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Growth rates of *Gracilaria* species (Gracilariales, Rhodophyta) from Tosa Bay, southern Japan

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The daily growth rates (DGR, % increase in wet weight day^{-1}) of *Gracilaria chorda, G. gigas, G. "verrucosa", G. incurvata* and *G. textorii* from Tosa Bay, southern Japan were measured. Intact, young unbranched fronds arising from new discoid basal discs were collected from natural attached populations of *Gracilaria* and grown for two months in net cages suspended in the bay. DGRs of these fronds varied during the culture period but were generally 2–4% day^{-1}, and grazers were a problem. Growth response to seawater temperature (10–33°C) was investigated by growing segments of thalli of each species for two weeks in a closed-circulating system (aquatron). *G. chorda* showed its maximum DGR at 15°C (3.82 ± 1.00% day^{-1}), while *G. gigas* (4.74 ± 1.02% day^{-1}), *G. incurvata* (4.19 ± 1.16% day^{-1}) and *G. textorii* (2.91 ± 0.70% day^{-1}) showed their maximum DGR at 20°C. *G. "verrucosa"* showed its maximum DGR (1.54 ± 0.63% day^{-1}) at 18°C, being lowest among the species investigated, and did not exhibit a clear response to temperature. *G. "verrucosa"* showed growth at 30 (0.64 ± 0.30% day^{-1}) and 33°C (0.79 ± 0.33% day^{-1}).

Key Index Words: *agarophytes*—Gracilaria—*growth rate*—*seasonality*—*southern Japan*—*temperature.*

Species of *Gracilaria* are major sources of the phycocolloid, agar (Santelices and Doty 1989, McLachlan and Bird 1986). They are distributed world-wide, and 16 species have been reported from Japan (Yamamoto 1978).

Six species of *Gracilaria* occur in Uranouchi Inlet of Tosa Bay in Shikoku, southern Japan. Five of them, *Gracilaria chorda* Holmes, *G. gigas* Harvey, *G. "verrucosa"**, *G. incurvata* Okamura and *G. textorii* (Sur.) DeToni, represented more than 90% of the total macroalgal standing stock in Uranouchi Inlet during the growing season in January 1987 to December 1988, reaching a maximum value of 1,296.5 g (dry wt) m^{-2} in May 1987 (Orosco and Ohno, in press).

We are presently doing ecophysiological and biochemical studies on these *Gracilaria* species. In this paper, we report on growth rates of the five species when cultured in net cages in the field and in a temperature-controlled, closed-circulating system (aquatron).

Materials and Methods

Two growth-rate experiments using the three terete species (*G. chorda, G. gigas* and *G. "verrucosa"*) and the two flabellate species (*G. incurvata* and *G. textorii*) were carried out. All samples were collected from natural populations of *Gracilaria* in Uranouchi Inlet of Tosa Bay. Attached thalli were collected, transported to the laboratory in seawater-filled buckets and kept in running seawater.

Outdoor cage culture. For each species, five to eight healthy, intact, young unbranched fronds arising from new discoid basal discs were cleaned of sediments and epiphytes, tagged and placed in two nylon net-covered

* The taxonomic status of different populations referred to as *G. verrucosa* is uncertain (Abbott *et al.* 1985). We use this name based on the recommendation of Yamamoto and Sasaki (1988) to continue using this name for the Japanese taxon until the status of this species is resolved by crossing experiments with other Japanese populations.

cages ($10 \times 20 \times 20$ cm). The cages were hung from a raft in the harbor to a depth of 0.5 m. Wet weight of individual thalli was measured weekly. Thalli were cleaned of epiphytes and debris during each measurement. This experiment was carried out for a period of 56 days (Jan. 31, 1987 *et seq.*). Growth-rate data are expressed as the mean±S.D. of five to eight thalli. Seawater temperature, salinity, and nutrients (phosphate-phosphorus, nitrate-, nitrite- and ammonium-nitrogen) were measured during each sampling period. Nitrogen is expressed as dissolved inorganic nitrogen (DIN=nitrate+nitrite+ammonium). Salinity was measured using a conductivity meter, while nutrients were determined by colorimetric methods (Meteorological Agency 1970).

