it was illustrated that these morphological characteristics can easily change, and phylogenetic trees did not support the validity of the current taxonomy of *Microcystis* species.

While the cyanobacteria have traditionally been described under the Rules of the Botanical Code, some attempts to describe cyanobacteria under the Rules of the Bacteriological Code have recently been made. For the consideration of the problem mentioned above, the genomic DNA homology, an important criterion for the species delimitation in bacteriological taxonomy, was examined for *Microcystis* major species (*Microcystis aeruginosa*, *Microcystis ichthyoblabe*, *Microcystis novacekii*, *Microcystis viridis*, and *Microcystis wesenbergii*). DNA-DNA reassociation values between *M. aeruginosa* and the other major species exceeded 70%, which is considered high enough for them to be classified within the same bacterial species. Considering the present results and some other previous reports, we propose to unify the species examined into *M. aeruginosa* under the Rules of the Bacteriological Code, and propose NIES843 (= IAM M-247) as the type strain. Tow other species, *Microcystis flos-aquae* and *Microcystis pseudofilamentosa*, should be regarded as morphological variations of this unified *M. aeruginosa.*

We also give the description of the genus *Microcystis*, and consider the taxonomic position of phycoerythrin-containing *Microcystis* sp.

P21

MORPHOLOGY AND TAXONOMY OF MARINE ATTACHED DIATOM GENUS *GRAMMATOPHORA* KÜTZING (BACILLARIOPHYCEAE) IN JAPAN

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The genus *Grammatophora* (Pennales, Araphidineae) was established by Ehrenberg (1839) and composed of 47 species (Van Landingham1971), and it is characterized by the conspicuous septum in copula. Species of this genus secretes muciligenous substances from apical pore fields at both ends of valve and their cells joined with each other to make a zig-zag colony. The taxonomic characteristics at the rank of species are the shape of septa, and valve, and density of striae. Fine structures of frustule of the following 5 taxa were observed using light and scanning electron microscopies : *Grammatophora angulosa* Ehrenberg, *G. oceanica* Ehrenberg, *G. hamulifera* Kützing, *G. marina* (Lyngbye) Kützing and *G. subtilissima* Ralfs. They were collected from littoral regions in Japan. The common characteristics were among them were as follows;

1) Septum is formed in valvocopula. 2) Plaques are present on the edge of valvocopula. 3) Copulae are open type, while valvocopula is closed one. 4) Prominent areas of areolae are present around apices of valvocopula. 5) Rimoportulae are arranged on the apical axis.

P22

TAXONOMY OF THE DIATOM *CHAETOCEROS* RESTING SPORE GENERA *DICLADIA* EHRENBERG, *SYNDENDRIUM* EHRENBERG AND ITS ALLIED GROUP

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Chaetoceros is one of the largest marine planktonic diatom genus and plays an important role in marine bioproduction in near-shore upwelling regions. The vegetative frustules of this genus are so delicate that they are dissolved after their death and generally not preserved as fossils. As the resting spores, however, are silicified heavily enough, fossils of *Chaetoceros* resting spores frequently occur in near-shore sediments in association with other fossil diatom valves. However, there has been no systematic study on the taxonomy of the resting spores. In this study, the morphology and taxonomy of fossil diatom *Chaetoceros* resting spore genera *Dicladia* Ehrenberg, *Syndendrium* Ehrenberg, and its allied species are examined. The genus *Dicladia* is characterized by its primary resting spore valve with two conical protuberances, and includes three species, *D. capreola, D. mitra,* and *D. japonica.* The genus *Syndendrium* bears *S. diadema* and a fossil new species, and is characterized by its two or more branched processes on the primary valve. A new genus, which closely related to *Dicladia,* is here proposed to receive two fossil new species. The new genus differs from *Dicladia* by having only one protuberance of primary valve. The three genera *Dicladia, Syndendrium* and the newly proposed genus are very similar to each other in possessing the dichotomously branched processes on the protuberance, and therefore they may have phylogenetic relationship. Some of the fossil species recognized in this study are short ranging and have potential utility in determining the age of near-shore marine sediments.

P23

FINE STRUCTURE OF AUXOSPORE IN THE FRESHWATER MONORAPHID DIATOM *COCCONEIS PLACENTULA* EHRENB. (BACILLARIOPHYCEAE)

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Auxospore structure has proved a useful characteristic in formulating new definitions of diatom taxa, and the relevance of auxospore studies to an understanding of diatom morphogenesis is discussed. The structure of auxospore and the morphology of initial cell in *Cocconeis placentula* were observed by the light and electron microscopy, found on a water grass *Elodea canadensis* from the Egawa River, Kamojima Town, Tokushima Prefecture, Japan. The mature auxospore covered with a perizonium is spherical, and in which the initial valves (an epi- and a hypovalve, a raphid and an araphid valve) are laid down. The perizonium consists of only converging bands, and lacks the longitudinal bands or the apical caps as seen in several pennate diatoms. The converging series consists of narrow, plain strips of silica, which taper towards ends on the center of the epi- and hypovalve sides and is not fimbriate. The initial epivalve shows several extraordinary morphological peculiarities, in having a domed section, no raphe and pseudoraphe, striae radiating from a central part of valve face and a wide hyaline marginal strip. The initial hypovalve is convex and has radiate or branched pseudoraphes.

Comparisons are made with auxospore structure of the other *Cocconeis* species, and the uniqueness of the genus *Cocconeis* among pennate diatoms is discussed.

P24

A PHYLOGENETIC STUDY OF FRESHWATER DINOFLAGELLATES

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The dinoflagellates inhabit both marine and freshwater environments. However, most of them are marine and only about 10% of all dinoflagellate (approximately 2000 extant species) are the freshwater representatives. In Japan, 54 species of freshwater dinoflagellates have been recognized so far, but still extensive taxonomic surveys are needed to clarify the Japanese freshwater dinoflagellate flora. Furthermore, phylogenetic affinities of most of the freshwater dinoflagellates have not been studied. We have little knowledge on origin(s) and evolutionary history of freshwater dinoflagellates. The current taxonomic system applied to the freshwater dinoflagellates relies mainly on gross morphology of the cells and little taxonomic re-evaluations have been made based on molecular phylogenetic information. The aims of our study are, then, 1) to clarify species diversity of freshwater dinoflagellates in Japan, 2) to study phylogenetic affinities of freshwater forms by means of molecular phylogenetic techniques, and 3) to evaluate present taxonomic system based on molecular phylogenetic information.

So far, we have found 45 species of dinoflagellates representing 10 genera; *Amphidinium*, *Ceratium*, *Cystodinium*, *Glochidinium*, *Gymnodinium*, *Katodinium*, *Peridiniopsis*, *Peridinium*, *Tetradinium* and *Woloszynskia* from lakes and ponds of various parts of Japan. For the molecular phylogenetic analyses, we used SSU rRNA gene sequences and a total of 53 strains, representing 38 species have been analysed.

The resulting phylogenetic trees revealed that the genera *Gymnodinium*, *Peridiniopsis*, *Peridinium* and *Woloszynskia* were polyphyletic. While *Gymnodinium palustre*, *G. acidotum* and *G. uberrimum* formed a distinct clade with a type species of the genus, *Gymnodinium helveticum* was resolved as a distinct lineage. The morphological study demonstrated that *G. helveticum* possessed *Gyrodinium* type of cellular features. Some of the species assigned to *Amphidinium*, *Gymnodinium* or *Katodinium* were shown to possess *Woloszynskia* type of thecal plates and they were clustered with some of the *Woloszynskia* species in the molecular trees.

P25
PHOTOBIONT OF *MULTICLAVULA CLARA* (CLAVARIACEAE, BASIDIOMYCETES)

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Multiclavula belongs to the Basidiomycetes. It forms symbiotic associations with the protonema of Musci, Myxomycetes, unicellular green algae or cyanobacteria. Three species are recognized as the lichenized *Multiclavula* in the world. *Multiclavula clara* is one of the lichenized species. It grows on the soil or the weathered rocks. The thallus of *M. clara* is thin. Photobiont cells are only in the mycelium. Basidiocarps never have photoibont. In the present study, a photobiont of *M. clara*, which was collected from the soil wall by the mountain trail in Kochi Prefecture was isolated and identified. As the result, vegetative cells of the photobiont algae were spherical. They had numerous disk-shaped chloroplasts without pyrenoids. They produced autospores and zoospores as reproductive cells. These morphological features agreed well with those of the genus *Bracteacoccus* (Neochloridaceae, Chlorophyta). So far, photobionts of *Multiclavula* have been reported as *Coccomyxa* sp. (Oberwinkler 1984). This is the first report that *Bracteacoccus* has been recognized as the photobiont of the genus *Multiclavula* and also as lichen photobiont.

Bracteacoccus is well known genus as the aerial algae. In the present study, aerial algae were also isolated from the soil surface near the thalli of *Multiclavula clara*, and were identified in order to confirm the possibility of getting its photobiont from the soil algae. As the result, about 10 species of soil algae were isolated. Among them *Coccomyxa gloeobotrydiformis*, *Klebsormidium flaccidum* and *Pseudococcomyxa simplex* were mainly recognized. *Bracteacoccus* sp. was rarely isolated. Visible algal colony observed on the soil surface was *Coccomyxa* sp. These results suggest that *M. clara* never gets dominant algal species as its photobiont but specifically selects the compatible photobiont in its habitat.

P26
MORPHOLOGY OF THE EPIPHYTIC DIATOM, THE TAXA *ACHNANTHES JAVANICA* GRUNOW VARIATION (BACILLARIOPHYCEAE)

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The genus *Achnanthes* Bory 1822 are the main constituents of the marine and brackish waters. And they attaches to some substrates with other species such as *Cocconeis* and *Grammatophora*. This study investigates the morphology of some prominent marine and brackish water *A. javanica* Grunow 1878 complex including three subdivisions of species were collected from littoral regions in Japan.

They include *Achnanthes javanica* var. *javanica* f. *javanica* Grunow 1878 (forma *javanica*), *A. javanica* var. *javanica* f. *subconstricta* (Meister) Hustedt 1932 (forma *subconstricta*) and *A. javanica* var. *rhombica* Grunow 1880 (var. *rhombica*). Syntype slide of forma *javanica* from the Natural History Museum (P. T. Cleve & Möller No. 147 made by Grunow) were used for comparisons. Specimens were examined using LM and SEM.

Forma *subconstricta* was most abundant in Japan, while forma *javanica*, found only in Okinawa (24°27'N, 124°11'E). In forma *javanica* the valve face was spindle shaped, and in var. *rhombica* it is rhombic to spindle and in forma *subconstricta* it is panduriform, gently constricted in central area. All taxa had marginal ridge and forma *subconstricta* and var. *rhombica* had terminal ridges and hyline areas.

Marginal and terminal ridges are the structure of frustule used for connecting valves in chain. It is concluded that in present study forma *javanica* was not a forma of *A. javanica* var. *javanica*, based on the difference in frustule structures such as terminal ridge, costa and band.

P27

MASS CULTURE OF AN ENDANGERED SPECIES 'MARIMO (*AEGAGRROPILA LINNAEI*)' FOR ITS CONSERVATION

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'Marimo' is a fresh water green alga that is designated as endangered species in Japan. Although the development of large-scale culture method is expected to contribute for progress of Marimo conservation, there is no successful example until now because growth rate of this alga is very slow. In this study, conditions for culturing the Marimo filaments by use of a 100 liters scale water tank were investigated. The culture system was mainly composed of the following units: circulating filtration equipment for eliminating micro algae as well as microbes, aeration unit for stirring, fluorescent lamps as light source and pH control unit. The ASP-6 medium, an artificial seawater, was diluted by addition of fresh water at 0.34% salinity, which was used for culture experiments as base medium. In the experiments, optimum growth condition being expressed by increase of fresh-weight of Marimo filaments was surveyed on the various environmental factors and additional nutrients. Good growth was observed in the follows. 1) Temperature: 20-25 °C. 2) Light intensity: 70-90μmol/m^2/sec on the surface of the medium. 3) pH control: no significant difference within the range pH7.0-9.0. 4) Supplementary quantity of nutritive salts: NO_3-N; 0.4-0.5mg/day/l, PO_4-P; 0.060-0.075mg/day/l. 5) Nitrogen source: sodium nitrate is more effective than ammonium nitrate, ammonium sulfate and urea the same concentrations. Finally, by the combination of the above-mentioned optimum conditions, maximum growth rate attained to about 200% per month.

P28

HEMIFLAGELLOCHLORIS KAZAKHSTANICA GEN. ET SP. NOV.: A NEW CHLOROCOCCALEAN SOIL ALGA FROM A FARM FIELD IN KAZAKHSTAN (CHLOROPHYCEAE, CHLOROPHYTA)

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From a soil sample of the Ili River Basin in saline irrigation land of Kazakhstan, a unicellular green alga was isolated, whose vegetative cells are spherical and chloroplast is parietal with pyrenoids. Reproduction occurs by means of biflagellate zoospore formation. Zoospores are long ovoid with a relatively large anterior end and a gently tapered posterior end. One flagellum is 1.8~2.3 times longer than the body length, and the second one is 0.7~1 time of body length. Electron microscopy revealed that the pyrenoid is penetrated by thylakoid membranes, and that the zoospore is covered with a cell wall of single layer. The basal bodies are shifted in the clockwise orientation (CW), and connected to one another by distal and proximal fibers. Rootlets are arranged in the cruciate pattern with s-rootlets comprised of four microtubules in 3-over-1 configuration, and d-rootlets of 2 microtubules. Molecular phylogenetic analyses using 18SrDNA sequence data showed that this isolate is included in one of the clades of CW group.

P29

GENETIC VARIATION OF THE POPULATIONS OF MARIMO (*AEGAGROPILA LINNAEI*) IN LAKE AKAN

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In Japan, the marimo (*Aegagropila linnaei*) is found in 17 lakes and marshes; viz. Lake Akan, Lake Chimikeppu, Kushiro Marsh, Lake Shimokita, Lake Ogawara, Fuji Five Lakes, and Lake Biwa.

The marimo is famous for the shape of spherical aggregation, but in addition to the spherical aggregation form, the marimo shows two growth forms; epilithic filaments and free-floating filaments. Epilithic filaments attach on rocks and stones of various sizes. The marimo is distributed rather widely through Japan as mentioned above, though, the population of spherical aggregations is restricted to the northern part of Lake Akan.

In Lake Akan, there are nine populations and all of these three growth forms are seen. Two populations consist of spherical aggregations and free-floating filaments; five populations consist of epilithic filaments and free-floating filaments; one population consists of free-floating filaments; and the another population consists of epilithic filaments only. All populations are loosely isolated one another. Because of the different appearance, these growth forms have been regarded as different species. Therefore, the genetic relationship among the populations has not been investigated.

To elucidate the genetic diversity of the marimo in Lake Akan, and infer the gene flow among populations, we conducted allozyme analysis. If each population has different genetic variation, it is supposed that gene flow among populations is restricted. And if there is no genetic difference among populations, there is a possibility of gene flow.

In the present study, most loci are monomorphic through all the populations of Lake Akan. However, some unique alleles found in a certain population suggest the existence of the restriction of gene flow.

P30

MORPHOLOGICAL OBSERVATIONS AND MOLECULAR PHYLOGENY OF *SCHIZOCHYTRIUM* CF. *OCTOSPORUM* (LABYRINTHULEA, STRAMENOPILES)

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The class Labyrinthulea in the stramenopiles is characterized by monocentric holocarpic thallus and presence of the ectoplasmic net produced by the sagenogenetosomes. The Labyrinthulea include a single order and two families. The Labyrinthulaceae contains one genus *Labyrinthula,* whose cells move inside the ectoplasmic net. While the cell of the Thraustochytriaceae produce the rhizoid-like ectoplasmic net from a single sagenonetetosome. Classification of eight thraustochytrid genera is based on cell morphology of various stages in the life cycle. However, recent molecular phylogenetic works suggested that the taxonomical rearrangment is needed, because some genera did not form monophyletic groups.

The thraustochytrids strain SEK-0122 was isolated from Iriomote Is., Okinawa, Japan. This organism formed cell clusters via successive binary divisions. This growing process is a key character of the genus *Schizochytrium*. Each vegetative cell transformed to one spherical zoosporangia, producing eight ovoid zoospores. These morphological features agreed with the description of this species. Thus, this strain was identified as *Schizochytrium* cf. *octosporum.*

Molecular phylogenetic analysis of 18S ribosomal RNA gene sequences showed that the Labyrinthulea was comprised of three distinct groups. The first group consisted of all examined strains of *Labyrinthula*. Thus, the Labyrinthulaceae is suggested to be a natural taxon. The second is the biggest group and including the type species of *Schizochytrium*, *S. aggregatum* (ATCC 28209), and *S. limucinum*. Whereas *S. octosporum* located in the third group, and especially form a monophyletic group with *S. minutum* and *S. aggregatum* (T91-7). It was strongly suggested that the genus *Schizochytrium* was an artificial group, and should be separated into at least two taxa. However, two strains identified as the type species located in different groups. Therefore, at this time we must wait to work the taxonomical treatment until accumulating information of *S. aggregatum* strains and the other thraustochytrids.

P31

PHOTOSYNTHETIC PIGMENTS IN THE SIPHONOCLADALES AND *AEGAGROPILA* RELATIVES AND THEIR ENVIRONMENTAL ADAPTATION

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Aegagropila linnaei Kütz. is a freshwater siphonocladalean alga that is called "Marimo" in Japanese. A recent molecular phylogenetic analysis has revealed that *A. linnaei* forms an independent sister-clade together with several species belonging to the Siphonocladales from rest members of the Siphonocladales (referred here to the Siphonocladales *sensu stricto*). The latter clade is comprised of two subclades. These results are inconsistent with present taxonomic system constructed mainly based on ultrastructural features. Further analyses are therefore necessary to settle the argument.

We analyzed photosynthetic pigments in *Aegagropila* relatives and the members of the Siphonocladales *s.s.* using HPLC. The carotenoid composition of these algae can be classified into 3 types: lutein type characterized by containing lutein as a major carotenoid and lacking loroxanthin and siphonaxanthin: loroxanthin type characterized by containing loroxanthin (and lutein): siphonaxanthin type characterized by containing siphonaxanthin (and lutein). All the *Aegagropila* relatives examined belonged to the loroxanthin type. In the Siphonocladales *s.s.*, three carotenoid types distributed. One of two subclades of the Siphonocladales *s.s* included loroxanthin type and the other included lutein type and siphonaxanthin type. Distribution of carotenoid types was likely congruent with molecular phylogenetic analysis.

Loroxanthin type carotenoids in *A. linnaei* collected from various sites of Lake Akan was further analyzed. The results show that loroxanthin/lutein ratio was dependent on environmental light conditions, *i.e.* the ratio was low in the plant grown in the bright areas and high in those grown in the shaded or dark areas. Because loroxanthin-lutein conversion has been suggested to be a xanthophyll cycle to respond to various light intensities and then lutein could defend plants against photoinhibition, loroxanthin type species could survive under various light conditions as does *A. linnaei*. From these results, we will discuss taxonomic and evolutionary implication of the carotenoid types and their environmental adaptation to light conditions.

P32

PHYLOGENETIC ANALYSIS OF COCCOID GREEN ALGAE MAINTAINED IN THE MICROBIAL CULTURE COLLECTION AT THE NATIONAL INSTITUTE FOR ENVIRONMENTAL STUDIES (NIES-COLLECTION).

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Taxonomic position of coccoid green algae has been revised in recent years by analyzing 18S rDNA sequences. This advance in taxonomy, especially set up of the class Trebouxiophyceae, directly impacted on the strain list of the Culture Collection. Because of polyphyly in some families, and even in genera, it became difficult to classify Trebouxiophyceae and Chlorophyceae without sequence data. Thus, we started to analyze 18S rDNA sequences, as well as ultrastructure and morphology of vegetative cells, and to verify the taxonomic position of the strains maintained in the NIES-Collection. Corresponding green algae maintained at NIES-Collection amounted to 300 strains. 40 NIES strains were analyzed in this study. DNA extraction was conducted by CTAB protocol after freezing and crushing of cells, and direct sequencing was conducted. For phylogenetic analysis, 150 sequences comprehensively selected from Chlorophyta were aligned by Clustal W and eyes with SeAl program, and the phylogenetic tree was constructed with PAUP* Version 4.0b4a. The 40 strains scattered in the Trebouxiophyceae (22 strains), the Chlorophyceae (15 strains) and ambiguous clade (*Michonastes* and *Chlorosarcinopsis*). 4 genera of the Oocystaceae, *Oocystis*, *Lagerheimia, Makinoella* and *Eremosphaera,* and *Tetrachlorella* formed a monophyletic group with high bootstrap support, while 4 species of *Oocystis* and 2 species of *Eremosphaera* were separated in the different clades. It was found that three morphologically distinctive genera, *Micractinium*, *Dictyosphaerium* and *Actinastrum,* were closely related to *Chlorella vulgaris*. Among the 15 strains placed to the Chlorophyceae, *Coelastrum astroideum* and *C. proboscideum* were related to *Graesiella emersonii*. Present study showed several new combinations in taxonomy, suggesting necessity of re-evaluation and more detailed studies of the groups.

P33

TAXONOMICAL STUDIES OF *SARGASSUM PILULIFERUM* AND *S. PATENS*; A PRELIMINARY REPORT OF MORPHOLOGICAL ASPECTS

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A study of morphological characters in *S. piluliferum* and S. *patens*, collected along Japanese coasts (more than 20 localities in each), were established. More than ten individual plants of each population were collected and utilized for morphological, DNA and stable carbon isotope analysis. This is a preliminary report of morphological analysis.

Morphological characters in *Sargassum* plants were established for the description of each population, and statistical analysis of leaves and vesicles were studied. Seasonal variations for one year were also studied in some localities. A part of results are below:

(1) Morphological characters in the same population was strongly effected by some environmental factors.

(2) However, broader variations were observed in the seasonal study of same populations. These characters may be more variable in their developmental stages than their geographical variations.

(3) Morphological variations among the populations (geographical factors) were also recognized. Some populations were clearly distinguished from others by their characteristics.

(4) These results of morphological study were comparable with the results of DNA analysis.

P34

PHYLOGENETIC RELATIONSHIP AND BIOGEOGRAPHY OF THE FAMILY BATRACHOSPERMACEAE

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Recently, molecular techniques have been employed in some studies on systematics and biogeography of the family Batrachospermaceae in North America (Vis and Sheath 1997, 1998, 1999, Vis et al. 2001) and Australia (Vis and Entwisle 2000). Based on *rbc*L gene sequence data, the present study deals with about ten Japanese taxa of the family Batrachospermaceae in order to evaluate their sectional classification within the genus *Batrachospermum* and reveal their phylogenetic status within the order Batrachospermales. These taxa newly sequenced in this study represent the genus *Sirodotia* and the genus *Batrachospermum*, including the section *Aristatae*, the section *Batrachospermum*, the section *Setacea*, the section *Thrfosa*, and the section *Virescentia*. Special reference to *B. helminthosum* within the section *Virescentia*, the samples from more than ten localities from northern Fukushima Prefecture to southern Okinawa Prefecture in Japan, are analysed in order to better understand its biogeography. We determined their *rbc*L gene sequences and analysed them with previously published sequences of twelve samples from North America (Vis et al. 2001). There were four genotypes among the Japanese samples. The majority of samples represented one genotype (genotype 1), and the other genotypes were represented by one sample, respectively. Phylogenetic analysis using Maximum Likelihood method showed two major clades within *B. helminthosum*; one clade contains three genotypes from Fukushima etc. (genotype 1), Kyoto (genotype 2) and Ehime (genotype 3), which are well supported by high bootstrap value, and the other clade comprises the genotypes from Okinawa (genotype 4) and from North America. Though there was little confidence in the latter clade, such phylogenetic relationships indicated that two phylogenetic groups exist in Japan. These two groups, the genotype from Okinawa (genotype 4) and other three genotypes (genotype 1, 2, and 3), might be immigrated into Japan through different routes.

P35

THREE STRAINS OF *ENTEROMORPHA PROLIFERA* (ULVALES, CHLOROPHYTA) WITH DIFFERENT LIFE HISTORY: THEIR SEQUENCES AND NUCLEAR GENOME SIZE ESTIMATED BY MICROSPECTROPHOTOMETRY

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Populations of *Enteromorpha prolifera* (O. F. Müll.) J. Ag. collected from four rivers on Shikoku Island in Japan had three different types of life history. One of them was a typical sexual life history in genus *Enteromorpha*, which is isomorphic with quadriflagellate zoospores and biflagellate gametes. The other two types were asexual life histories by biflagellate or quadriflagellate swarmers. Both biflagellate and quadriflagellate asexual swarmers were intermediate in size to gametes and zoospores in the sexual life history, and exhibited negative phototaxis. The microspectrophotometric study showed that DNA values of nuclei in the vegetative thalli asexually reproducing by biflagellate or quadriflagellate swarmers were the same level to those in diploid sporophyte (2C) in the sexual life history. Furthermore, both types of asexual swarmers were also estimated to have the same DNA value (2C). That shows the asexual swarmers to be produced without meiosis. The three strains with different life history were determined to be conspecific on the basis of similarity of the morphological characteristics and low sequence divergence of the nuclear ribosomal DNA internal transcribed spacer (ITS). However, there was no correlation between ITS sequences and the life histories. Although a single type of strain with the sexual life history predominated in one river, two different types of strains intermingled in the same population in the other three rivers. Our results showed that the ITS analysis, morphology and distribution would not distinguish the three strains.

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PHYLOGENETIC ANALYSIS OF THE DICTYOTALES BASED ON 18S RIBOSOMAL DNA

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Dictyotalean algae (Phaeophyceae) have been classified into 2-3 groups based on the numbers and arrangement of apical cells, and 13-18 genera are recognized by the thallus organization and the distribution of reproductive organs. This traditional classification has been regarded as questionable and expected the approach of molecular phylogeny.

11 genera and 23 species in Dictyotales were collected from Japan and adjacent regions (Guam, Palau and India), and they were analyzed with 18S ribosomal DNA sequences (*ca.* 1800bp). From the phylogenetic tree constructed by NJ, ML and MP methods, it can be pointed out that Dictyotalean algae are monophyletic and clearly separated from the other brown algae. It is also obvious that the two distinctive groups were recognized in this order; *Zonaria-Lobophora* (ZL-) clade and the other (including 9 genera 19 species), and the latter was divided into six lineages, 1) *Dictyota-Dilophus-Pachydictyon* (DDP-), 2) *Stypopodium* (S-), 3) *Dictyopteris-Spatoglossum* (DS-), 4) *Distromium* (D-), 5) *Homoeostrichus* (H-) and 6) *Padina* (P-) lineages. These phylogenetic relationships led us the following morphological significance. The ZL-clade is distinct from the other Dictyotalean algae with tetraspores by its possessing octospores in sporogenesis. On comparing with their morphological characteristics, the flabellate thallus and furnishing with multiple apical cells forming terminal apical row are symplesiomorphy of this order and the dichotomy including pseudodichotomy commonly observed in DDP-, S- and DS-lineages is synapomorphy. Within these three lineages, a single apical cell in DDP-lineage is autapomorphy toward multiple apical cells in S- and DS-lineages.

Based on the present molecular results, it is hard to support the widely accepted taxonomic system in which Dictyotalean algae examined here are classified into two tribes (or families), Dictyoteae (DDP-) and Zonarieae (ZL-clade and other five lineages), however, it is possible to distinguish another two groups, ZL-clade and the other. Although the monophyly of DDP-, DS- and S-lineages were supported by high bootstrap values, the taxonomic relationship among them and the generic autonomy of *Dictyota* and *Dictyopteris* were still unclear.

P37

MORPHOLOGY, ONTOGENY AND PHYLOGENY OF FRESHWATER GREEN ALGA, *BLIDINGIA MINIMA* VAR. *RAMIFERA* FROM NIKKO, TOCHIGI PREFECTURE, JAPAN

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Freshwater *Enteromorpha*-like green alga *Blidingia minima* var. *ramifera* was collected from Yukawa River, Nikko, Tochigi Prefecture, Japan and investigated by laboratory culture and molecular phylogenetic analysis.

Frond morphology was mostly the same as the previous report (Yamada and Hirose 1943 as *Enteromorpha nana* var. *subsalsa*). In ontogeny of marine *Blidingia minima* from Japan there are three types of early development: Compact disc type (D-type), filamentous microthallus type (F-type) and middle type (M-type)(Iima 1989). In the present study freshwater *Blidingia minima* var. *ramifera* was similar to M-type development which have been mainly found in Japan Sea coast strains. Molecular phylogenetic analysis based on chloroplast DNA *rbc*L gene sequence showed that this freshwater strain is the fourth group. The *rbc*L gene sequence of the freshwater strain is different from other three groups of marine *Blidingia minima* from Japan, which represent the above three types (F, M, D). D-type group is located at the base of the *B. minima* clade composed of the above four groups, the next branching group is the freshwater strain, and M-type group and F-type group form a most derived clade. This phylogenetic relationship suggests that the freshwater strain was not derived from the three marine groups presently distributed in Japan.

P38

SPECIES COMPOSITION AND DISTRIBUTION OF *BOSTRYCHIA* AND *STICTOSIPHONIA* IN MANGROVE AREA OF SOUTHEAST ASIA

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Species in the genus *Bostrychia* and *Stictosiphonia* (Ceramiales, Rhodophyceae) are the main algae growing in brackish water. They grow in a mangrove forest and a mouth of river in temperate to tropical regions in the world. They often grow on mangrove roots associated with species of *Rhizoclonium*, *Caloglossa*, *Catenella* and *Cladophora*.

Species composition and distribution of *Bostrychia* and *Stictosiphonia* are compared among Southeast Asia and Japan as follows. 8 taxa in eastern Indonesia: 4 taxa in Bali Isl., Indonesia: 5 taxa in Iriomote Isl. and Ishigaki Isl., Okinawa Pref.: 1 taxon in Amami Isl., Kagoshima Pref.: 3 taxa in the Philippines: 3 taxa in Thailand: 2 taxa in Vietnam.

Judging from their species composition and distribution of Southeast Asia, *Bostrychia radicans* and *Stictosphonia kelanensis* are more widely distribute than the other taxa. Conversely, *Bostrychia pinnata* and *Bostrychia tenella* ssp. *flagellifera* distribute in the restricted areas, such as eastern Indonesia, Iriomote Isl. and Ishigaki Isl. The species diversity became higher in the following order; Indonesia, Japan, the Philippines, Thailand, Vietnam.

