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Age and growth of mitre squid *Photololigo chinensis* in the Tonkin Gulf of Vietnam based on statolith microstructure

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Abstract: A total of 254 mitre squid *Photololigo chinensis* collected in the coastal area of Tonkin Gulf was subjected to daily increment of statolith analysis to determine the ages. The oldest individuals observed were 194 days old for specimens collected in the summer–autumn season. Size–at–age relationship was best described by power function for both individuals hatched in spring–summer and autumn–winter periods. *P. chinensis* exhibited sexual dimorphism in the relative growth of the mantle length: males grew faster in length compared with females while weight growth rates were similar for both sexes. Seasonal factors strongly influenced the growth of squid, and the growth rates in the warm water season was higher than those in the colder season.

Keywords: *Photololigo chinensis, age, growth, statolith, increment*

1. Introduction

Mitre squid *Photololigo chinensis* is a neritic species, occurring from the shallow coastal area. The species is distributed in the western Pacific, from the South and East China Sea to Japanese waters, Gulf of Thailand, Arafura, Timor Sea and northern Australia (DUNNING *et al.*, 1998). Mitre squid has a wide range of distribution and high abundance in Vietnamese waters especially in the Tonkin Gulf (DUC, 1991), and is considered one of the most important species in the cephalopod fisheries in the area with 40–50% of the total catch of all squid species in the area (CHU *et al.*, 1998).

The daily growth increments were first detected in fish otoliths (PANELLA, 1971) and statoliths analyses of squids have provided information on growth rates, hatching dates, life–spans and even short term fluctuations in growth performance (JACKSON and MOLTSCHANIWSKYJ, 1999). Since the growth of statolith increments of *P. chinensis* was validated daily by JACKSON (1990), growth of tropical loliginid squids have been reported from Australian waters (JACKSON and CHOAT, 1992; JACKSON, 1995) and the Andaman Sea (SUKRAMONGKOL *et al.*, 2007). The statolith increments and somatic growth of *P. chinensis* in Australian water were influenced by seasonal factors, and the growth rate of male squid was relatively

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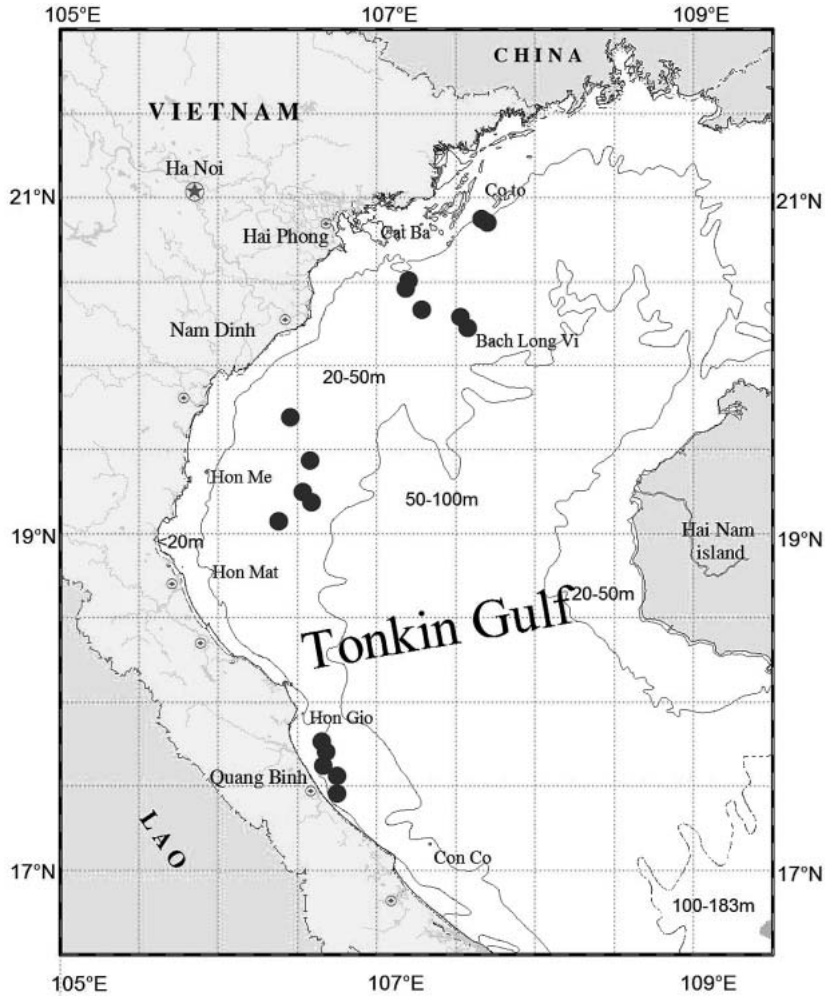

Fig. 1. Sampling locations (solid circles represent fishing areas) in the depth zone from 20–50 m in the Tonkin Gulf.

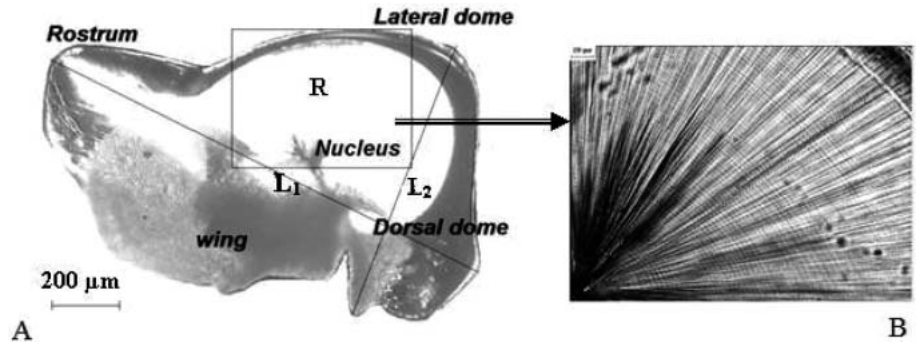

Fig. 2. Statolith increments of *Photololigo chinensis* (120 mm ML) observed under the light microscope. L_1, L_2 are Statolith length and width. R_1 is rectangle area where every increment was counted and measured using the Caliper tool in Image Pro Plus Software (lines annotations).

Table 1. Size (ML mm) of male and female *Photololigo chinensis* specimens with readable statolith increments in nearshore of the Tokin Gulf, Vietnam from April to October 2001.

Month of sampling	Fishing gear	Mesh size (mm)	ML of Male				ML of Female			
			No.	Range	Mean	SD	No.	Range	Mean	SD
April	OT*	25	8	76–197	130.7	41.2	7	84–191	125.9	39.1
July	OT, S.H.D.N.**	25,20	19	75–313	153.8	78.5	24	70–215	117.5	38.7
August	OT, S.H.D.N.	25,20	24	95–200	127.0	31.4	27	74–155	109.2	18.8
September	OT, S.H.D.N.	25,20	33	82–250	152.2	45.7	50	74–330	122.5	47.9
October	S.H.D.N.	20	32	70–400	139.9	68.4	30	67–220	114.2	32.0

OT* (otter trawl) and S.H.D.N.** (stick held dip net).

higher than that of females at the same age (JACKSON and CHOAT, 1992; JACKSON, 1995).

Although mitre squid is a valuable species in the cephalopod fisheries in the Tonkin Gulf of Vietnam, there have been no studies on the growth and life history of this species in the area. In the southern area of Vietnam, growth parameters of *L. formosana* (referred as synonym of *P. chinensis*) had been calculated by the ELEFAN program (Electronic Length–Frequency Analysis) (DINH *et al.*, 1998) which suggested that *P. chinensis* has a life span exceeding 3 years with a very fast growth in the first year. However, this length frequency analysis is an inadequate means of describing growth for a rapid growing organism with multiple cohorts like squids (JACKSON *et al.*, 2000).