Indoor culture. This experiment was done to study the effect of seawater temperature on the growth rate.

One- to two-gram cuttings of apical portions were prepared from the collected samples and were kept in flowing seawater for 24 h before start of the experiment. Fifteen cuttings for each species, except for *G. textorii*, were inserted between the braids of rope hanging from a glass rod. These ropes were weighed down by another glass rod. *G. textorii*, because of its brittle and wide, flat thallus, was tied to the ropes by a thread passing through the thallus near its cut end. Samples were cultured in a temperature-controlled closed-circulating system, aquatron (Fig. 1, Ohno 1977). Medium used was plankton net-filtered seawater (450 *l*) from the bay. Light was provided by white fluorescent tubes at a 12 : 12 h light : dark cycle at a photon fluence rate of 65 $\mu E\, m^{-2} sec^{-1}$. The system was run for 24 h at the set temperature before the start of the experiment. Temperatures tested were 10, 12, 15, 18, 20, 22, 25, and 28°C. Growth rates of *G. "verrucosa"* were measured also at 30 and 33°C. Wet weight of each sample was taken after the 14-day incubation period. Data represent the mean±S.D. for 15 samples. This experiment was carried out from April 19 to July 8, 1987.

Daily growth rate (DGR, % day^{-1}) was calculated using the formula of Rosenberg and Ramus (1981): $DGR = 100\ (\ln n_t/n_o)t^{-1}$,

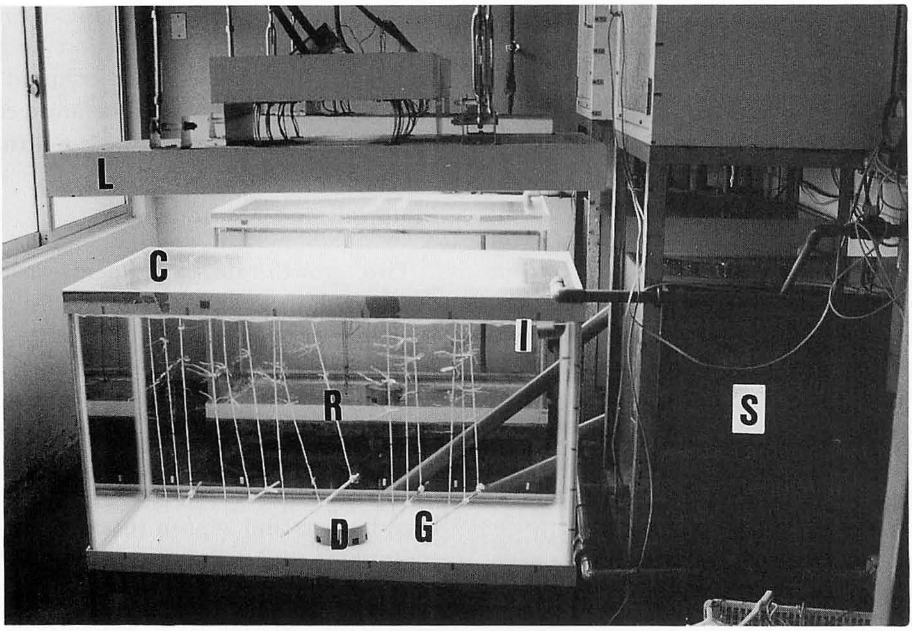

Fig. 1. Closed-circulating system (aquatron) used in the temperature-growth study. C, plexiglass cover; D, drain; G, glass rod; L, light panel; I, water inlet; R, rope; S, sand filter (recirculation tank).

where n_0 is weight at the beginning of each period, n_t is weight after t days, and t is the number of days.

Results and Discussion

Ambient seawater temperature, salinity and phosphate-phosphorus varied little during the experimental period. DIN had slightly higher values in March owing to increase in the three nitrogen species measured (Fig. 2).

In the outdoor cage-culture experiment, *G. textorii* showed the highest long-term DGR over the 56-day culture period, 4.47±0.51% day^{-1}, followed by DGR of *G. "verrucosa"*, 3.86±0.85% day^{-1}. *G. chorda* and *G. incurvata* had similar DGRs, 2.97±1.36 and 2.96±0.67% day^{-1}, respectively, while *G. gigas* had the lowest DGR, 2.07±0.32% day^{-1} (Figs. 3 & 4).