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THE ORDER LAMINARIALES (PHAEOPHYCEAE) FROM THE FAR EASTERN SEAS OF RUSSIA

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According to the previous taxonomic revision of the order Laminariales from the Russian Far Eastern seas (RFES), 37 species were distributed in this area (Petrov, 1975). The present study presents the new taxonomic revision of Laminariales from RFES. Presently, the laminareacean flora of RFES appears to be counted as 44 species and 23 genera. Also, 2 new species belonging to new genera were established, and 1 genus and 2 species were found in this region for the first time. The number of some taxa was reconsidered. As suggested, 11 genera include only one species, and 7 genera are endemics for RFES. 10 genera are distributed along the coasts of the Bering Sea and Southeastern Kamchatka. Floras of the continental coast of the Sea of Ochotsk, Kurile Islands and Sea of Japan include 8, 15 and 8 genera, respectively. The analysis of kelp distribution along 40 different areas of the Russian Far Eastern coast showed that the most abundant and specific laminareacean flora is present on the Kurile Islands, especially on Simushir Island (11 genera and 22 species), Iturup Island (10 genera and 24 species), and Kunashir Island (9 genera and 24 species). In other areas, only 5-15 species and 4-10 genera are found. Many species (e. g. *Tauya, Costularia, Undariella* etc.) have very narrow distribution and inhibit places with very specific hydrological conditions. During the past two centuries, distribution of some species had changed significantly. Currently, penetration of the American and Japanese species into the Russian algal flora is observed. The comparative morphological analysis and florogenetic investigations of the members of Laminariales showed 5 centres of species diversity in the northern Pacific hemisphere. The laminareacean flora of RFES was formed under the influence of 3 centers. Possible ways of the historical development of this order were also suggested.

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SEXUAL AND ASEXUAL POPULATIONS OF *SCYTOSIPHON LOMENTARIA* (PHAEOPHYCEAE) IN HOKKAIDO, JAPAN

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Thalli of *Scytosiphon lomentaria* (Lyngbye) Link were collected in Oshoro, Asari (near Otaru) and Muroran, Hokkaido, Japan. In a laboratory, their sex (female or male or asexual) were determined by observations of gamete fusion. Both sexual thalli and asexual thalli were obtained from each locality. Asexual thalli were strongly constricted, whereas, sexual thalli were rarely slightly constricted. Unialgal cultures were established from the thalli whose sex was determined. In the cultured thalli, DNA sequences were determined by a PCR direct sequence method for genetic comparison between sexual and asexual thalli. In the RUBISCO spacer region, all sequences were identical except for three sexual thalli of Muroran with a single nucleotide difference. In the ITS2 region of the nrDNA, five sequence types were found. Four sequence types of them were in sexual thalli: A and B type in Oshoro, C and D type in Asari, and C type in Muroran. Asexual thalli had E type. To investigate if plural ITS2 sequence types are in a single thallus, PCR primers specific to each sequence type were made, and a part of samples were checked by PCR with the sequence-type specific primers. The result suggested that thalli of A and E type did not include other four sequence types. However, thalli of B, C and D type included three types of B, C and D. In the mitochondrial cox3 gene, four sequence types (K, L, M and N; 554 bp) were found. Cox3-K type was found in ITS2-A-type thalli, and cox3-L type in ITS2-B type, and cox3-M type in ITS2-C and D type, and cox3-N type in ITS2-E type. In comparison of cox3 sequences, nucleotide differences were 0-4.5 % among sexual thalli (K, L and M type), and 8.8-10.4 % between sexual thalli and asexual thalli (N type). These results suggest that thalli determined as asexual are genetically different from sexual thalli.

P41

PECULIAR FLORA AND EVOLUTION OF MARINE MACROALGAE INHABITING MARINE LAKES IN PALAU

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It is presumed that the marine lakes in Palau were formed by geological activities seven thousand to thirty thousand years ago. They are basically isolated from the open sea, however, they retain the salinity from 29-36‰ . Although the numbers of species were restricted, marine macroalgae were found in all of the five lakes we examined; Jellyfish Lake (JFL): 7spp., Milky Way Lake (MWL): 11spp., Bablomekang Island Lake (BIL): 1sp., Ngerktabel Island Lake 1(NIL1): 3spp., Ngerktabel Island Lake 2(NIL2): 3spp. Only three species were common to more than two lakes; *Caulerpa verticillata, C. serrulata,* and *Halimeda gigas.* The dominant species in each lake were different; *C. verticillata, C. fastigiata* and *Avrainvillea amadelpha* in JFL, *C. lentillifera* in MWL, *Rhipilia orientalis* in BIL, *H. simulans* and *H. gigas* in NIL1, *Lobophora* sp. and *Acrosorium* sp. in NIL2. From these results, it can be pointed out that all of the dominant macroalgae are multinuclear siphonous green algae with the exception of *Lobophora* sp. and *Acrosorium* sp. in NIL2. It is also significant that the species which are dominant in each lake could not be found in the open sea outside of the lakes. Of course, there were some cases, when the algae in the lake could not be easily identified because of their considerably differentiated gross morphological appearance, caused by their adaptation to the calm environmental conditions in the lakes.

Using *rbc*L gene of *A. amadelpha* collected from JFL and the open sea in Palau, the evolutionary distance was analyzed. The result shows that eleven nucleotide substitutions have occurred between them. *H. simulans* from both inside and outside of NIL1 were also analyzed by the sequences of ITS regions, however, no conspicuous differences were encountered between them.

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MORPHOLOGICAL AND MOLECULAR PHYLOGENETIC ANALYSES OF THE THOREACEAE 2. A VARIETY OF *THOREA GAUDICHAUDII* FROM THE MIYAKO ISLAND, JAPAN

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The Miyako specimen was found growing on gravel, shells of gastropod and submerged wall in the Nu-gusuku-ga Spring, situated on cliff of pleistocene limestone terrace in the Miyako Island, Okinawa Prefecture, Japan. The Miyako specimen shows the following combination of features: (1) plant filamentous, dark wine-red, 9-30 cm long (up to ca. 80 cm), 400-1200 μm in diameter; (2) ramification often found at the base, but rare at middle and distal portions of thallus; (3) assimilatory filaments chantransia-like, rarely branched, 210-560 μm long, consisting of 10-22 cells; (4) reproductive structures such as large and small types of sporangia are obsevred; the former may be assigned to spermatangia and the latter carposporangia. The Miyako specimen resembles the specimens of *Thorea gaudichaudii* from the Okinawa Island in many morphological features, but differs from the latter in the outward appearances such as the ramification and the diameter of thallus. Of the specimen from the Miyako Island, the ramification is less, the length and cell number of assimilatory filaments are shorter and less than those of *Thorea gaudichaudii* from the Okinawa Island. In the morphological features, the Miyako specimen is identical with the Cebu specimen, which was found growing on vertical submerged walls near the headwaters of the Kawasan Falls in the Cebu Island, the Phlippines, and identified as *Thorea violacea* by Liao and Young (2000). Molecular analyses based on 18S rDNA and rbcL gene show that there are two phylogenetic groups with high bootstrap values, the samples of *Thorea gaudichaudii* from the Okinawa Island and the samples from the Miyako and the Cebu Islands. Based on the morphological differences and the phylogenetic relationships, the Miyako specimen with the Cebu specimen is regarded as a new variety of *Thorea gaudichaudii.*

P43

MORPHOLOGICAL VARIATION OF *PROROCENTRUM MINMUM* IN THE BRACKISH LAKES NAKAUMI AND SHINJI, JAPAN

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Prorocentrum minimum has often dominated and made red tide in the brackish Lakes Nakaumi and Shinji. Cell shapes in valve view vary from heart-shaped to triangular in populations of these lakes. These forms have been considered as morphological variation of *P. minimum* (Dodge, 1975; Horiguchi, 1990). However, critical analyses of ranges of morphological variation based on field and culture populations and genetial analysis have not been carried out. In this study, we report morphological characteristics of four populations of *P. minimum* collected from Lakes Nakaumi, Honjo area (a part of Lake Nakaumi) and Lake Shinji.

Samples were collected on October, 2000 and May, 2001 in Lake Nakaumi, August, 2001 in Honjo area and on June, 1999 in Lake Shinji, when red tides occurred. Surface water samples were fixed with 1.25 % glutaraldehyde at the field and postfixed with 5 % of formaldehyde and/or 1 % of osmium tetroxide in laboratory. After freeze-dried using t-butanol, detailed structure of cells was observed by a SEM.

In populations of Lake Nakaumi on May 2001 and Lake Shinji, cells were heart-shaped in valve view, narrowly obovate in side view. Cells in population of Lake Nakaumi on October 2000 were heart-shaped in valve view, obovate in side view. These cells had wide intercalary bands. Cells in population of Honjo area were almost triangular in valve view, narrowly obovate in side view.

There were no qualitative differences in valve and side views between four populations. Only spine density was different among these populations. Spine number per 25 mm^2 of valve views of Lake Shinji (mean 94.6, sd=12.8) and Lake Nakaumi on October, 2000 (mean 91.5, sd=7.5) was statistically higher than those of populations of Lake Nakaumi on May, 2001 (mean 79.8, sd=9.5) and Honjo area (mean 80.5, sd=8.6)

P44

CAMPYLAEPHORA BOREALIS ISOLATED FROM THE *C. CRASSA* COMPLEX (CERAMIACEAE, RHODOPHYTA)

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Campylaephora crassa is a ceramiaceous red algal species that occurs exclusively in the northwest Pacific Ocean. The species is so variable in morphology and subdivided into five infraspecific categories based on ramification and structure of cortication. *C. crassa* f. *borealis* is annual and occurs in the lower intertidal in spring to summer on the East coast of Korea. It is morphologically diagnosed by evident main axis tending to form percurrent axis and ecologically epiphytic on various red algae. Phylogenetic analysis of plastid Rubisco spacer region sequences, determined from 29 samples of *C. crassa* f. *borealis,* f. *crassa,* and other putative relatives, shows monophyly of *C. crassa* f. *borealis* with strong supports. Since our morphological, ecological, and molecular evidences point at its independent taxonomic position and natural entity isolated from the *C. crassa* complex, we propose to rename *C. crassa* f. *borealis* as *C. borealis* at species level. The phylogenetic relationships between *C. borealis* and related taxa are discussed.

P45

MORPHOLOGICAL AND TAXONOMIC STUDIES OF *GRACILARIA FIRMA* AND *G. CHANGII* FROM TRAT, EASTERN THAILAND

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Morphological taxonomy of the two Thai species of *Gracilaria*, *G. firma* and *G. changii*, distributed in Trat, eastern Thailand were studied. In this area, the two species are generally distributed in the same habitat. The two taxa can be distinguished morphologically as followed: 1) *G. firma* has two forms of plants (the first form with few orders of branching; branches long, terminal branches without or with some furcations and blunt apices, the second form with many orders of branching, branch intervals of the last two orders are short and form a cluster with acute or blunt apices) while *G. changii* has only one form (thallus with many branches constricted at the base, inflated at the middle and tapering toward apices). 2) *G. firma* has first-order of branches always constricted at bases, others with or without constrictions, in contrast with *G. changii* of which all branches abruptly constricted at bases. 3) Fronds in transverse section, *G. changii* consists of large medullary cells with thick cell walls, transition of medulla to cortex abrupt, two to three layers of cortical cells, whereas *G. firma* consists of medulla with many layer of thin-walled cells, transition of medulla to cortex gradual, cortex only one to two cells thick. Although *G. firma* and *G. changii* can be clearly distinguished in their morphology, some specimens which have intermediate characters between the two species were collected among their natural populations, such characters occurred probably due to environmental variation or hybridization between the two species. Identities of these specimens have not been determined clearly and require further studies.

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PHYLOGENETIC STUDIES IN THE GENUS *CODIUM* (CHLOROPHYTA) FROM JAPAN

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The genus *Codium* (Chlorophyta) currently includes 113 species, 9 subspecies, 18 varieties and 17 formae and of these, seventeen species have been reported from Japan. Although gross morphology, as well as utricle and gametangial morphologies have been used for species identification, it is difficult to make accurate identification due to lack of stable morphological characters. Most morphological characters used for identification have been found to be extremely variable even within an individual. Consequently, the taxonomy of *Codium* remains confused. This study evaluates the usefulness of the plastid encoded *rbc*L gene of *Codium*, as a tool to distinguish *Codium* species, by comparing the results with morphological features. Another aim of our study is to resolve phylogenetic relationships among the species of *Codium*, For this purpose, we have determined *rbc*L sequences for eighty-eight specimens belonging to nineteen species from twenty-nine localities. Pairwise distances among the *rbc*L sequences revealed intra-specific divergence was from 0% to 1.283%, and inter-specific divergence was in the range between 1.705% and 11.209%. It seems that the *rbc*L sequences could be useful as a marker to discriminate species of *Codium*. Phylogenetic tree indicated that: 1) four large clades were recognized within the genus; 2) species of subgenus *Tylecodium* and *Schizocodium* were not clustered as a monophyletic clade, respectively; 3) *C. minus* was the earliest diverging species within the genus; 4) four adherent species were recognized, and one of them was separated from other three; 5) the specimen identifiable as *C. pseudolatum* Yamada (herbarium name) was clustered with *C. latum*; 6) *C. inerme* Silva (herbarium name) possesses similar gross morphology with *C. fragile* but different in flatted utricles, had identical sequence with *C. fragile*; 7) three entangled species were recognized, and one of them was newly recorded from Japan

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MORPHOLOGICAL AND MOLECULAR PHYLOGENETIC ANALYSES OF THE THOREACEAE 1. A SPECIES OF THE GENUS *THOREA* FROM INAWASHIRO-CHO, FUKUSHIMA PREFECTURE, JAPAN

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The specimen of the genus *Thorea* is collected from a small stream, emanating from a volcanic hot spring pond, situated at latitude 37°N in Inawashiro-cho, Fukushima Prefecture. The water temperature of the stream was 21°C in March, higher than those of neighboring rivers.

The Inawashiro specimen shows the following combination of features: (1) Plant filamentous, dark olive green, 3-150 cm long, 2500-4000 μm in diameter; (2) Ramification frequently; (3) Assimilatory filaments chantransia-like, rarely branched, 1000-1700 μm long, consisting of 20-30 cells; (4) Plant monoecious: Spermatangia ovoid, 7-9 μm in diameter and 10 μm long, 2-3 in clusters, terminal on short branches arising from the basal part of the assimilatory filaments; Carpogonia urceolate, 11-12 μm in diameter and 18μm long at the base, with a slender and elongate trichogyne ; Carposporangia obovoidal, 14-16 μm in diameter and 16-20 μm long, single or in pairs, formed on the same position as those of spermatangia; (5) Monosporangia formed on the middle part of assimilatory filaments, similar in size and shape to carposporangia.

Molecular analysis based on 18S rDNA and *rbc*L gene showed that the Inawashiro specimen form a monophyletic group with *T. okadae* collected from several rivers in Japan, and this clade is sister to the clade composed of *T. gaudichaudii* and *T. violacea*. The Inawashiro specimen and the specimens of *T. okadae* differ by 9 bp in the 18S rDNA and 40 bp in the *rbc*L gene. On the other hand, the Inawashiro specimen differs from the latter in the length of assimilatory filaments, the sexuality (monoecious) and the presence of monosporangia. Considering such morpphological features and phylogenetic relationship, the Inawashiro specimen is regarded as a new species of the genus *Thorea*.

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COMPARATIVE MOROPHOLOGY OF SO-CALLED *GRACILARIA CORONOPIFOLIA* (GRACILARIALES, RHODOPHYTA) FROM JAPAN

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Japanese populations of the red alga presently known as *Gracilaria coronopifolia* (Gracilariaceae, Gracilariales) were reinvestigated by morphological and cultural studies. Plants of populations growing in Okinawa, southern Kyushu and Hachijyo Isl. (taxon A) produced multicavitied, *Polycavernosa*-type spermatangial conceptacles and are entirely different from genuine *G. coronopifolia* from Hawaii that bear deep pot-shaped, *Verrucosa*-type spermatangial conceptacles. Characteristic adhensive habits of the former are also not represented in genuine *G. coronopifolia*. The examined red alga can be distinguished from all known species of *Gracilaria* distributed in the southwestern Pacific by the following set of features: i) a discoid holdfast (basal disc) with rhizome-like processes; ii) adhesion of the fronds to one another or substrata; iii) dense branching in the upper part of the fronds; iv) reddish purple in color; v) multicavitied *Polycavernosa*-type spermatangial conceptacles; and vi) basal traversing filaments in the cystocarp.

The branching and color of taxon A is distinct from observed Malaysian and Japanese *G. edulis*, however the former is similar to the latter in features i), ii), v), and vi). Externally, the plants of populations growing in Tokyo Bay, Chiba Prefecture (taxon B) resemble genuine *G. coronopifolia*. The cortex of the former is thick in the basal to midsection of the main axes and first- (second-) order branches and is clearly different from the latter species and from taxon A. Although no conclusions were made upon the relationship between taxon A and *G. edulis*, due to the problematic taxonomy of genuine *G. edulis* and to the pending taxonomic status of taxon B because of sterile specimens, we suggest that taxon A and B are distinct species and that the name of *G. coronopiflia* should not be applied to these taxa.

P49

NEW RECORD OF FRESH WATER RED ALGAE (RHODOPHYTA) IN THAILAND *BATRACHOSPERMUM CAYENNENSE* MUNTANG EX KÜTZING

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Samples of *Batrachospermum cayennense* Muntang ex Kützing were collected from streams in Phu Kradung National Park, Loei Province. It is a new record of fresh water red algae in Thailand. The algae were attached to rocks and wood in shallow streams in coniferous forest and hill evergreen forest at about 1,300 meter above mean sea level with low light intensity and low water temperature. *Batrachospermum cayennense* is highly gelatinous and bluish green. We found both gametophyte plants and carposporophyte plants. Sexual reproduction produced spermatia and carpogonia. Carposporophyte development was trichogyne with gonimoblast filaments, carposporangia and carpospores.

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DIVERSITY OF INORGANIC ION CONCENTRATIONS AND PHYLOGENETIC RELATIONSHIP IN *PLOCAMIUM* SPP. (PLOCAMIALES, RHODOPHYTA) IN JAPAN

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Among the fifty species of the genus *Plocamium* described to date, five species have been reported from the Japanese coast. However, they are considerably variable in the morphology, and the taxonomic boundary among the species *P. telfairiae*, *P. cartilagineum* and *P. recurvatum* are not clear due to the occurrence of morphological intermediates. In this study, we investigated the morphological variations and evolutionary relationships of Japanese *Plocamium* species based on the morphology, intracellular inorganic ion compositions, and the molecular phylogenetic analysis using the spacer region sequences of Rubisco genes between *rbc*L and *rbc*S.

Detailed morphological observations revealed that the shape and number of branchlet, which are the diagnostic characters of these taxa, were considerably variable within each species. The width and length of branchlet were comparatively stable in *P. telfairiae* under various culture conditions, whereas *P. cartilagineum* showed great variability. Regarding the pH of cell extracts, three types were recognized: neutral type (average pH = 6.7±0.63), acidic type (pH 3.8±0.27) and highly acidic type (pH 2.1±0.38). *P. telfairiae* included all three types and *P. cartilagineum* included the neutral and acidic type.

The molecular phylogenetic data indicated that Japanese *Plocamium* species examined in the present study were divided into five genetic groups; group 1 and group 2 both including *P. telfairiae* and *P. cartilagineum*, group 3 including *P. telfairiae* and *P. recurvatum*, and group 4 and 5 including *P. ovicornis* and *P. serratulum* respectively. Contrasting the discrepancy between the molecular data and the conventional taxonomy based on the morphological features, the types of cellular pH (i.e., neutral, acidic and highly acidic types) agreed with the phylogenetic groups elucidated from the molecular data, indicating that this character is useful for reexamining the taxonomy of *Placamium* species.

P51

THE MORPHOLOGY AND REPRODUCTION OF *NEMALIONOPSIS TORTUOSA* (RHODOPHYTA)

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Nemalionopsis estabished by Skuja 1934 with *Nemalopnopsis shawii* Skuja as the type species. as known monosporangia are located at the tips of assimilatory filaments. *N. tortuosa* was esatabished by Segi and Yoneda 1949 from Okichi-izumi, Ehime Prefecture, Japan. The habitat, thallus structure and monosporangia formation have been described for the species, but no gametangia have been definitely ascribed to the genus. Since the taxonomy of the Florideophyceae is based on the structure of the carpogonia, the systematic position of the genus has been uncertain. Detailed morphological studies have been carried out on the vegetative thallus, gametangia and carposporophytes of *N. tortuosa* collected from Nagasaki Prefecture. Thallus structure is multiaxial, consisting of medullary filaments and assimilatory cortocal filaments. The upper part of the assimilatory filaments composed of barrel-shaped cells. The monosporangia are borne at the apex of barrel shaped assimilatory filament. Spermatangia are borne on the terminal single or two or three in short small cluster. The carpogonium is borne laterally or terminally on a cell of an assimilatory filament. The gonimoblast initials are produced directly from the fertilized carpogonium, dividing soon afterwards to form several filaments. The gonimoblast filaments produce branches which grow among the assimilatory filamets, resulting in the formation of a diffuse carposporophyte. On the basis of these and other data, *Nemalionopsis* is maintained in the Thoreaceae.

P52

TANDEM REPEATS OF 5S RIBOSOMAL RNA GENES AND SPACER SEQUENCES OF THREE LAMINARIALEAN GENUS *LAMINARIA*, *CYMATHAERE* AND *KJELLMANIELLA* FROM JAPAN

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5S ribosomal RNA genes (rDNA) occurred as tandem repeats, not linked with the 18S-5.8S-25S rDNA unit, in *Laminaria* (*L. japonica*, *L. religiosa*, *L. ochotensis*, *L. diabolica*, *L. longipedalis*, *L. angustata*, *L. longissima*, *L. coriacea*, *L. cichorioides*, *L. sachalinensis* and *L. yendoana*), *Cymathaere* (*C. japonica*) and *Kjellmaniella* (*K. gyrata* and *K. crassifolia*) . Nucleotide length of 5S rDNA was 118bp and there was almost no substitution among the sequences studied for several populations of these laminarialean species from Hokkaido and Tohoku, Japan. Spacer regions between such tandem repeats of 5S rDNA were 199-252 bp in length and contained several nucleotide insertions/deletions among these species and also between individuals of the same species. Phylogenetic analyses showed that five species of *Laminaria* (*L. japonica*, *L. religiosa*, *L. ochotensis*, *L. diabolica* and *L. longipedalis*) formed an intermingled complex of very short branches, although some presumed annual individuals of *L. japonica* and *L. religiosa* were out of this complex. Four species of *Laminaria* (*L. coriacea*, *L. cichorioides*, *L. sachalinensis* and *L. yendoana*), two species of *Kjellmaniella* (*K. gyrata* and *K. crassifolia*) and *Cymathaere japonica* were more closely related with each other than to the above mentioned five *Laminaria* species complex and also to *L. angustata* and *L. lonigissima,* of which 5S rDNA spacer sequences were identical.

P53

ALGAL SUCCESSION CORRESPONDED TO THE MIGRATION OF AYU *PLECOGLOSSUS ALTIVELIS* IN THE NEZUGASEKI RIVER

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Ayu *Plecoglossus altivelis* is a typical algivorous fish in Japanese rivers and streams. The ayu juveniles go up to the rivers from the seas early in spring and stay until they spawn and die late in autumn. In experimental conditions ayu grazing has the impact on algal succession to alter the dominance from diatoms to a filamentous blue-green alga *Homoeothrix janthina*. Present study investigated algal succession in the Nezugaseki River, Yamagata Prefecture, from April to December 2001, where migration of wild ayu has been observed every year. The river was divided into two sections with a wear that obstructed the upstream movement of the juveniles. Algal samples were collected monthly in each section and the density of ayu was also estimated by snorkeling senses. In April the juveniles had not gone upriver and the diatoms and *Phormidium* sp. predominated in every section. In May *H. janthina* predominated in the downstream section where the juveniles had arrived, although the diatoms kept predominating in the upstream section where the dispersion of the ayu individuals was obstructed by the wear. After the individuals were introduced into the upstream section in Jun, *H. janthina* predominated in each section until October. Thus, the replacement of the algal dominance was coincident with the migration of ayu. This result suggested that ayu would play an important part in algal succession in the Nezugaseki River.

P54

NESTS OF AMPHIPOD CRUSTACEANS ON LARGE BROWN ALGAE

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Nests of amphipod crustaceans are often abundant on large brown algae and can affect on the ecological nature of them, but phycologists have been paid little attention to their presence. The nests built on the algae are classified into two types: epiphytic nests and the ones formed in the branch or stipe of algae. Epiphytic nestlers are tube-builders such as corophiids and leaf-curlers such as ampithoids. Tube-builders usually build their nests of mud and mucoid substance and often foul the surface of the algae. While tube-builders are believed to be grazers of epiphytic microalgae and do not feed on their substrate itself, leaf-curlers are leaf-eaters. They curl the leaf of laminarian algae with using the silk-like thread secreted from two pairs of their legs, and use the curled leaf as their nestling and feeding site. Eophliantids and biancolinids bore into the branch or stipe of *Sargassum* or laminarian algae. They form tunnels in the stipe or form grooves or slits on the branch. *Najna consiliorum* in najnids form envelop-like nest on the leaf of laminariales. We observed that they often cut and damaged the stipe of *Undaria pinnatifida*. The relationships between these nest-forming amphipod crustaceans and the substrate brown algae are discussed.

P55

REMOVAL OF HEAVY METALS FROM WASTEWATER USING NATURAL BIOFLOCCULANTS PRODUCED BY MICROALGAE

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The development of industry has been accompanied by the release into the environment of tremendous amounts of wastewater contaminated with toxic metals, such as lead, cadmium and mercury. In many cases these untreated wastes are dumped directly into rivers and lakes or at otherwise unsuitable sites, from which the toxic elements percolate to underground water reservoirs. Unlike organic pollutants, which in most cases eventually disintegrate, waste containing heavy metals must be treated to remove the toxic substances. To solve this problem, we have developed a simple, fast and inexpensive method for ridding water of a series of pollutants. Our system is based on the use of a combination of natural anionic bioflocculants produced by microalgae (A) and natural cationic bioflocculants (B). Addition of these flocculants to contaminated water results in immediate flocculation of the suspended particles, with the formation of hard flocs that absorb, entrap or cause the aggregation of dirt particles and heavy metals. The flocs settle rapidly, and the clarified water is then easily decanted.

Using this method, we removed 75% of the arsenic, cadmium and boron, 83-85% of the zinc, nickel and cobalt, and 90% of the lead and copper at the first pass. Repeated treatment resulted in 90% removal of the metals.

P56

THE OCCURRENCE OF A TOXIC DINOFLAGELLATE *GYMNODINIUM CATENATUM* GRAHAM IN SURFACE SEDIMENTS OF THE WESTERN JAPAN

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A naked and colonial dinoflagellate *Gymnodinium catenatum* Graham is known to be PSP (paralytic shellfish poisoning) causative species. The first toxic event derived from this species was in Spain in 1976, and then have been expanding world-widely. In particular, Tasmania in Australia was first suffered with PSP by this species in1986. At that time, since this species had never been described from Australian coastal waters, it seemed that this species might be introduced from other regions by ballast waters. In Japan, a PSP event given by *G. catenatum* was first reported from Senzaki Bay, western part of the Sea of Japan in 1986. Since then, the geographical distribution of this species seems to expand around West Japan. Simultaneously, PSP events related with this species also increased in these days. However, this species was reported from Omura Bay, West Kyushu under a different and non-described name, *Gymnodinium* sp. 5 before 1986. To clarify a possibility of its artificial migration based on a historical (stratigraphical) occurrence of *G. catenatum* in Japanese coastal waters, sediments which preserve a historical record of this species were collected from Omura Bay, Nagasaki Bay, Imari Bay and Matoya Bay where this species has been observed. Consequently the oldest occurrence of this species was recorded in the sediment of Omura Bay dated in ca. 1700. It is different situation that *G. catenatum* invaded recently in Tasmania in Australia. The oldest occurrence age of this species was in Edo period in Japan. There is no possibility for Japanese *G. catenatum* to be artificially introduced with such as ballast waters from other areas. This fact strongly suggests that this species occurring in Japan is originated and not artificially introduced from different areas.

P57

ASSESSMENT OF BIOTECHNOLOGICAL POTENTIAL OF ANTARCTIC CYANOBACTERIUM *NOSTOC COMMUNE* AS BIOFERTILIZER

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Mass culture of dominant species of cyanobacteria, *Nostoc commune*, available in Schirmacher Oasis of East Antarctica, was prepared under artificial conditions inside a glass house under maintained temperature in Indian Station Maitri, Antarctica. The cyanobacterial culture was used as biofertilizer and its effect was monitored on 7 varities of 4 vegetables growing in hydroponic system with usual Hoagland solution (soil less culture), vegetables grown were Bean, Bottleguard, Cucumber and Muskmelon. The application of cyanobacteria minimize the nitrogen deficiency after fifth day of transplanting these plants. In view of the above the present study deals with the cyanobacterial isolation from Antarctic soil, its mass culturing in green-house of Maitri and study their effect as biofertilizer, in growth and performance of vegetables. It was the first successful attempt made during the XI Indian Scientific Expedition to Antarctica in 1991-92 to use the Antarctic cyanobacteria as biofertilizer for vegetable cultivation in ice covered continent.

P58

ANNUAL DYNAMICS OF PHYTOPLANKTON COMMUNITIES IN THE LOWER PART OF THE HAN RIVER

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The study was biweekly investigated the water quality and phytoplankton communities at 6 stations during the period from Mar. 2001 to Feb. 2002 in the lower part of the Han River.

Water temperature was entirely under the influence of the air temperatures and pH was slightly alkaline. Low salinity showed at St. 6 and DO was changed with months and stations. Mean chlorophyll-*a* and BOD concentrations at each station varied from 10.2mg/m^3(St. 1) to 40.9mg/m^3(St. 4), and changed from 1.26mg/l (St. 1) to 3.74mg/l (St. 6), respectively. TN was changed from 1.7367mg/l (St. 1) to 6.8928mg/l (St. 6), TP ranged from 0.029mg/l (St. 1) to 0.418mg/l (St. 6). Chlorophyll-*a*, BOD, TN and TP level of the lower river were increased more than that of the upper due to pollutant inputs of municipal and industrial wastewater from several streams.