The aim of this study is to estimate the age and growth rates of *P. chinesis* in the Tonkin Gulf of Vietnam to provide basic information for the fisheries management of the squid in the area.

2. Materials and methods

The Tonkin Gulf is located between 17°–22°N and 105°–110°E, covering a total area of 150,000 km^2 (TANG, 1997). There are two main seasons in this area: winter–spring and summer–autumn corresponding with northeast (November to April) and southwest monsoons (May to October), respectively. In 2000–2001, sea surface temperature (SST) of the Tonkin Gulf in winter–spring (December–March) was from 20 to 23°C (21.5°C in average) while those in summer–autumn (May–October) ranged from 26 to 30°C (28.6°C in average) (source of data from NOAA, 2009).

Squid specimens for the statolith analysis were obtained from the Tonkin Gulf (Fig. 1) in April and July–October, 2001 by otter trawls and stick held dip nets (Table 1). Dorsal mantle length (ML) and wet body weight (BW) of the specimens were measured to the nearest mm and g, respectively. The maturity of 131 female squid caught in July–October was classified into 6 developmental stages following the description of LIPINSKI and UNDERHILL (1995). Stage I and II were defined as immature, stage III as maturing, stages IV and V as mature and stage VI as spent.

Statoliths were extracted from fresh squids following the method of NATSUKARI *et al.* (1991). Statolith was mounted to glass slides by Crystal Bond (Aremco Products, Inc) with the anterior side of statolith pointing downward. Before grinding, photograph of whole statolith was taken under light microscope. Statolith length (SL) and statolith width (SW) were measured to the nearest 1 μm following CLARKE (1978) by the manual measurement tool in Image Pro Plus Software (Media Cybernetics) (L_1 and L_2 in Fig. 2A). A waterproof abrasive paper # 2000 was initially used to grind the statoliths on a Buehler machine, then the paper was changed to a finer one until the core was visible. The Caliper tool in Image Pro Plus Software was used for counting and measuring the increments (Fig. 2B).

All the statistical analyses were conducted by STATISTICA (software version 5.5., StatSoft, Inc). Differences in the regression slopes of the length–weight relationship, mantle length and estimated age, as well as body weight and estimated age between sexes and seasons were linearized by log transforming

(*ln*ML–*ln*BW; *ℓn*ML–estimated age; *ℓn*BW–estimated age). Tests and comparisions were conducted by analysis of covariance (ANCOVA) for squid ranging from 70 to 280 mm in ML (because squid bigger than 280 mm in ML was mainly males). The first step was to test the homogeneity of slopes. If the slopes were not significantly different from each other, the homogeneity of intercepts was tested. Statolith width at estimated age was tested by ANCOVA for any differences among seasons. Moreover, to analyse the effect of sex and season on statolith increment width, residuals from polynomial estimation were used in ANOVA. Power function as $Y=aX^b$, where Y and X are variables and a and b are constant, was used for the analysis of the length–weight, length–age and weight–age relationships.

Daily growth rates (DGR, mm d^{-1} or g d^{-1}) and instantaneous growth rates (G, %) were calculated after Ricker (1958):

$$DGR = (W_2 - W_1) / T,$$
$$G = [(\ln W_2 - \ln W_1) / T \times 100,$$

where W_1, W_2 are calculated mantle length or body weights based on the length or weight and estimated age relationships at the beginning and end of the time interval (T =10 days).

3. Results

3–1 Mantle Length and Body Weight

The length–weight relationships for males and females were $BW=1.80\times10^{-4}ML^{2.16}$ (n=116, p<0.01, r^2=0.95, size range 70–400 mm ML) and $BW=5.00\times10^{-4}ML^{2.42}$ (n=138, p<0.01, r^2=0.95, size range 67–330 mm in ML), respectively (Fig. 3). Slopes of the regression equations between males and females were significantly different (*ln*ML–at–*ln*BW homogeneity of slopes test, ML ranged 70–280 mm, F=7.5, p<0.01). Mean weight of females squid at the same ML larger than 160 mm was significantly heavier than males (ANCOVA test, F=12.8, p<0.01).

3–2 Age, hatching date and maturation

Estimated age of the squid based on the statolith increments ranged from 63 (72 mm in ML) to 190 days (400 mm in ML). Back calculation from the number of statolith increments and catching dates showed that hatching dates

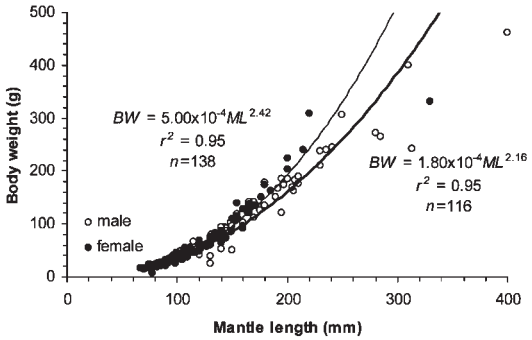

Fig. 3. Length–weight relationship of male (open circles) and female (solid circles) *Photololigo chinensis* from the Tonkin Gulf in 2001.

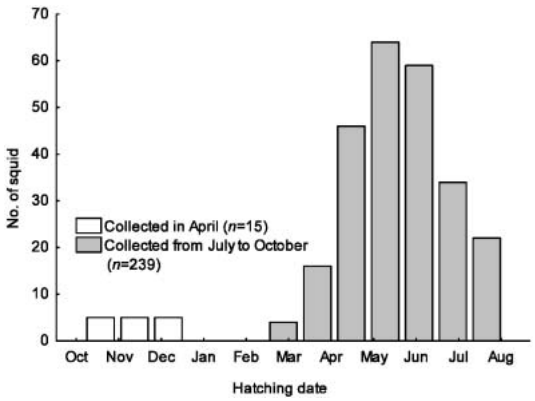

Fig. 4. Estimated hatching date of all individual (n=254) of *Photololigo chinensis* collected from the Tonkin Gulf in April (transparent background bars) and from July to October (grey background bars).

of individuals sampled in July–October ranged from March to August with a peak from April to June (Fig. 4). Squid in spring–summer hatching group which was a main cohort in the Tokin Gulf was collected from July to October and autumn–winter hatching group was collected only in April. Based on the hatching and sampling periods, *P. chinensis* hatched throughout the year and the hatching groups of *P. chinensis* in this study was classified into two separate groups, namely spring–summer hatching and autumn–winter hatching groups.

Observed maturation stages of female squid varied from stage 1 to 5 for specimens ranging

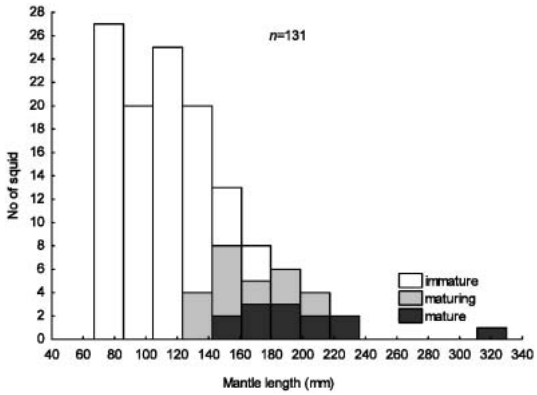

Fig. 5. Maturation of female *Photololigo chinensis* hatched in spring–summer season.