However, daily growth rates varied from week to week during the 56-day culture period. *G. textorii* exhibited the highest short-term DGR of 7.90±1.26% day^{-1} at the beginning, which, however, continually decreased to a lowest DGR of 2.87±0.80% day^{-1} at the end of the culture period. *G. incurvata* had a DGR of 5.32±0.89% day^{-1} during the first week but the rate decreased to about 60% of the initial rate (3.20±1.84% day^{-1}) in February, and in March the rate was only 26-37% of the initial DGR. *G. "verrucosa"* had a high DGR during the first week of culture, but the rate decreased and remained at about 50% of the initial DGR until the beginning of March; then the rate decreased further. The DGR of this species ranged from 0.76±0.91 to 6.86±4.76% day^{-1}. *G. gigas* had the lowest DGR, 1.76±0.86 to 2.56±0.52% day^{-1}, among the five species. Over the two-month period, however, the rate was never less than 80% of the initial DGR. *G. chorda* had growth rates, equal to or slightly higher than the initial DGR (3.37±1.98% day^{-1}) in February, but in March DGR was only 62-87% of the initial rate. DGR ranged from 2.04±0.77 to 3.70±2.01% day^{-1}.

One problem encountered during the field growth study was grazing of the samples by

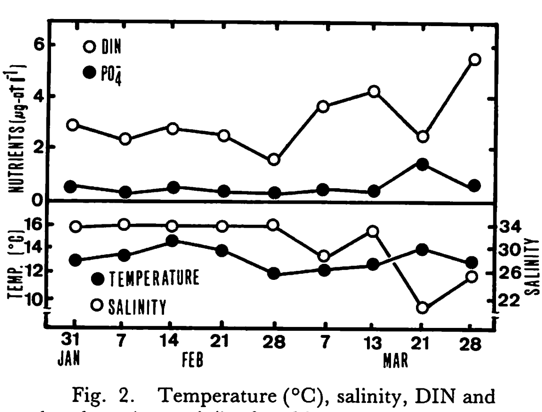

Fig. 2. Temperature (°C), salinity, DIN and phosphate (µg-at l^{-1}) of ambient seawater during the outdoor cage culture experiment.

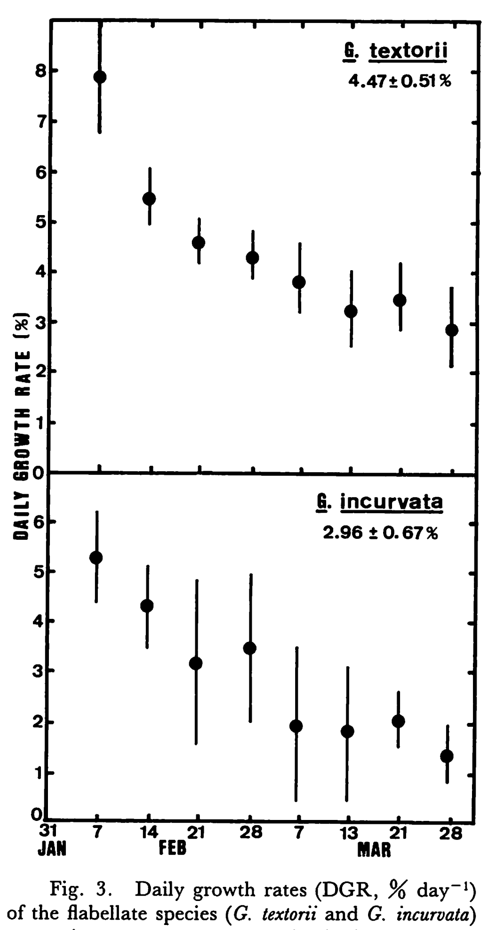

Fig. 3. Daily growth rates (DGR, % day^{-1}) of the flabellate species (*G. textorii* and *G. incurvata*) grown in net cages suspended in the bay. Plotted values represent the mean±S.D. at weekly intervals (t=7). Daily growth rate over the whole culture period (t=56) is given in the upper right-hand corner of each graph.