Phytoplankton communities were composed of diatoms, cyanophytes, chlorophytes, euglenoids, dinoflagellates and silicoflagellates. Phytoplankton standing crops were extensively fluctuated by months and stations, ranging from min. 6.5x10^5 cells/l (Sept. 8, St. 1) to max. 4.6x10^7 cells/l (Apr. 7, St. 4). Phytoplankton blooms were abundant from winter to the late spring due to the dry seasons, and those were rare in summer due to the rainy. Seasonal variations of the dominant species were comparatively obvious; *Asterionella gracillima* and *Aulacoseira granulata* var. *angustissima* in Spring, *A. granulata* var. *angustissima* in Summer to Autumn, *A. gracillima* and *Stephanodiscus hantzschii* in Winter.

The Han River are now eutrophicated owing to the heavy terrestrial and waterborne pollutants loads during the last three decades. If it is maintained, phytoplankton blooms could be increased frequency.

P59

WATER QUALITY AND ALGAE IN CHIANG MAI MOAT IN 1998 (I)

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The studies on the diversity of algae and water quality in Chiang Mai moat in 1988. Water samples and benthic algae were collected bi-weekly from four sampling sites during March to December 1998. From this study 48 genera in 5 divisions of benthic algae were recorded. Number of genera were as follows: Cyanophyta 11 genera, Chlorophyta 17 genera, Chrysophyta 14 genera, Cryptophyta 3 genera and Euglenophyta 3 genera. The common genera of benthic algae in all sampling sites, were *Chlamydomonas, Chlorella, Chlorococcum, Closterium, Crucigenia, Scenedesmus, Anabaena, Chroococcus, Lyngbya, Merismopedia, Oscillatoria, Phytolyngbya, Cycrotella, Cymbella, Fragilaria, Gomphonema, Melosira, Navicula, Pinnularia, Chilomonas, Cryptomonas, Euglena* and *Trachelomonas.*

Statistical analysis showed that the number of genera of benthic algae were not significantly difference among months and sampling sites. Division Euglenophyta was negatively correlated with pH (r=-0.7874). Amount of DO and BOD_5 indicated that Chiang Mai moat was in the fourth to fifth category; suitable for consumption and use after proper treatment especially and for communication, according to the standard value of surface water recommended by National Environment Committee Announcement of Thailand.

P60

STANDING CROPS OF PHYTOPLANKTON AND ITS RELATION TO CYANOBACTERIAL TOXINS IN A KOREAN EUTROPHIC LAKE

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The production of microcystins by bloom-forming cyanobacteria in eutrophic lakes and reservoirs is a common phenomenon in many countries of the world. The standing crops of phytoplankton, microcystin concentration, and physicochemical water quality were investigated from June to November 2001 in the Daechung Reservoir, Korea. The total standing crops of phytoplankton varied in a range of 2.5- 211.8 x 10^3 cells/ml. Cyanobacterial species such as *Anabaena* sp., *Microcystis* sp., and *Oscillatoria* sp. were identified as dominant species during the surveyed period. The microcystins were analyzed using a protein phosphatase (PP-1) inhibition assay with *p*-nitophenylphosphate as a substrate. The microcystin concentration in water ranged 0.05-0.16 $\mu g \cdot L^{-1}$, and in algae ranged 5.11-32.53 $\mu g \cdot g^{-1}$. The microcystin concentration exhibited a positive correlation with the standing crops of cyanobacteria. The seasonal variation of microcystin concentration in relation to the physicochemical parameters of water will be also discussed.

P61

CHARACTERISTICS OF PSP TOXIN PRODUCTION OF MARINE TOXIC DINOFLAGELLATES *ALEXANDRIUM TAMARENSE/CATENELLA* COMPLEX FROM THE KOREAN COASTAL WATER AND OPEN SEA

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Thirty-six isolates of toxic *Alexandrium tamarense/catenella* complex from the coastal vegetative cells and sediment resting cysts in Korea were subjected to analysis of paralytic shellfish poisoning (PSP) toxin by high performance liquid chromatography (HPLC). *A. tamarense/catenella* complex isolates were characterized on the basis of PSP toxin composition and contents to reveal correlation between toxin profiles and geographical distribution.

Six isolates from Sujeongri in Jinhae Bay had NEO, C1+2 and GTX1+4 as major components but GTX2+3 as minors. Seven isolates from Jindongri in Jinhae Bay had GTX1+4 and C1+2 as major components but GTX2, NEO and STX as minors. Four isolates from the southwestern open sea had GTX3/4 and C2 as major components. Five isolates from the southeastern open sea had GTX1+4 and C1+2 as major components but GTX3/5 and NEO as minors. Seven isolates from the south central open sea had C2 as a major component. There were three characteristics points based on the PSP toxin analysis, as follows; 1) the coastal isolates were more toxic than open sea; 2) isolates from the south central open sea showed low toxicity; 3) only ten isolates (five isolates from St. I, SOCD01j, SOCD01h, SOCF01i, YOC98a and YOC98c) had GTX5.

Cluster analyses, based on HPLC determinations of the suite of toxins produced by each isolate, revealed four distinct groups. First group was comprised of C1+2 toxin, second group was comprised of C1+2 and GTX1+4, third group was comprised of GTX1+4, and the fourth group was comprised of NEO as major components. Among the four groups, the third and fourth groups that composed of GTX1+4 and NEO toxins included more coastal isolates (70.58%) than the open sea ones. These results confirmed that toxin composition could be used as a marker to discriminate different geographical populations of these species.

P62

INCRESSING OF BIOMASS AND EFFICIENCY OF NUTRIENT ABSORPTION BY FRAGMENTATION OF *SPIRULINA PLATENSIS*

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Spirulina platensis was cultivated in Zarrouk's medium under the light intensity of 10,000 lux with aeration by air pump. Until the log phase was obtained. The algae was then sonicated at 47 kHz with increments of 10 seconds up to 120 seconds in order to break the filaments. The algae filaments were then cultivated continuously under the same conditions. It was found that the highest yield was obtained from the fragment sonicated for 90–100 seconds. The O.D. being 33-41% higher than that of the control and the dry weight was also higher: i.e. 1.17 and 1.11 grams compared to 0.84 gram in the control (from the cultivation volume of 400 ml). It was possible that fragmentation caused an increasing in the surface area of the filaments. Subsequently, absorption of nutrients increased which in term, increased the growth rate.

P63

SEASONAL DYNAMICS OF TOXIC CYANOBACTERIA AND THE CYTOTOXINS OF THE NAKTONG RIVER, KOREA

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Seasonal and spatial variations of cyanobacterial community were monitored at 16 sites along the Naktong River. The abundance of cyanobacteria was outstanding from May through November with dominant genera of *Microcystis, Anabaena, Oscillatoria* and *Gomphosphaeria.* Frequency and biomass of the genera became greater at lower reaches of the river. The *Microcystis* were observed from May to October, forming scum at the surface in some sites and *M. aeruginosa* was most dominant. There were significant relationships between biomass of *Microcystis* and NO_3^-, TP and pH in water column. However, NH_4^+, PO_4^{-3} and N/P were not critical in successions to the *Microcystis* dominated community. *Microcystis* blooms were notable at water temperature higher than 25°C. *Microcystis* density in sediment was 3 orders of magnitude higher than water column. *Anabaena* density ranged up to 11,220cells/ml. Four species of *Anabaena* were identified and *A. flos-aquae* was most dominant. *Anabaena* biomass was not related to temperature, NO_3^-, TN, PO_4^{-3}, TP and N/P of water column and the frequency of trichome with akinete and/or heterocyte were not related to these parameters. Microcystins were detected from May to November with yearly fluctuations. Microcystin-RR was most dominant. Total 84.2% of bloom materials with *Microcystis* exhibited toxicity of microcystin with maximum of 3,292μg/g dry wt. Total 12.5% of water column with *Microcystis* exhibited dissolved microcystin up to 3.3μg/l. Microcystin concentrations were positively related to *Microcystis* biomass and pH of water column. Anatoxin-a was determined by FD-HPLC analysis with NBD-F and all concentrations were below the detection limit of 0.1mg/l.

P64

COCHLODINIUM BLOOMS ALONG THE SOUTHERN COAST OF KOREA

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Cochlodinium polykrikoides blooms along the southern coast of Korea in 2000 were examined by *in situ* data and NOAA satellite images of sea-surface temperature (SST). In general, the *Cochlodinium* blooms, which have occurred annually in Korean coastal waters since 1995, initiate between late August and early September around Narodo Island and expand to the whole area of the southern coast. However, initiation and short-term change of the bloom of 2000 were quite different from the pattern observed up to now. In mid-August, thermal fronts in SST were formed; 1) between the Tsushima warm current water (TWCW) and the southern Korean coastal waters (SKCW), 2) between the Jindo cold water mass and the Southwestern coastal waters, and 3) between the upwelled cold waters in the Southeast coast and the offshore warm waters. Free-living cells of *C. polykrikoides* were concentrated in these frontal regions. In late August, the thermal front TWCW-SKCW approached the mouth of Yoesu Haeman where Seomjin River water and anthropogenic pollutants from Gwangyang Industrial Complex are discharged. The *Cochlodinium* bloom of 2000 initiated in the Yeosu Haeman in late August and coexisted with *Alexandrum* sp., *Gymnodinium* sp., *Skeletonema coastatum* and *Chaetoceros* spp. Two typhoons, 'Prapiroon' and 'Saomai', during the *Cochlodinium* bloom probably affected short-term changes in this species. After the former typhoon passed the Korean peninsula, cell growth of *C. polykrikoides* was maximal, but after the latter typhoon, the *Cochlodinium* bloom disappeared (20 September). Two weeks later (5 October), secondary *C. polykrikoides* bloom broke out within the coastal bay of Jinhae Bay and Hansan-Koje Bay. NOAA satellite images showed that the isothermal line of 22°C extended into Jinhae bay. This bloom also occurred simultaneously with *Akashiwo sanguinea(=Gymnodinium sangunium)*, a common red tide-forming dinoflagellate species in these coastal bays.

P65

MONITORING OF HARMFUL ALGAE IN MOLLUSC FARM IN MINONOSOK BAY (SEA OF JAPAN)

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Monitoring of harmful algae carried out in mollusk farm in Minonosok Bay during the spring-autumn period of 1997 and 1999-2001 included phytoplankton survey on three stations and dinoflagellates resting cysts study on seven stations. Forty-one types of cysts were identified to species level, representing 12 genera. The abundance of cysts varied from 542 to 1069 cysts/ml. The most common cysts were those of *Gonyaulax grindleyi, G. spinifera, Protoperidinium conicoides, Scrippsiella precaria* and *S. trochoidea.* Cysts of two dinoflagellates capable of producing paralytic shellfish poisoning (PSP) toxins (*Alexandrium tamarense* and *Gymnodinium catenatum*) were detected in the cyst survey. The study of phytoplankton samples revealed 133 species and intraspecific taxa of microalgae. The overall numbers of phytoplankton varied from 0.03 to 19.6 million cells/liter and biomass varied from 0.02 to 19 g/M.^{3}. The greatest density of phytoplankton was observed in June and August. Nine species of harmful microalgae known to be toxic were founded. These microalgae were diatoms *Pseudo-nitzschia multiseries* and *P. pungens*; the dinoflagellates *Alexandrium pseudogonyaulax, A. tamarense, Dinophysis acuminata, D. fortii, D. rotundata, Karenia brevis, K. mikimotoi, Prorocentrum minimum.* Species of the genera *Pseudo-nitzschia, Alexandrium* and *Dinophysis* were more abundant and exceeded the harmful concentration during the summer. Cases of human poisoning were not recorded.

P66

DIVERSITY OF DESMIDS IN NONG HAN RESERVOIR, SAKOLNAKORN PROVINCE, THAILAND

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Desmids are attractive and well known group of freshwater algae, belonging to Division Chlorophyta, Family Desmidiaceae and Family Mesotaeniaceae. Sampling of desmids in fresh water reservoir, Nong Han, Sakolnakorn province was carried out between March 2000-March 2001. They were collected using a plankton net (meshsize 10μm), squeezing aquatic macrophytes.They were preserved with Lugol's solution. Identification was done with light compound microscope (100X, 400X).It was found that the water quality classified by tropic level was oligo-mesotrophic. Ninety-four taxa were found, identified and classificated into 18 genera, i.e. *Netrium*(1), *Closterium*(5), *Pleurotaenium*(6), *Gonatozygon*(3), *Triploceras*(1), *Micrasterias*(6), *Euastrum*(7), *Cosmarium*(26), *Cosmocladium*(1), *Staurodesmus*(11), *Xanthidium*(2), *Staurastrum*(14), *Sphaerozosma*(1), *Onychonema*(1), *Spondylosium*(3), *Hyalotheca*(1), *Bambusina*(2), *Desmidium*(3).

P67

INFECTION OF LYSOGENIC VIRUSES (CYANOPHAGES) IN CULTURED STRAIN OF LPP GROUP CYANOPHYTES (CYANOBACTERIA)

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Many cyanophages (viruses that attack cyanobacteria) have been reported since their discovery by Safferman and Morris in the early 1960s. Some of them enter the lysogenic cycle after infection. We previously reported that more than half of the marine LPP group isolates kept in our laboratory may harbor lysogenic cyanophages. The cyanophages infecting these strains appear to belong to the families Cyanomyoviridae and Cyanostyloviridae from their morphologies. Cultures of strains infected with lysogenic cyanophage tend to be unstable; spontaneous cell lysis often occurs under stressed conditions, e.g. a temperature shift or unsuitable light conditions. Furthermore, horizontal gene transfer by viruses, which has been suggested in bacteria may also occur in cyanobacteria. However infection of cultured strains of cyanobacteria by lysogenic cyanophases has not received much attention. One of the hosts of lysogenic cyanophage that is in our collection is *Phormidium persicinum*, which was isolated by Pintner in 1958. This strain was obtained via private collection, and was found to be infected with a lysogenic cyanophage of the family Cyanostyloviridae. Sudden and synchronous cell lysis occurs when cells of *P. persicinum* are treated with mitomycin C (5μg/ml) or briefly illuminated with UV-light. Last year I obtained the same strain from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP). *P. persicinum* CCMP638 is not infected with the same cyanophage that is infecting our strain, because it is completely lysed by the cyanophage isolated from our *P. persicinum*. These findings strongly suggest that the infection and lysogenization of cyanophage of this strain occurred after isolation, though the route and the time of infection are unclear. The possibility of cyanophage transfer among marine LPP group isolates will be discussed.

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DIVERSITY OF PHYTOPLANKTON AND WATER QUALITY IN THE RESERVOIR OF LAMTAKONG DAM, NAKORN RATCHASIMA PROVINCE, THAILAND

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Phytoplankton and water quality in the reservoir of Lamtakong Dam, Nakorn Ratchasima Province were investigated between April 2000 – March 2001. It was found that phytoplankton was present in 6 divisions in 105 species. The dominant species were *Pseudanabaena limnetica* Komárek, *Monoraphidium contortum* (Thur.) and *Fragilaria ulna* var. *acus* (Kützing) Lange - Bertalot. Four rare species of planktonic algae, *Acanthoceras zachariasii* (Brun) Simonsen, *Fragilaria arcus* (Ehrenberg) Cleve, *Navicula trivialis* Lange – Bertalot and *Fragilaria ulna* var. *acus* (Kützing), which have never been recorded in Thailand were discovered. The water quality in the reservoir of Lamtakong Dam was classified to be in the mesotrophic status and in the category 2 – 3 due to the assessment of water quality by comparison to the standard water quality of Thailand. The water could be used for household consumption after being properly treated.

P69

SEASONAL DYNAMICS OF PHYTOPLANKTON COMMUNITY IN SOME INLAND WATERS OF PUSAN METROPOLITAN CITY, KOREA

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A total of 95 phytoplankton species belonging to 7 phyla and 49 genera were identified at 17 sites in inland waters of Pusan Metropolitan City during the period from April 2001 to March 2002. Phytoplankton biomass ranged from 336 cells/ml to 1.39 x 10^6 cells/ml throughout the experimental period. The average of chlorophyll *a* concentrations ranged from 26μg/l to 89μg/l, and most of the sites were hypertrophic according to the OECD guideline. During the period from July to August, the phytoplankton community was dominated by cyanophycean algae, showing relative frequency of *Microcystis* up to 99.9%. During the period from November to March the community was dominated by chrysophycean algae, showing relative frequency of *Stephanodiscus* up to 99.8%. Hoedong Reservoir, which supplies about 10% of drinking water demand of Pusan Metropolitan City, showed algal biomass up to 1.39 x 10^6 cells/ml and 261μg chl. *a* /l, mainly due to *Microcystis* bloom during the summer period. The tributaries to the Hoedong Reservoir did not show the *Microcystis* abundance, and presumably the water column stratification enhanced the bloom formation in the reservoir.

P70

VIABILITY ASSAY BY USING SYTOX GREEN AND AUTOFLUORESCENCE IN CYANOBACTERIA

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A fluorescence method to evaluate the viability of cyanobacteria, *Synechocystis* sp. PCC 6803, using fluorescence of SYTOX and autofluorescence equipment with CLSM was tested. SYTOX green is high-affinity nucleic acid stain that easily penetrates cells with damaged plasma membrane and will not cross the membrane of live cells. After 5 min incubation with 1 μM SYTOX, the dead cells fluoresce bright green when excited with the 488 nm spectral line of the argon-ion laser. Whereas all live cells and almost dead cells fluoresce bright red caused by autofluorescence when excited with the 633 nm spectral line of the He-Ne laser, and a part of dead cells were colorless. Consequently, we could recognize live cells colored in red, and dead cells colored in yellow or green in merged image. Data obtained by fluorescence measurement of cyanobacteria were compared with those obtained by plate counting for *Synechocystis* sp. PCC 6803. A good correlation was obtained between percentages of live bacteria obtained by fluorescence measurement and those obtained plate counting (R^2 = 0.98). This indicates the fluorescence measurement is effective quantitative technique for calculating the proportion of viable to dead cyanobacterial cells. This technique might be useful not only unicellular cyanobacteria but also filamentous cyanobacteria and eukaryotic microalgae.

P71

THE DIATOM GENUS *CHAETOCEROS* EHR. FROM THE NORTHERN PART OF THE SEA OF JAPAN

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Based on the original and literature data the species of the genus *Chaetoceros* from the northern part of the Sea of Japan are reviewed. Sixty *Chaetoceros* taxa were recorded in the samples (47 species and 13 varieties and forms). A morphological description of 37 species and microphotographs obtained using bouth light and electron microscopy are given. The seasonal groupings of the *Chaetoceros* species are analyzed. *C. debilis* and *C. pseudocrinitus* were the co-dominant species in the winter season and accounted for 55% of the total population. *C. compressus, C. debilis, C. decipiens, C. pseudocrinitus* were the dominant species in spring and comprised 80% of the phytoplankton present. *C. affinis* and *C. salsugineus* made up the greater part of the summer population and caused yearly summer blooms. Neritic temperate species *C. compressus, C. danicus, C. debilis, C. didymus* could be found throughout the year. Warm-water species *C. anastomosans, C. coarctatus, C. messanensis, C. pseudocurvisetus, C. rostratus* were rare and could be found only in the warmest period – late summer–yearly autumn. *C. affinis, C. convolutus/C. concavicornis, C. debilis, C. decipiens, C. pseudocrinitus, C. salsugineus* were bloom-forming species.

P72

CHARACTERIZATION OF A NOVEL VIRUS (THV) WHICH CAUSES LYSIS OF *SCHIZOCHYTRIUM* SP. (LABYRINTHULEA, STRAMENOPILES)

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The Labylinthulea is a class of the stramenopiles (Kingdom Chromista). They are marine fungoid protists, characterized by ectoplasmic nets and the cell wall composed of non-cellulosic, sulfrylated scales. This class consists of a single order and two following families: Labyrinthulaceae and Thraustochytriaceae. Recent studies suggest that the thraustochytrids play important roles in the coastal ecosystem as the decomposers as well as the producers of polyunsaturated fatty acids and the pathogens to marine mollusks.

We succeeded in isolating a lysis factor specifically killing *Schizochytrium* sp. that belongs to thraustochytrids, from the Kobe harbor, Japan. Inoculation of this factor caused cell lysis of *Schizochytrium* sp. and specific increase of virus-like particles (VLPs), which were icosahedral, lacking a tail, and ca. 20 nm in diameter. They formed crystalline arrays and random assemblies in the cytoplasm of host cells. The lysis factor was repetitively transferable to a fresh culture of *Schizochytrium* sp. On the growth experiment, host cell density was decreased in an order by inoculation of the factor. Whereas the VLPs have never been detected in healthy host cells. Therefore, fulfilling Koch's Postulate, the lysis factor was considered to be a virus, and designated "ThV (Thraustochytrid Virus)".

ThV has several properties that are common with the virus family Picornaviridae: the size, the morphological feature, and formation of crystalline arrays in the cytoplasm. Genomic studies on ThV would give further information on its classification and nomenclature.

Viruses are highly abundant in the marine environment (10^8-10^{11} ml^{-1}), and have been recognized important in controlling plankton community and composition. However, there have been few reports on viruses infecting eukaryotic decomposers in marine environments. Thus, ThV is considered a noticeable biological specimen to study the ecology of decomposition processes in coastal environments.

P73
EFFECTS OF THERMAL DISCHARGES ON THE DISTRIBUTION OF BENTHIC MARINE ALGAE

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Marine algae, or seaweeds, have long been served as important food sources in Orient, especially in Korea. As far as the effects of cooling water and thermal discharges are concerned, marine algae are likely to be vulnerable to a discharge.

The goals of this studies are: (1) to understand the qualitative and quantitative aspects of marine algae in the vicinity of nuclear power plants; (2) to clarify the effects of thermal discharges on the succession of marine algal community.

As a result, a total of 96 species of marine algae (7 blue-green, 12 green, 25 brown and 52 red algae) were investigated at Kori nuclear power plant and reference site. The distance of ecological effect of thermal discharges on the intertidal benthic marine algae was estimated as 70-90 m from the outfall of Kori unit 1, probably because of the influence of seawater input from the adjacent Wollae Bay.

Differences in successional patterns of marine algal community were observed between the outfall and control site. It is interesting to note that several coralline algae, particularly crustose coralline algae or melobesioidean algae, appeared rather abundantly in the permanent quadrats established at the control area. These coralline algae have been considered as the causative organisms of algal whitening, which is one of the significant ecological deterioration of coastal marine ecosystem in the East Sea in recent years.

P74
DISAPPEARANCE PROCESS OF *ECKLONIA* BEDS DUE TO GRAZING OF HERBIVOROUS FISH OFF THE COAST OF MIYAZAKI PREFECTURE, SOUTHWEST JAPAN

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The *Ecklonia* beds were developed off the coast of Kawaminami town and Tsuno town in Miyazaki Pref. central part. The area of *Ecklonia* beds was 314ha in 1976 (Yurino *et* al. 1976). The area was decreased to 99ha in 1989. Therefore, various researches were done in 1990 and afterwards. We transplanted *E. kurome* plants of 11cm in the average length inside and outside the cage in Tsuno in February 1990. The plants in the cage grew up to 20cm in length one year later. The plants outside the cage disappeared by the grazing of the herbivorous fishes in November. The same phenomenon occurred to a natural *Ecklonia* beds in Tsuno in 1991 and Kawaminami in 1992. The area of the *Ecklonia* beds was decreased to 0.1ha. In addition, *Ecklonia* beds had disappeared off Hyuga City, Kadokawa town, and Nobeoka City in the prefecture northern part from 1993 to 1996. It was confirmed by the document investigation that the *E. kurom*e had grown on Nichinan City in 1913 and Miyazaki City in 1954 in the southern part of the prefecture. Thus, it is clear that this phenomenon expanded from the southern part to northern part in Miyazaki Prefecture. The large schools of rabbit fishes were sometimes observed to be grazing the laminae. It was concluded that *Ecklonia* beds disappeared by overgrazing of the rabbit fish. The surface water temperature off the coast of Tsuno from 1966 to 2001 is rising tendency in the winter. The water temperature after 1989 was higher than 18.1 degrees of the average value at all periods in winter. It is thought that the amount of the resource of the rabbit fish increases since a high water temperature in the winter enables rabbit fish's passing the winter and the overgrazing occurs.

P75

FIELD PREDOMINANCE OF TETRASPOROPHYTES OF *GELIDIUM SESQUIPEDALE*: SEARCHING FOR AN ECOPHYSIOLOGICAL EXPLANATION

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Most of the individuals of *G. sesquipedale* in the population located in Albufeira (Portugal) are tetrasporophytes and are fertile throughout the year. To estimate whether the unequal distribution of the two phases is related to a better fitness of diploids, their physiological performances were studied. Tetrasporophytes (diploids), carposporophytes and male individuals (haploids), were cultured in a spray chamber on artificial substrates, under short and long photoperiod. Diploid thalli presented a higher growth only during the first week of culture, independently of the photoperiod, and their apical parts attached to the calcareous substrate more rapidly than haploid individuals (almost 100% of re-attachment within 7 days). After four weeks of culture, new germlings appeared on some rhizoids. Photosynthesis, nitrate-reductase activity and nutrient uptake of diploid and haploid individuals will be compared.

P76

INHIBITING SUBSTANCE OF THE SPORULATION FROM VEGETATIVE FROND OF THE GREEN ALGA *ENTEROMORPHA PROLIFERA* (MÜLLER) J. AGARDH

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Vegetative cells of *Enteromorpha prolifera* are matured in zoosporangia or gametangia by cutting the frond into small fragments (1-3 mm in diameter). When the small fragments were incubated in the various concentrations of the filtered solution from homogenization of vegetative fronds for 2-3 days, the inhibiting of sporulation for fragments increased in accordance with increasing in the concentration of the filtered solution. The presence of the sporulation inhibitor (SI) was suggested to have been in vegetative fronds by these results. The relation between SI and the small fragments were investigated on the following items: distributions of SI in the frond, SI productions at various developmental stages of frond, changes of the growth (length of frond) and morphology of the frond after incubated in the mediums added the various concentrations of filtered solution. From these experiments, higher concentration of SI was distributed inside of a hollow structure with a single layer of cells. Furthermore, the concentration of SI increased from apical region to basal region along the axis of frond. The SI production in frond was increasing during growing periods, but it was decreasing or stagnated during non-growing periods. The young fronds of *E. prolifera* showed the lower growth rates in the culture with the high concentration of filtered solution. The abnormal cells of frond also appeared at the higher rates in the culture with higher concentration of filtered solution.

P77

ANALYSIS OF GRAZING EFFECTS ON THE BIOMASS DYNAMICS OF *LAMINARIA JAPONICA*

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Local distributions of kelp are often restricted by grazers, which have even destroyed beds. Sea urchins have been recognized as major grazers of *Laminaria*. The feeding equation of Ivlev (1961) was combined to the photosynthetic production model (Honda, 1999) for mathematical analysis of grazing effects on the biomass dynamics of *Laminaria japonica*. Less than two solutions in standing biomass were given to the equation,

Photosynthetic production – Feeding = Zero.

One solution is a stable equilibrium of maximum biomass. Another one is an unstable equilibrium of minimum biomass required for a persistent *L. japonica* stand. Relationships between the rate of biomass change and standing biomass of *L. japonica* are classified into three types: 1) the rate of biomass change is positive under the maximum biomass, 2) the rate of biomass change is positive between the maximum biomass and the minimum biomass required for a persistent *L. japonica* stand, and 3) the rate of biomass change is negative for all biomasses. The maximum biomass decreased with increasing feeding rate and depth. The minimum biomass required for a persistent *L. japonica* stand was found with a feeding rate which exceeded the threshold value. The feeding rate on the threshold value was affected by water depth and the attenuation coefficient that characterized the amount of light reaching the seafloor. In the absence of grazers, the minimum biomass required for a persistent *L. japonica* stand was not achieved. The value of the minimum biomass required for a persistent *L. japonica* stand increased with increasing feeding rate and water depth.

P78

A NON-DESTRUCTIVE MEANS OF OBTAINING FIELD PRODUCTION DATA FOR *ZOSTERA MARINA*

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Through a numerical simulation model analysis, which incorporates the available seagrass information, it may be possible to develop a better understanding of the complex seagrass ecosystem. Clarification of seagrass production is important in the material circulation analysis within the ecosystem. The comparison between the calculated and observed results is indispensable in confirming the effectiveness of the seagrass productivity simulation model. Marking techniques previously used are helpful in *Zostera marina* production estimation. Production data, however, cannot be taken repeatedly in the same point since it entails seagrass collection within the place. Hence, we are attempting to develop a productivity measurement method for *Z. marina* without needing collection.

Shoot weight and the weight of rhizome with leaves were expressed as the function of shoot length and number of leaves. The number of new nodes indicates the number of new leaves. Therefore, weight increase in leaf and rhizome can be extrapolated from shoot length, number of leaves, and number of new nodes in the *Z. marina* bed and incorporating relative growth relations. Four quadrats (1 square-meter) within the *Z. marina* bed in Aburatsubo Bay, Kanagawa Prefecture were selected. The number of shoots within each quadrat was counted. Twenty shoots of each quadrat were marked using small tag. Shoot length, number of leaves, and number of new nodes of tagged shoots were measured monthly.

P79

ANALYSIS OF LIGHT ATTENUATION EFFECTS ON THE PRODUCTIVITY OF *LAMINARIA JAPONICA*

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The water in which aquatic plants occur both absorbs and scatters light. As a result of absorption and scattering of the solar flux, irradiance of the light field attenuates in an exponential manner with water depth.

If you see photosynthesis-irradiance curve, you can easily understand how much the photosynthetic rate decreases with decreasing irradiance. Therefore, you might think that you can easily predict how much the productivity in the seaweed bed decreases for the attenuation of light in sea water.

There was no big difference (the relative value is less than 5 % in any irradiance) in photosynthesis-irradiance curves in August and November of *Laminaria japonica*. Nevertheless, the computed autumnal productivity of the algal production model (Honda 1999) was more sensitive to attenuation than the computed summer productivity. The main cause of the sensitive autumnal productivity of *L. japonica* bed for light attenuation was not on the seasonal photosynthetic characteristic but rather on lesser irradiance and shorter day length.