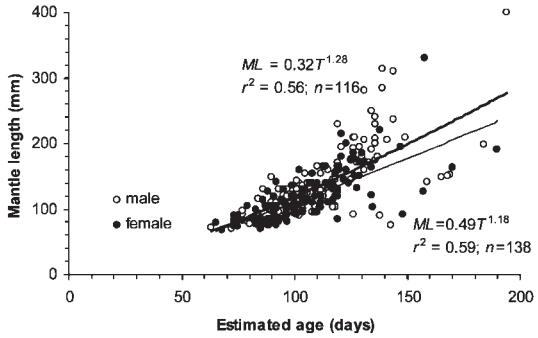

Fig. 6. Relationships between estimated age and mantle length of male (open circles) and female (solid circles) *Photololigo chinensis*.

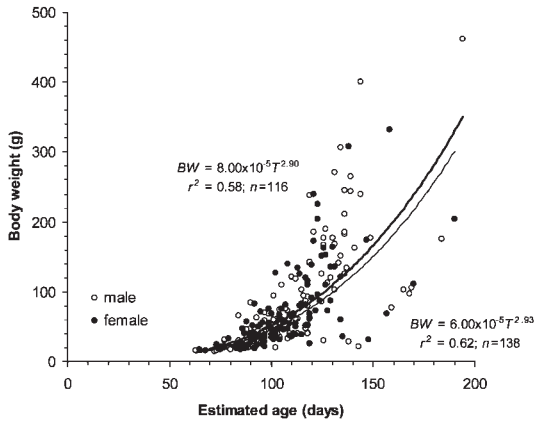

Fig. 7. Relationships between estimated age and body weight of male (open circles) and female (solid circles) *Photololigo chinensis*.

from 67 to 330 mm in ML (n=131). All females larger than 220 mm in ML were matured when older than 126 days. The smallest squid in maturing stage was 130 mm in ML (about 116 day old), while the biggest immature specimen was 182 mm (124 day–old) (Fig. 5).

3–3 Size and estimated age

Estimated age of squid ranged from 63 (72 mm in ML) to 194 day–old (400 mm in ML) for males, and 65 (78 mm ML) to 190 day–old (204 mm ML) for females (Fig. 6). The relationship between ML and esimated age (T days) was expressed as $ML=0.32T^{1.28}$ (r^2 =0.56, n=116, p<0.01), and $ML=0.49T^{1.18}$ (r^2 =0.59, n=138, p<0.01) for male and female, respectively (Fig. 6). The ML and estimated age relationships were significantly different between male and female (ANCOVA test, p<0.003). Growth in males varied considerably compared to females. At the same range of age, from 65 to 190 day–old (for both male and female), daily growth in ML ranged from 1.35 mm to 1.82 mm for males, and 1.22 mm to 1.47 mm for females (Equations in Fig. 6 were used for the estimations).

The relationship between BW and esimated age was expressed as $BW=8.00\times10^{-5}T^{2.90}$ (r^2=0.58, n=116, p<0.01), and $BW=6.00\times10^{-5}T^{2.93}$ (r^2=0.62, n=138, p<0.01) for male and female, respectively (Fig. 7). Based on these equations, daily growth rates of the body weight were estimated to be from 0.7 g to 5.46 g, and 0.61 g to 5.18 g for male and female squid, respectively, at the age range from 65 to 190 day–old. There were no significant differences in BW and estimated age between males and females of *P. chinensis* in this study (ANCOVA test, F=3.37; p=0.067) (Fig. 7).

The ML and estimated age relationships combining male and female were $ML=6.60\times10^{-3}T^{1.96}$ (n=15, r^2=0.78, p<0.001) and $ML=8.00\times10^{-2}T^{1.58}$ (n=239, r^2=0.73, p<0.001) for the autumn–winter and spring–summer hatching groups, respectively (Fig. 8). There was a highly significant difference between ML and estimated age of the two hatching groups (ANCOVA test, age ranging from 115 to 190 day–old, p <0.001, Fig. 8).The ML growth of the squid of

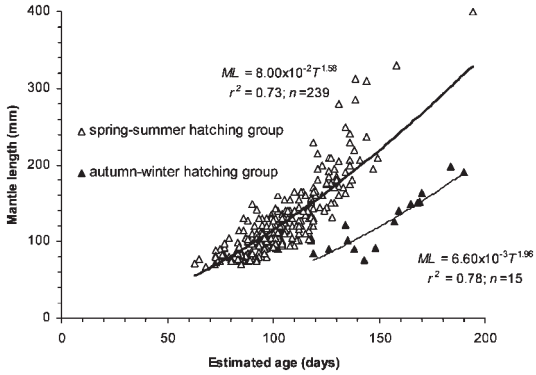

Fig. 8. Relationship between estimated age and mantle lengths of *Photololigo chinensis* in different seasons.

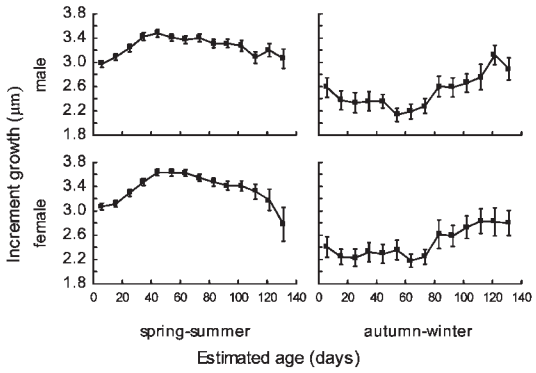

Fig. 9. Mean statolith–increment growth at estimated age of male and female squid from spring–summer and autumn–winter hatching groups. Whiskers represent 95% confidence intervals.

spring–summer hatching group was faster than that of autumn–winter group. Within the same age range (115 to 190 day–old), instantaneous growth rates of spring–summer hatching squid decreased from 0.59% to 0.36% of the mantle length compared with 0.34% to 0.15% of the mantle length in the autumn–winter hatching group.

3–4 Statolith growth

The statolith width (SW) ranged from 632 to 1208 μm, and 657 to 1122 μm for male and female squids, respectively. However, there was no significant difference in SW and estimated age relationships between male and female (ANCOVA test, p=0.09).

The relationships between the estimated age and statolith increment width of the squid from the same hatching group were not significantly different between male and female (ANOVA test on residuals from polynomial regression estimation of increments, p=0.19; Fig. 9). As for spring–summer hatching group, statolith increment width increased from a mean of 3 μm at day 1 to 3.5 μm at age 50–60 days. Then, increment width gradually decreased to the mean of about 2.8–3.0 μm at age 120–140 days. But for the autumn–winter group, increment width decreased from a mean of 2.4–2.6 μm (at day 1) to 2.2 μm at day 50–60. Then, increment widths gradually increased to the mean of about 2.8 μm at age 120–140 days (Fig. 9).

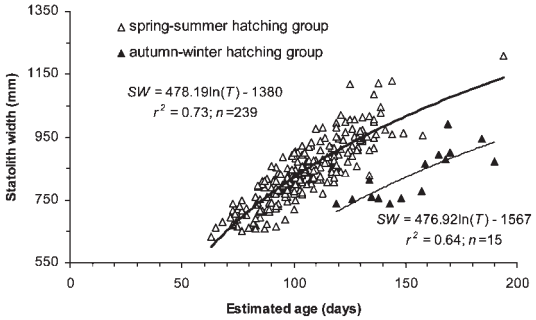

Fig. 10. Statolith widths and estimated age relation for spring–summer and autumn–winter hatching groups

Regressions between the estimated age and SW of squid hatching in spring–summer and autumn–winter were SW=478.19ln (T) −1380 (r^2=0.73; n=239; p<0.01) and SW=476.92ln (T) −1567 (r^2=0.64; n=15; p<0.01), respectively. There was a highly significant difference in slopes of SW and estimated age between these two hatching groups (ANCOVA test in the estimated age ranging from 115 days to 190 days, p<0.001, Fig. 10).