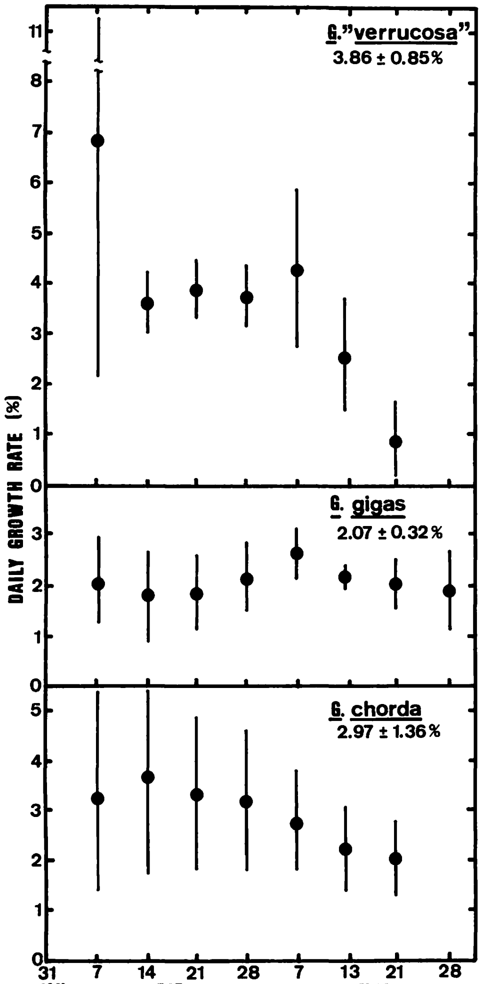

Fig. 4. Daily growth rates (DGR, % day^{-1}) of the terete species (*G. chorda, G. gigas* and *G. "verrucosa"*) grown in net cages suspended in the bay. Details are the same as in Fig. 3.

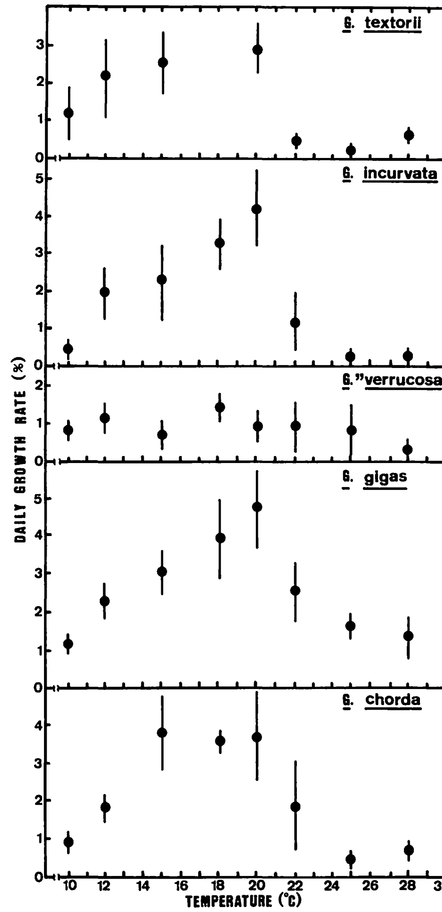

Fig. 5. Daily growth rates (DGR, % day^{-1}) of *Gracilaria* species in response to seawater temperature when grown in an aquatron for 14 days. Plotted values represent the mean ± S.D.

copepods. Van Dover and Kirby-Smith (1979 in Rosenberg and Ramus 1981) noted that the amphipod *Caprella penantis* occurred among the branches of *Gracilaria* but it did not consume the host seaweed. During our field growth experiments, some copepods built their "homes" on the thalli by cementing silt around the main axis with a fibrous material and lived in the space between the thallus and the silt. Removal of these homes by forceps revealed white grazing marks on the thallus where the medulla had been exposed. Most susceptible to this attack was *G. "verrucosa"*. Some animals were also found on *G. gigas* and the fewest number was found on *G. chorda*. When these animals were found on *G. textorii* and *G. incurvata*, they formed patches of silt on the upper surface of the thallus. These structures were well-attached to the thallus and could not be removed by washing with seawater; they had to be forcefully removed by forceps. Heavy infestation resulted in fragmentation of the thallus, although in some instances the broken branches were cemented together by the silt structures.

