

Diurnal vertical migration and dark uptake of nitrate and phosphate of the red tide flagellates, *Heterosigma akashiwo* HADA and *Chattonella antiqua* (HADA) ONO (Raphidophyceae)¹⁾

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Diurnal vertical migration of *Heterosigma akashiwo* and *Chattonella antiqua*, which form heavy red tides during the summer periods, when the thermal stratification is striking, was observed in laboratory cultures. These species concentrated at the surface in the daytime and at the bottom at night. The timing of descent and ascent did not coincide exactly with the onset or end of the light phases. Dark uptake by *H. akashiwo* was 41-100% NO_3^- and 43-100% PO_4^{3-} of that in the light, whereas that by *C. antiqua* was 49-100% NO_3^- and 75-100% PO_4^{3-} of that in the light. Nitrate and phosphate uptakes were less affected by light conditions in these species than in coastal diatoms. The combination of diurnal vertical migration and the ability to take up nitrate and phosphate at night probably gives these species double ecological advantages over the coastal diatoms in the thermally stratified waters. The migratory ascent at daytime maintains them in the euphotic zone and the descent at night makes sufficient nutrients available to them from the nutrient-rich bottom water.

Key Index Words: *Chattonella antiqua*; diurnal vertical migration; *Heterosigma akashiwo*; nitrate and phosphate uptakes; red tide.

The raphidophycean flagellates, *Heterosigma akashiwo* HADA and *Chattonella antiqua* (HADA) ONO, have often formed extensive red tides during the summer periods, when the thermocline was observed in the water body, in the Seto Inland Sea, Japan (ONO and TAKANO 1980, YAMOUCHI *et al.* 1982). As these red tides develop, the surface waters rapidly become turbid and reddish brown. Such changes often run their courses within a few days and while a dramatic increase in cell division rates does occur, such high concentration of organisms must be explained by other mechanisms as well.

Many red tide flagellates migrate vertically (HASLE 1950, 1954, EPPLEY *et al.* 1968,

BLASCO 1978, STAKER and BRUNO 1980, HEANEY and EPPLEY 1981, KAMYKOWSKY 1981, CULLEN and HARRIGAN 1981). Their ascent during the daytime usually results in more rapid increases of the cell concentrations in surface waters than can be accounted for by cell division (cf. IWASAKI 1979). Furthermore, the ability to assimilate nitrate at night observed in *Gonyaulax polyedra*, together with the capacity to migrate between the euphotic zone and the nutrient-rich bottom, has been recognized as possibly giving *G. polyedra* an ecological advantage over coastal diatoms and allowing growth of its population to bloom dimensions (EPPLEY and HARRISON 1975, HARRISON 1976).

This paper describes the diurnal vertical migration and dark uptake of nitrate and

¹⁾ Dedicated to Prof. Munenao Kurogi on the occasion of his academic retirement.

phosphate of *Heterosigma akashiwo* and *Chattonella antiqua* in laboratory cultures and discusses the ecological role of diurnal vertical migration in the development of red tides caused by these species in the Seto Inland Sea.

Materials and Methods

Axenic clones of *H. akashiwo* (OHE-1) and *C. antiqua* (Ho-1), used in the previous studies (WATANABE *et al.* 1982a, NAKAMURA and WATANABE 1983a, b), were maintained under a light intensity of $0.04 \text{ ly} \cdot \text{min}^{-1}$ and a photoperiod of 12:12 LD (lights on at 0800 and off at 2000), at 22.5 and 25°C, respectively, throughout the course of the present experiments.

Diurnal vertical migration: Each organism was cultured in two sets of 1000 ml Erlenmeyer flasks with 500 ml f/2 medium (GUILLARD and RYTER 1962). A 1000 ml culture of exponentially growing cells of *H. akashiwo* (cell concentration = ca. $1 \times 10^4 \text{ cells} \cdot \text{ml}^{-1}$) or *C. antiqua* (cell concentration = ca. $1 \times 10^8 \text{ cells} \cdot \text{ml}^{-1}$) was transferred into a cylindrical glass tube 25 cm high by 8 cm internal diameter, whose sides and bottom were blackened. After the cultures had settled for 3 hrs, 2 ml water samples from 3 depths were taken every 1–3 hrs over the diel cycle. Cell concentrations were measured under a microscope (WATANABE *et al.* 1982a).