4. Discussion

In the Tonkin Gulf, the relationship between mantle length and body weight of male and female *P. chinensis* was significantly different,

especially in larger squid (ML>160 mm) where female was heavier than the male at the same mantle length. This may be explained by sexual dimorphism at maturity size and is agreed with previous studies of *P. chinensis* in the Gulf of Thailand (CHOTIYAPUTTA 1994), Amadan Sea (SUKRAMONGKOL *et al.*, 2007) and North Queensland, Australia (JACKSON and CHOAT 1992; JACKSON 1993). Power coefficient (b) of the length–weight relationships ($ML=aW^b$) for female (b=2.42) was higher than that for male (b=2.16) in this study, which is compatible with this species in the Gulf of Thailand with b=2.2–2.4 and b=2.0–2.1 for female and male, respectively (CHOTIYAPUTTA 1994). While the results of *P. chinensis* from Andaman Sea was comparable to our result for female (b=2.39), the result for male squid was different (b=1.79) (SUKRAMONGKOL *et al.* 2007).

In this study, the oldest squid was estimated to be 194 day–old (400 mm ML). Our result shows a remarkably shorter life span compare to the previous study of *P. chinensis* in the southern of Vietnam which exceeding 3 years with maximum ML of 340 mm (DINH *et al.* 1998). The main reason for this difference of previous study in Vietnam was base on the length–frequency relationship, which was an inadequate means of describing growth for a rapid growing organism with multiple cohorts like squids (JACKSON *et al.*, 2000). The oldest individual (400 mm ML) collected in October in this study is estimated hatching in March and grew up most of the time during the warm water period, suggesting a faster growth rate compared to that from cold period (Fig. 8). Previous studies based on the statolith increment aging method in Andaman *sea* and North Queensland, Australia reported that the life span of many loniginid squids was estimated to be less than 9 months (JACKSON 1993; JACKSON 2004; SUKRAMONGKOL *et al.* 2007).

P. chinensis has power growth function in sizes and estimated age relationships both in spring–summer and autumn–winter hatching groups (Fig. 8). At the age range of 115 to 190 days, the average daily growth rates varied from 0.59% to 0.36% of mantle length for summer–autumn and 0.34% to 0.15% of mantle length for the winter–spring season. There have been a number of studies about the effects of seasons on the growth of squid (RODHOUSE and HATFIELD, 1990; JACKSON and CHOAT, 1992; JACKSON *et al.*, 1997; JACKSON and MOLTSCHANIWSKYJ, 2002). FORSYTHE and HANLON (1989) indicated that temperature has a strong influence on growth rates of the loliginid squids especially in the winter time. Experimental studies on temperature effects for growth in squids (*L. vulgaris, L. opalescens and L. pealeii*) supported the idea that there was a significant change in growth rate at different temperatures (TURK *et al.*, 1986; YANG *et al.*, 1986; HATFIELD *et al.*, 2001). In the Tonkin Gulf, sea surface temperature from December to March in 2000–2001 ranged from 20 to 23°C (21.5°C in monthly average) while that from May to October ranged from 26 to 30°C (28.6°C in monthly average), which will be a cause of the seasonal differences in growth of seasonal hatching groups of the squid.

Increment growth patterns also give strong support to the variations of the seasonal growth of *P. chinensis*. Spring–summer hatching group had mean statolith increment width ranging from about 2.2 μm to 3.5 μm which exhibits larger increment growth compared to squid hatched in autumn–winter season (means of statolith increment widths range from about 2.2 – 2.8 μm) (Fig. 9). Experiments on *Lolliguncula brevis* (DURHOLTZ and LIPINSKI, 2000) also indicated a strong influence of temperature on statolith growth rate. Both somatic and statolith growth in this study support previous findings that there is a strong difference in seasonal growth pattern in *P. chienensis*. Therefore, growth modelling of loliginid squids needs to take seasonal patterns into an account.

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道東沿岸流の水塊の季節変化

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Seasonal variations of the water mass of the East Hokkaido Coastal Current

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Abstract: In our previous paper (NAGATA *et al.*, 2009), we concluded that the depth range from 50 to 75m is the best level to analyze the revolution of the East Hokkaido Coastal Current and its water mass properties. We used in this paper the same data base of the Hokkaido Kushiro Fisheries Experiment Station as in the previous paper. We selected two stations nearest to the coast, of the north–south observation lines P1, PK0, P2, PK1, P3. These stations are located inside of the current zone. We used the temperature and salinity data at 50m depth in order to analyze seasonal variations of the water mass of the East Hokkaido Coastal Current. We classified the data into three groups: case (1) that the East Hokkaido Coastal Current can be recognized both in the temperature and salinity cross–sections, case (2) that the Coastal Current can be recognized in either of the temperature and salinity cross–section and case (3) that no Coastal Current can be recognized both in the temperature and salinity cross–sections. However, the distribution areas of the water type of three cases for each month are well overlapped with one another on the TS surface, and the distribution natures of three cases appear to be almost identical. So, the distributions of two extreme cases (1) and (3) were examined. The distribution of case (1) is overlapped with that of case (3) for each month, but the overlapping manner is different one another. In the first half of the year (the season of the Coastal Oyashio), the area of overlapping is minimum in February when the Coastal Oyashio is strongest, is a little smaller in April, and is maximum in June. In the second half of the year (season of the East Hokkaido Warm Current), the areas are almost separated in October, indicating that the East Hokkaido Warm Current is strongest in October. The considerable overlapping area is seen in August, and two distributions are almost identical in December. The distribution of the water type of case (1) in the first half of the year is well separated to that of the second half of the year ; the temperature value in the first half is higher than 4℃、and

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that in the second half is lower than 4℃. The results obtained here indicate that the east Hokkaido Coastal Current Water cannot be generated by the water mass which has been carried directly from the Okhotsk Sea.

Keywords: *the East Hokkaido Coastal Current, the Coastal Oyashio, the East Hokkaido Warm Current, water mass analysis, seasonal variations*

1. はじめに

KURODA *et al.*（2006）は、日高湾沖を中心に沿岸親潮の流速場を数値実験をもとにして論じており、沿岸親潮がその沖合部に顕著なフロントを持ち、そこに速い流速場が存在することを示し、それをADCPによる実測流速場と対比している。また、坂本圭（私信）も道東沖についても同様のフロント構造が認められることを示している。前回の論文（永田ら、2009：以下では前論文と呼ぶ）において、水温・塩分の鉛直断面に現れる等値線の形状だけから、道東沿岸流の出現状況の季節変化を考察したが、沿岸親潮の外縁にフロント構造が見られることは、水温・塩分の鉛直断面から容易に沿岸親潮の存在・非存在を判定できることを示唆している。前論文では、水温・塩分の鉛直断面に現れる形状に、沿岸に接して低温・低塩分の帯が現れている場合を沿岸親潮が出現しているとし、高温・高塩分の帯が現れている場合を道東暖流が出現しているとして、その現れる頻度を調べ、年の前半（2月、4月、6月）は沿岸親潮の現れる季節であり、年の後半（8月、10月、12月）は道東暖流の現れる季節であることを示した。このように鉛直断面に現れる等値線の形状だけに焦点を絞ったのは、小笠原（1990）が指摘しているように、道東沿岸流の水の水温・塩分値が、非常に大きな季節変化を示すために、一年を通しての水塊特性を系統的に議論することが難しかったためである。しかし、前論文において、道東沿岸流の厚さは、沿岸親潮の場合も道東暖流の場合も、100m程度であり、75m深を超すと出現頻度が減少することが示された。また、道東暖流の場合、夏季においては、この海域を広く覆う高温・低塩分の表層水が出現するために、30m以浅ではその構造が見難くなることが多いことが示された。そこで、この論文では一年を通して道東沿岸流の構造が見易い50m深を選んで、その季節変化を論ずることにする。