In the aquatron experiment, growth rates increased with increase in temperature from 10 to 20°C and decreased when temperature exceeded 20°C (Fig. 5). *G. chorda* had its highest DGR at 15°C (3.82±1.00% day^{-1}), while *G. gigas* (4.74±1.02% day^{-1}), *G. incurvata* (4.19±1.16% day^{-1}) and *G. textorii* (2.91±0.70% day^{-1}) had their maximum DGR at 20°C. *G. gigas* was the most responsive to temperature, while *G. "verrucosa"* did not show a clear response to temperature although the maximum DGR occurred at 18°C (1.54±0.63% day^{-1}). The maximum DGR was lowest in *G. "verrucosa"* among the species investigated. The growth of *G. "verrucosa"* was also measured at 30 and 33°C as its thalli were found throughout the year in the intertidal zone where the temperature attains 30°C or more. DGR of this species was 0.64±0.30% day^{-1} at 30°C and 0.79±0.33% day^{-1} at 33°C.

Our previous study on the seasonal abundance of natural populations of the same *Gracilaria* species in Uranouchi Inlet (Orosco and Ohno, in press) showed changes in biomass corresponding to changes in seawater temperature. Sporelings and new growth from perennating holdfasts or stumps are observed in late autumn to early spring when seawater temperature is 13-15°C. There is a large biomass from March to June-July, when temperature increases to about 25°C; however, biomass peaks in April-May (15-18°C). Senescence occurs after the reproductive season when water temperatures are above 23°C. Plants pass the summer as holdfasts, stumps, or spores attached to substrata covered by sand; *G. "verrucosa"* in the upper intertidal area, however, continues to grow even when water temperature is 28-30°C or slightly higher (Orosco and Ohno, in press). The present results from the aquatron experiment on *G. "verrucosa"* showed that growth is possible at 30 and 33°C. In the natural habitat, temperatures as high as these values may be reached as the sites are located in intertidal areas which are often exposed during low tides.

Thus, the growth response of the five *Gracilaria* species to temperature in the indoor culture experiment coincides well with the natural seasonal growth cycle of *Gracilaria* species in Uranouchi Inlet of Tosa Bay. Except for *G. "verrucosa"*, growth rates increase as temperature increases towards the optimum at 15-20°C above which growth rates decrease and senescence occurs.

Growth rates of *Gracilaria* species in this study are slightly lower than the general values of 5-10% day^{-1} compiled by McLachlan and Bird (1986). Short-term DGRs in the outdoor cage culture were lower than the maximum growth rates obtained in the aquatron for *G. chorda, G. incurvata* and *G. gigas*, although growth rates in the outdoor cage culture were expected to increase in the later months as the optimum temperature for growth had just started at the termination of the experiment.

G. textorii is the least tolerant to high temperature. In the aquatron, there was a drastic decrease in growth rate at temperatures higher than 20°C. It has the shortest growth period in Uranouchi Inlet; it is usually found only until June when seawater temperature reaches 22°C (Orosco and Ohno, in press).

Temperature for optimum growth of *G. "verrucosa"* in these experiments is generally lower than that of *G. verrucosa* from the Philippines (optimum: 25-30°C) grown in the laboratory between 15 and 30°C (Hurtado-Ponce and Umezaki 1987). Growth rates of *G. verrucosa* in the intertidal (5.0-16.4% day^{-1}) and in an abandoned fishpond (1.5-8.4% day^{-1}) at temperatures of 28-32°C in the Visayas, Philippines (Largo *et al.* 1989) were relatively higher than the growth rates we obtained at temperatures above 20°C. Further comparison of growth rates of *G. verrucosa* is difficult because of the uncertain status of the species from other localities (Abbott *et al.* 1985).

Based on the experimental assessment of the geographic distribution of *Gracilaria* species in relation to temperature (McLachlan and Bird 1984), the species from Tosa Bay, southern Japan still seem to belong to the temperate-water species which showed maximum growth rates at 15 or 20°C, although none of the species reported in this paper was included.