Nitrate and phosphate uptakes: *H. akashiwo* was cultured in 1000 ml Erlenmeyer flasks with 500 ml N- or P-limited modified ASP-7 medium (WATANABE *et al.* 1982a) containing $100 \mu\text{M}$ nitrate or $10 \mu\text{M}$ phosphate, respectively, and *C. antiqua* with 500 ml N- or P-limited H medium (NAKAMURA and WATANABE 1983a) containing $50 \mu\text{M}$ nitrate or $5 \mu\text{M}$ phosphate, respectively. When the cell concentrations of *H. akashiwo* reached ca. $2 \times 10^4 \text{ cells} \cdot \text{ml}^{-1}$ and of *C. antiqua* ca. $1 \times 10^8 \text{ cells} \cdot \text{ml}^{-1}$, the exponential phases, samples were taken every 3 hrs over the diel cycle. Sampling procedures and analytical methods were the same as those previously reported (WATANABE *et al.* 1982a).

Results

Diurnal vertical migration: *H. akashiwo* and *C. antiqua* concentrated at the surface in the daytime and at the bottom at night (Figs. 1 and 2). Maximum cell concentrations of *H. akashiwo* and *C. antiqua* were $1 \times 10^6 \text{ cells} \cdot \text{ml}^{-1}$ and $7.8 \times 10^8 \text{ cells} \cdot \text{ml}^{-1}$, respectively, at the surface in the daytime. These reached $2.5 \times 10^6 \text{ cells} \cdot \text{ml}^{-1}$ and $6 \times 10^8 \text{ cells} \cdot \text{ml}^{-1}$, respectively, at the bottom at night, whereas cell concentration at the surface dramatically decreased. The timing of descent and ascent did not coincide exactly with the onset or end of the light phases but, rather, began to descend from the surface before the light was extinguished and ascended before the light came on.

Nitrate and phosphate uptakes: Cell division of *H. akashiwo* occurred between 0500–1100 and of *C. antiqua* 0200–0800 (Figs. 3–6). Ambient nitrate or phosphate concentration in the cultures of both species decreased monotonously throughout the light and dark periods. Light and dark uptake rates of nitrate and phosphate were determined by the following equation;

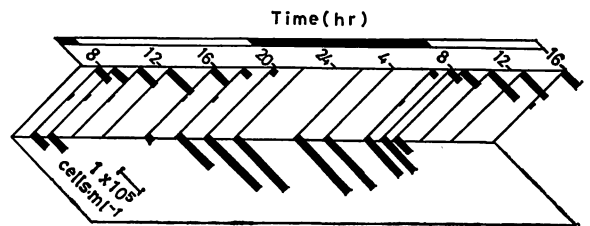


Fig. 1. Diurnal vertical migration of *Heterosigma akashiwo*.

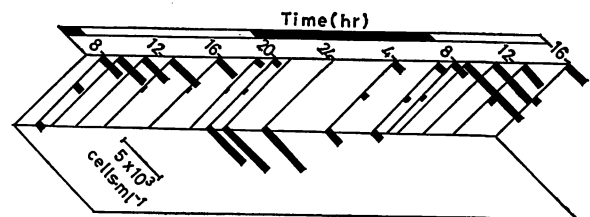


Fig. 2. Diurnal vertical migration of *Chattonella antiqua*.

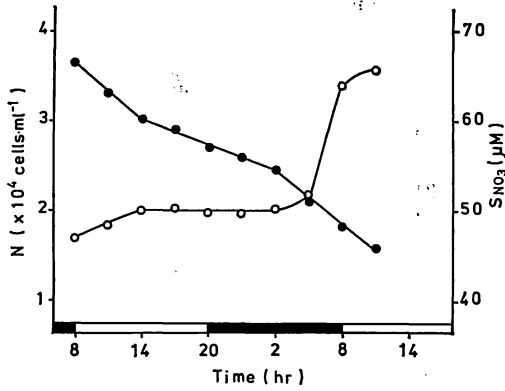


Fig. 3. Increase of cell concentration of *Heterosigma akashiwo* and decrease of ambient $\text{NO}_3\text{-N}$ concentration under a light-dark cycle. \circ : cell concentration. \bullet : ambient $\text{NO}_3\text{-N}$ concentration.