2. 使用したデータと解析方法

年6回観測が実施されている北海道立釧路水産実験所の定期観測資料の中から、1990年から1996の7年間のデータを使用した。観測は原則として、2月、4月、6月、8月、10月、12月に行われる

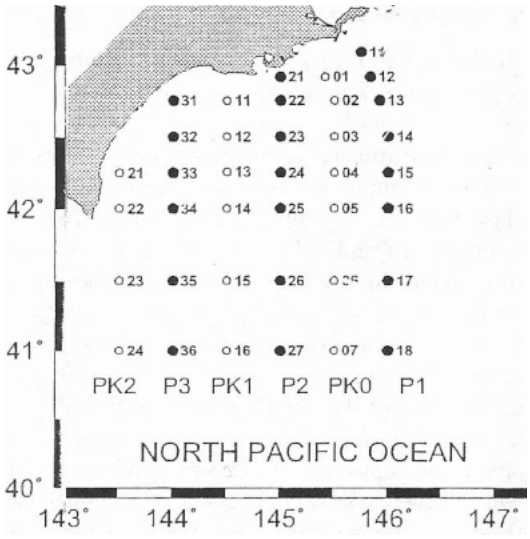

Fig. 1. Routine observation network of the Hokkaido Kushiro Fisheries Experiment Station. Oceanographic observations are conducted six times per year: basically in February, April, June, August, October and December. P1, PK0, P2, PK1, P3, and PK2 are the names of the north–south observation lines from east to west.

が、この期間では1990年から1992年の3年間は、6月の代わりに5月に、1992年には8月の代わりに7月に観測されている。これらについては便宜上翌月に観測されたものとして取り扱う。測点の配置はFig. 1 に示すが、PK2 測線は42°N以北には2測点しかなく、欠測も多いため、この論文では、P1、PK0、P2、PK1、P3の南北測線における水温・塩分の断面分布を中心に検討する。前論文で、一年を通して道東沿岸流の特性が最もよく現れることが示された50m層の水温・塩分値を解析の対象に選び、各測線で最も岸よりの2測点のデータを選び出して用いる。これらの測点は、水温・塩分断面形状に道東沿岸流が現れる場合には、ほとんど例外なしに、道東沿岸流域内に含まれる。なお、本論文においても、前論文同様、断面図における等値線は、水温の場合には1℃間隔で、塩分の場合には0.1間隔で引き、その形状から、道東沿岸流の存否を判断した。

出現状況の分類は、前論文の場合よりも簡略化し、場合（1）水温・塩分の断面の双方に沿岸親潮あるいは道東暖流が現れており、選ばれた観測点の50m層がその内部に含まれている場合、場合（2）水温・塩分の断面の片方だけに沿岸親潮あるいは道東暖流が現れており、選ばれた観測点の50m層がその中に含まれている場合（沿岸親潮型では塩分分布に注目、道東暖流型では水温分布に注目する)、場合（3）水温・塩分の断面の双方に沿岸親潮あるいは道東暖流が現れていないか、現れていても選ばれた観測点の50m層がその外部にある場合に分けた。最初、これらの場合それぞれについて、月別にデータを一枚のTS図上にプロットして見たが、データの存在範囲はどの月でも互いに大きく重なり合っており、明確な差は認め難かった（NAGATA, 2009)。このことは、道東沿岸流の有無によっては水塊特性がほとんど変わらないことを意味し、他の海域（例えばオホーツク海）から特徴的な水塊特性を持つ海水の流入によって道東沿岸流が形成されるとは考え難いことを示唆している。

3. 道東沿岸流の水塊の季節変化

沿岸親潮の水はその沖合の水に比べてより低温・低塩分であり、道東暖流の沖合の水に比べてより高温であり高塩分である。もしも、道東沿岸流のすぐ沖側の水が、道東沿岸流水が流入してこない場合の、この海域に特徴的な水であるとすると、場合（3）の水塊特性は、この海域に特徴的な水であるはずである。上述のように、道東沿岸流が存在する場合と、存在しない場合で、沿岸流の位置の水塊の性質があまり変わらないことは何を意味するのであろうか。そこで、上記に分類で、両極端である場合（1）と場合（3）の場合を比較して、道東沿岸流の有無によって、道東沿岸流域の水塊特性にどのような差が現れるかを詳細に検討して見よう。

沿岸親潮

沿岸親潮の季節である2月、4月、6月の場合について（前論文参照)、TS図上に水型の分布をそれぞれFig. 2、Fig. 3、Fig. 4に示す。これらの図では、沿岸親潮が水温・塩分の両方の断面に明確に現れた場合（1）を●で、両断面の両方に認められない場合（3）を△で示している。それぞれの図において、●のデータ点の分布が△の分布に比べて低塩分側に偏っていることが認められる。しかし、いずれの月でも分布域は互いに重なっていることは注目すべきである。前論文で、2月が沿岸親潮の最強期であり、ほぼ100%その出現が認められること、出現率は4月から6月に向かって順次減少していくことを示したが、面白いことに、分布域の重なりは2月で最も少なく4月から6月に向かって順次重複域が増大していく。6月

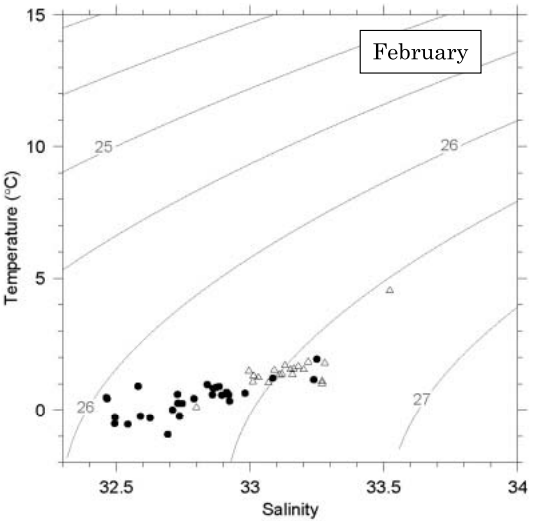

Fig. 2. Scatter diagram of water types on TS surface in February: solid circles (●) indicate the case (1) when cold and fresh water belt (the Coastal Oyashio) can be seen along the coast on both of temperature and salinity cross-sections, and open triangles (△) the case (3) when cold and fresh belt cannot seen on both of temperature and salinity cross-section. Numerals attached on curves indicate density σ.

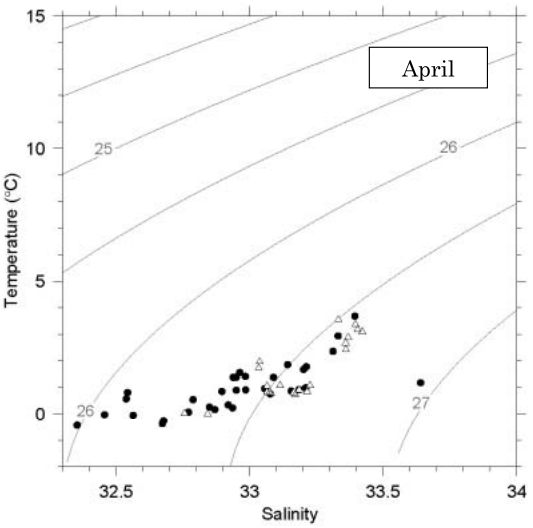

Fig. 3. Same as in Fig. 2 except for April.