P80

CHEMICAL ENVIRONMENT AND BIOMASS OF SEAGRASS (*ZOSTERA MARINA*) BED IN ABURATSUBO BAY

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A quantitative environmental impact prediction method in the seagrass bed and the wetland ecosystem is an important problem considering costal biology. Especially, the seagrass bed ecosystem in sand-clay area is important for the benthic organism in the deposit, and the material circulation analysis. Further, production of seagrass and taking up the nutrient in sediments making use of seagrass root were impact for sediment quality and some of environmental factor (suspended solid flow, surface sediments remove, benthic organism) around this area.

Circulation of nutrients in seagrass ecosystem, however, was difficult for seeing relation of all each other. So that it is necessary to construct the model by which an organic relation between them is appreciable. The purpose of this paper was to clarify the relation to the biomass of the benthic organism and the biochemical reaction in the sediments. We observed the biomass of the *Zostera marina*, the biomass of the benthic organism and the sediment and the water quality for the area with the *Zostera marina* and the area without the *Zostera marina* of Aburatsubo bay in Japan.

As a result, in both areas the difference was seen in the mechanical composition of the sediments, the nutrient concentration in the sediment pore water, and the amount of the algae on the surface of sediments. Especially, the settling rate of suspension matter and the amount of the algae on surface of sediments in seagrass bed was greatly influenced by the growth rate of the *Zostera marina*. In addition, the subsidence of the suspension particle was promoted, and an increase in the amount of the organism and a steady benthic organism appeared in the near place of seagrass bed.

P81

LIFE HISTORY OF *PORPHYRA KUNIEDAE* (BANGIALES, RHODOPHYTA) IN CULTURE

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Culture studies of *Porphyra kuniedae* from Korea were carried out at various conditions. Mature foliose thalli of *Porphyra kuniedae* collected on 10 March 1995 at Wando from Korea. Zygotospore germlings were cultured at the constant temperatures of 5-30°C and with photon irradiances 10-80 μmol m^{-2} s^{-1} under photoperiods of 14L:10D and 10L:14D. Conchospores were cultured at the condition of 5-30°C, 40 μmol m^{-2} s^{-1} under both photoperiods. Higher growth of conchocelis was observed at 20°C and 40 μmol m^{-2} s^{-1} under 14L:10D, and conchosporangial branches were produced at 20 and 25°C and 10-80 μmol m^{-2} s^{-1} under both photoperiods. Foliose thalli grew well at the conditions of 5-10°C under 10L:14D. At 30°C, the foliose thallus could not survived. Archeospores were observed in all temperature conditions except for 5°C under both photoperiods. Spermatangial and zygotosporangial sori were formed at the upper and marginal parts of mature thallus. Spermatia and zygotospores from foliose thallus were released at 5-25°C under 14L:10D and 10L:14D. Anatomical examination revealed that the matured spermatangial sorus was 64(a/4, b/4, c/4) and zygotosporangia sorus was 8(a/2, b/2, c/2) according to the calculation of Hus's formula(1902)

P82

GROWTH AND MATURATION OF *ECKLONIA STOLONIFERA* OKAMURA IN TONGYOUNG IN THE SOUTHERN COAST OF KOREA

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Perennial alga *Ecklonia stolonifera* Okamura is distributed in Korea from the Eastern coast to the southern coast. Field observations were carried out from September 1998 to August 1999 in Tongyoung in the southern coast of Korea. Fifty *Ecklonia stolonifera* plants from covered rocks and large gravels at depth of 6-8m were collected by SCUBA diving. Individual blade length, blade width, stipe length and stipe diameter were measured for fifty plants sampled. Blade weights were measured after excess water was blotted by paper towel. The age of the plant was determined by annual ring of the stipe of *Ecklonia stolonifera*. The annual variations of seawater temperature in Tongyoung coast were 9 and 26°C. Mean blade lengths of the plants were 45.2 and 96.2cm and the maximum values obtained in May. Mean blade width ranged from 13.7cm to 28.6cm. Stipe length ranged from 4.3 to 11.9 in average. Mean stipe diameters were 5.11mm in the maximum value (June) and 4.26mm in the minimum value (February), respectively. Formation rates of zoosporangial sori were 16 and 78 percentage and the highest rate was observed in September. In conclusion, *Ecklonia stolonifera* from Tongyoung are greater than those of other sites in Korea in size and weight.

P83

MARINE ALGAE FOUND IN THE DISCHARGE CANALS OF THREE NUCLEAR POWER PLANTS IN THE EAST COAST OF KOREA

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Heated effluents from nuclear power plants, with the temperature rises of 8-12°C under normal operating and design conditions, are discharged through the discharge canal and into natural water bodies. Thus, the benthic marine algae grown at the discharge canal can be regarded as warm tolerant species. This study is intended to clarify the structure and seasonal dynamics of warm tolerant benthic marine algal community in Korea. The species composition of marine algae at the discharge canals of three nuclear power plants (Kori, Wolseong and Uljin) in the east coast of Korea were investigated seasonally from spring 1991 to autumn 2000. As a result, a total of 152 species of marine algae was found during the past 10 years. In general, the number of species observed was abundant in spring or summer and less in autumn or winter. 37 species (2 blue-green, 10 green, 9 brown and 16 red algae) of marine algae occurred more than 20% frequency and thus can be categorized as warm tolerant species.

P84

BIOLOGY AND ECOLOGICAL VARIABILITY OF *LAMINARIA BONGARDIANA* P. ET R. (PHAEOPHYTA) ON THE COASTS OF THE RUSSIAN FAR EAST

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The development of the brown alga, *Laminaria bongardiana* P. et R. (Phaeophyta) under different ecological factors and different levels of the anthropogenic pollution were investigated in various areas of the Eastern Kamchatka and some northern Kurile Islands from 1996 up to 2001. *L. bongardiana* appeared to have many morphological variations, and it was capable of forming abundant kelp in different environmental conditions. The investigations on the influence of oil-products, detergents and phenols on *Laminaria* development were carried out in the Avacha Bay (Southeastern Kamchatka). The peculiarities of sporogenesis, age, size and weight of different parts of lamina were studied. The life history of *L. bongardiana* usually completes in three years. However, high speed of the water movement and low annual temperature may reduce the terms of vegetation to one year. The plants growing in the heavily polluted areas had shorter life history as well. The dramatic changes of width and weight were observed along the lamina, due to the movement of assimilated substances to the place where sporangia sori developed. In the one-year-old plants, these changes were especially conspicuous. The changes of developmental strategy of the blade resulted in earlier sporogenesis, therefore ensuring survival of the populations in harmful environmental conditions. In less polluted areas, the life history of the sporophytes did not become shorter. The dramatic changes of width and weight along the lamina were not observed. Maturation of the one-year-old plants occurred later, and therefore reproductive strategy of populations did not change.

P85

IMPACT OF OCEANOGRAPHIC CLIMATE ON PRODUCTION OF *LAMINARIA JAPONICA* ARESCH. IN NORTH-WESTERN REGION OF THE SEA OF JAPAN

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Kelp *Laminaria japonica* belongs to the species of boreal group. Its optimal growth conditions are (1) the water temperatures not over 16-18°C, (2) good water exchange with flow speed above 30-40 m/sec, and (3) saturation with biogenic nutrients, particularly with nitrates, more than 15-20 microgram per liter. The north-western part of the Sea of Japan with the cold Primorye current meets all this demands, and productivity of kelp inhabited this region is stable. But there are years, when the current velocity lowers and, as a result, this kelp status changes.

In 2000, phenological autumn in northern Primorye was belated and high water temperature kept till mid-November. Such a situation is explained by the positive setup of warm water mass caused by delay of shift from southern winds to winter-type coastal ones that in turn was induced by the global atmosphere phenomena. Lasting warm fall caused the destruction of both first-year thalli and maternal ones with reproductive tissues. As a result, there were almost total lack of second-year thalli and decrease in the population productivity in whole in 2001.

The more temperature-tolerated second-year maternal thalli were not damaged such severe in 2000, but before the water temperature has become favorable for sporification, maternal thalli lost up to 2/3 of their plate areas and, consequently, of considerable amounts of zoospores. This resulted in the decrease of the first–year thalli spreading in 2001 that in turn caused a drop in the kelp production in 2002.

Thus, the oceanographic conditions strongly affect the population dynamics of *Laminaria japonica* and have to be taken in consideration for aquaculture purposes.

P86

THE RESULTS AND PROSPECTS OF BROWN ALGAE' CULTIVATION IN THE SEAS OF JAPAN AND OKHOTSK

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Kelps (*Laminariales*) form vast forests in the seas of Far-Eastern Russia. In the Sea of Japan, kelp forests are dominated by *Laminaria japonica, L. cichorioides* and *Costaria* sp., whereas *L. gurjanovae, L. inclinatorhisa, L.appressirhiza, L. bongardiana, Alaria* sp., *Arthrotamnus* sp., *Lessonia* sp. are prevalent in the Sea of Okhotsk.

In the Sea of Japan with the strongly marked seasonal thermostratification of the costal waters macroalgae adopt their life cycles (gametophyte, sporophyte) to the definite periods of year. The germination of the zoospores into gametophytes takes place in fall and young sporelings appear in early spring. These features of kelp life history are taken into account at field cultivation. About 5000 tons of raw *Laminaria japonica* are annually grown from seed, but the crop only can be taken once a year for a limited period of time, which brings some difficulties in organization of the cultivation process.

In the Sea of Okhotsk with the water temperature being relatively constant all year round reproduction period of *Laminariales* algae lasts much longer and sporelings appear all the year round. Thus close to adult plants of *Laminaria gurjanovae* and *Alaria* sp. reaching length of 5–12 meters their sporelings of 1–5 centimeters in length can grow. It opens good prospects for kelp cultivation, as the settlement of zoospores on available substrata and the harvesting can be done the whole year round.

P87

DIVERSITY OF MACROALGAE IN TWO RIVERS OF NORTHERN THAILAND

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A study on the diversity of macroalgae in two rivers of northern Thailand was carried out from April 2001 to March 2002. The samples were collected from five different sites along the Ping and Nan Rivers. The macroalgae were classified in to 5 divisions: Cyanophyta, Chlorophyta, Charophyta, Xanthophyta and Rhodophyta. The prominent species were *Spirogyra* spp., *Cladophora glomerata* Küetzing and *Microspora floccosa* West & West. The physical and chemical parameters were also examined to study the coloration with diversity of macroalgae. The study revealed that some species of macroalgae could be used as water quality indicators.

P88

MACROALGAE GROWTH RESPONSE TO DEEP SEAWATER

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It is known that primary productivity is high in oceanic areas where deep seawater is upwelling to the euphotic zone. Phytoplankton seem to rapidly proliferate in regions where deep seawater mixes with surface seawater, because phytoplankton can not efficiently utilize the nutrientsalts in deep seawater without the presence of certain trace elements. In order to examine the effects of deep seawater on growth and uptake of nutrientsalts by macroalgae, growth and uptake of nutrients (NO_3-N and PO_4-P) by *Ulva* sp. (Ulvales, Chlorophyta) were investigated by cultivation in deep seawater (taken from 687 m depth at Yaizu, Shizuoka Prefecture, central Japan in August 2001), surface seawater (taken from 24 m depth) and a combination of the two. Culture was carried out in aerated 500 mL flasks with 4 blades of *Ulva* sp. (cut sections of ca. 2 cm^2) at 20 °C water temperature, 100 $\mu mol\ m^{-2}s^{-1}$ light intensity and a 14:10 LD cycle.

Growth (frond area, dry weight and chlorophyll) and uptake of nutrients by *Ulva* sp. were greatest in deep seawater and lowest in surface seawater. Uptake rates of nutrients by *Ulva* sp. cultivated in mixed seawater were half of those cultivated in deep seawater, but growth rates in mixed seawater were higher than half. Thus it is clear that growth rate is not proportional to nutrient uptake rates in *Ulva* sp.

From these results, it can be seen that macroalgae may be able to efficiently utilize deep seawater, which may lack or have low concentrations of certain trace elements, and that their growth is not always proportional to the quantity of nutrients uptaken.

P89

TEMPERATURE CHARACTERISTICS IN GROWTH AND MATURATION OF THREE *UNDARIA* SPECIES IN JAPAN

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The Japanese annual kelp *Undaria pinnatifida*, *U. undarioides* and *U. peterseniana* have difference distributional patterns in Japan. *U. pinnatifida* has a widely distribution around the coast of Japan. On the other hand, *U. undarioides* distributes in more southern coastal area than *U. pinnatifida*. *U. peterseniana* distributes in deeper water than the other two species. In this study, we test the factors controlling the distributional patterns in the three species, such as critical and optimal temperatures for growth and maturation of gametophytes and sporophytes.

Optimal and higher critical temperatures for growth of gametophytes were quite same in the three species, 20 and 28°C, respectively. On the other hand, optimal temperature for maturation between *U. pinnatifida* and *U. undarioides* were quite different. Optimal temperatures for maturation of *U. pinnatifida*, *U. undarioides* and *U. peterseniana* were near upon 10 to 15, 20 to 21 and 10 to 20°C, respectively. Optimal temperature for maturation of *U. peterseniana* was covered the temperature ranges of *U. pinnatifida* and *U. undarioides*. Optimal and higher critical temperatures for growth of young sporophytes of *U. pinnatifida* and *U. undarioides* were almost same, 20°C and 26 to 27°C, respectively. On the other hand, lower critical temperatures were quite different between the both species, below 5°C for *U. pinnatifida*, and 15°C for *U. undarioides*.

The major factors controlling the distributional patterns between *U. pinnatifida* and *U. undarioides* were difference in the both species of maturation temperature range of gametophyte and lower critical temperature for growth of young sporophytes. As for *U. peterseniana*, factors controlling the distributional pattern were not clear concerning in the water temperature for growth or maturation of gametophytes.

P90

ANNUAL VARIATION AND PRODUCTIVITY OF *SARGASSUM YEZOENSE*

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Growth, maturation and productivity of *Sargassum yezoense* were studied in a rocky coastal area on Oshika Peninsula, northern Japan, from June 1999 through February 2001. The alga was perennial, its holdfast persisting even after its main branches had decayed. In winter, the plant was small and its growth was very slow. With rising water temperature, the alga grew progressively both in length and weight. From June to July, the alga grew rapidly, and the main branches reached maximum length (141 cm) and standing crop (1,825 g dry weight/m^2) in July. Concurrently, most main branches over 40 cm in length formed receptacles. Yearly water temperatures were the highest in August, and the main branches rapidly decayed. Afterwards, only the holdfasts and short main branches (under 10 cm in length) remained in October. The stratified clip technique was applied to estimate the annual net production, which was estimated to be 1,959 g dry weight/m^2/year (737 g C/m^2/year). The relative value of the annual net production to the maximum standing crop was approximately 1.1.

P91

DISTRIBUTION PECULIARITIES AND PHYTOMASS PRODUCTIVITY OF THE MAIN SPECIES OF BROWN ALGAE IN SUBLITTORAL ZONE OF THE NORTHERN KURIL ISLANDS (PARAMUSHIR, SHUMSHU, ATLASOV)

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A sublittoral zone of some northern Kuril Islands has been studied in July 1993 by the diving method. The analysis of the obtained results have shown that in the coastal zone of the islands a strong algae belt, which width can change from several tens meters to a half of kilometer, is well developed at the depth of 1 to 20 m.

The absolute domination of the brown algae phytomass was observed in the macrophyte community. The base was made by the representatives of genera *Alaria, Laminaria, Arthrothamnus, Thalassiophyllum. Laminaria bongardiana* P. et R., *Alaria fistulosa* P. et R., *Arthrothamnus bifidus* (Gmel.) P. et R., *Laminaria longipes Bory, Thalassiophyllum clathrus* P. et R., *Alaria angusta* Kjellm., and *Cymathere triplicata* (P. et R.) J. Ag. were the most frequent. The average phytomass of these algae varied from 0.1 to 2.9 kg/km^2 with the maximum 145 kg/km^2. Shallow sites of the sublittoral zone of 1 to 6 m depth were the most favorable for the brown algae development. *L. bongardiana* and *A. bifidus* were the most productive; their phytomass was 11 kg/m^2. The phytomass of *A. fistulosa, L. longipes, T. clathrus, C. triplicata,* and *A. angusta* at this depth was 4.8 kg/m^2. At the depth of 7-12 m a productivity of *A. fistulosa* increased to 2 kg/m^2 on the average. The productive ability of algae *L. bongardiana, A. bifidus, L. longipes, T. clathrus, C. triplicata,* and *A. angusta* significantly decreased here, averaged 2.5 kg/m^2. With the increase in depth up to 18-20 m, the *A. fistulosa* phytomass also increased and reached 4.4 kg/m^2. *L. bongardiana, A. bifidus, T. clathrus*, and *C. triplicata* were not frequent at these depths; their phytomass did not exceed 1 kg/m^2. *L. longipes* and *A. angusta* were not observed at these depths. Large brown algae practically were not found below 20 m.

P92

THE DECLINE OF REEF-CORAL COMMUNITY AND THE LUXURIANT GROWTH OF BENTHIC MARINE ALGAE IN THE MOAT OF ISHIGAKI-JIMA ISLAND, RYUKYUS (SOUTHERN JAPAN)

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A large-scale event of coral bleaching occurred throughout the tropical Pacific and Indian Ocean in 1998. During September of 2000 and 2001, a survey of reef-corals, damselfishes and marine plants was carried out on Ishigaki-jima Island, Ryukyus, southern Japan in order to assess general health and recovery. Belt transects were used to investigate the species richness and abundance at 16 stations between the east and south coasts of the island. Three 50-m transects were laid parallel to the shore (near, mid-reef and outer-reef moat). Here we focus on benthic marine algae, seagrasses and cyanobacteria. Among 135 taxa encountered, 46 were chlorophytes, 21 phaeophytes, 58 rhodophytes, 6 cyanophytes and 4 seagrasses. The demise of dominant reef-corals such as *Acropora* has opened space for colonization and a new successional sequence, especially in the moat area. Perennial, overstory species such as *Turbinaria ornata, Hormophysa cuneiformis* and *Sargassum* spp. dominated on the open space composed of rock flat. Predominantly annual, understory species such as *Dictyota friabilis, Lobophora variegata,* Melobesioideae spp. and *Peyssonnelia* spp. have filled in the coral rubble area derived from dead branching *Acropora.* Monitoring results over the past two years following the bleaching disturbance event, indicate a complex pattern in which both ephemeral species and more long-lived taxa are able to invade together as a result of different life-history strategies that include rapid recolonization modes. According to some reports, the *Acropora* community around Ishigaki-jima Island was subjected to severe grazing pressure by *Acantherstar planci* in the 1980s. Twenty years later the moat has still not recovered. This suggests that moat biotope has remained in a permanent state of early succession.

P93

GROWTH AND SURVIVAL RATES OF LARGE TYPE SPOROPHYTES OF *ECKLONIA CAVA* TRANSPLANTED IN A GROWING ENVIRONMENT OF SMALL TYPE SPOROPHYTES

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Stipe length of sporophytes in *Ecklonia cava* Kjellman (Laminariales, Phaeophyta) is apparently longer along the southeast than southwest coast of Izu Peninsula, central Japan. Transplanting experiments were carried out in order to clarify whether large type sporophytes with long stipe growing in Nabeta Bay (southeast of Izu Peninsula) turn into small type with short stipe when transplanted in Nakagi Bay (southwest of Izu Peninsula). Ten individuals of juvenile *E. cava* sporophytes having a stipe shorter than 5 cm long were collected at 6-9 m depth in Nabeta Bay in December 1995. These sporophytes were attached to artificial concrete reefs immersed at the bottom 9 m depth in Nakagi Bay by using water-resistant epoxy resin adhesive. For comparison, juvenile *E. cava* sporophytes of almost similar size growing in Nakagi Bay were also transplanted in the same way to the same experimental site. Growth and survival rates of the sporophytes were monitored monthly for 3 years from December 1995 to December 1998. The transplanted Nabeta and Nakagi sporophytes showed an increase in their stipe length and diameter from winter to spring, whereas almost no increase was observed from summer to autumn. But the elongation was larger in Nabeta sporophytes than in Nakagi sporophytes. The primary blade length increased mainly from winter to early spring and decreased largely in autumn, which was almost the same in average of both Nabeta and Nakagi sporophytes from the end of the first year of transplanting. Although ca. 70 % of both Nabeta and Nakagi sporophytes were survived during the first two years after transplantation, only two individuals of Nabeta sporophytes were finally survived. Nabeta sporophytes came to surpass in plant length (stipe length + primary blade length) attained in Nakagi sporophytes under the same environmental conditions.

P94

HERITABILITY AND CORRELATION COEFFICIENT OF CULTIVATED *UNDARIA*S IN KOREA

Shin, J-A* (1); Jeoung, J-H (2); Lee, W-K (1); Chin, P-D (2); Seo, T-H (1); Jun, Y-H (2); Choi, S-J (2)
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The heritabilities of the total weight, the total blade length, the longest pinnate blade length, the pinnate blade number and the blade thickness in the block A were 0.67, 0.62, 0.46, 0.01 and 0.00, those of the characters were 0.57, 0.27, 0.11, 0.08 and 0.22 in the block B.

The phenotypic correlation coefficients(r_P) of combinated two characters in the block A were all positive (r_P=0.51-0.91) in March, were positive (r_P=0.30-0.93) except for combination of the total blade length and the longest pinnate blade length (r_P=-0.09) in April, and were all positive (r_P=0.51-0.93) in May. The r_P in the block B were all positive in March (r_P=0.52-0.94) and in May (r_P=0.28-0.99), and positive (r_P=0.36-0.93) except for the combination of the total blade length and the longest pinnate blade length(r_P=0.2-0.23) in April.

The genetic correlation coefficient(r_A) of combined two characters in the block A and B were negative or positive from March to May. What the r_A were more than 0.90 in block A and B was combinations of the total blade length and the total weight, the longest pinnate blade length and the total weight in March, correlation between the stipe length and the sporophyll length in April and May, while what the r_A was more than –0.90 in the two blocks was correlation between the pinnate blade number and the pinnate blade thickness in March. What the r_A in the block A was greater than 0.90 were the correlation between the longest pinnate blade length and the pinnate blade thickness in April, and between the longest pinnate blade length and the pinnate blade thickness, and between the pinnate blade thickness and the pinnate blade number in May. What the r_A in the block B was greater than 0.90 was combination of the total weight and the pinnate blade number in March, and of the pinnate blade thickness and the pinnate blade number in March.

The environmental correlation coefficient(r_E) of combination of two characters were all positive in the block B in March and in the block A in May. What the r_E was more than 0.90 did not exist in this test.

P95

GENOTYPE BY ENVIRONMENT INTERACTION OF CULTIVATED *UNDARIA*S IN KOREA

Shin, J-A* (1); Jeoung, J-H (2); Lee, W-K (1); Chin, P-D (2); Seo, T-H (1); Jun, Y-H (2); Choi, S-J (2)
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The performance of the total blade length of the three cultivars in May showed quantitative genotype (cultivar) x environment (block) interaction, and the cross-environment genetic correlation between character states (r_G) of the three cultivars in May was very high (r_G =0.99). The performance of the longest pinnate blade length for three months showed quantitative GxE interactions in all cultivars, and r_G =0.90-0.99. In the total weight, the performances of the three cultivars in March and May showed the quantitative GxE interactions, and the r_G =0.79 and 0.98in March and May. The performances of the blade length of the Kohung and the Kijang cultivar in April and May showed the Kohung and the Kijang cultivar in April and May showed the qualitative GxE interactions, and the respective r_G=0.89 and 0.59. The performance of the sporophyll number in Kohung and the Kamaishi cultivar in May showed the qualitative GxE interaction, and the r_G=0.59. The GxE interactions of the pinnate blade thickness if the three cultivars in March, April and May were qualitative and/or quantitative, and the respective r_G=0.47, 0.04 and 0.04. The interactions of the pinnate blade number of the threes in March and April were qualitative and/or quantitative, and the respective r_G=0.28 and 0.75. The interaction of the midrib width of the Kohung and the Kamaishi cultivars in April, that of the sporophyll length, and that of the lobation widthof the twos in May, were qualitative, and the respective r_G=0.88, 0.87 and 0.67. The interactions of the stipe length of the threes from March to May were quantitative, and the r_G=0.97-0.98. The interactions of the midrib thickness of the Kohung and the Kamaishi ones from March to May were qualitative and those of the two and the Kijang one quantitative, and the r_G=0.87-0.99. The performance of the upper stipe length of the Kohung and the Kamaishi cultivars in May was the quantitatibe GxE interaction, and that of the twos and the Kijang one was the qualitative, and the r_G =0.01.

P96

HERITABILITY AND CORRELATION COEFFICIENT OF CULTIVATED *LAMINARIA*S IN KOREA

Shin, J-A* (1); Jeoung, J-H (2); Chin, P-D (2); Seo, T-H (1); Jun, Y-H (2); Choi, S-J (2)
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To perform basically statistico-genetic analysis of several subcharacters of *Laminaria*s, the two cultivars of *L. japonica* were cultivated at two aquafarms in Kohung area from December 2000 to July 2001.

Ten subcharacters between the two cultivars in *L. japonica*. Variations of some subcharacters of them between the two cultivars existed as shown in the previous data; the heritability of the fascia thickness in the Myungchon aquafarm was -0.23 and the value was not significant at 5% level. and the heritability of the others was 0.40-0.80 and the value was significant at 5% level. In the Gyedol aquafarm the heritability of the total blade length, blade length and blade thickness was 0.23-0.29 and these were not significant at 5% level; the heritability of the others was 0.49-0.88.

The heritability of fascia width, fascia thickness and total weight was high in general. The coefficient of phenotypic, genotypic and environmental correlations for each month was very high in general.

P97

GENOTYPE BY ENVIRONMENT INTERACTION OF CULTIVATED *LAMINARIA*S IN KOREA

Shin, J-A* (1); Jeoung, J-H (2); Chin, P-D (2); Seo, T-H (1); Jun, Y-H (2); Choi, S-J (2)

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To perform basically statistico-genetic analysis of several subcharacters of Laminarias, the two cultivars of *L. japonica* were cultivated at two aquafarms in Kohung area from December 2000 to July 2001.

The genetic variation in plasticity of the total blade length of the two cultivars of *L. japonica* plotted in two ways : genotype (cultivar) x environment (block A and B) interaction plots and scatter plots from which the cross - environment genetic correlations between character states(r_G) can be estimated. In the left-hand panels, each line corresponds to the mean phenotype that the two genotypes (cultivars) expresses in each of the two environment (block A and B). The performance of the two cultivars in the two environments in February, April and May showed that the genotype x environment (G x E) interaction was due to a change in ranking, that is, qualitative G x E. The performance of the two cultivars in January, June and July showed that the G x E interaction was due entirely to a change in scale, that is, without reversal of ranks of genotypes, quantitative G x E. The performance of the twos in March one seemed to be no G x E interaction, that is, the two genotypes responded to environment change in a parallel fashion.

Total blade length, total weight, blade width, fascia width and blade thickness showed the qualitative and/or quantitative genotype by environment interactions monthly, and consequently these differences resulted in a negative and/or positive genetic correlation across environment.

P98

GROWING TEST OF CULTIVATED *UNDARIA*S IN KOREA

Shin, J-A* (1); Jeoung, J-H (2); Chin, P-D (2); Seo, T-H (1); Jun, Y-H (2); Choi, S-J (2)

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For a basic study on selection of *Undaria pinnatifida*, the growing test of Kohung, Kamaishi and Kijang cultivars was performed at two blocks in Myungchon aquafarm, Kohung, Korea, from December 1999 to May 2000. During the test environmental condition and character states of thirteen characters were measured.

The sporophyll number of the three cultivars in the block B, and the sporophyll length in the two block increased continuously from February to May 2000; the pinnate blade number in the block A decreased. The other characters increased or decreased with the month.

Ratio of the blade length to the total blade length of the three cultivars during the three months was 0.69-0.87 in the block A, and 0.71-0.84 in the block B; that of the stipe length to the total blade length was 0.13-0.31 in the block A, and 0.16-0.29 in the block B; that of the lobation width to the longest pinnate blade length was 0.09-0.27 in the block A, and 0.16-0.33 in the block B; that of the longest pinnate blade length to the total length was 0.26-0.47 in the block A, and 0.21-0.43 in the block B; and that of the upper stipe length to the stipe length was 0.00-0.75 in the block A, and 0.06-0.79 in the block B.

P99

GROWING TEST OF CULTIVATED *LAMINARIA*S IN KOREA

Shin J-A* (1); Jeoung, J-H (2), Chin, P-D (2); Seo, T-H (1); Jun, Y-H (2); Choi, S-J (2)

(1) Yosu National University, KOREA; (2) Koheung Fisheries Technology Institute, KOREA

To perform basically statistico-genetic analysis of several subcharacters of Laminarias, the two cultivars of *L. japonica* were cultivated at two aquafarms in Kohung area from December 2000 to July 2001. Of environmental condition investigated, the water current speed between surface- and sub-layer showed clearly significant difference.

Characters that are studied by biologists are of three types: meristic, metric and all-or-none(or binary) characters. Of the threes, the metric character of interested is a continuously distributed variable, and this quantity is described by the probability density function which is the probability that a variable lies within a specific range of values. The amount of variation is measured and expressed as the variance: when values are expressed as deviations from the population mean the variance is simply the mean of squared values. The components into which the variance is partitioned are the same as the components of values, i.e. the genotypic variance is the variance of genotypic values, environmental variance is the variance of environmental deviations, and total variance is the phenotypic variance, or the variance of phenotypic values.

The total blade length increased to June, and decreased in July due to blade-end loss. The total weight and substantiality continuously to July. Of the relationship between blade width and fascia width only did not show the significant difference at 1 and 5% level.