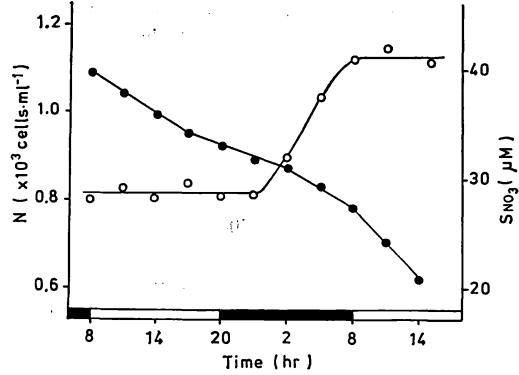


Fig. 5. Increase of cell concentration of *Chattonella antiqua* and decrease of ambient $\text{NO}_3\text{-N}$ concentration under a light-dark cycle. \circ : cell concentration. \bullet : ambient $\text{NO}_3\text{-N}$ concentration.

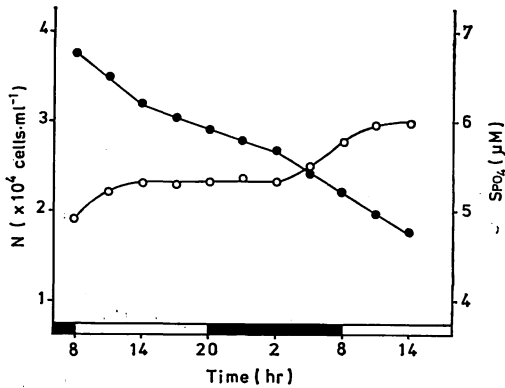


Fig. 4. Increase of cell concentration of *Heterosigma akashiwo* and decrease of ambient $\text{PO}_4\text{-P}$ concentration under a light-dark cycle. \circ : cell concentration. \bullet : ambient $\text{PO}_4\text{-P}$ concentration.

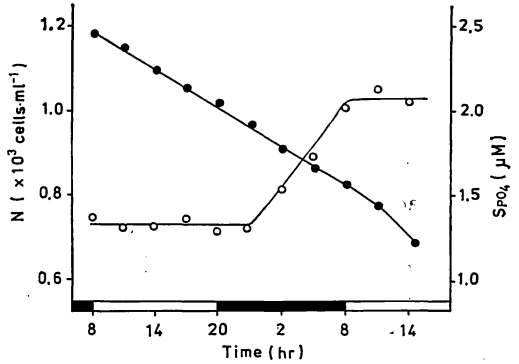


Fig. 6. Increase of cell concentration of *Chattonella antiqua* and decrease of ambient $\text{PO}_4\text{-P}$ concentration under a light-dark cycle. \circ : cell concentration. \bullet : ambient $\text{PO}_4\text{-P}$ concentration.

$$V = -\frac{dS}{dt} / N,$$

where V = uptake rate, $-\frac{dS}{dt}$ = disappearance rate of nitrate or phosphate, and N = cell concentration. The results are summarized in Table 1. These two species took up nitrate and phosphate in both light and dark. The dark/light uptake ratios were estimated at 41-100% NO_3^- or 43-100% PO_4^{3-} in *H. akashiwo* and at 49-100% NO_3^- or 75-100% PO_4^{3-} in *C. antiqua*.

Discussion

Vertical distribution of flagellates is a function not only of their migration patterns but also of other factors such as zoo-plankton grazing and tidal transport (cf. SOURNIA 1974). Experiments with pure cultures demonstrate that flagellates migrate vertically according to a diurnal rhythm. The patterns of diurnal vertical migration of cultures of *H. akashiwo* and *C. antiqua* correspond well with the periodic changes of vertical distribution of the natural popu-

Table 1. Uptake rates of nitrate (V_N) and phosphate (V_P) of *Heterosigma akashiwo* and *Chattonella antiqua* at the light (L) and dark (D).

Species	hrs	L or D	V_N ($\text{fmol} \cdot \text{cell}^{-1} \cdot \text{hr}^{-1}$)	V_P ($\text{fmol} \cdot \text{cell}^{-1} \cdot \text{hr}^{-1}$)
<i>H. akashiwo</i>	0800—1100	L	59	4.7
	1100—1400	L	54	4.2
	1400—1700	L	24	2.0
	1700—2000	L	24	2.0
	2000—2300	D	24	2.0
	2300—0200	D	24	2.0
	0200—0500	D	52	3.1
	0500—0800	D	39	2.8
<i>C. antiqua</i>	0800—1100	L	791	51
	1100—1400	L	791	51
	1400—1700	L	791	51
	1700—2000	L	409	51
	2000—2300	D	409	51
	2300—0200	D	389	48
	0200—0500	D	655	43
	0500—0800	D	586	38

lations of these species observed in the Seto Inland Sea (YAMOCHI *et al.* 1982, HAMAMOTO *et al.* 1979). In nature they concentrate at the surface during the daytime and at lower zones at night. This suggests that the diurnal changes of vertical distribution in the natural population of these species were mainly due to their movement.