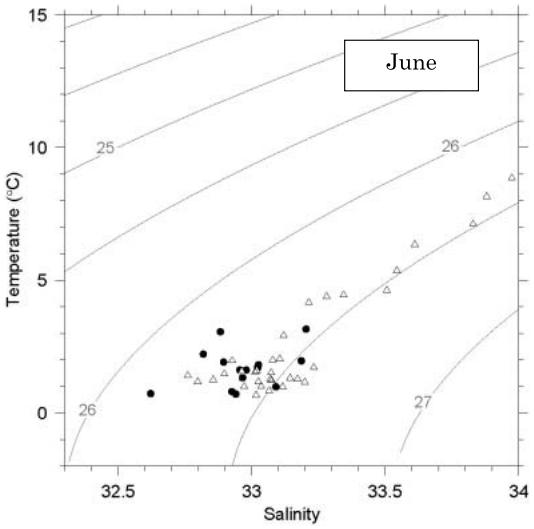

Fig. 4. Same as in Fig, 2 except for June.

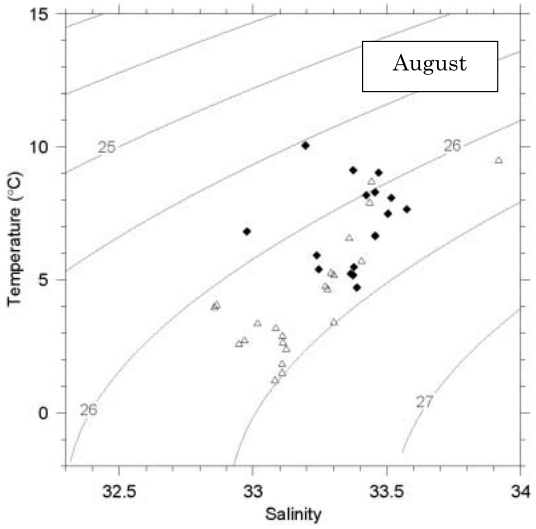

Fig. 5. Scatter diagram of water types on TS surface in August: solid diamonds (◆) indicate the case (1) when warm and saline water belt (the East Hokkaido Warm Current) can be seen along the coast on both of temperature and salinity cross-sections, and open triangles (△) the case (3) when warm and saline belt cannot seen on both of temperature and salinity cross-section.

では、場合（3）のデータ（△）が、33.2以上の高塩分側に分布域を伸ばしているが、この部分を除くと、両者の分布域は全く重なっている。分布域は全体として4月から6月に向かって高塩分側にシフトする傾向があるが、分布域の重複の度合いが沿岸親潮の強度と逆相関を示すことは興味深い。

Fig. 2、Fig. 3の水型の分布（場合（1））は、いわば沿岸親潮の内部の水型をランダムに採集したものである。もしも水塊特性が季節によってきまっているならば、水型の分布は沿岸親潮の中の水塊分布をあらわしていることになり、データ点がその周りに分布する右上がりの直線は、平均的な水塊分布を示すことになる。右上がりということは、沿岸親潮内で岸から沖に向かって水温・塩分が増加していくことに対応していると考えられる。大谷（1971）は沿岸親潮の水塊を水温2℃以下、塩分33.0以下で定義しているが、これは2月と4月の場合（1）のデータ分布に対応すると考えられるが、今回の解析結果では、より高塩分側にもデータ点が存在する。33.3以下ではデータ点は1つのやや右上がり直線周りに分布するが、塩分33.3以上ではこの直線から離れるように見える。典型的な親潮水の定義には33.3以下に取るべきかもしれない。しかし、場合（3）のデータもほぼ同じ範囲に現れるから、沿岸親潮の水塊を水温・塩分の範囲を与えて定義出来るかどうか、かなり疑問がある。

前論文で、水温、塩分の等値線をそれぞれ1℃、0.1の間隔で引いた場合、沿岸親潮は水温断面よりも塩分断面により明確に現れることを示した。データ点の分布が、若干右上がりであるが、かなり塩分軸に平行な直線の付近に分布することに対応していると考えられる。また、沿岸親潮の力学

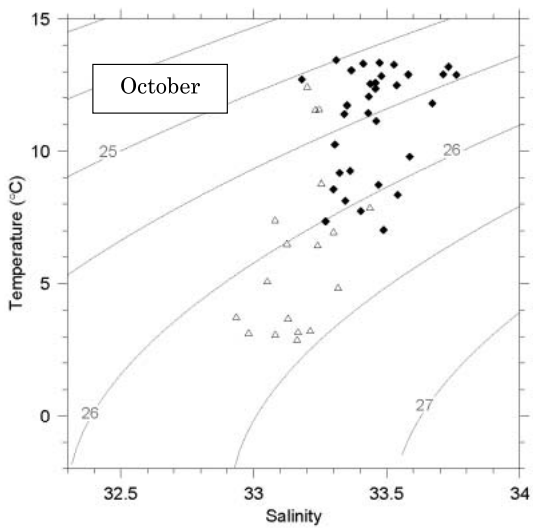

Fig. 6. Same as in Fig. 5 except for October.

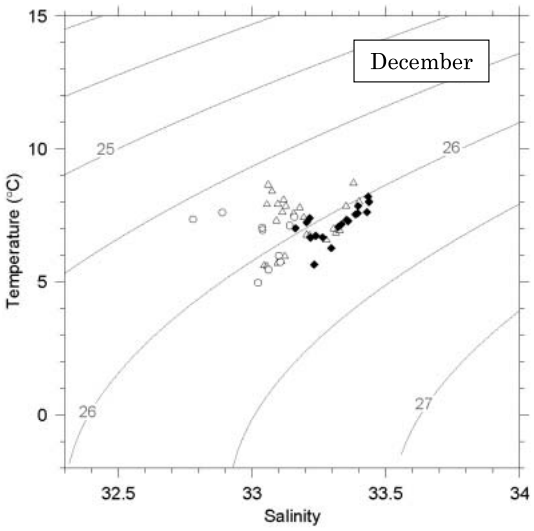

Fig. 7. Same as in Fig, 5 except for December. Water types for the case when cold and fresh water belt (the Coastal Oyashio) can be seen along the coast both on temperature and salinity cross-sections are also plotted with open circles.