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References

Abbott, I. A., Chiang, Y. M., Fredericq, S., Norris, J. N., Tsuda, R. T., Bangmei, X. and Yamamoto, H. 1985. The red alga *Gracilaria* Greville (Gracilariaceae, Gigartinales): Introduction. p. 67–68. *In*: I. A. Abbott and J. N. Norris (eds.), Taxonomy of Economic Seaweeds. California Sea Grant College Program, La Jolla, California.

Hurtado-Ponce, A. Q. and Umezaki, I. 1987. Growth rate studies of *Gracilaria verrucosa* (Gigartinales, Rhodophyta). Bot. Mar. **30:** 223–226.

Largo, D. B., Bacolod, P. T., Cusi, M. A. V., Orosco, C. A. and Ohno, M. 1989. Growth rates of *Gracilaria verrucosa* and *Gracilaria salicornia* (Gracilariales, Rhodophyta) in an intertidal and semi-enclosed pond system in the Visayas, Philippines. Bull. Mar. Sci. Fish., Kochi Univ. **11:** 95–100.

McLachlan, J. and Bird, C. J. 1984. Geographical and experimental assessment of the distribution of *Gracilaria* species (Rhodophyta: Gigartinales) in relation to temperature. Helgol. Meeresunters. **38:** 319–334.

McLachlan, J. and Bird, C. J. 1986: *Gracilaria* (Gigartinales, Rhodophyta) and productivity. Aquat. Bot. **26:** 27–49.

Meteorological Agency 1970. Kaiyo Kansoku Shishin. The Oceanographical Society of Japan, Tokyo, 432 pp. [Manual of Oceanographic Methods].

Ohno, M. 1977. Effect of temperature on the growth rate of seaweeds in an aquatron culture system. Bull. Jap. Soc. Phycol. **25** (Suppl.): 257–263.

Orosco, C. A. and Ohno, M. (in press) Seasonal abundance and growth of *Gracilaria* species (Gracilariales, Rhodophyta) in Tosa Bay, southern Japan. Aquat. Bot.

Rosenberg, G. and Ramus, J. 1981. Ecological growth strategies in the seaweeds *Gracilaria foliifera* (Rhodophyceae) and *Ulva* sp. (Chlorophyceae): The rate and timing of growth. Bot. Mar. **24:** 583–589.

Santelices, B. and Doty, M. S. 1989. A review of *Gracilaria* farming. Aquaculture **78:** 95–133.

Yamamoto, H. 1978. Systematic and anatomical study of the genus *Gracilaria* in Japan. Mem. Fac. Fish., Hokkaido Univ. **25:** 97–152.

Yamamoto, H. and Sasaki, J. 1988. Interfertility between so-called *G. verrucosa* (Huds.) Papenfuss and *G. vermiculophylla* (Ohmi) Papenfuss in Japan. Bull. Fac. Fish. Hokkaido Univ. **39:** 1–3.

C. A. Orosco・大野正夫：日本南岸土佐湾産オゴノリ属海藻の成長速度

日本南岸の高知県土佐湾に生育するオゴノリ属のツルシラモ，オオオゴノリ，オゴノリ，ミゾオゴノリ，カバノリについて，浦の内湾および室内培養によって日成長率 (daily growth rate, DGR) を測定した。湾内では試料をカゴに入れて水深 0.5 m につるして 2 ケ月実験を行ったが，小形甲殻類による食害の影響がみられ，すべての種類において，DGR は，2–4％の範囲であった。室内では循環式恒温水槽（アクアトロン）により水温 10–33°C の範囲で 2 週間培養を行い，成長速度を求めた。ツルシラモでは最大 DGR は 15°C でみられ，3.82±1.00％ であった。オオオゴノリ，ミゾオゴノリ，カバノリでは，最大 DRG は 20°C でみられ，それぞれ 4.74±1.02％, 4.19±1.16％, 2.91±0.70％ であった。オゴノリの最大 DGR は 18°C でみられ，その値は低かった (1.54±0.63％) が，DGR の温度による際は明瞭でなく，30°C では 0.64±0.30％, 33°C では 0.79±0.33％ であった。（781-11 土佐市宇佐町井尻194　高知大学海洋生物教育研究センター）