P100

DIVERSITY OF MACROALGAE AND BENTHIC DIATOMS IN HUAY KHAYANG WATERSHED, THAILAND

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Diversity of macroalgae and benthic diatoms was studied during a 2 years period (November 2001 – October 2003) at Huay Khayang watershed, Thong Pha Poom District, Kanjanaburi Province, Thailand. The watershed covered 1,200 Square Kilometers, the geographic and climatic conditions of this area are clearly different from other parts of Thailand. Thus, the biodiversity in this area is higher than another part of Thailand. The relationship between physico – chemical parameters and the presence of macroalgae and benthic diatoms were investigated. Freshwater red algae, *Batrachospermum macrosporum* (Montague), were found from the preliminary study of the water source. The dominant species of freshwater epilithic diatoms, *Frustulia romboides* (Ehrenberg), *Stauroneis phoenicenteron* (Nitzsch)Ehrenberg and *Eunotia* spp. indicated clean water.

P101

PRELIMINARY EXPERIMENTS ON SEA GRASS AND SEAWEED CULTURE IN SEMI-CLOSED OUTDOOR-TANK SYSTEMS WITHOUT CLEANING

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Sea grass and seaweed culture in semi-closed outdoor-tank systems is an effective method to study physiological and ecological features of them or for a practical purpose. However, it is sometimes difficult to operate those systems, because increase of periphyton in them often inhibits the growth of objective plants. Remove of periphyton is troublesome, time-consuming work. The purpose is the technique improvement for sea grass and seaweed culture in outdoor tanks. Stable conditions were maintained without cleaning through control of light intensity and grazing pressure of herbivorous animals. Beach sand composed of granite fragments and oyster shell were placed into outdoor-tanks of 200 *l* in volume at Aug., 1995 and 2 tons in volume at Jun., 1997. Sea water supply pipes were set into the paved sand at bottom of the tanks filtrate the seawater. Seawater was over flowed and drained out from drainpipes. The turnover ratio of seawater in the tanks was 10 - 20 times/day. No air was supplied in the tanks. Several numbers of small herbivorous snails *Omphalius rusticum* were thrown into the tanks to control the propagation of periphyton. The tanks were covered using several layers of shielding nets and/or black sheets to control the light intensity. After the setting of the whole system, culture of various sea grass and seaweeds were tried repeatedly. Healthy growth and clean plants have been obtained in the tanks as follows; transplanted and artificially seeded *Zostera marina* (sea grass) on the sandy bottom, artificially seeded *Sargassum filicinum*, *S. confusum* and *S. macrocarpum* on the concrete blocks, transplanted *S. patens*, *S. fusiforme*, *Caulerpa okamurae* and *C. scalpelliformis* var. *scalpelliformis*, and naturally occurred thick crustose coralline algae on surface of the tank walls.

P102

ECOLOGICAL STUDIES OF *ULVA* SPP. IN HIROSHIMA BAY, THE SETO INLAND SEA

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Green tide, caused by Ulvaceae plants, is now supposed to affect the ecological status of the inland sea areas. Ecological studies on the Ulvaceae plants have been conducted at the inner Hiroshima Bay (Ohno-seto strait), Seto Inland Sea, Japan. Seasonal changes in biomass, growth rates and other ecological features have been studied and compared for floating type *Ulva* spp. and *U. pertusa* population growing on the hard substratum.

1) Floating type *Ulva* spp.

Surveys were conducted from May 2000 to February 2002 at a small inlet (5.6 ha). Accumulation of *Ulva* thalli was observed from intertidal to subtidal zone over 5m in depth. Total biomass in the study area decreased from summer to autumn and increased during winter reaching peak in spring. The highest biomass, 10.1 ton w.w./ha, was observed in April 2000, and the lowest, 2.26 ton w.w./ha in December 2001. The abundance in intertidal zone was relatively constant during the year (2-3 kg w.w. /m^2 at the most accumulating points), however the abundance in deeper, subtidal zone dramatically changes, which affected the seasonal change in the total biomass. In situ culture study indicated that seasonal availability of light in water column severely affected the seasonal growth of *Ulva* thalli at each depth.

2) *U. pertusa*

The *Ulva* bed was formed vertically between + 1.5 - + 0.5 m D.L. along the stone-built seawall mound of the coast. Thalli of *U. pertusa* on the substratum appeared in autumn and grew during winter. Maximum biomass of the *Ulva* bed was 450 - 750 g d.w./m^2 at the end of spring, with a subsequent decrease in summer. Fruiting thalli of *U. pertusa* were observed during all seasons.

P103

ECOTYPIC DIFFERENTIATION IN SEASONALITY AMONG LOCAL POPULATIONS OF *SARGASSUM HORNERI* (FUCALES, PHAEOPHYTA) IN HIROSHIMA BAY, THE SETO INLAND SEA

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Sargassum horneri (Fucales, Phaeophyta) is one of the common seaweeds along the coast of Japan. In Hiroshima Bay, the Seto Inland Sea, *S. horneri* is a dominant species in the subtidal zone and forms dense underwater forests at the depth of 0-2m. Differentiation in maturation seasonality is observed among local populations of *S.horneri* in the Bay and its adjacent area, and the seasonal variation is attributable to the ecotypic differentiation among them.

Ecological surveys were conducted for 8 populations in the Bay and its adjacent area. Plant growth advanced earlier for the populations inhabiting inner area of the Bay, and delayed seasonally for populations inhabiting near the entrance and exterior area of the Bay. Receptacle formation was also initiated seasonally earlier in late autumn and egg release was observed from November to January for the populations inhabiting in inner area of the Bay. On the contrary, receptacle formation was begun during winter and egg release was observed in spring, from March to May for the populations inhabiting near the entrance and exterior area of the Bay.

Laboratory and outdoor culture studies was conducted for seedlings of 4 *S. horneri* local populations with different maturation seasonality. Seedlings of 2 populations with maturation initiation in late autumn, growth (stem elongation) was promoted under wide ranges of photoperiod conditions. On the other hand, seedlings of the other 2 populations with maturation seasonin spring, growth was promoted only under short day conditions (shorter than 12 hrs of lightperiod) and inhibited under long day conditions. The difference in seasonality among local populations of *S. horneri* is attributable to the difference in inherent characteristics, which appeared in different reactions to photoperiod.

P104

MORPHOLOGY AND REPRODUCTION OF AN EPIZOIC DIATOM, *PSEUDOHIMANTIDIUM PACIFICUM* HUST. & KRASSKE (BACILLARIOPHYCEAE: PROTORAPHIDACEAE)

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An epizoic diatom *Pseudohimantidium pacificum* Hustedt & Krasske has been found attached only to marine copepods of the genera *Corycaeus*, *Farranula* and *Euterpina*. The life history of it has not been fully revealed yet. In the present study, we report morphology and reproduction of this diatom based on culture strains.

Individuals of *Corycaeus affinis* McMurrich attached with this diatom were collected with a plankton-net in the coast of Takehara city, Hiroshima prefecture, and in Oashi fish port and Kaga fish port, Shimane prefecture. Attached diatoms were isolated from the hosts. Culture strains of this diatom were maintained on f/2 medium under standard conditions; ca. 500 or 4000 lux on a 12 : 12 h light : dark cycle at 20 °C. Live and peroxide-cleaned preparations were examined using a LM equipped with Nomarski differential interference contract optics. Detailed structures of valves were observed with a SEM.

This diatom has grown well in this medium, and growth rate was much higher in 4000 lux than in 500 lux. Several cells attached to the bottom of the dish by stalk consisted of mucilage and formed branching colonies as well as on the hosts. Length of valves reduced from 77.5 μm to 9.5 μm through vegetative cell division. Valves were lunate in normal cells, but were oval in small cells. Sexual reproduction has not been observed.

Although, this diatom has been attached only to marine copepods in the field. Our result showed that attachment on host copepods is not necessary to this diatom in this medium and culture conditions.

P105
MORPHOLOGY OF *DICTYOPTERIS LATIUSCULA* (DICTYOTALES, PHAEOPHYCEAE) ESPECIALLY ON REPRODUCTIVE ORGANS

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In *Dictyopteris latiuscula* (Okamura) Okamura, sexual reproductive organs had never been reported, and only sporangia have been known (Okamura 1907). Sporophytes and male and female gametophytes of *D. latiuscula* were collected at Izu and Boso Peninsulas, Japan. Blades with reproductive organs exist in the upper to middle parts of a thallus. Every reproductive organ forms sori along the midrib on both surfaces of the blade, and projects above the cortical layer. Sporangial sori, at first, are looked as brownish spots or lines to the naked eye. As maturation progresses, they form large belt-shaped sori by their expansion and unify. Sporangia on one to four stalk cells are spherical or ellipsoidal, 121 ± 12 μm across and 141 ± 9 μm high. Oogonial sori are light brown and irregular in outline. In sori, a number of oogonia in almost the same maturation stage are clustered together. Oogonia with only one stalk cell are spherical, obovoid or ellipsoidal, 58 ± 8 μm across and 85 ± 9 μm high. Antheridia differentiate from cortical cells in the elliptical or rectangle area of sori. The sori expand by producing antheridia outward, and unite with the adjacent sori. Antheridia with one or rarely two stalk cells are quadratic prism shaped, 20 ± 3 μm across and 63 ± 6 μm high and contain 13 to 24 layers that consist of four to eight loculi. The reproductive structures of *D. latiuscula* are quite similar to *D. prolifera* in the arrangement and the structure of sori. These two species grow new blades from the midrib, and endemically distribute around Japan and the adjacent waters. It is considered that the similarity of reproductive structures supports the closer relation between *D. latiuscula* and *D. prolifera.*

P106
BACTERIAL EFFECTS ON THE EARLY DEVELOPMENT OF *ENTEROMORPHA COMPRESSA*

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Growth and morphology in early development of axenic thalli of *Enteromorpha compressa* were compared with that of the thalli cultured with bacteria. To accomplish axenic culture, gametes released from the thalli (strain MGEC-1) were repeatedly washed with sterilized seawater by their phototaxis. The resultant axenic gamete-suspension was inoculated in a 24-well plate, and serially diluted with PES medium in the ratio 1:3. Serial dilutions of the gamete-suspension including bacteria was also prepared at the same density as the axenic series. Growth was monitored by cell number per individual. Both of the axenic and bacteria-including cultures accelerated their growth rates with decrease in thallus density. However, the increases in growth rates of the axenic cultures were lower than that of the bacteria-including cultures, and the axenic cultures stopped growing within 20 days. Cell morphology of the axenic cultures became abnormally long, and chloroplasts of the cells shrank. Thallus density affected the cell morphology of the axenic cultures, and the abnormality was conspicuous in the wells where 2.2×10^3 to 1.4×10^5 gametes were inoculated. Various bacteria were examined on activities to restore the axenic cultures to normal morphology and to make the cultures restart growing. Bacteria were isolated from the medium where the thalli of *E. compressa* were cultured and surfaces of macroalgae growing in the field. Isolated bacteria were inoculated in the wells where 3.9×10^4 gametes had been inoculated and cultured for 2 weeks. All the examined bacteria (ca.100 strains) had the activity to restore the cell morphology of the axenic cultures to normal, and the cultures mixed with bacteria restarted growing within 5 days.

P107

NEW MOLECULE LOCALIZED TO THE CONTACT SITE DURING NET FORMATION IN *HYDRODICTYON RETICULATUM*

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In the green alga *Hydrodictyon reticulatum* (Chlorococcales, Chlorophyceae), zoospores are arranged in a regular fashion to form an intricate hexagonal network during the asexual reproductive cycle. A monoclonal antibody that was raised against a homogenate of zoospores recognized new molecule localized to the contact sites between zoospores. Antigenic polypeptide was constantly expressed in zoospores during net formation and decreased after the completion of the adhesion between zoospores. The subcellular localization of the molecule during net formation was analyzed by indirect immunofluorescence microscopy. The protoplasmic region of the parental cell was stained in a punctated manner. The peripheral regions of the spherical zoospores and the contact regions of the polyhedral zoospores were stained brightly. Immediately after cessation of the movement of polyhedral zoospores, the molecule was located on the closely apposed surfaces of two cells. The staining intensity in the adhering regions of net cells became faint, but was maintained for several hours; however, it could not be detected 24 h after net formation. In order to examine the precise spatial distribution of the molecule, zoospores and net cells were double-stained with this monoclonal antibody and a monoclonal antibody against chicken β-tubulin. The molecule closely colocalized with the microtubule bundles at the contact regions of the polyhedral zoospores and the net cells. Immunoelectron microscopy revealed the presence of gold particles on a Golgi stack and the plasma membrane in polyhedral zoospores and the contact regions of net cells. I treated isolated zoospores with this monoclonal antibody. Treatment with the antibody inhibited formation of hexagonal nets. These results suggest that this molecule localized to the contact site may participate in the adhesion of zoospores during hexagonal net formation.

P108

INDUCTION OF GAMETOGENESIS IN *ENTEROMORPHA COMPRESSA*

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Exchanging growth medium has been known to induce gametogenesis in the cells of Ulvales. It is considered that the cells excrete gametogenesis-inhibiting substances into the growth medium, and decrease in concentration of the substances by medium exchanging is a cue of the gametogenesis. The present study was undertaken to clarify the mechanism by which the vegetative cells of *Enteromorpha compressa* transfer into generative phase. The thalli (strain MGEC-1) were cultured at 15°C with a photoperiod of 10:14 h LD. To induce gametogenesis, the thalli were thoroughly rinsed with fresh growth medium and transferred into a flask containing fresh growth medium at 21°C. When the induction of gametogenesis was performed during a light phase, gametes were released from the thalli on the second day after the induction. Regardless of induction timing, gamete-release started around noon. Gamete-release also occurred on the following days, with the amount of released gametes decreasing. In such cases gamete-release also started around noon. When the induction was performed after the transition to the following dark phase, the amount of gametes released on the second day decreased with delaying induction, and the maximal gamete-release tended to occur on the third day. Temperature did not affect the timing of gamete-release although induction of gametogenesis was insecure at 15°C. To examine the dependence of gametogenesis upon light, the thalli were transferred to continuous dark immediately after the induction of gametogenesis. No evidence of gamete-release during continuous dark was found. When the thalli were returned to light-dark cycles, gamete-release occurred on the second day after the return regardless of the duration of dark. The present data suggest that light has a significant role in the gametogenesis of *E.compressa.*

P109

ISOLATION AND CHARACTERIZATION OF PHASE-SPECIFIC cDNAS FROM CARPOSPOROPHYTES OF *GRACILARIOPSIS LEMANEIFORMIS* (RHODOPHYTA)

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Carposporophyte stage is unique to florideophycean algae, and its morphology and developmental pattern have been investigated well. The carposporophyte, however, is very tiny and mostly embedded in female gametophyte tissues, and there are few molecular, genetic, or biochemical data about this stage. Although conventional methods to isolate tissue-specific genes require a lot of tissues, recent advanced techniques have allowed the subtractive cloning of differentially expressed genes from small amounts of tissue or cells. We applied the subtractive hybridization method using magnetic beads and PCR to analyze phase-specific cDNAs from carposporophytes of *Gracilariopsis lemaneiformis* (Gracilariales, Rhodophyta). A hundred cystocarps were dissected to isolate gonimoblast tissues, and total RNAs were extracted from the gonimoblast tissues and the female gametophyte branches, respectively. Messenger RNAs were captured on paramagnetic oligo-dT beads, followed by first-strand cDNA synthesis on the beads. Several rounds of subtractive hybridization between the amplified second-strand carposporophyte cDNA in solution and the first-strand gametophyte cDNA attached to magnetic beads were sufficient to remove common genes present in both gametophyte and carposporophyte stages. A specific PCR product from the nuclear GAPDH gene was readily amplified from gametophyte and carposporophyte cDNA, but no amplification was observed using the subtracted carposporophyte cDNA as template. This control PCR product demonstrates that the hybridization steps successfully removed the common GAPDH cDNA, giving confidence that the remaining genes cloned from the subtracted carposporophyte cDNA library are phase-specific. Some of the subtractive cDNA fragments sequenced in this study were significantly homologous to animal, plant or fungal proteins which are known to be differentially transcribed during active cell division, so these proteins may be intimately related to the carposporophyte development. We also tried to characterize the genes that are specifically expressed in the early developmental stage of the carposporophyte by using the same technique.

P110

MORPHOLOGY AND REPRODUCTION OF *CALOGLOSSA OGASAWARAENSIS*

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Members of the genus *Caloglossa* (Delesseriaceae, Ceramiales) are one of the most typical components in mangrove environment. *Caloglossa ogasawaraensis* Okamura 1908 is especially widespread in brackish- and fresh-waters in the temperate to tropical regions. Despite of the wide distribution, mature gametophytes have rarely been observed in the field. Yosizaki *et al.* (1986) examined the occurrence of gametophytes of *C. ogasawaraensis* in Chiba Pref., Japan for one year and three months, and reported the rare occurrence of gametophytes (1.5%). On the other hand, tetraspores of *C. ogasawaraensis* grew into mature male and female gametophytes in laboratory culture (Tanaka and Kamiya 1993).

Mature male and female gametophytes of *C. ogasawaraensis* were collected from mangrove in Serangan Isl., Bali Isl., Indonesia. Carpospores released from a wild thallus grew into mature plants. Among 23 isolates, 17 produced tetrasporangia and cystocarps (some of them were observed having spermatangia), 4 produced tetrasporangia and procarps, and 2 produced only tetrasporangia. Gross morphology was examined in field-collected Indonesian specimens and it was compared with the Japanese specimens. Blades in Indonesia were 1.3-4.6mm long and 56-224μm wide, and one axial cell produced 2-4 wing cells at the middle of blades and 2-4 cell rows at margin. On the other hand, blades in Japan were 2.1-6.7mm long and 210-448μm wide, and one axial cell produced 4-8 wing cells at the middle of blades and 3-6 cell rows at margin.

P111

BIOLOGY OF REPRODUCTION IN *GRACILARIA CORTICATA* J.AG.

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The coastal ecosystem of Port Okha (Gujarat) has distinct supratidal, intertidal and subtidal zones which demand specific or particular developmental and reproductive adaptations of existing algae. *Gracilaria corticata*, a phycocolloid taxon of red algae, grows along the intertidal zone at Port Okha in the Gulf of Kutch(Gujarat), India. The intertidal zone that gently slopes towards the sea is exposed and gets flooded during low and high tides respectively. The presence of discoid holdfast with modified internal cellular arrangement is a specialized adaptation which enables the plants to anchor firmly and withstand tidal fluctuations. The internal structure of alga is characterized by wavy outline, compact arrangement of cortex and medulla, wall ingrowths in the medullary cells at thallus-holdfast transition zone as well as sulphated polysaccharides in the intercellular mucilage. These features provide strength and flexibility to the thallus in addition to protection of this seaweed against physical damage and stringent environmental conditions.

The plant is dioecious, with three distinct season specific phases of diplobiontic life cycle. The male and female plants grow in favourable environmental condition after onset of monsoon on west coast of India. The carposporophyte phase is concerned with amplification of the diploid stage, because fertilization chances are meagre due to non-motile male gametes. The tetrasporophyte plants are robust, heavily infested with epiphytes and survive throughout the year. A knowledge of seasonal distribution of different stages and biology of reproduction of *Gracilaria corticata* will be useful for its cultivation on commercial basis.

P112

PREFERENTIAL DESTRUCTION OF CHLOROPLAST AND MITOCHONDRIAL DNA AT EARLY MALE GAMETOGENESIS OF THE ANISOGAMOUS GREEN ALGA *DERBESIA TENUISSIMA* (CHLOROPHYTA)

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Gametogenesis in male and female gametophytes was studied by light and electron microscopy in the dioecious multinucleate green alga *Derbesia tenuissima* (Moris & De Notaris) P. Crouan & H. Crouan, where male and female gametes differ in size. Gametogenesis was divided into 5 stages: 32 h (Stage 1), 24 h (Stage 2), 16 h (Stage 3), 8 h (Stage 4), and 0.5 h (Stage 5) prior to gamete release. At Stage 1, the first sign of gametogenesis observed was the aggregation of gametophyte protoplasm to form putative gametangia. At Stage 2, gametangia were separated from the vegetative protoplasm of gametophytes. Morphological changes of nuclei and organelles occurred at this early stage of male gametogenesis, and organelle DNA degenerated. At stage 3, male organelle DNA had completely degenerated, while in female gametangia, organelle DNA continued to exist in both chloroplasts and mitochondria. Gametogenesis was almost completed at Stage 4 and fully at Stage 5. Small male gametes had a DNA-containing nucleus and a large mitochondrion and one or several degenerated chloroplasts. The mitochondria and plastids were devoid of DNA. The large female gametes had a nucleus and multiple organelles, all of which contained their own DNA. Thus, degeneration of chloroplast DNA along with morphological changes of organelles occurred at male gametogenesis in anisogamous green algae (*Bryopsis* and *D. tenuissima*; Kuroiwa and Hori 1986, Ogawa 1988, Kuroiwa et al. 1991, present study), in contrast with previous studies in isogamous green algae (*Chlamydomonas*, *Acetabularia caliculus* and *Dictyosphaeria cavernosa*; Sager and Lane 1972, Kuroiwa et al. 1982, Coleman 1984, Kuroiwa et al. 1985), in which degeneration of chloroplast DNA occurred after zygote formation.

P113

ANATOMICAL STUDY OF SOME JAPANESE LAMINARIAN SPECIES

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Sporophyte of laminarian species has an epidermis in outermost part, a cortex with cubic cells and a medulla with hyphae and sieve elements in innermost part.

Longitudinal sections were made from several parts of the thalli of nine species (*Agarum clathratum, Arthrothamnus bifidus, Costaria costata, Ecklonia cava* (juvenile), *E. kurome, Kjellmaniella gyrata, Laminaria angustata, L. coreacea, L. longissima*). The thickness of medulla and cortex, and the number, length, width and size of cortical cells along the transect line between epidermis and medulla were measured.

In most species, medulla was thicker at the base of thallus and became thinner at the upper part. Although these differences were not clear in those of *K. gyrata, A. clathratum, E. cava* and the primary blade of *E. kurome*. Correlating with it, the number of cortical cells increased at the base and decreased at the upper part.

Cortical cells were larger and spherical at the middle part of cortex and smaller and ellipsoidal near epidermis and medulla. At the upper part of thallus, cortical cells were strongly elongated than those at the base. It suggested active cell divisions occurred frequently in meristoderm located in outer side of cortex, and it caused the elongation of outer cortical cells. No remarkable elongation was observed in the cortical cells at the base of thallus. The cortical cells under cell division were observed near the medulla in all species.

P114

MORPHOGENESIS INDUCERS OF *MONOSTROMA OXYSPERMUM* (CHLOROPHYTA) IN MARINE ENVIRONMENT

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Many green algae cannot develop normally when they are grown under aseptic conditions. *Monostroma oxyspermum*, for example, proliferates unicellularly in an aseptic culture, but develops into a normal foliaceous gametophyte in the presence of some marine bacteria. More than 1,000 bacterial strains were isolated from marine algae, sponges and other marine organisms, and assayed for their ability to induce the morphological differentiation of unicellular *M. oxyspermum*. Fifty bacterial strains exhibiting morphogenesis-inducing activity against unicellular *M. oxyspermum* were isolated. The partial *gyr*B (*ca.* 1.2 kbp) and 16S rDNA sequences (*ca.* 500 bp) of about 40 active strains were determined, and their phylogenetic relationships were analyzed. All of these strains were located within to the *Cytophaga-Flavobacterium-Bacteroides* (CFB) complex, and most of these strains were clustered in a clade comprising [*Cytophaga*] UP7 and *Zobellia uliginosa*. These bacteria also had morphogenetic activity against germ-free spores of *Ulva pertusa*, *Ulva conglobata* and *Enteromorpha intestinalis*. Moreover, these bacteria induced the release of spores from the leafy young gametophyte of *M. oxyspermum*. These results indicate that some strains belonging to the CFB complex play an important role in the normal development of green algae in the marine coastal environment.

P115

PERIODIC GAMETOGENESIS OF *BRYOPSIS MAXIMA* IN RELATION TO THE ENVIRONMENT

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We examined the relation of gametogenesis of the siphonous green alga, *Bryopsis maxima* Okamura, to water temperature fluctuations at the shores of Oarai, Japan, over a period of two years, 2000-2001. During the winter-spring (Feb.-Apr.) of 2000, the water temperature was constantly low (8-11°C). Formation of the gametes was not observed in the field during this period. When the water temperature increased from 11°C to 12.5 °C at the beginning of April, synchronous gametogenesis and gamete liberation occurred at the end of the first spring tide. In the spring (Apr.-Jun.), the water temperature increased from 13°C to 17°C. Gametogenesis and gamete liberation was at regular fortnightly intervals and occurred only during spring tide. In the summer (Jul.-Aug.) when the water temperature was high (18-24°C), gametogenesis and gamete discharge occurred at irregular intervals. In the winter of 2001(Feb.-Mar.), in which the water temperature was slightly higher (10-12°C) than that of 2000 (Feb.-Mar.), gametogenesis and gamete liberation occurred at regular fortnightly intervals during spring tide. By the laboratory culture experiments on the effect of temperature on the gametogenesis, it was found that (1) the gametophytes, which collected in the winter-spring (Feb.-Apr.) of 2000 and cultured at 8.3°C, did not produce gametes but did at 11.4°C and 13.6°C, (2) most of the gametophytes, which collected in the spring (Apr.-Jun.) of 2000 and cultured at 13.5°C, did not produce the gametes but did at 16.3°C and 19.3°C. These results suggest that (1) the water temperature is the cue to initiate gametogenesis, (2) the lower water temperature in the winter-spring inhibited the gametogenesis in nature and (3) the critical temperature for the gametogenesis changes with seasonal fluctuation of water temperature.

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PYRENOID FORMATION ASSOCIATED WITH THE CELL CYCLE IN THE BROWN ALGA, *SCYTOSIPHON LOMENTARIA* (SCYTOSIPHONALES, PHAEOPHYCEAE)

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Pyrenoids are proteinaceous structures in chloroplasts observed in many eukaryotic algae and hornworts. It has been confirmed that the main component of the pyrenoids in all algae is ribulose-1, 5-bisphosphate carboxylase/oxygenase (RUBISCO), a key enzyme involved in carbon dioxide fixation. RUBISCO consists of eight large and eight small subunits, and in brown algal cells, both the large (*rbc*L) and small (*rbc*S) subunit of RUBISCO is chloroplast-coded. At present, it is unclear why and how RUBISCO proteins gather locally in the pyrenoid.

Scytosiphon cell characteristically has only one chloroplast with a prominent protruding pyrenoid though the life cycle. In this study, we examined how the number of pyrenoid in cell is maintained, and when new pyrenoid appears within chloroplast during the cell cycle. We prepared a polyclonal antibody against the *rbc*L extracted from *Scytosiphon,* and observed the behavior of pyrenoid though the first and second cell cycle of zygotes which developed synchronously, by immunofluorescence and electron microscopy. Just fertilized zygotes had two chloroplasts derived from male and female gametes. At the first cell division, each of the chloroplasts was distributed into the daughter cells, respectively. From observations by immunofluorescence and electron microscopy, it became clear that the new pyrenoid was formed during mitosis. At this time, the old pyrenoid remained, and several masses which were detected by anti-*rbc*L antibody, appeared at some regions of chloroplast stroma. Under electron microscopy, these were observed as electron dense material. Eventually, they gathered in one place and chloroplast had two protruding pyrenoids. It was also suggested that the new pyrenoid formation would be depended on the cell cycle rather than the light and dark cycle in culture as the result of experiments using some cell cycle inhibitors.

P117
CULTURE AND PYRENOID STRUCTURE OF A SYMBIOTIC *CHLORELLA* SPECIES ISOLATED FROM *PARAMECIUM BURSARIA*

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The pyrenoid structure has been regarded as a stable feature worthy of the taxonomic recognition of species or genus for unicellular free living or symbiotic green algae of the ciliate, *Paramecium bursaria*. We compared the pyrenoids of *Chlorella* in a symbiotic association with *P. bursaria* with those cultured in artificial conditions after isolation from the ciliate. We did not find any difference in the morphology of the chloroplast between symbiotic and isolated cells under light microscopic (LM) observation. The axenically isolated strain was cultured on CA medium solidified by 1.5% agar, containing NH_4NO_3 as nitrogen source and no carbon source. Under LM observation, all algae observed have the pyrenoid surrounded by two starch sheaths in a cup-shaped chloroplast in symbiotic condition, while every strain has no or degenerated pyrenoids without starch sheaths in isolated condition. We failed to keep the culture of the isolates for longer than three months in spite of frequent transfers of the isolates to fresh media. These results suggest that the symbiotic algal strains show a strong dependency on the host. Further comparative study is needed using TEM for confirmation of the observations on the pyrenoid morphology made under LM.

P118
FREQUENCY AND TIMING OF CELL DIVISION OF *ENTEROMORPHA COMPRESSA*

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Diurnal gating of cell division was recently found in the early development of *Enteromorpha compressa*. This regulation system specified a certain phase in which cell division was allowed to occur and the other phase in which cell division was forbidden, even if the cells had attained sufficient size. The phase in which cell division was allowed to occur lay mainly in the dark phase, and a part of the resulting daughter cells subsequently divided until the following light phase. The present study was designed to define the regulation point in a cell cycle, and to clarify the mechanism by which the cell cycle progression is controlled. Gametes released from the thalli (strain MGEC-1) were inoculated on a slide glass. The resultant thalli were identified and observed with photomicrography. 5-Fluoro-2'-deoxyuridine (FdUrd), which is an inhibitor to DNA synthesis, completely inhibited the cell cycle progression when it was added to the cultures at 14:00-16:00. However, cell division occurred until the following noon when FdUrd was added to the cultures at and after 18:00. The percentage of the cells that remained undivided until the following noon decreased with delaying addition of FdUrd. The present data suggest that the cell cycle progression was regulated by gating at the G1-to-S phase transition. Coupling a circadian oscillator with the gating of cell division was examined under constant conditions. After transfer from light-dark cycles to continuous dark, the rhythm of cell division disappeared, with the cells dividing only during the first 24 h. After transfer to continuous light, the rhythm of cell division also disappeared, with the cells being freed from the forbiddance of division. Disappearance of the phase in which cell division was forbidden occurred regardless of light intensity.

P119

MORPHOLOGY AND RAPD VARIATION OF *UNDARIA*S IN KOREA

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To obtain basic data on RAPD-based variation of cultivated *Undarias* in Korea, the study on RAPD-based analysis Undarias used in this study were five cultivars, the Kamaisi and naruto cultivars from Japan, and the Kijang, Myungchon and Pungnam cultivars, which were cultivated at Myungchon aquafarm, Kohunggun, Jeollanamdo, Korea in 2001 was performed. For the DNA isolation, 100mg thalli were suspended in TE buffer two times, DNA were isolated by the method of in TE two times. DNA were isolated by the method of CTAB.