を考える場合、密度構造に注目する必要があるという指摘があるが（河野時廣：私信)、この分布特性は沿岸親潮が明確な密度構造を伴っていることを示唆している。

6月の水型の分布では塩分が33.3以下の部分では、場合（1）と場合（3）のデータの分布範囲がほぼ完全に重なっている。33.3以上の部分では、場合（3）のデータの存在範囲が高温・高塩分側に等密度線に沿うような形で伸びている。この分布特性の意味合いは良く分からないが、6月は沿岸親潮の季節から、道東暖流の季節への遷移時期に当たっており、道東暖流的な構造が現れ始めているのかもしれない。遷移時期の水塊変化については、さらに検討する必要がある。

道東暖流

道東暖流の季節である8月、10月、12月の場合について、TS図上に水型の分布をそれぞれFig. 5、Fig. 6、Fig. 7に示す。これらの図では、道東暖流水温・塩分の両方の断面に明確に現れた場合（1）を◆で、両断面の両方に認められない場合（3）を△で示してある。前論文で示したように12月には、岸沿いに低温度・低塩分の沿岸親潮に対応するような水の帯が現れる場合がある。12月のFig. 7には、その場合の水型を、○で合わせて示してある。

場合（1）と場合（3）の水型の分布範囲は各月ともに、相互に重なり合っている。しかし、その重なり方は月によって大きく異なり12月（Fig. 5）では全く重なりあっているのに対して、8月（Fig. 7）においては、場合（3）のかなりの数のデータ点が場合（1）のデータ域よりも低温・低塩分側に存在しており、分布域に若干の違いが見られる。10月（Fig. 6）では、一部に重複する部分はあるが、場合（1）のデータは、場合（3）のデータとはほとんど分離されている。前論文では8月も、10月も同様に道東暖流が強勢となる時期と判断したが、この水塊分析の結果は、10月に道東暖流が最も強勢になる時期と考えるべきことを示している。

12月を除くと、場合（1）のデータ点（◆）は、低温（低塩分）側から高温側（高塩分側）に延びている。沿岸親潮の場合に比べて、データの散らばりが大きく、ある直線の周りに集まっているとは言えないが、塩分変化よりも水温変化が大きく、全体として温度軸に平行する形になっている。前に述べたように、これを道東暖流内の水塊構造を表すものと考えると、道東沿岸流が、塩分断面よりも水温断面に現れ易いという、前論文の結論を支持する形になっている。データの分布する高温部分では、等密度線の傾きが小さくなっており、塩分軸にかなり平行する形になっているため、データ分布は道東暖流は、沿岸側（高温側）から、沖合（低温側）に密度が増大することになり、沿岸親潮の密度構造の場合と同様の密度構造を示すことになる。道東沿岸流域の水深が浅いので、完全に地衡流とみなせるかどうか分からないが、道東沿岸では1年を通して南西流が卓越していること（日下ら、2009）と整合性がある。

12月の水型の分布（Fig. 7）は、8月、10月の分布とは、非常に違っている。場合（3）のデータ点（△）は、場合（1）のデータ点（◆）より僅かであるが、高温側、低塩分側に現れる傾向があるが、ほとんど重なっている。また、場合（1）のデータ点は、温度軸に平行に分布するのではなく、むしろ塩分軸に平行（ないしは等密度線に平行）に分布している。前論文で、12月は全体として道東暖流の季節であると考えられることを述べたが、この分布形状は沿岸親潮の分布形状に対応している。12月には、時として、沿岸親潮の形状（岸沿いに低温・低塩分の帯）が認められることがあるが、その場合の水型のデータを○で示してある。この場合の水は、場合（1）に比べても、明らかに低塩分側に現れる。しかし、その水温値は5℃以上あり、通常の沿岸親潮の水温よりはるかに高温である。前論文で、12月には沿岸親潮の前駆現象が現れることがあると考察したが、水温

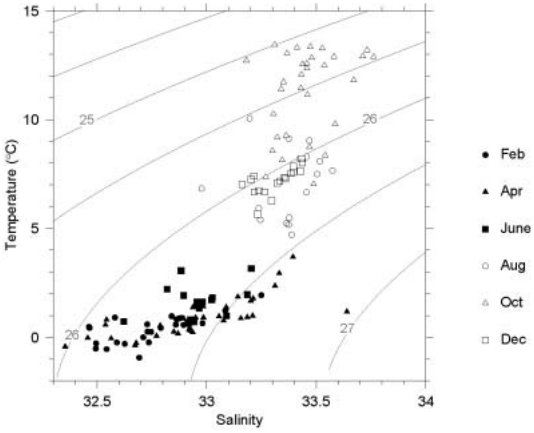

Fig. 8. Scatter diagrams of water types on TS surface in the case (1) that the Coastal Oyashio (solid symbols) or the East Hokkaido Warm Current (open symbols) can be seen both in temperature and salinity cross-sections. Solid circles (●) are for February, solid triangles (▲) for April, solid squares (■) for June, open circles (○) for August, open triangles (△) for October and open squares (□) for December.

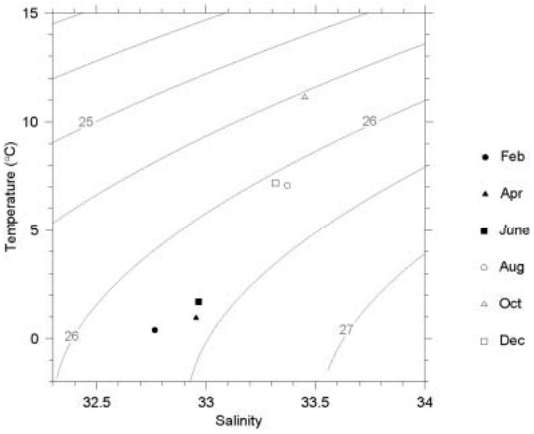

Fig. 9. Seasonal variation of the water type averaged for each month. Same symbols are used just as same as in Fig. 8.

値が非常に高いことには留意すべきであろう。

水型の季節変化

道東沿岸流の水塊の季節変化を見るためFig. 2からFig. 7の場合（1）の水塊分布を1枚の図に示したものがFig. 8である。また、各月について、場合（1）の水温・塩分の平均値から平均的な水塊の季節変化を示したものがFig. 9である。

先ず注目すべきことは、沿岸親潮の季節である年の前半（2、4、6月）と、道東暖流の季節である年の後半のデータが4℃を境に完全に分離されていることである。上述のように転換期の6月あるいは12月の分布特性が同じ季節の他の月に比べて若干異なった特性を持っているものの、やはり6月は沿岸親潮の季節に属し、12月は道東暖流の季節に属していると考えるのが自然であろう。Fig. 9の結果は、道東沿岸流の水塊特性は平均的にはTS図上を一定の曲線上を往復するように変化することを示している。（曲線の形状を決定するには、月別のデータを使用して分解能を細かく、せめて一か月にすることが望ましい。）

ここで注意しておきたいのは、水温・塩分の両断面に道東沿岸流が認められた場合（1）と両断面ともに認められなかった場合（3）を比較して、両者の差異が全般的に小さかったことである。実際には、水温・塩分の断面の一方（沿岸親潮では塩分断面、道東暖流では水温断面）のみに認められる場合（2）があり、この場合のデータはTS図上では場合（1）、場合（3）の中間にあり、両者と非常に重なって分布する。そのため、全体は1つのグループに属するようにしか見えない。また、Fig. 8、Fig. 9に見られるように、2か月ごとに見た季節変化は、各月の場合（1）の水型分布範囲に匹敵するか、それ以上に変化している。言いかえれば、道東沿岸流域水の水塊特性は、沿岸流の有無よりは、季節によって決まる傾向にあることを示す。前論文で示したように道東沿岸流の厚さは100m程度であるから、道東沿岸流水は、その源泉水の特性よりは、この海域にもたらされる途中での、大気との相互作用や、陸水の影響で決まっていることが強く示唆されていると考えられる。

4. 道東沿岸流の水塊の形成機構に関する考察

この研究は根室市水産研究所とロシアのサハニロ（SakhNIRO）研究所との共同研究ハナサキ・プログラムの一環として実施されたものである。このプログラムの中で、東北大学のグループは、ハナサキガニのDNAの解析を通して、知床半島の西側の斜里で得られるハナサキガニがサハリンで得られたカニと同じ集団に属するが、根室周辺および国後島と色丹島・歯舞諸島に挟まれたユジノクリリスク海峡（通常三角領域と呼ばれる）に生息するハナサキガニとは異なった集団に属することを示している（池田実：私信）。しかも、この2つの集団は遺伝学的に見て数万年前に分離させられたと推定される。このことは道東沿岸流水が、その源泉を直接オホーツク海に直接求め得る