The migratory descent and ascent of some dinoflagellates preceded light changes (EPPLEY *et al.* 1968, HEANEY and FURNASS 1980, KAMYKOWSKI 1981, CULLEN and HOLLIGAN 1981). The importance of circadian rhythms in the diurnal vertical migration of the dinoflagellates, *Cachnia niei* and *Ceratium hirundinella*, seems clear, because in continuous dark they migrated with a similar periodicity to that in the light-dark cycle. In continuous darkness, *H. akashiwo* and *C. antiqua* migrated with not only a different periodicity from that of a light-dark cycle but also different from each other (WATANABE *et al.* in press., NAKAMURA and WATANABE in press.). Further metabolic studies are necessary to explain these results.

Red tides caused by flagellates are commonly associated with nutrient-depleted surface waters and steep, shallow thermoclines,

below which nutrients are rich (HOLMS *et al.* 1967). This is true in the case of *H. akashiwo* or *C. antiqua* red tide (MURAKAMI 1978, SATO *et al.* 1979). Under such conditions, it has been reported that diurnal vertical migration gives flagellates double survival opportunities. The migratory ascent in the daytime places them in the euphotic zone and the descent at night positions them in the nutrient-rich bottom waters (HOLMS *et al.* 1967, EPPLEY *et al.* 1968, EPPLEY and HARRISON 1975, HARRISON 1976). Descent is advantageous only if the organisms can take up nutrients at night. Since N or P is limiting nutrient in the Seto Inland Sea (YAGI *et al.* 1982, NAKAMURA and WATANABE 1983b), dark uptake of these nutrients should be a significant adaptive feature for *H. akashiwo* and *C. antiqua*. As shown in the results, the dark/light ratios of nitrate or phosphate uptakes were 41–100% NO_3^- or 43–100% PO_4^{3-} in *H. akashiwo* and 49–100% NO_3^- or 75–100% PO_4^{3-} in *C. antiqua*. On the other hand, nitrate or ammonium uptake and nitrate reductase activity in natural assemblages of marine diatoms showed maximal activity about noon and minimal activity about

midnight and the amplitude of the variation from day to night was 5 to 10 fold (EPPLEY and HARRISON 1975). *Skeletonema costatum*, which often competes with *H. akashiwo* or *C. antiqua* in the Seto Inland Sea (MURAKAMI 1978, YAMOCHI *et al.* 1982), assimilated less nitrate at night than in the daytime (EPPLEY *et al.* 1971) and did not take up phosphate at night (WATANABE *et al.* 1982b). It seems that nitrate or phosphate uptake of *H. akashiwo* and *C. antiqua* is less affected by light conditions than that of the diatoms. These findings provides a basis for speculating that diurnal vertical migration provides *H. akashiwo* and *C. antiqua* an ecological advantage over the coastal diatoms.

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渡辺 信・中村泰男・木幡邦男：赤潮鞭毛藻, *Heterosigma akashiwo* と *Chattonella antiqua* の日周垂直移動と夜間における硝酸塩、磷酸塩の摂取

瀬戸内海にて夏期、成層が発達した時に赤潮を形成する *Heterosigma akashiwo* と *Chattonella antiqua* の日周垂直移動と硝酸塩・磷酸塩摂取の経時変化を純粋培養下で観察した。両種とも日中は表層に、夜間は底層に集積し、その上下の移動は明暗切り換え時刻に先行して行われた。*H. akashiwo* の夜間における硝酸塩、磷酸塩の摂取速度は日中のそれの各々 41~100%, 43~100% であり、*C. antiqua* では各々 49~100%, 75~100% であった。この値は、これらの種と競合関係にある硅藻と比べると大きく、従って両種の硝酸塩、磷酸塩の摂取は明暗条件で硅藻ほど影響をうけていないといえる。

H. akashiwo と *C. antiqua* に確認された有光層と栄養塩を豊富に含む底層の間を日周期的に移動しうる能力と夜間に硝酸塩、磷酸塩を摂取しうる能力は、成層期においてこれら2種の個体群の発達に大きな役割を果すものであることが推論された。(305 茨城県筑波郡谷田部町小野川 国立公害研究所水質土壌環境部)