The value of band sharing index(BSI) was 0.43-0.49 between Kijang and Myungchon-Naruto-Pungnam cultivars, 0.55-0.57 between Kamaisi and Kijang-Myungchon-Pungnam cultivars. The level of BSI was high between 0.82-0.87 between Myumgchon and Pungnam, Naruto and Myungchon-Pungnam cultivars.

P120

ELECTRON MICROSCOPY OF A CELL WALL STRUCTURE OF A ACHNANTHACEAEN DIATOMACEOUS ALGA *ACHNANTHES BREVIPES*

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Cell wall structure of pennate diatom *Achnanthes brevipes* was examined using scanning and transmission electron microscopy. *A. brevipes* belongs to the monoraphid group; one valve (raphid valve) has raphe and the other (rapheless valve) has central sternum. The raphid valve is concave and thinner than the convex araphid valve. Since the areolar structure was less studied, we targeted structural details of *A. brevipes* by scanning and transmission electron microscopy. Two areolar forms were commonly found: peanut-type and bean-type structures. The peanut-type areolar averages 1,000 nm in length and that of the bean type about 600 nm. Both areolar structures had many small cavities. Treatments with different heatings (800-1150 °C) showed a transformation from amorphous silica to crystalline silica and a tetragonal α-cristobalite.

P121
BIOCHEMICAL STUDY ON CULTIVATED *LAMINARIA JAPONICA* ARESCH

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The chemical composition of cultivated *Laminaria japonica* Aresch. at Far Easten plantation has been investigated. Cyclic accumulation of basic metabolites in the process of *L. japonica* two-years growing has been defined. The increase of total mineral elements' and proteins' content takes place in winter-spring period, the decrease – in summer-autumn period; it was found the reverse dependence for mannitol and alginic acid. The abrupt change of *L. japonica* biosynthesis is to be seen at the change of the cultivation depth. The mineral elements and proteins' content increases, alginic acid structural composition changes while algae raising on the sea surface in Febrary-March. The abrupt increase of guluronic acid content in alginates from 23,2 to 50% is to be seen, its molecular weight increases from 191 to 333 Pa. The investigations show that the mannuronic acid predominate in alginic acid during two-years algae cultivation. The method of two-years *Laminaria* deeping in summer at the depth of 6-10 m creates the favorable conditions for prolongation of algae life. Here the process of alginic acid biosynthesis is changing in the direction of guluronic acid increasing to 50-58%.

P122
DISTRIBUTION AND CHEMICAL COMPOSITION OF COMMERCIAL SEAWEEDS OF THE JAPAN SEA (PRIMORYE COAST)

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Laminaria japonica Aresch. is a dominating seaweed of Primorye region. It is the unique commercial species among brown algae of the Japan sea at present time. Two-year's algae contain 33-35% of alginic acid, 5-8% of mannitol in July-August. Iodine content is higher than in all other investigated algae (0,2-0,3%). *Cystoseira crassipes* (Turn.) C. Ag., *Sargassum pallidum* (Turn.) C. Ag., *Costaria costata* (Turn) Saund. *Laminaria cichorioides* Miyabe are the perspective brown algae for trade. Chemical composition of *L. cichorioides* and *C. costata* is compared with chemical composition of *L. japonica*. C. crassipes and *S. pallidum* differ by the increased content of mineral matters and protein. Seaweeds of Laminareaceae differ by the increased content of carbohydrates, *C. crassipes* and *S. pallidum* – mineral matters and protein. Red algae of the Japan sea (Primorye coast) present such mass species as *Gracilaria verrucosa* (Huds.) Papenf, *Chondrus pinnulatus* (Harv.) Okam., *Tichocarpus crinitus* (Gmel.) Rupr., *Ptilota filicina* (Farl.) J. Ag., *Ptilota phacelocarpoides* A. Zin., *Odonthalia corymbifera* (Gmel.) J. Ag. However there are only two economic significant species – *Ahnfeltia tobuchiensis* (Kanno et Matz.) V. Mak. and *Chondrus armatus* (Harv.) Okam., which chemical composition has been studied most of all. *A. tobuchiensis* – commercial species, used for agar receiving (13% agar). *C. armatus* is a source of polysaccharide carrageenan (40% carrageenan). *Zostera marina* L. and *Phyllospadix iwatensis* Makino contain polysaccharides of pectin's nature and are the economic significant species among seagrasses. *Z. marina* form pure kelps, *P. iwatensis* form the mixed with brown algae kelps. The investigations of chemical composition show their similarity by the content of minerals, nitrogen's matters and physical-chemical properties of main polysaccharide.

P123

HEMAGGLUTININ AND ANTIBIOTIC ACTIVITY OF FRESHWATER MICROALGAE

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In this study, we used 22 strains of laboratory-cultured microalgae, including 13 strains of *Chlorella* spp. We screened those microalgae for hemagglutinating activity with different animal and human erythrocytes, including cow, sheep, pig, human A, B, and O types RBC which native or treated with enzyme. The dry cell powder was pretreated with liquid nitrogen, ground with aluminum oxide and extracted with 20% ethanol. Distinct high hemagglutination activity was detected in some of algal extracts. Variety of agglutinating titers were shown when algal extracts reacting with native or enzyme-treated erythrocytes. The results indicated that remarkable titer was obtained when papain-treated erythrocytes were used in this test. Comparisons of hemagglutinating activity using various animal and human native erythrocytes, the algal extracts were presented different titers clearly. The results revealed that remarkable hemagglutination was obtained when papain-treated erythrocytes were used in this test. This screening indicated that almost freshwater microalgal species have hemagglutinins, especially *Chlorella* spp.

We also assayed the antibiotic activity of those 7 algal extracts against bacteria or fungi. Antibiotic abilities were found in all algae, especially the *Chlorella* sp. I and 21. This result indicated that agglutinins were distributed in several kinds of freshwater microalgae and they might be involved in some physiological function of antibiotic activity in the algae. For identifying the *Chlorella* sp. (V, I, 21, and 226) isolates, we used the traditional taxonomic methods with morphology, ultrastructure and physiological characters to identification. Their phylogenetic position and relationships were determined by analysis of 18S rDNA sequence. Their morphology features indicated that they might have close relationship with *C. vulgaris*, *C. sorokiniana*, and *C. kessleri*. The physiological characters, those isolates could growth up to 37 °C and utilizing lactose or sucrose as carbon source, indicated above 3 strains belong to *C. sorokiniana*.

P124

EXTRACTION AND CHARACTERIZATION OF LAMINARAN FROM BROWN ALGAE IN NORTHERN SEA OF JAPAN

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Some species of the brown algae have been removed for better growth of other eatable algaes from the seaside of eastern Hokkaido, the northern area of Japan. They are assumed to be good sources of one of beta-(1→3)-linked D-glucan "laminaran", which have been reported to have various biological activities. In the present work, laminaran was extracted from a specie of those algaes, *Alaria praelonga*, which have not been reported yet, and characterized.

Water soluble polysaccharide (WSP) was extracted with acidic water from the algae, followed by decolorization by 85% methanol. Laminaran is significantly lost to methanol if the algae is milled before decolorization, which might be due to its low molecular weight. The yield of WSP was 0.35% of starting decolored algae as dry weight. The content of laminaran in WSP was estimated to be 47% as glucose content. Then WSP was purified to remove high molecular weight polysaccharides such as alginic acid and fucoidan. Laminaran is neutral and low molecular weight polysaccharide, the D-glucose residues of which are up to 50. Therefore, ultrafiltration membrane (UM; the pore sizes are different from 10 - 200KDa as molecular weight of residues), and anion-exchange column (AEC) on Ecteola-cellulose (Cl^-) were employed for purification. It appears that most of laminaran is trapped by the membrane which has small pore size, as well as other high molecular weight polysaccharides. On the other hand, the yields and purities are about 40% of starting WSP and about 90% in resulting laminaran, respectively, by UM(200KDa) and AEC. Gas chromatograph shows that AEC is better for purification than UM(200KDa) due to its low impurities. ^{13}C NMR spectrum of laminaran obtained by AEC is identical to that of commercial one (Sigma Chemical Co.). This indicates that the laminaran might be a beta-(1→ 3)-linked D-glucan with occasional beta-(1→ 6)-linked branches and termination of D-mannitol as was previously reported on commercial one. Mass spectrometry and 2D-NMR should be applied for further structural analysis.

P125

SORPTION OF CADMIUM AND LEAD BY *NOSTOC PALUDOSUM* AND *PHORMIDIUM ANGUSTISSIMUM*

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Screening for mercury (Hg), cadmium and lead (Pb) removal ability in aqueous solution by 43 strains of microalgae. The result showed that most of microalgae had high heavy metals removal ability such as green algae; *Chlorella vulgaris* var. *vulgaris* had Hg, Cd and Pb removal capacity 94%, 89% and 88%, respectively. *Chrococcum* sp. had 96%, 94% and 71%, respectively. Blue green algae *Nostoc paludosum* and *Phormidium angustissium* were selected for futher experiment because of rapid growth, easy to separate from aqueous solution because of its filamentous strain and high Cd and Pb removal ability. Cd removal were 92% and 81%, respectively and Pb removal were 92% and 72%, respectively. *Nostoc paludosum* had maximum adsorption (q_{max}) of Cd and Pb at 2.71 and 3.86 mg/g dry wt., respectively and *Phormidium angustissium* had maximum adsorption (q_{max}) at 2.77 and 3.13 mg/g dry wt., respectively.

Alkaline treatment by NaOH could increase the Cd and Pb removal ability. The maximum adsorption (q_{max}) of Pb by non-treated cells of *Nostoc paludosum* and *Phormidium angustissimum* were 166.67 mg/g dry wt. and 93.46 mg/g dry wt. and were 101.01 mg/g dry wt. and 116.28 mg/g dry wt., respectively in Cd adsorption. NaOH-treated cells of *Nostoc paludosum* and *Phormidium angustissimum* were increased q_{max} of Pb to 312.50 mg/g dry wt. and 140.85 mg/g dry wt., respectively and q_{max} of Cd were increased to 156.25 mg/g dry wt. and 238.10 mg/g dry wt., respectively. From this studies suggested that cyanobacteria, *Nostoc paludosum* and *Phormidium angustissimum* could remove Pb and Cd in wastewater because of their selective binding with Cd and Pb which this advantage could be compete with ion exchange resin in hard water condition.

P126

CHARACTERIZATION OF MANNITOL SYNTHESIZING ENZYMES FROM THE RED ALGA, *CALOGLOSSA CONTIMUA*

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Mannitol is synthesized as a compatible solute in response to changes in the intracellular water balance brought by drought, low temperature and high salinity in many plants and algae. It is known in the genera *Caloglossa* that mannitiol is synthesized from fructose-6-phosphate (F6P) by sequential two enzymes, mannitol-1-phosphate dehydrogenase (M1PDH) and manniol-1-phosphatase (M1Pase). However, there is a limited information on its regulatory mechanism because of the lack of enzymological and molecular information on these enzymes. The present study aimed to accumulate such information by purification and characterization of both enzymes from *C. continua.*

Gel-filtration and SDS-PAGE patterns showed that M1Pase and M1PDH are monomer. The molecular masses of native forms of M1Pase and M1PDH were 28,500 and 51,000, respectively. M1Pase showed narrow substrate specificity to mannitol-1-phosphate (M1P) and K_m(M1P) was 0.41 mM. M1PDH also showed narrow substrate specificity to M1P and NAD in oxidative reaction and to F6P and NADH in reductive one. K_m values of M1PDH for M1P and NAD were 51 mM and 19 mM, respectively, and those for F6P and NADH were 150 mM and 5.8 mM, respectively. Optimum pH was 7.4 for the catalytic activity of M1Pase, and that of M1PDH was 8.2 and 7.0 for oxidative and reductive reactions, respectively. M1Pase was activated by Mg^{2+}, but strongly inhibited by Ca^{2+}, NaF, *N*-ethylmaleimide, and *p*-hydroxymercuribenzoic acid (PCMB). NaCl and mannitol also inhibited the activity, but almost a half of the activity was remained even at 500 mM and 250 mM, respectively. As for M1PDH, both oxidative and reductive activities were strongly inhibited by PCMB and Zn^{2+}, however the reductive activity was activated by 100 mM of cations, such as Na^+, K^+ and NH_3^+, whereas the oxidative activity was not. These findings suggest that the biosynthesis of mannitol might be regulated by changes in the concentrations of such cations and mannitol.

P127

PHYTASE ACTIVITY FROM THERMOTOLERANT BLUE-GREEN ALGAE FROM HOT SPRING IN NORTHERN THAILAND

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Four isolates of thermotolerant Blue-green algae including *Synechococcus lividus* Copeland strain SKP50, *S. lividus* strain DSK74, *S. bigranulatus* Skuja and *Chroococcidiopsis thermalis* Geitler, isolated from the hot spring in northern Thailand, were cultivated at 50°C in the phosphate-free medium containing 0.1%(w/v) calcium phytase as a sole phosphorus and carbon source. The phytase activity was found in intracellular fraction of *Synechococcus lividus* strain SKP50, *S. lividus* strain DSK74 and *S. bigranulatus* with the maximum level at 1.83 , 1.68 and 1.77 mU/ml respectively, after cultivation for 18 days, while 0.99 mU/ml was found intracellular of *Chroococcidiopsis thermalis* at 20 days. The enzyme activity found was corresponding with the algal growth characteristics and the phytase activity in culture broth was found in trace amount in all isolates. The induction mechanism of enzyme production and some characterization of the phytase from those are in under investigation.

P128

BIOSYNTHESIS OF BETAINE LIPIDS IN MARINE MICROALGAE

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Betaine lipids are detected in the limited species of plants including algae. Three types of betaine lipids have been reported up to now; DGTS (1,2-diacylglyceryl-*O*-4'-(*N,N,N*-trimethyl) homoserine), DGTA (1,2-diacylglyceryl-*O*-2'-(hydroxymethyl)-(*N,N,N*-trymethyl)-β-alanine) and DGCC (1,2-diacylglyceryl-3-(*O*-carboxyhydroxymethylcholine). The chemical structure of DGCC derived from *Pavlova lutheri* (Haptophyceae) was determined in 1994. DGCC was detected to be one of the common constituents of Haptophyceae. The aim of present work was to clarify the biosynthesis of betaine lipids in Haptophyceae. We analyzed fatty acids and polar lipids in CCMP504 (Haptophyceae). The principal polar lipid components in CCMP504 were galactolipids (MGDG, DGDG and SQDG) and betaine lipids (DGCC and DGTA). DGTS was not detected. The prominent fatty acids were 16:0, 18:1, 20:5 (EPA) and 22:6 (DHA). DGCC and DGTA contained high proportions of these polyunsaturated fatty acids. In order to investigate the biosynthesis of the polar moieties in betaine lipids, cells were incubated with [methyl-^{14}C] methionine, [methyl-^{14}C] SAM, *b*-[1-^{14}C] alanine, [methyl-^{14}C] choline chloride and [1,2-^{14}C] choline chloride in pulse-chase manners. The results of pulse-chase experiments indicated that methionine might be a precursor of polar moieties of DGCC and DGTA. ^{14}C-DGTS as an intermediate was not detected, suggesting that the biosysnthetic pathway of DGTA in CCMP504 was different from that in *Ochromonas danica*. On the other hand, ^{14}C-choline was converted to only DGCC. The biosynthetic pathway of DGCC seems to be independent of that of DGTA in CCMP504. In order to confirm these results, feeding experiments with ^{13}C-labelled compounds are now in progress.

P129

CELL WALL EXTENSIBILITY IN THE TIP-GROWING XANTHOPHYCEAN ALGA *VAUCHERIA TERRESTRIS*

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Cell growth in plants involves cell wall loosening and extension, which are coincident with the secretion of wall materials from the cytoplasm.In the present study, the apical cell walls that were isolated from the giant-cellular xanthophycean alga *Vaucheria terrestris* were used to clarify extensibility in the tip growing walls. The apical wall was inflated with silicon oil under various pressures ranging 0.4 to 0.7 MPa, and the time-course of the extension of the wall was analyzed. The wall did not show any significant elastic deformation but exhibited gradual plastic extension, resulting in rupture at apical or subapical portion. Wall extensibility was dependent on pH in the buffer used for infiltration before inflation. The optimum pH for wall extension was about 8.0, whereas the cell wall was much less extensible after infiltration with an acidic buffer. The pH-dependent changes in wall extensibility were completely reversible. Moreover, boiling the wall in methanol before buffer infiltration had little effect on extensibility in the wall. It is known that some cell wall proteins may regulate acid growth in diffuse-growing cells. However, the present results suggest that extensibility in the wall of the tip-growing algal cell may be controlled by mechanisms distinct from those in such diffuse-growing cells.

P130

BIOCONCENTRATION OF SELENIUM BY *EMILIANIA HUXLEYI*

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Coccolithophorids are abundant and widely distributed components of marine phytoplankton. They are significant globally for worldwide production of $CaCO_3$. They have contributed substantially to the limestone sediments of the ocean floor, and had been a long-term sink of inorganic carbon. We reported previously that nanomolar level of selenium (Se) is essential for growth of coccolithophorids but it was toxic at high concentration (micromolar level). Therefore, selenium can be a very important growth-regulation factor for coccolithophorids. In this study, we examined the uptake and the concentration mechanism of selenium by a cosmopolitan coccolithophorid, *Emiliania huxleyi.*

^{75}Se-radiolabelled selenite was used as a substrate for the uptake experiment. At 3.2-nM, nearly same as seawater-level, intracellular concentration of selenium reached 7.7 μM that is about 2400-fold higher concentration to the initial concentration of selenium in medium.

Time course pattern of Se-uptake was clearly divided into three phases. Namely, a rapid absorption process that continued only the first few minutes, a middle-speed absorption phase continued the subsequent nine hours and a slow absorption that continued longer. The kinetics of the selenite absorption at the second phase showed a typical Michaelis-Menten type curve. This is an evidence that the process is mediated by an active transport system.

Protein-bound selenium concentration linearly increased with time. At 48-h after addition of ^{75}Se-selenite, 40% of the intracellularly accumulated selenium was protein-bound form. By the analysis of ^{75}Se-labelled proteins by SDS-PAGE, six kinds of ^{75}Se-labelled proteins were detected. Their molecular weights were 61, 29, 27, 21 and 15-kDa. By using newly constructed ^{75}Se/^{35}S-double labeling method, it was confirmed that ^{75}Se-labelled proteins with molecular masses of 29 and 27-kDa were selenium-specific, but were not proteins in that Se was not specifically replaced with S.

P131

SEAGRASSES OF THE RUSSIAN FAR EASTERN SEAS COAST: STOCK, BIOCHEMICAL CHARACTERISTICS, UTILIZATION OF POLYSACCHARIDES

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Zostera marina, Z.asiatica, Z.nana and *Phyllospadix iwatensis* growing in the Far East seas belong to the family of Potamogetonaceae. They occupy the littoral and sublittoral zones down the depths of 21 m, practically along the whole coast of the Sea of Okhotsk and the Sea of Japanese up to the Bering strait. They form large areas of biomass 0,2 to 12 kg/m^2. According to the expert estimate the seagrasses stock is evaluated at 450-600 ths. t. The studies have shown that content of organic substances in the seagrasses can reach 77-86% of dry matter. It consists of the cellular (12,3-21,3%), proteins (6,5-13,8%), pectins (10-15%) and others. The content of mineral components can reach 14-23% of dry matter seagrasses and consist of polyvalent elements connected with polysaccharides. Water dissolved salts containing iodine 0,002 to 0,017%. The main polysaccharide of seagrasses is a linear polymer containing of residues of D-galacturonic acid. The yield of polysaccharide is 10-16% depending on the period of seagrasses harvesting and species. The main trends of seagrasses utilization are as follows: as stabilizer of food products and biologically active additives for preventing organism intoxication with heavy metal and medical treatment of gastrointestinal tract diseases.

P132

ANTIOXIDANT MOLECULES OF PHYTOPLANKTON: QUANTIFICATION AND PHYSIOLOGICAL ASPECTS OF GLUTATHIONE, CYSTEINE AND ASCORBIC ACID

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Glutathione and cysteine are major sources of nonprotein thiols in most organisms including plants, animals and microorganisms. They protect cells from environmental stresses such as oxidative stress, and from xenobiotic electrophiles and heavy-metal ions. However, their relation to differential tolerance to heavy metals or oxidative stresses is controversial so far. In this study we measured cellular contents and concentration of nonprotein thiols of six species of microalgae, *Tetraselmis tetrathele* (Prasinophyceae), *Porphyridium purpureum* (Rhodophyceae), *Heterocapsa circularisquama* (Dinophyceae), and three Haptophycean species (*Pavlova* sp., *Isochrysis* sp., and *Pleurochrysis carterae*). And their relations with susceptibility to mercury were examined. Contents (and concentrations) of the nonprotein thiols per cell of the six species were 119-2230 amol (0.66-12.0 mM) in cysteine, 31-677 amol (0.12-1.25 mM) in GSH. Oxidation ratios of cysteine and glutathione were various among the five species, and there was a tendency that the higher in concentration of the reduced molecules, the lower they were oxidized. We investigated susceptibility to $HgCl_2$ using a fluorochrome FDA by flow cytometry. Exposure to $HgCl_2$ caused decrease in the fluorescence intensity in the five species, and there was a correlation between the 50%-effect concentrations (EC_{50}s) of $HgCl_2$ and concentrations of GSH.

Ascorbic acid (vitamin C) is also a major component in plant defences against oxidative stress. Although it is known that ascorbic acid accumulates at high concentrations in photosynthetic tissues in higher plants (millimollar order), there is a few information about its content in phytoplankton. We also quantified ascorbic acid in the phytoplankton described above. The contents (and concentrations) of ascorbic acid per cell were 3120 amol (5.77 mM) in *Tetraselmis*, 116 amol (1.27 mM) in *Pavlova*, 35.5 amol (0.44 mM) in *Porphyridium*, and 13.3 amol (0.19 mM) in *Isochrysis*. Ascorbic acid could not be detected in *Pleurochrysis*.

P133

CHANGES IN STAINABILITY OF CORTICAL MEMBRANES IN THE DINOFLAGELLATE *SCRIPPSIELLA HEXAPRAECINGULA*

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The stainability of the plasma membranes and amphiesmal vesicle membranes in motile and non-motile cells in the dinoflagellate *Scrippsiella hexapraecingula* was examined by electron microscopy using the periodic acid-thiocarbohydrazide-silver proteinate test (PATAg) and a silicotungstic acid-chromic acid mixture (STA).

In motile cells, PATAg staining demonstrated the plasma membrane, but only a slight density was evident in amphiesmal vesicle membranes. When ecdysis occurred with the motile cells becoming non-motile cells, the plasma membrane and the outer amphiesmal vesicle membranes, which separated from the non-motile cells, stained with PATAg. However, a new plasma membrane that formed in the non-motile cells stained less with PATAg. About half an hour after ecdysis, the new plasma membrane became electron opaque, whereas newly developed amphiesmal vesicle membranes stained less with PATAg.

The plasma membrane was unstained with STA in motile cells. However, the inner amphiesmal vesicle membranes were stained with STA stronger than the outer ones. Just after ecdysis, a new plasma membrane in non-motile cells was positive with STA. Contrasting of the plasma membrane completely disappeared about an hour after ecdysis. Instead, developing amphiesmal vesicle membranes stained positively with STA, the inner membranes being stronger than the outer membranes. Patterns of these three membranes stained with STA in the non-motile cells were retained in the motile cells.

The stainability of the inner amphiesmal vesicle membranes with PATAg and STA in the motile cells was the same as that of the new plasma membrane in the non-motile cells just after ecdysis. This suggests that although such the early plasma membrane originates from the inner amphiesmal vesicle membranes morphologically, it does not transform into the plasma membrane functionally. The fact that the early plasma membrane changed its staining reaction later suggests the occurrence of rapid conversion in membrane properties, compositions and functions.

P134

INVESTIGATION OF THE POLYSACCHARIDES AND PHYCOBILIN PIGMENTS FROM RED ALGAE OF THE RUSSIAN PACIFIC COAST COLLECTED IN ONTOGENETIC PROCESS

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Red seaweed biosynthesize different pigments and a great variety of sulfated galactans - the major components of the extracellular matrix, wich have important value for biomedical and industrial application. The influence regular biological and ecological factors on quantitative and qualitative characteristics of sulfated polysaccharides of carrageenans and phycobilin pigments from algae belonging to the *Gigartinaceae* and *Tichocarpaceae*, collected from Russians Pacific coast have been investigated.

The variation in the pigment content and carrageenans characterization were observed under two different irradiances 10 and 80 % PAR. The high phycocyanin and phycoerythrin yield was found for four species of carrageenanophytes of *Gigartinaceae* and their accumulation is negatively correlated with photon irradiance. The content of pigments in *Tichocarpaceae* dependent on the life stage of seaweed.

The chemical structure, molecular weight, gelling and viscoelactic properties of different samples of carrageenans were study using methods by spectroscopy, viscosimetry, reolometry and analytical ultracentrifuge. Variations in polysaccharide yield and composition have been shown to be considerably influenced by irradiance conditions in natural beds of algae and thus to reflect limiting steps in the biosynthesis of these cell wall constituents. According to results obtained by ^{13}C-NMR and TFIR- spectroscopy polysaccharides were composed predominantly of kappa-carrageenan (30-40%) with trace iota-type with good gelling properties and small quantity (10%) of lambda-carrageenans for *Gigartinaceae*, and hybrid kappa-beta carrageenan, that formed a very weak gel for *Tichocarpaceae*. The study of carrageenans from different phases of the life cycle of algae allowed us to observe the formal pattern of the systems biosynthesized by each stage of the seaweed. The biological properties of carrageenans «in vivo» and «in vitro» were investigated.

The obtained dates have demonstrated the relationships between chemical structures, rheological and biological properties of carrageenans. The results will be useful in selection of potential industrial sources of carrageenan and pigments from the most abundant algae growing on the Russian Pacific coast.

P135

EXTRACTION AND CHARACTERIZATION OF SULFATED POLYSACCHARIDES FROM BROWN ALGAES IN NORTHERN SEA OF JAPAN

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Some species of the brown algae have been removed for better growth of other eatable algaes from the seaside of eastern Hokkaido, the northern area of Japan. They are assumed to be good sources of sulfate polysaccharide "fucoidan", which have been reported to have various biological activities. For example, antitumor activity, anticoagulant activiy and anti-HIV activity have been reported. In the present work, fucoidans were extracted from two species of those algaes, *Costaria costata* and *Alaria praelonga*, and characterized.

Water soluble polysaccharides (WSPs) were extracted with acidic water from decolored and dried algaes. The yield of WSP and the content of fucose from *C.costata* were much higher than those from *A.plaeronga*. Acidic polysaccharide fractions in WSPs were prepared with $CaCl_2$ solution and aqueous cetylpyridinium chloride (CPC) on various concentrations. WSPs were dissolved in 4.0M $CaCl_2$ solution, and the precipitated polysaccharide was named Fraction No.1. The supernatant solution was dissolved in CPC solution to be 5%(w/v) and 0.5M of final CPC and $CaCl_2$ concentration, respectively. The polysaccharide in the supernatant of the CPC/$CaCl_2$ solution was named Fraction No.2. The precipitation of the solution is CP-polysaccharide complex. Fraction No.3, No.5 and No.4 were dissociated from the complex stepwise in 2.0M, 3.0M and saturated $CaCl_2$ solution, respectively.

Each fractions were examined for acidity by electrophoresis on pH7.5 calcium acetate buffer and for characterization by chemical analyses. Fraction No.1 was identified to water soluble alginic acid. The yields of fraction No.4 and No.5 were quite low (<2%). Fraction No.2 and No.3 showed good electrophoretic migration and had higher content of fucose and sulfate ester, which were identified to crude fucoidans. This indicates that most of crude fucoidans are dissociated from CP-polysaccharide complex in 3.0M $CaCl_2$ solution.

The yields of crude fucoidans were about 35% and 27% of the starting WSPs from *C.costata* and *A.praelonga*, respectively. It is apparent that *C.costata* is better source of fucoidan. Fraction No.3 from *C.costata*, having highest content of fucose and sulfate ester, were analyzed by anion-exchange chromatography on Ecteola-cellulose (Cl^-). The chromatographic patterns showed five subfractions, indicating that fraction No.3 have at least five subfractions, the sulfate ester content of which are different each other. Further fractionation by anion-exchange and gel filtration chromatography will necessitate to obtain the purified fucoidan, having homogenious structure.

P136

LIGHT REQUIREMENT IN GROWTH OF YOUNG *ZOSTERA MARINA* IN CENTRAL JAPAN

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Zostera marina is a marine angiosperm and have a wide distribution over the Northern Hemisphere. Generally, light is one of the most important factors which affect vertical distribution of marine plants. This study was designed to estimate the critical depth for growth of *Z. marina*.

Young *Z. marina* of 10cm in total length were used for photosynthetic study. They were cultured for a week under water temperature from 10 to 25℃, and light intensity of $50\mu Em^{-2}s^{-1}$. Then, photosynthetic activity of young *Z. marina* was measured. The daily light compensation points for young *Z. marina* were estimated with mathematical model based on photosynthetic activity, light conditions in growing site of *Z. marina*.

Estimated relative critical light intensity of young *Z. marina* were from 7.5 to 7.9% of the sea surface in all seasons. Light compensation depths were calculated from relative critical light intensity above mentioned and typical values of extinction coefficient of seawater on *Z. marina* bed based on Beer-Lambert's law. Estimated compensation depths were affected by the extinction coefficients. For example, in the clear coastal area (the extinction coefficient of 0.2) the compensation depth is reached to about 12m. Although, in the turbid inner sea area (the extinction coefficient of 0.5) the compensation depth is only about 5m. Almost of all data about growing depth of *Z. marina* reported from various points in the world were shallower than the results of our estimation. Moreover, the lowest limits of *Z. marina* distribution were also agreed well with the compensation depths of our estimation. So, this model used in this study was effective method to estimate the critical depth in growth of *Z. marina*. Lower limit of the growing depth of *Z. marina* was mainly restricted by light condition in growing site.

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PRESENCE OF CHLOROPHYLL *D'* IN A CHLOROPHYLL *D*-DOMINATING CYANOBACTERIUM *ACARYOCHLORIS MARINA*

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In 1996, a novel oxygenic alga *Acaryochloris marina* was isolated from a species of colonial ascidians. Although in all previously known organisms capable of oxygenic photosynthesis the dominating chlorophyll is Chl *a*, in *A. marina* the major one is Chl *d*. The Chl *a* content in *A. marina* is very low. The primary donor of PS1 was named P740, corresponding to P700 in other oxygenic photosynthetic organisms, because isolated PS1 particles showed a flash-induced absorbance loss maximum at *ca.* 740 nm. Here, we have performed a pigment composition analysis of *A. marina* cells by silica normal-phase HPLC. Chl *d'* and Phe *a* were detected as minor pigments. No Chl *a'* and Phe *d* were detected. The molar ratios were Chl *a*/Phe *a* = 2, Phe *a*/Chl *d'* = 1 and Chl *d*/Chl *d'* = 70, suggesting that the RC of PS2 consists of 4Chls *a* and 2Phes *a*, and that P740 consists of Chl *d*/*d'* heterodimer, like P700 is Chl *a*/*a'*. Time-resolved florescence spectroscopy on the *A. marina* cells showed that a delayed fluorescence peak from PS2 was in the Chl *a* emission range, and not in the wavelength region of Chl *d* emission; hence, the electron donor of PS2 in *A. marina* was most probably identical with that of other oxygenic photosynthetic organisms, namely, P680 consisting of Chl *a*. Homology of amino acid sequences of PS1 RC proteins, PsaA and PsaB, and those of PS2 core antenna proteins, CP47 (PsbB) and CP43 (PsbC), in *A. marina* to those of general cyanobacteria was low, due to the substitution of chlorophyll associated with these proteins in *A. marina* from Chl *a* to Chl *d*. In contrast, homology of amino acid sequence of D1(PsbA) and D2(PsbD) were high, intending that D1 and D2 proteins in *A. marina* have to use Chl *a* even in that antenna chlorophyll in PS2 had been replaced to Chl *d*.

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CALLUS-LIKE TISSUE CULTURE OF *GRATELOUPIA TURUTURU* (GRATELOUPIACEAE, RHODOPHYTA) AND ITS APPLICATION IN MACROALGAE STOCK CULTURE

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In order to set up a method to hold germplasm of marine macroalgae in laboratory, unialgal culture of callus-like tissue of *Grateloupia turuturu* was established in laboratory. Samples of *G. turuturu* were collected at the coast of Qingdao, China. Thallus was first rinsed with sponge in sterilized seawater, then cut into small pieces of about 3mm, which were incubated in enriched seawater at 18°C and a photon flux density of 10 μmol $m^{-2}s^{-1}$. After two-weeks' culture, the uncontaminated pieces were picked out under microscope and cultured in 100ml flask. Callus cell reached out from the edge of thallus pieces and formed filamentous callus-like tissue after 3 to 4 weeks. This filamentous callus-like tissue was then cut down and cultured in lab. It grew continuously at 18°C, 10 μmol $m^{-2}s^{-1}$ and remained filamentous status. When photon irradiance was enhanced to 40 μmol $m^{-2}s^{-1}$, the filaments merged with each other, then the young thallus grew out. Callus-like tissue can be induced, cultured and regenerated into new thallus provides a stock culture method for marine macroalgae.

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EFFECT OF SALINITY ON GROWTH, PHOTOSYNTHESIS AND OSMOLARITY IN THE FRESHWATER GREEN ALGA *RHIZOCLONIUM RIPARIUM*

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Effect of salinity on several physiological characteristics of a green alga *Rhizoclonium. Riparium* collected from a rice field at vicinity of, Tokyo, was investigated. The alga grew well in seawater media from 0.1 to 40 Practical Salinity Unit (PSU). In particular, higher growth rate was observed in 10 PSU rather than in 0.1 PSU (the salinity of its original habitats). Although no distinct growth was observed under the higher salinity than 40 PSU, photosynthetic activity was observed in from 0.1 to 100 PSU of seawater reaction media. Intracellular osmolarity corresponded to 1.2 M NaCl, and was much higher than that of *Spirogyra* (0.35 M NaCl) living in the same freshwater environments. Although the plasmolysis occurred in more than 30 PSU (0.5 M NaCl) of seawater, the alga was deplasmolysed within 2hrs in the same NaCl solution. The alga in seawater medium synthesized more amount of a protein than that in freshwater medium. Molecular weight of the protein was almost the same as that of carbonic anhydrase. Photosynthetic activity was kept more than 27 weeks of cultivation in seawater medium (31 PSU), and salinity range capable of photosynthesis became broader as from 0.1 to 110 PSU.

These results suggest that the freshwater algae *R. riparium* are able to grow in seawater medium for a long time by increasing the osmolarity of cells, and their photosynthetic activities are tolerable to hyper salinity stress. Increase in the amount of carbonic anhydrase like protein in cells seems to be one of adaptive mechanisms to salinity stress.

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MUROTO DEEP SEA WATER ACCELERATES THE GROWTH OF THE RED ALGA *GRACILARIA CHORDA* HOLMES IN CULTURE

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Growth of *Gracilaria chorda* Holmes from Shikoku Island, Japan, was investigated using several culture media in unialgal culture. Unialgal cultures of *G. chorda* were originated from spores liberated by fertile plants harvested in nature. Growth of the alga cultured in Muroto deep sea water (MDSW) was compared with those cultured in Muroto surface sea water (MSSW) and Yashima surface sea water (YSSW). Algal fresh weight was measured weekly at intervals coinciding with the renewal of culture media. Data were analyzed by single factorial analysis of variance (ANOVA) and Tukey's multiple comparison test. The statistical analysis indicated that MDSW was the most effective accelerator of the algal growth among the seawaters tested. Algal fresh weight cultured in MDSW for four weeks was approximately twice as heavy as that cultured in MSSW. Chemical analysis indicated that MDSW contained large amounts of inorganic nutrients such as NO_3^- and PO_4^{3-}. Because fresh weight of the alga cultured in enriched MSSW (EMSSW: final concentration: 30 μM NO_3^- and 2 μM PO_4^{3-}) corresponded to approximately 1.3 times of that cultured in MSSW, such nutrients probably contribute to the algal growth. This result also suggested that MDSW contained any accelerators of the algal growth except for NO_3^- and PO_4^{3-}. Effect of sterilized MDSW on the growth of the alga was similar to that of non-sterilized MDSW. Concentration of two vitamins, cyanocobalamin and biotin, in seawater determined by bioassay. The result of bioassay showed that MDSW contained cyanocobalamin shorter than MSSW, and biotin in seawater was not detected. Although algal growth accelerators except for inorganic nutrients in MDSW are not clear to date, the results suggest that MDSW is a most effective culture medium for the red alga, *G. chorda*, among seawaters tested.

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ANALYSIS OF GENETIC VARIATIONS OF *UNDARIA PINNATIFIDA* BY USING RAPD

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RAPD is employed to analyze genetic variations of seven lines gametophyte clones of *Undaria pinnatifida*, which from different locations of China. Twenty primers have been used and sixteen of them can give reproducible amplified fragments, of which 56 are polymorphic. Dendrograms constructed from RAPD data showed that there is a high variation in *Undaria pinnatifida* (D=0.4419~0.7420) and the genetic distance has no direct relation to the geographic.

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GROWTH OF PRUNED KELP *LAMINARIA JAPONICA* AND SORUS FORMATION OF THE CUT-OFF BLADE TIPS IN DEEP-SEA WATER PUMPED FROM TOYAMA BAY

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As a multiple/cascade utilization of deep-sea water (DSW, known as a cold, clean and nutrient-rich seawater) pumped from Toyama Bay, present authors (Matsumura & Fujita 2002) proposed a self-sustainable abalone culture system. In the system, DSW enhances the intercalary growth of kelp after cutting off its blade tip, which is fed to abalone reared in the warmed drainage of kelp culture. The advantages of pruning are 1) shortening of culture tanks, 2) keeping high kelp densities without forming canopies, 3) preventing deterioration by air exposure 4) minimizing the waste of produced kelp materials. In addition, sorus formation of cut-off blade tip in DSW culture provides year-round kelp seed production. A quarter of cultured kelp is rotationally pruned every week to supply sufficient blade tips to abalone. Here we report the details of growth rates and sorus formation of *Laminaria japonica* Areschoug in DSW culture. In exp.1, blade tips were cut off at 15 cm above the stipe-blade transition every month. Growth was examined using a hole punching method from March 2001 to April 2002. All of the pruned kelps continued to grow in warmed DSW (w-DSW, 11°C) even after the repeated cut-off by 14 times. The mean elongation varied from 1.3 to 8 cm/week in length, recording the maximum 16.7 cm/week in March The cut-off blade tips formed sori in 2 weeks every month when cultured in w-DSW. In exp. 2, in which growth rates were compared among kelps of various pruning sizes (15, 20, 30, 50 or 100 cm in blade length) and between two water temperatures using intact DSW (3°C) or w-DSW from March 2002. At the first measurement in April 2002, mean growth rates were higher in longer pruned kelps and in kelps cultured in lower temperature.

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THE PHYSIOLOGYCAL ROLE OF ULTRAVIOLET ABSORBING SUBSTANCES IN RED ALGAE

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UV irradiation has various harmful effects on living organisms. One of the remarkable effects of UV irradiation is the lesion of a DNA molecule. The energy of UV irradiation absorbed by adjacent pyrimidine bases in DNA rearranges the covalent bond of bases and produces pyrimidine photodimers that cause errors in gene expression or DNA replication.

Specific red algae living in shallow water contain water-soluble UV absorbing substances (UVAS), which absorb mainly the region of UV-B and UV-A in sunlight. The major group of UVAS found in red algae was identified as mycosporine-like amino acids (MAAs) with an absorption maximum of 300 to 350 nm. Although various chemical characteristics and the distribution of MAAs are studied, the physiological role of these compounds is little known. We have tried to understand the physiological/biochemical role of UVAS in algal life.

As the fluorescence of UVAS is too weak for photosynthesis and no energy transfer from UVAS to photosynthetic pigments are detected, the main role of UVAS should be the protection of DNA. Since UVAS absorb harmful UV-B region in sunlight, they may inhibit the production of pyrimidine photodimers of DNA. The experiment for the protective function of UVAS to a thymine molecule suggested that, in addition to the UV screening activity, UVAS have a significant function in quenching the excited state of thymine or thymidine molecules. These results suggested that UVAS play an important eco-physiological role in protecting algal DNA against UV irradiation not only by the UV-screen but also by the inter-molecular energy transfer process from thymine bases to UVAS.

The protective function of individual MAA also will be discussed.

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EFFECT OF UV OR GAMMA RAY IRRADIATION ON SOME MICROALGAE

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UV-B irradiation (optimum 314nm) causes the decrease of growth rate and photosynthetic oxygen evolution of some microalgae. Two cyanobacteria; *Synechococcus leopoliensis*, *Synechocystis* sp. are the most stable for the UV-B irradiation in both photosynthesis and growth. Green algae; *Dunaliella tertiolecta, Chlamydomonas reinhardtii* are less tolerant for the irradiation in photosynthetic activity. Other green algae; *Chlorella vulgaris, C. sorokiniana*, and red algae; *Cyanidium caldarium, Galdieria sulphuraria, Cyanidioschyzon merolae* are intermediately tolerant among these algae for the irradiation.

On the other hand, gamma ray radiation of cobalt 60 also inhibits some algal photosynthetic oxygen evolutions and their growths. The photosynthetic activities of a green alga, *D. tertiolecta* was mostly inhibited by 1000 Gy gamma radiation, and those of other algae; *C. reinhardtii, C. vulgaris, C. sorokiniana*, and a red alga, *G. sulphuraria* were not affected by the same radiation. The growth of *D. tertiolecta* was also inhibited by 200 Gy radiation, but those of other algae were not affected by the same radiation.

As a result, *D. tertiolecta* was one of the most damageable algae by both UV-B and gamma irradiations. The algal cell size is larger than other algal ones tested and has two flagella and no cell wall. Such morphological properties are thought to be one of the cause of the weakness for the irradiations. There may be no clear correlation between UV-B and gamma irradiation effects on the other algae in these experiments.

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TISSUE CULTURE OF BROWN ALGA *SARGASSUM*

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Stems and leaves developed from the fragment tissue of brown alga genus *Sargassum* axenically cultured in multi-well dishes. The cubic specimen cut from *Sargassum duplicatum* and *S. cristaefolium* collected in Kagoshima, Japan were cut into small cubic fragments and transferred to PES enriched seawater medium. The specimen were cultured at 20 C, 10,000 lux, 12:12 hrs LD light cycles for 90 days. Stems and leaves developed after 4 weeks. Receptacles developed from the tissue of both species. Holdfast were also elongated from the cultureed tissues of *S. duplicatum*. The maximum temperature for the development of these organs were 10,000 lux in *S. duplicatum* and 3 - 5,000 lux for *S. cristaefoium*. After the development of receptacles, eggs were released from the ostiole but were sterile and did not develop into young embryos. The procedure used for our study may be useful for the developmental or cytological studies of *Sargassum* in the future.

P146

GROWTH OF ABALONE FED ON *UNDARIA PINNATIFIDA* CULTIVATED AS FREE LIVING FORM "GERMLING AGGREGATION" IN DEEP SEAWATER

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One of the characteristics of deep seawater (DSW) is greater quantity of nutrient. Nutrient-rich DSW can provide a good environment for the cultivation of seaweed. *Undaria pinnatifida* was continuously cultured in the DSW. When highly dense fertilized eggs of *U. pinnatifida* were placed in a small Petri dish and cultured for few weeks, several holdfasts of the sporophytic germlings were connected each other and made a cluster. The free living form, "germling cluster", allow cultivation of higher density in the tank than the attaching form to substratum. *U. pinnatifida* was cultured in 7t and 1t tank from April 2001 to February 2002. DSW was poured into the 7t and 1t tanks at a rate of 21t and 3t/day, respectively. Water temperature in the tanks changes at 16.2-23.1°C in the summer and 7.6-13.8°C in the winter. The average daily growth rates for *U. pinnatifida* also showed 20% from the spring to the autumn and showed 15% at the winter. Abalone, *Haliotis sieboldii*, fed on the blades of *U. pinnatifida* from November 1, 2001 to April 1, 2002. Rate of monthly increase in weight for Abalone also showed 20% from November 2001 to January 2002, 5% from January to March and 10% after April. Survival rate was 97.5%. Finally, we discussed polyculture between the abalone and *U. pinnatifida* using DSW.

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PHOTOSYNTHETIC LIGHT-RESPONSE CURVES IN COLD WATER SPECIES OF LAMINARIALES FROM THE EASTERN PACIFIC COAST OF HOKKAIDO

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Photosynthetic response to light intensity was examined in sporophytes of cold water species of Laminariales, i.e. *Agarum cribrosum*, *Alaria praelonga*, *Arthrothamnus bifidus*, *Costaria costata*, *Cymathaere japonica*, *Kjellmaniella gyrata*, *Laminaria angustata*, *L. coriacea*, *L. diabolica*, *L. longissima*, *L. sachalinensis*, *L. yezoensis* from the eastern Pacific coast of Hokkaido. Photosynthesis and respiration were measured at *in situ* temperatures (0-16°C) by a Clark-type oxygen electrode system with discs cut out of the middle part of a blade. Photoinhibition did not occurred within the investigated range up to 400 μmol m^{-2} s^{-1}. The cold water species of Laminariales showed higher light-saturated net photosynthetic rates (22.6-46.4 μL O_2 cm^{-2} h^{-1}), which were equal to those at *in situ* temperatures (14-24°C) of warm water species of Laminariales. Dark respiratory rates of cold water species were between 1.6 and 7.7 μL O_2 cm^{-2} h^{-1}. I_k and compensation points ranged from 36.6 to 62.5 μmol m^{-2} s^{-1} and from 2.1 to 7.9 μmol m^{-2} s^{-1}, respectively. Initial slope were between 0.509 and 1.120 μL O_2 cm^{-2} h^{-1} (μmol m^{-2} s^{-1})$^{-1}$. Almost parallel trends can be seen between the changes in the light-saturated net photosynthetic rates and the *in situ* temperature. On the basis of the photosynthetic light-response curves, productivity and photoacclimation of cold water species of Laminariales were discussed.

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THE RELATIONSHIPS BETWEEN TEMPERATURE AND DARK RESPIRATION OF THE STIPE OF *ECKLONIA CAVA* (LAMINARIALES, PHAEOPHYTA)

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In order to clarify the relationship of dark respiration to temperature, sporophytes of *Ecklonia cava* Kjellman (Laminariales, Phaeophyta) with a stipe of 22-102 cm long were collected at 6-9 m deep in Nabeta Bay, Shimoda, central Japan by SCUBA diving in February (winter) and in August (summer) 1998. Dark respiration of the stipe of *E. cava* was measured at various water temperatures ranging 15-27.5 °C in winter and 15-30 °C in summer in a closed system by using a dissolved oxygen meter. The stipe respiration was compared on whole stipe, length, surface area, volume, wet weight and dry weight bases. On each basis, the stipe respiration usually increased with water temperature rise. The whole stipe respiration increased with the stipe length. On length and surface area bases, however, there were a little differences of the respiration rate irrespective of the stipe length. On volume and weight bases, longer stipes had lower respiration rate than shorter stipes.

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CULTIVATION OF *TINOCLADIA CRASSA*, EDIBLE BROWN SEAWEED, AT THE SOUTHERN COAST OF TOKUSHIMA PREFECTURE, JAPAN

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The study of the cultivated techniques for *Tinocladia crassa* (Japanese, huto-mozuku) was carried out at the Pacific coast part of southern Tokushima. Neutral plurispores and filamentous thalli of this alga were settled on the cultivated nets in a tank. The nets were adjusted to a height of 20 cm from the surface of waters with iron pilings, when the juvenile thalli appeared on the nets. The young sporophyte plants were visible to the naked eye on a part or all of the nets after one month. Their plants grew up to the length of 15-25 cm for 2-3 month, and they covered over a part of the nets. The sporophyte plants matured in formation of unilocular sporangia. They became similar to the shape of wild sporophyte plants. The effects of friction by sands were remarkable to scrape sessile organisms such as diatoms during the period of the cultivation. Their seeds and seedling on the nets were removed together by the sands. After then, other seaweeds, *Colpomenia sinuosa* and *Enteromorpha prolifera* grew well on the nets. These seaweeds blocked out the light and the flow of water over the nets. The condition of lower light and lower nutrient contents has inhibited to growing cultivated *T. crassa* plants. Optimum conditions for the cultivation of *T. crassa* are shown that the water temperature ranged among 13°C and 19°C during growing periods and that the depth of the nets is 5 m under surface of waters with irregular water currents.

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SEAWEEDAFRICA : A NEW (ELECTRONIC) RESOURCE FOR INFORMATION ON THE USES OF SEAWEED

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SeaweedAfrica is a project to expand AlgaeBase (www.AlgaeBase.org), a biodiversity database of seaweed information, to include ecological, commercial and technology data from the whole of Africa. The project receives funding from the European Union under the INCO-DEV section of the Fifth Framework Programme. SeaweedAfrica brings together the combined expertise of scientists from Ireland, Sweden, France, Portugal, Kenya, Moçambique, South Africa, Namibia and Brazil.

Following a detailed literature survey, we have identified the following uses of seaweeds and their extracts under such categories as: i) aesthetics, ii) agriculture, horticulture and agronomy, iii) aquaculture, iv) cosmetics, v) environmental health, monitoring and remediation, vi) food, vii) health, thalassotherapy and wellness, viii) industry, ix) pharmaceutical and pharmacology, x) science, technology and biomedicine, xi) and miscellaneous uses.

The database already indicates that one area for the greatest potential of future development of seaweed utilisation is in the arena of pharmaceutical applications; in particular due to biologically-active secondary metabolites, and the scientific/biomedical sectors incorporating novel uses and applications of hydrocolloids, e.g. alginates. Clearly studies on seaweed biodiversity, and a detailed understanding of seaweed physiology and biochemistry, in tandem with cultivation, of potentially useful species will be required to successfully harness the full potential of seaweed resources. This is particularly true in Africa where successful examples from South East Asia can be responsibly modelled, and seaweed utilisation has the potential to contribute to sustainable employment opportunities and socio- economic upliftment in emerging economies. African coasts have a generally high biodiversity of seaweeds where opportunities for specialisation of utilisation and development of niche products based on seaweed raw materials from pollution free environments may be key marketing advantages.

SeaweedAfrica will contribute by gathering relevant information in both European and African contexts and constructing a user-friendly database for ease of use of the information by practitioners and decision makers.

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ALGAEBASE (WWW.ALGAEBASE.ORG) PROVIDING TOOLS FOR THE DEVELOPMENT OF SEAWEED BIOTECHNOLOGY

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Seaweeds have long been used in biotechnology and new applications are being developed continuously. Compounds commonly used include alginates, agars, carrageenans and laminarans, but many other components of seaweeds show potential for development, such as phycobilins and carotenoids. Product or process development will be based on accurate species information and detailed knowledge of the ecology of those species. Commercial development in the long term will require facilitative tools such as information on distribution, aquaculture, harvesting, relevant legislation etc.

AlgaeBase is an Internet based searchable database which, at present, contains information on over 9,000 currently accepted species of algae and seagrasses and nearly 11,000 synonyms. At present, the main focus of AlgaeBase is taxonomic and nomenclatural information. This valuable resource, however, is currently being expanded dramatically via a project known as "SeaweedAfrica" and this expansion will serve to increase the usefulness of AlgaeBase to the marine biotechnology community.

SeaweedAfrica is an EU-funded project entitled "Underpinning Sustainable Ecosystem Management of Seaweed Resources in Africa: Expanding the Seaweed Database". This three-year project began in November, 2001, and is being coordinated from NUI, Galway. Four EU countries, five African countries and one South American country are involved. Partners include both marine botanists and applied phycologists as the project will expand AlgaeBase to ecological, commercial and technology data concerning seaweeds from Africa and the EU. This information will be available via the Internet instantly and will also be produced in CD-ROM format which will be distributed, free-of-charge at the end of the project to targeted end-users in the development of seaweed based marine biotechnology.

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REFLECTIONS ON BIOTECHNOLOGICAL POTENTIAL OF ARID ZONE CYANOBACTERIA

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The two components of nature, microorganisms and their environment are much complex and dynamic but also interdependent, mutually reactive and interrelated. The vegetation cover is spare in the desert and the ground surface is thus exposed to atmosphere and the associated physical forces. The climate of Indian desert is characterized by extremes of temperature (<50 °C), severe drought accompanied by high wind velocity, low relative humidity, evaporation and too scanty infrequent and irregular rainfall.

Topographically the Rajasthan, one of largest state of India encompass and dunes, sandy and rocky planes, river beds, marshy and saline habitats and their organic and inorganic conditions certainly effects the existence and distribution of different form of cyanobacteria. The conspicuous presence of cyanobacterial taxa like *Lyngbya, Scytonema, Phormidium, Tolypothrix, Microcoleus, Calothix, Oscillatoria, Gloeocapsa, Aphanothece, Schizothrix, Spirulina* have been found in the various stress conditions. Various bioactive compounds produced by cyanobacteria have proved their phyto-pathological and clinical potential. Thus the study of cyanobacteria index the arid zone has been revealed their vital role in ecological balancing, protein production, and novel bioactive compound. The discovery of bioactive molecules has enhanced the economic value and has created new vistas in biotechnological potentials of cyanobacteria. In the present communication, Cyanobacteria have been taken up for the scientific approach to the study of its distribution and abundance in respect of desert climatic conditions and their future prospects in the area of algal biotechnology.

P153
COMPLEX USING OF CYANOBACTERIAL BIOMASS: BIOCIDAL SUBSTANCES

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Cyanobacterial biomass (containing about 98% *Microcystis aeruginosa*) can be used for forage protein-vitaminous concentrate production: the technology was developed in the Institute of Hydrobiology (Kiev). Technological process includes steaming of decarbohydrated paste, condensation and hydrochloric acid hydrolysis, purification of hydrochloric acid hydrolysate, its dechlorination, condensation and drying. In obtaining 40 t of protein-vitaminous concentrate, 90 m^3 of waste liquid products exude (L.A.Sirenko & V.N.Kozitzkaya, 1988; N.I.Kirpenko, 1999).

Our suggestions in this sphere are turned to using of those waste materials as the natural pesticides. Herbivorous insect larvae (Colorado potato beetle, fall webworm and lackey moth) of the 2nd and 3rd instars were tested in a series of experiments. Their results displayed high level of deterrent, growth inhibition and insecticidal activity of waste materials. A treatment of host-plant leaves by waste liquid (in 1:1; 1:2 and 1:4 concentrations) led to strong inhibitory action. The larval nutrition was suppressed in comparison with control individuals. These rates were 1.25-1.68 times for lackey moth, 2.1-7.9 ones for fall webworm and 1.5-1.9 ones for Colorado potato beetle. The larval mass in experimental versions was quite less than in control – in 57.0% for lackey moth, in 67.6% for fall webworm and in 77.9-84.3% for Colorado potato beetle. The normal process of metamorphosis was broken. In a number of cases the total mortality of fall webworm and Colorado potato beetle (2nd instar larvae) was observed on the 5th and 15th day accordingly. Lackey moth larvae were steadier: their elimination made up 72.0% on the 15th day and 80.0-88.0% on the 20th day. The most of the 3rd instar larvae died off during 15-20 days. In addition to intestinal action, the waste materials also possessed by the contact effect: treatment of larval surface caused the mortality of fall webworm (80.0%) and Colorado potato beetle (76.7%) during 19 days.

P154
EXPRESSION OF A GENE ENCODING A HIGH-CO_2-INDUCIBLE PROTEIN IN *CHLAMYDOMONAS REINHARDTII*

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A unicellular green alga, *Chlamydomonas reinhardtii*, can grow auto-, hetero-, and mixotrophically. Under autotrophic conditions, we newly found a high-CO_2-inducible protein named H43 located in periplasmic space of *C. reinhardtii*. cDNA clones for H43 were already isolated. The cloned cDNA fragments encoded a 362-amino acid polypeptide consisting of N-terminal hydrophobic signal peptide of 16 amino acids. Northern blot analysis was carried out using cDNA fragment as a probe. The level of H43 mRNA increased within 1 hour after the transfer from low (0.04%) to high (3%) CO_2 conditions in the light, but H43 mRNA was not accumulated in the dark. In addition to high CO_2 conditions, H43 was also induced under hetero- and mixotrophic conditions (in the presence of 17.4 mM acetate). H43 mRNA increased in 4 hours after addition of acetate in the light, and 2 hours in the dark. These results showed that light was not needed for H43 biosynthesis, but that the transcription of H43 was delayed by acetate. In the presence of acetate, the level of H43 mRNA was much higher under dim light than strong light. The same experiment was carried out using the mutant *dum-1* that was defective in cytochrome respiration. H43 mRNA in *dum-1* was not accumulated under strong light, and increased slightly under dim light. However, the rate of respiration was not influenced by changing light intensity in both wild type and *dum-1*. In the same light intensity, the level of H43 mRNA was higher in wild type than in *dum-1*. These results indicate that H43 mRNA is not induced by acetate itself, but induced by CO_2 generated from acetate metabolism. Therefore, we concluded that H43 biosynthesis was regulated by changes in CO_2 concentration at the level of transcription.

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CULTIVATION OF *SPIRULINA PLATENSIS* (*ARTHROSPIRA*), CMU1 AND CMU2, USING BIOGAS EFFLUENT WATER FROM PIG FARM

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Spirulina platensis (*Arthrospira*) strain CMU1 and CMU2 were isolated from the oxidation pond at Nakornrajchasrema and Sakornnakorn provinces. In winter season they were cultured in biogas effluent water. Because the nutrient concentration and turbidity were high, dilutions of medium were prepared and some dilutions had added nutrients which contained $NaHCO_3$, $NaNO_3$, K_2HPO_4 and fertilizer. All treatments are as follows: dilution 0%, 1%, 3%, 5%, 10%, 20%, 30% and dilution 3% with nutrients, 5% with nutrients, 10% with nutrients and 20% with nutrients. Then added NaOH to adjust the pH which ranged between 10±1 and was cultured in glass greenhouse. The temperature range between the studies fell into the range of 15 to 26 °C. The results showed that both strains of *S. platensis* can grow in biogas effluent water. The highest average specific growth rate and dried cell matter in strain CMU1 were 0.601 day^{-1} in 5% dilution with nutrients and 1237.5 $mg.l^{-1}$ in 3% dilution with nutrients. For strain CMU2, the highest average specific growth rate and dried cell matter were 0.504 day^{-1} and 2892.5 $mg.l^{-1}$ in 3% dilution with nutrients. Treatments with nutrients are suitable for the enhancement of both strains of *S. platensis*. The winter season slowed the growth of *S. platensis* in biogas effluent water.

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BIOTECHNOLOGY OF CULTIVATION OF *LAMINARIA JAPONICA* AND SEA URCHIN *STRONGYLOCENTROTUS INTERMEDIUS*

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By the early 90s the natural beds of *Laminaria japonica* in the costal waters of Primorye (the Sea of Japan) have considerably decreased because of unfavorable environmental conditions and of dramatic industrial impact in 1980–1985. Certainly the disturbances obliterated much of the structure in the kelp forest. Despite the banning of the kelp harvesting the recovery of its forests occurs slowly. Moreover, in some areas kelp self-reproduction cannot take place because of absence of maternal plants with reproductive tissues. It is evident the steps should be taken to restore *L. japonica* populations. Otherwise the natural substratum (the sea bottom) will be inhabited by seaweed species of low value and, as a result, irrevocable change of biocenosis will occur. In other words, there will give rise to so-called "Isoyake" situation observed in many nearshore sublittoral ecosystems world around. The problem has become more actual one with the activation of the sea urchin catch. The commodity value of sea urchin is well known to be dependent on the state of kelp bed which accelerates reproductive maturation and growth rate, and enhances gonad production and survival in juvenile and young adult of sea urchin.

To restore the populations of *L. japonica* the unique inexpensive biotechnology was elaborated in TINRO-Center. The maternal thalli of *Laminaria* specially treated are placed on the sea bottom at the period optimal for a given Primorye area. The bottom formation of the selected site and hydrodynamic regime of the costal waters are taken into consideration. By request, kelp can be cultivated as a foodstuff in aquaculture for sea urchin or as raw material for kelp industry. The daily feeding rate for sea urchin feeding on *L. japonica* was experimentally estimated as 20 kilograms of kelp per 1 ton of sea urchin.

The proposed method can help to store the natural biological resources and to increase their productivity.

P157

ISOLATION AND THERMOTOLERANT CHARACTERIZATION OF BLUE GREEN ALGAE FROM SOME HOT SPRING AREAS IN THE UPPER PART OF NORTHERN THAILAND

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Thermotolerant blue-green algae have special properties and their products can be utilized for industrial, medicinal and agricultural purposes. The purpose of this research was to isolate blue-green algae from hot spring as axenic culture and study their characteristics. The samples were collected from 9 hot springs in the upper part of northern Thailand, that have temperatures ranging from 40° to 80°C and pH levels from 7 to 10. All samples were cultivated on BG-11 medium at 30°, 50° and 70°C under fluorescent light. Eight strains of blue-green algae were isolated in axenic cultures i.e. *Chroococcidiopsis thermalis* Geitler, *Leptolyngbya* sp., *Mastigocladus laminosus* Cohn, *Phormidium* sp., *Pseudanabaena galeata* sensu Anagnostidis, *Synechococcus bigranulatus* Skuja, *Synechococcus lividus* Copeland strain SKP50 and strain DSK74. In order to characterize them, dark chemoheterotrophy, optimum growth temperature and the optimum growth pH level were examined. It was observed that no strains showed chemoheterotrophy. The optimum growth temperatures were 30°C for *Leptolyngbya* sp., *Mastigocladus laminosus*, *Phormidium* sp. and *Pseudanabaena galeata* and 50°C for *Chroococcidiopsis thermalis, Synechococcus bigranulatus*, *Synechococcus lividus* strain SKP50 and strain DSK74. Their optimum growth pH levels were 7 for *Mastigocladus laminosus* and *Phormidium* sp., 9 for *Chroococcidiopsis thermalis, Leptolyngbya* sp., *Pseudanabaena galeata* and *Synechococcus lividus* strain SKP50 and 10 for *Synechococcus bigranulatus* and *Synechococcus lividus* strain DSK74. From examining their optimum temperature for growth, it can be concluded that *Synechococcus lividus* strain SKP50 is thermophilic blue-green algae and the others are thermotolerant blue-green algae.

P158

DIFFERENTIAL EXPRESSION OF CHLOROPLAST GENES IN NITROGEN-STARVED RED ALGA *PORPHYRA YEZOENSIS*

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Phycobilisomes (PBS) are light-harvesting complexes of cyanobacteria and red algae. Imposing nitrogen starvation on cultures of cyanobacteria triggers the rapid and complete degradation of the PBS, which causes the cells to look yellow or bleached. Mutants unable to degrade the PBS during nitrogen-limited growth have been isolated from cyanobactria. The mutant phenotype was complemented with the *nblA* (non-bleaching) gene, which encodes a polypeptide of 59 amino acids. The transcription of *nblA* was dramatically induced by nitrogen starvation. NblA shows no homology to proteins with known function and its exact role in the degradation process is not clear. A homologous gene to *nblA, ycf18*, is also present in chloroplast genome in red algae, but it remains unclear whether the gene is involved in phycobilisome degradation in red algae.

To elucidate the mechanism in regulating phycobilisome degradation in red algae, we investigated the expression pattern of genes for Ycf18 and phycobiliproteins in the red alga, *Porphyra yezoensis*. Gametophytic blades of *P. yezoensis* (TU-1 strain), which were kindly provided from Prof. N. Saga (Hokkaido Univ., Japan), were grown in the modified ESS2 medium containing 2.8 mM nitrate at 15°C on a 10-hr light: 14-hr dark photoregime with illumination from cool white fluorescent lamps (4500 lux). For nitrogen starvation the gametophytes were transferred to the medium without nitrate and incubated under the same conditions. After 5 days the level of phycocyanin, phycoerythrin and chlorophyll in the nitrogen-starved gametophytes decreased to a half of that in the control. Nucleotide sequences of chloroplast DNA in *P. purpurea* were completely identified, but those in *P. yezoensis* are poorly known. Then, we isolated chloroplast genes encoding Ycf18 and phycobiliproteins and identified their structures. Northern blot analysis revealed that the expression of phycocyanin and phycoerythrin genes decreased under nitrogen starvation, while that of *ycf18* was not.

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MOLECULAR EVIDENCE FOR PLASTID ROBBERY IN *DINOPHYSIS*, A DINOFLAGELLATE CAUSING DIARRHETIC SHELLFISH POISONING

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The dinoflagellate genus *Dinophysis* contains species known to cause diarrhetic shellfish poisoning. Although the majority of photosynthetic dinoflagellates have plastids with a characteristic carotenoid, peridinin, most photosynthetic *Dinophysis* species have cryptophyte-like plastids containing not peridinin but phycobilin. We cloned and sequenced nuclear- and plastid-encoded SSU rDNA from three photosynthetic species of *Dinophysis* collected in the marine environment, and performed molecular phylogenetic analyses based on their sequence data. In the tree of nuclear SSU rDNA from dinoflagellates, the monophyletic group of *Dinophysis* was a recently diverged lineage nested with peridinin-containing dinoflagellates. However, in the tree of plastid SSU rDNA, the *Dinophysis* lineage was apparently within the radiation of cryptophytes, and was closely related to a species in the family Geminigeraceae. These data indicate that an ancestor of *Dinophysis* which may have originally possessed peridinin type plastid had lost it subsequently, and had adopted others from a cryptophyte. Gene sequences from the fully integrated plastids of dinoflagellates have been reported to have exceptionally high evolutionary rates. However, the plastid SSU rDNA sequences from the three species were identical, while there were some base substitutions in their nuclear SSU rDNA sequences. Blast search of the DNA database showed that the plastid SSU rDNA sequence of *Dinophysis* is almost identical to that of an environmental DNA clone of a <10 μm sized plankter, possibly a cryptophyte. The present findings strongly suggest that these *Dinophysis* species had engulfed and temporarily retained plastids from cryptophyte.

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STUDY ON RECIPIENT SYSTEM FOR TRANSGENIC MANUPULATION IN *SPIRULINA PLATENSIS* (*ARTHROSPIRA*)

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1. Preparation and regeneration of single cells from axenic *Spirulina platensis* (*Arthrospira*)

We explored a method for preparation of axenic strain of *S. platensis*, filaments were treated with 0.4 % NaClO for 5 min, then were cultured in Zarrouk medium containing Kanamycin, Gentamicin and Neomycin. The final concentration of the three applied antibiotics were 150μg /μL respectively, and were lasted for 48 hr at 35°C. Finally, the filaments were rinsed with Zarrouk medium containing 1.5 mol /L NaCl for 1 min to eliminate the bacteria inside the sheath of *S. platensis*.

By a lysozyme-free method for preparation of single cells from axenic *S. platensis*, filaments of *S. platensis* were washed with Zarrouk medium containing 0.75 mol /L NaCl, and it is showed that many single cells were obtained with regeneration rate of 28.6%.

2. Study on sensitivies of *Spirulina. platensis* to six antibiotics (*Arthrospira*)

The sensitive tests of *S. platensis* to six antibiotics were conducted, and it was showed that *S. platensis* (S_6) was not sensitive to Kanamycin (10-700 μg/mL) and Neomycin (10-300μg/mL). The inhibitory concentration of Gentamicin to S_6 was 300 μg/mL in liquid culture medium. S_6 strain was sensitive to Ampicillin and Streptomycin, the lethal concentration of Ampicillin were 5.0-50.0 μg/mL on solid culture medium, while to Streptomycin, it were 5.0 μg/mL in liquid culture medium and 50 μg/mL on solid culture medium. But S_6 strain was highly sensitive to Chloramphenicol, and were inhibited at 0.1 μg/mL in liquid culture medium, 1.0 μg/mL Chloramphenicol was the lethal concentration to S_6 on solid culture medium. Our conclusive remark indicated that Chloramphenicol would be the most useful antibiotic employed if CAT gene were applied as selection marker for transgenic analysis in *S. platensis*.

3. Effects of electroporation on the ultrastructure of *Spirulina platensis* (*Arthrospira*)

Effects of electroporation on cell ultrastructure of *S. platensis* were also studied. Compared the normal cell, the ultrastructure of electroporated cell exihibited thick cell wall, and disappearance of thylakoid and phycobilisomes. However, ultrastructures of the electroporated cell were recoverable after senen days' treatment. Fresh regenerated sawtooth sheath and thylakoid were observed, and these phenomena for electroporated *S. platensis* cell indicated electroporation would be a valid method of transforming foreign gene into *S. platensis*. **Key word**: *Spirulina platensis*, single cell, regeneration, antibiotics, electroporation, ultrastructure

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THE FRITSCH COLLECTION OF ILLUSTRATIONS OF FRESHWATER ALGAE

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Traditionally used by researchers to assist in identification and for the resolution of taxonomic problems, the Fritsch Collection comprises sheets collating by species over half a million published figures and taxonomic entries on fresh- and brackish-water algae from worldwide distributions. The sheets are organised by genus within the major groups, e.g. Diatoms. The Collection contains floristic information from a variety of papers, not only major works, which have been published worldwide from the eighteenth century to the present day. Constantly updated, the Fritsch Collection illustrates the diverse and changing views that have been or are still held about the identification and taxonomy of many algae. Entries on the sheets are supported by a full author index, and a searchable computer database of citations entered into the Collection is being compiled. Its size and wide scope make the Fritsch Collection a valuable research tool, and its role as a source of data on morphology and taxonomy is being extended by its growing ability to provide other types of information, such as data relating to geographical distribution. We appreciate receiving authors' reprints, which help to keep us up to date in their areas of algal research.

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THE USE OF CRYOPRESERVATION TO CONSERVE A LIVING RESOURCE: THE COBRA PROJECT

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The primary remit of Biological Resource Centre's (BRC's) worldwide is to act as depositories of "biological resources" (cell-lines, microorganisms, plants, animals etc.) and to make the material and its associated data accessible for end-users, including those in the biotechnology sector. In all such collections the maintenance of the organisms in a viable, secure, unchanged state is fundamental to the successfully fulfilling their primary remit. The COBRA project (The COnservation of a vital european scientific and Biotechnological Resource: microAlgae and cyanobacteria) is a recently launched European Union, RTD Infrastructures project (Contract No. QLRI-CT-2001-01645) which has the objective to develop a physical and virtual European Biological Resource Centre based on existing algal culture collections. COBRA's central aim is to apply cryopreservation methodologies to currently "preservation recalcitrant" strains of microalgae and cyanobacteria. Although cryopreservation has been found to be effective as a preservation technique for some algal and cyanobacterial strains many organisms have, to date, proved difficult or impossible to conserve using conventional cryogenic methods. In addition, molecular and biochemical stability tests will be developed to ensure that the equivalent strains of microorganisms supplied by the culture collections will give high quality and consistent performance. Fundamental and applied knowledge of stress physiology will be an essential component of the project and will assist the optimisation of methods for preserving a wide range of algal diversity. COBRA's "Resource Centre" will utilise Information Technologies (IT) and Knowledge Management practices to assist project coordination, management and information dissemination and to facilitate the generation of new knowledge pertaining to algal conservation. This project will, for the first time utilize cryopreservation as a standard technique to ensure the genetic stability of conserved strains of algae, thus providing the European biotechnology community with the organisms they require, as well as guaranteeing their long-term availability and stability.

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MICROBIAL CULTURE COLLECTION AT NIES: MICROALGAL RESOURCES FOR SCIENCE, INDUSTRY AND THE ENVIRONMENT

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The Microbial Culture Collection at the National Institute for Environmental Studies (NIES-Collection) was founded in 1983 as the first collection center of microorganisms in relation to environmental science in Japan.

At present, NIES-Collection has more than 1,000 strains in total (including unopened strains) and has become one of the largest algal culture collections in the world. Although it had a starting point as the collection of microalgae which cause red tides, water blooms or pollution, and toxin reproduction, it now covers almost all classes of algae: Cyanophyceae (193 =number of opened strains), Glaucophyceae (4), Rhodophyceae (4), Cryptophyceae (36), Chrysophyceae(7), Raphidophyceae (40), Bacillariophyceae (45), Phaeophyceae (1), Xanthophyceae (2), Haptophyceae (8), Dinophyceae (60), Euglenophyceae (7), Chlorarachniophyceae (1), Prasinophyceae (21), Chlorophyceae (271), Trebouxiophyceae (20), Charophyceae (147), Ulvophyceae (3), and Chlorophyta *incertae sedis* (5).

Most of the strains in the NIES-Collection are maintained by subculture, but this method takes a lot of spaces and manpower, and growth conditions must be strictly monitored. Moreover, it sometimes causes changes in morphology, physiology or genetic characteristics of strains. In order to avoid such conditions, we have developed more stable preserving method for microalgae. In 2002, 169 strains of cyanobacteria are already preserved in a frozen condition.

Strains of the NIES-Collection are supplied worldwide as research material and well utilized in both basic and applied fields. In recent five years, more than 400 strains have been distributed per year. They are used not only for research of environmental science (*e.g.*, AGP test, developing of water-bloom inhibitor, toxin analysis, and so on), but also as materials for physiological analyses (growth characteristics and metabolism), or targets for new useful substances. Some are also used for educational purposes. Recently gene sequencing studies and phylogenic analysis of strains are markedly increasing and becoming to one of the important activities for the culture collection too. We are engaged in these studies in order to clarify the taxonomic position of our strains that are still unidentified.

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A REPORT ON THE EXHIBITION "MESSAGE FROM MARINE FORESTS" HELD IN AN INLAND PREFECTURE

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The people living in an inland area such as Tochigi prefecture have little information on the sea although they have a hankering after the sea. Therefore, it is natural that almost all the people of this prefecture are not informed of the existence of the marine forests, which have productivity larger than that of the forests on land. However, the people of Tochigi prefecture are related with the sea since many rivers rising from this area affect on the environment of the sea, while the people receive the effects and the favors from the sea as residents on the planet of water as well as the Japanese, a nation of ocean. The Exhibition "A Message from Marine Forests", which was planned mainly for the people of Tochigi prefecture, also proposed a new items of exhibition and experience for the whole nation. One of features of the Exhibition was the presentation of wonderful specimens of *Laminaria* species. Although *Laminaria* species had been difficult to be made up in to specimens since the plant of every species is too long to be set on a board, we could make the specimens of them similar in shape to living plants using resin and Japanese lacquer. A plant, water in which was replaced with resin, was lacquered with Japanese. The Japanese lacquered specimens of 20 species or more of *Laminaria* were exhibited on a wall. Another feature of the Exhibition was the beauty of seaweeds. Many wonderful works of pressed algal designs and botanical arts were exhibited, and a short course was held every day to make a beautiful postcard or bookmark with pressing pieces of various seaweeds. The short course as well as the exhibitions on the beautiful seaweeds attracted attention of many people.

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PRESERVATION OF FRESHWATER RED ALGAE INCLUDING 3 ENDANGERED SPECIES

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Species of the freshwater red algae distinctively inhabit clean, cool and shaded streams. They are known as delicate organisms easily influenced by agricultural chemicals and environmental changes such as increase of irradiance due to clear cutting of riparian vegetation and eutrophication. Thus, THE THREATENED WILDLIFE OF Japan –RED DATA BOOK- edited by the Environment Agency of Japan nominated 26 species of the freshwater red algae as endangered species.

We started the presearvation of the freshwater red algae in 2001, in addition to another endangered group, the charophytes. We focused on three species, Nemalionopsis tortousa, Thorea okadae and Compsopogonopsis japonica at the moment, the occurrence and the distribution of which have been confirmed based on the previous records. Collected samples were tentatively maintained in an open tank with artificial water current and used for experiments. Three species grew successfully in BOLD3N medium at 15-20°C under the 1000-2000 Lux (12:12 LD cycle). Preliminary experiments of the cryopreservation were conducted using fresh field samples. Both T. okadae and N. tortuosa showed relatively high viability under the standard condition, i.e. 5-20% of DMSO as cryoprotectant, freezing till -40°C at -1°C/min, keeping 15 min at -40°C, then stored in the liquid nitrogen. While, C. japonica was always damaged by freezing and did not show any viability, though this species could be easily maintained by inoculation of monospore. The present work suggests a possibility of ex situ conservation of the endangered freshwater red algae.

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ALGAL EDUCATION AT THE COMPULSORY LEVEL IN JAPAN

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Algal names were surveyed in five major science textbooks for elementary schools and junior high schools in Japan, which were first published in the early 1950s and thereafter revised almost every 10 years in conformity with the official science curriculum revision. In textbooks for elementary schools, the number of algal species was largest in the 1962 version and thereafter decreased steeply. No seaweed appeared after 1971. In junior high school textbooks, the number of algal species was largest in the 1972 version, and thereafter decreased but not so much as in the elementary school textbooks. In any case, the number of seaweeds has decreased, and in the last ten years seaweeds are briefly taught only in taxonomy. According to the newly revised science curriculum which is being enacted this year, algae will not be taught in elementary schools and seaweeds will not be taught at the compulsory level.

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APPLICATION OF THE FDA METHOD TO MEASURE SURVIVAL RATES OF MICROALGAE AFTER FREEZING AND THAWING

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At the NIES-Collection, Tsukuba, Japan, we have conducted experiments on the cryopreservation of cyanobacteria and green algae using a two-step cooling method employing dimethyl sulfoxide as a cryoprotectant. Because high levels of cell viability after thawing reduce the risk of the undesirable selection of freeze-tolerant subpopulation, viability estimation using a convenient and reliable method is routinely required. In a previous study using a fluorescein diacetate (FDA) staining method, which measures esterase activity, we found that 166 strains of cyanobacterial strains examined had post-thaw viabilities higher than 60%. This was approximately equal to the values obtained on directly measuring cell growth. Thus, 166 strains of cyanobacteria are currently maintained only by cryopreservation at NIES. However, viability levels determined by the FDA method and by cell growth were not consistent in green algae, with some strains not surviving after freezing and thawing (as determined by re-growth) although viabilities measured by FDA method just after thawing were high.

In this study, we examined the viability of 6 strains, 3 cyanobacteria, *Microcystis wesenbergii* (NIES-111), *Spirulina subsalsa* (NIES-527) and *Synechococcus* sp. (PS-767), and 3 green algae, *Chlorella vulgaris* (NIES-227), *Coelastrum astroidum* (NIES-342) and *Scenedesmus acutus* (NIES-94) using FDA method at time intervals from 0 to 44 hours after freezing and thawing. The viabilities were constant with time in all cyanobacteria examined. However, the viability levels of green algae decreased to very low levels 5.5 hours after thawing. The data obtained suggests that viability of green algae should be measured at least 6 hours after thawing when using the FDA method.

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PRELIMINARY APPLICATION OF DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE) TO THE ANALYSIS OF LICHEN PHOTOBIONT

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Lichen is composed of a lichenized fungi and photobiont(s). It is known that the combination of lichen symbionts is not always specific, although the nature of their combinations and/or photobiont diversity in lichen has not been well studied. Because the method of isolation-culture of lichen photobionts takes time and needs a large space and equipments, few researchers tried to investigate many samples of photobionts. Therefore, we study the application of Denaturing Gradient Gel Electrophoresis (DGGE) method to analysis of many samples of lichen photobionts without culture.

Materials for this study are Trebouxia sp. (ST-YO4774) isolated from Usnea bismolliuscula (YO4774), lichen thalli of Usnea bismolliuscula (YO4774) and Rimelia clavurifera (YO4777), and Prorocentrum dentatum (K117). Total DNA of samples were extracted by Benzyl chloride method. PCR products for the DGGE analysis were obtained by the following primer sets: "Euk1A" and "Euk516r-GC" are for the parts of 18S rDNA both lichenized fungi and photobiont; "ITS1-T" and "ITS4-GC" for the ITS regions in rDNA of photobiont, and "ITS1-F" and "ITS4-GC" for lichenized fungi. DGGE was performed using a DCode system (Bio-RAD) with 8% acrylamide gel (20 to 70% denaturant gradient) in 0.5X TAE for 18 hr at 35V. After running, the Gel was stained by the SybrGreen nucleic acid stain, and then it was observed under UV light. As the result, parts of 18S rDNA of ST-YO4774, YO4774 and YO4777 show the same band position, but that of K117 shows different position in DGGE although the length of PCR product is the same as ST-YO4774, YO4774 and YO4777. The bands of algal ITS regions of ST-YO4774, YO4774 and YO4777 also show the same position, but that of K117 and fungal ITS regions of YO4774 and YO4777 show the different position. Thus, the DGGE method can detect different taxa as the different bands in DGGE.

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UNIVERSITY OF MALAYA ALGAE CULTURE COLLECTION (UMACC)

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The University of Malaya Algae Culture Collection (UMACC) is the only culture collection of microalgae in Malaysia, consisting of more than 150 microalgal isolates. It serves as a national collection of microalgae and supplies selected cultures to both the research community and the industry. The collection consists of unialgal cultures, of which many are axenic, representing strains from different families, including Cyanophyceae, Chlorophyceae, Prasinophyceae, Euglenophyceae, Haptophyceae and Bacillariophyceae. Many are indigenous strains, isolated from diverse aquatic habitats of Malaysia, ranging from freshwater lakes, wastewater ponds, mangrove swamps, coastal waters to estuaries. There are also several strains of aerial microalgae isolated from wall scrapings (e.g. *Chlorococcum*). In addition, some cultures deposited in the UMACC are from other Culture Collections such as CCAP, CCMP, CSIRO and NIES.

Many of the microalgae in the UMACC have been used in various studies conducted by the Algal Biotechnology Group of the University of Malaya, and these include the following :

1) screening of the microalgae for high-value chemicals such as polyunsaturated fatty acids, carotenoids and phycobiliproteins.
2) use of the microalgae for treatment of agro-industrial wastewaters such as rubber and palm oil mill effluents, and sago factory wastewater.
3) use of the microalgae as biomonitors for heavy metal pollution and nitrogen enrichment in freshwater ecosystems.
4) use of the microalgae as mosquito larvicidal agent.
5) use of the microalgae as aquaculture feed.

The UMACC has expanded with the recent addition of 15 isolates of Antarctic microalgae. The microalgae were isolated from snow, seawater and soil samples, and water from waste treatment pond collected around Casey Station, Antarctica during the expedition to there last year. Characterisation of the morphology and growth of the Antarctic microalgae are in progress. This first Malaysian collection of Antarctic microalgae is useful for our studies on the physiological adaptation of such microalgae under extreme conditions, especially in comparison to tropical species, in attempts to understand impacts such as global warming and increased UV radiation.

The UMACC is important for the development of research in algal biotechnology in Malaysia. Our future research trends are directed towards genomic studies, which include research on phylogenetics, microarray analysis and genetic transformation.

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MICROALGAL CULTURE COLLECTION OF MARINE BIOTECHNOLOGY INSTITUTE CULTURE COLLECTION (MBIC)

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Since 1989, Marine Biotechnology Institute (MBI) conducted many research projects financed by the Ministry of Economy, Trade and Industry (METI), New Energy and Industrial Technology Development Organization (NEDO), Research Institute of Innovative Technology for the Earth (RITE), and 24 member companies of MBI. During these research projects, MBI made many microbiological sampling trips, isolated diverse microorganisms from marine and/or salt water environments and preserved them. For example, nine sampling trips on the research vessel Sohgen Maru were conducted between 1989 and 1995 to visit Australia, Palau and other countries. Currently, more than thirty thousand marine bacterial and microalgal strains are preserved in MBI. We selected scientifically interesting strains of our isolates to establish the MBI culture collection (MBIC). The URL of MBIC is http://www.mbio.co.jp/mbic.

Currently, MBIC microalgal strains are all original isolates from the Japanese coast, international waters, the Antarctic Ocean and coast and territorial waters of the Republic of Palau. The MBIC got the acknowledgement about distribution of strains which were collected from coast and territorial waters of the Republic of Palau by the government of Republic of Palau. Presently, the MBIC catalog contains about 350 strains of microalgae. The strain numbers will increase periodically. The MBIC catalog is available on-line. Scientific name, sampling information, phenotypic characteristics, culturing conditions and publication information are shown about each strain. The contents of the catalog are searchable. Micrographs of each strain for morphological information can be viewed. In addition, SSU rDNA sequences of many strains are included in the catalog.

We distribute MBIC strains only for research purposes under Material Use Agreement (MUA). The MUA fixes in writing the rights and obligations governing the distribution of MBIC strains.

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DETECTION OF MICROCYSTIN SYNTHETASE GENES FROM JAPANESE STRAINS OF *MICROCYSTIS*.

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Microcystis spp. are among the most major toxic cyanobacteria found in water blooms. *Microcystis* spp. produce lots of variants of microcystins, which are hepatotoxic cyclic heptapeptides that are of worldwide concern due to their toxicity to both human and animals. Genes responsible for microcystin synthesis had been identified as a polypeptide/polyketide synthetase gene cluster, consisting of 10 genes (mcyA-J) that sequentially catalyze peptide chain elongation and modification. These genes had been indicated to be useful as a target to identify toxic strains of *Microcystis*. Here we investigated 17 Japanese isolates (NIES strains) of *Microcystis* for the presence or absence of microcystin synthetase genes. Using previously reported primers directed to amplify mcyA and mcyB, PCR experiments successfully detected these genes from all toxic strains (4 strains), but did not from non-toxic strains (11 strains) with two exceptions. This result suggests a potential utility of these primers for genetically discriminating toxin strains among those recovered from Japanese water environments. Phylogenetic analyses based on these two protein (mcyA, mcyB) and PC-IGS (Phycocianin Intergenic Spacer) sequences indicate some discordance between organismal phylogeny and toxin gene genealogy of Japanese isolates as well as those from other countries, which implicate possible dynamic exchange of microcystin synthetase genes among diverse populations of *Microcystis*.

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TOXIC CYANOBACTERIA IN LAKE BIWA AND THE PRODUCTION OF HEPATOTOXIC MICROCYSTINS

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In recent years, water bloom caused by cyanobacteria occurs in eutrophic lakes. Water bloom is also observed in Lake Biwa, especially in its south basin. It has been reported that cyanobacteria contains a heptapeptide toxin called microcystin. The main target organ of the toxin is the liver and the recent studies have demonstrated that microcystins have a potent tumor-promoting activity. In this research, water samples were taken from Lake Biwa and the seasonal variations of microcystin concentration and toxic cyanobacteria in Lake Biwa were investigated. Moreover, cyanobacteria strains were isolated from Lake Biwa and the toxin production by the cyanobacteria strains was investigated.

The microcystin was detected from Lake Biwa water and its filtrate. The concentrations were from 90 to 250pg/mL in July and August, and then it increased to 4,400pg/mL in September. At that time water bloom was observed around the sampling point. Regarding the cyanobacteria *Microcystis* species in Lake Biwa, *M. aeruginosa* and *M. wesenbergii* were mainly identified. Although *Microcystis* species did not increase extremely in July and August, the species increased in September together with the cyanobacteria *Anabaena* species in Lake Biwa.

On the isolated *Microcystis* strains, *M. aeruginosa*, *M. ichthyoblabe*, *M. novacekii*, and *M. wesenbergii* were isolated from Lake Biwa. The microcystin was detected from most of *M. aeruginosa* and *M. novacekii* strains. However, microcystin was not detected from all the *M. wesenbergii* strains. Among the *Anabaena* species, *A. affinis*, *A. crassa*, and *A. flos-aquae* were isolated from Lake Biwa. Microcystin was not detected from all the *Anabaena* strains, which demonstrated that *Anabaena* species do not contribute to the microcystin production in Lake Biwa.

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TOXIC EFFECTS OF TWO CHEMICALS ON MICROALGAE CONTAMINATED IN *PORPHYRA* CONCHOCELIS CULTURE

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The toxic effects of two chemicals on four kinds of bluish microalgae often contaminated in cultures of free-living conchocelis of *Porphyra yezoensis* Ueda were studied. After being treated with the chemicals for 24 and 48h, LC50 to four kinds of microalgae and *P. yezoensis* conchocelis was evaluated to be significantly different. The endurance of the microalgae and conchocelis to the chemicals is in the order of microalga-2 < microalga-3 < microalga-1 < microalga-4 < *P. yezoensis* conchocelis. After the microalgae were completely killed by the chemicals, *P. yezoensis* conchocelis filaments were picked out, washed with seawater and transferred into new culture medium for confirming their recovery. The results showed that all of the conchocelis filaments treated with the chemicals grew well in culture of 10 days, indicating that the two chemicals can effectively remove the contaminated bluish microalgae and keep on the survival of *P. yezoensis* conchocelis. These chemicals can be safely applied to prevent contamination of the bluish microalgae in culture of free-living conchocelis of *Porphyra*.

P174: ISOLATION, PROPERTIES AND SPATIAL SITE ANALYSIS OF γ SUBUINITS OF B-PHYCOERYTHRIN AND R-PHYCOERYTHRIN

Wang, G*; Hu, S; Tseng, CK
Chinese Academy of Sciences, CHINA

Polysiphonia urceolata R-phycoerythrin and *Porphyridium cruentum* B-phycoerythrin were degraded with proteinase K, and then the nearly native γ subunits were isolated from the reaction mixture. The process of degradation of phycoerythrin with proteinase K showed that the γ subunit is located in the central cavity of $(\alpha\ ß)_6$ hexamer of phcoerythrin. Comparative analysis of the spectra of the native phycoerythrin, the phycoerythrin at pH12 and the and 565 nm, the fluorescence emission maximum at 580 nm; the absorption peak of phycoerythrobilins on the isolated r subunit is at 589 nm, the fluorescence emission peak at 620 nm which overlaps the absorption maximum of C-phycocyanin and perhaps contributes to the energy transfer with high efficiency between phycoerythrin and phycocyanin in phycobilisome; the absorption maximum of phycourobilin on the isolated r subunity is at 498 nm, which is the same as that in native phycoerythrin, and the fluorescence emission maximum at 575 nm. Meanwhile, the gene encoding the γ subunit was screened and cloned into pBluescript-SK, and then sequenced. The sequence of gene shows 2 reversed repeats and 627bp in length.

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