かどうかに疑問を与えるものである。また、三角領域でハナサキガニの幼生が多数補足されるが、そこでの循環流の中にトラップされた形になっており（N. GALANIN: 私信）、そのため、根室周辺ではほとんど捕捉されないことが示されている。このことは、根室水道あるいは三角領域から道東沿岸流を形作るような海水の供給が行われていないことを示している。

現在資料の整理中であるが、ハナサキ・プログラムの中で、根室の太平洋側の落石岬の近くの三里浜沖にあるハナサキガニ試験操業地で水深５mから60m至る８点の海底水温の連続観測を行っている。その結果によると、沿岸親潮の最盛期にあたる２月を中心とする冬季には、海底水温は沖に向かって増加し、道東暖流期に対応する晩春から夏季にかけての海底水温は沖に向かって低下するという結果が得られている。記録の回収作業時にSTD観測を実施しているが、この両時期においては、極表面層を除いて、水温は鉛直方向にほぼ一様であり、これらの水温の岸―沖変化は、水温場の水平構造に起因していることが示される。これらの水温の水平構造は、そのまま沿岸親潮または道東暖流の水温の水平構造につながっているようである。このような構造が出現する時期は、浅海部のほうが、沖合の沿岸親潮・道東暖流の出現する時期よりも１ヶ月くらい先行する。もしも、一般的に道東沿岸流の水平温度勾配が岸近くまで続いているとするならば、他の海域の特徴的な水塊特性を持つ水の流入によっては、道東沿岸流の水塊を形成するとは出来ないことになる。

永田（2009）は道東海域を30分メッシュの領域にわけ過去のデータからそれぞれの領域における水温の季節変化を調べている。42°N線沿いに根室沖の領域AIからAVまで東方へ並ぶ５つの領域での季節変化を比べると、２・３月の沿岸親潮期の最低水温は東に進むほど高くなる傾向を示し、また、８・10月の道東暖流期の水温は東に進むほど低くなる傾向を示す。この水温の東西勾配の特性が、東西に圧縮された形で道東沿岸域にもたらされるとすると、道東沿岸流の特性的な構造を説明できる。

例え、道東沿岸流の水の起源がオホーツク海の水であっても、根室水道や、三角領域を通るような短い道筋を通らないとすると、道東に至るまでに、水塊の性質が大きく変質することは十分予想できるところである。明確な結論を得るには、直接的な海況観測を含めた更に多くの研究が必要とされようが、そのような研究に対して、この論文の成果がよい指針を与えうると信じる次第である。

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High tolerance of phytoplankton for extremely high ammonium concentrations in the eutrophic coastal water of Dokai Bay (Japan)

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Abstract: The tolerance of phytoplankton in Dokai Bay for an extremely high ammonium concentration in culture media has been studied. Six species of phytoplankton, three diatoms (two clones of *Skeletonema* sp. and *Chaetoceros* sp.) and three flagellates (*Heterosigma akashiwo*, *Chattonella antiqua* and *Karenia mikimotoi*) were grown in various concentrations of NH_4Cl. The results suggested that high ammonium concentrations had negative effects on phytoplankton growth. Non-indigenous species in Dokai Bay, Japan, *C. antiqua* and *K. mikimotoi*, were unable to grow at 200 and 150 μM, respectively. Growth rates of *Skeletonema* sp. isolated from Harima Nada (Seto Inland Sea, Japan), *Chaetoceros* sp. and *H. akashiwo* were reduced significantly at higher ammonium concentrations compared to the control treatment. However, such a high ammonium concentration of even 1,500 μM could not produce a significant adverse effect on the growth rate of *Skeletonema* sp. isolated from Dokai Bay. Furthermore, the maximum chlorophyll fluorescence of tested species was also gradually decreased with an increase in ammonium concentration. The influence of a high ammonium level on phytoplankton growth observed in this study confirmed the phytoplankton species composition observed in Dokai Bay. Our results suggested that such a high ammonium concentration was an important factor in determining the species composition of the phytoplankton assemblage in that bay.

Keywords: *Ammonium toxicity, growth inhibition, Dokai Bay*

1. Introduction

Since the last century, the role of ammonium in phytoplankton growth has attracted the attention of scientists, and several studies have been published underlining its importance. Among dominant nitrogen sources, ammonium is known as an excellent nitrogen source for phytoplankton growth. Phytoplankton are believed to prefer ammonium to nitrate even when both substrates are available due to the higher energy cost of nitrate utilization. Phytoplankton resume the uptake of nitrate when ammonium concentration declines (e.g. SYRETT, 1981 and reference therein; FLYNN, 1991; FLYNN *et al.*, 1997; LEVASSEUR, *et al.*, 1993; WASER *et al.*, 1998). Although ammonium is an important factor related to primary productivity in aquatic environments (NATARAJAN, 1970; DORTCH and CONWAY, 1984), it is generally found in lower concentrations than nitrate in natural environments. The availability of nitrate determined a new primary productivity in aquatic environments (DUGDALE and GOERING, 1967).

However, both negative and positive effects

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of ammonium on the growth of phytoplankton have been reported. For example, the inhibitory effects of ammonium on the phytoplankton growth rate and photosynthesis have been variously reported (e.g. NATARAJAN, 1970; THOMAS *et al.*, 1980; AZOV and GOLDMAN, 1982; LIVINGSTON *et al.*, 2002). Moreover, several studies have reported in both culture and field experiments that the presence of ammonium significantly reduced the nitrate uptake rate of several phytoplankton species (e.g. DORTCH and CONWAY, 1984; DORTCH *et al.*, 1991; HORRIGAN and MCCARTHY, 1982; LOMOS and GLIBERT, 1999). SYRETT (1981) summarized that active nitrate reductase (NR), an important enzyme for nitrate uptake, is not formed in the presence of ammonium. A decrease in NR activity by ammonium reflects the inhibition of nitrate incorporation (BERGES *et al.*, 1995). Changes in NR activity are most likely mediated by changes in enzyme protein at longer time scales, but perhaps by an inactivation mechanism on a scale of only minutes.

Generally, though the amounts of ammonium in unpolluted coastal waters rarely exceed 5 μM, a high ammonium concentration could be observed in several coastal areas due to the discharge of high-loading or untreated wastewater from human activity (e.g. LIVINGSTON *et al.*, 2002; TADA *et al.*, 2001). In Dokai Bay, one of the eutrophic embayments in Japan, a high ammonium concentration (e.g. >200 μM) that was toxic to organisms (e.g. RANDALL and TSUI, 2002) has often been observed, and due to its negative effect on growth rates, ammonium might be one of the factors that have determined the species composition of the phytoplankton community in that bay. Species tolerant of such a high ammonium concentration should produce a bloom more frequently observed than in species with a lower tolerance. These hypotheses were derived from field observations of dominant phytoplankton species during phytoplankton blooms in Dokai Bay. YAMADA and KAJIWARA (2004) reported that *Skeletonema* spp. usually formed the major component of the phytoplankton assemblage in Dokai Bay, while only a few blooms of other species were observed. Blooms of flagellates seldom occurred even though sufficient nutrients were available. In addition, the phytoplankton assemblage in the bay was dominated all year around by *Skeletonema* sp.

Although TADA *et al.* (2001, 2004) had already shown that the growth rates of phytoplankton were an important factor in species composition because of the rapid advection of the surface water mass by a strong estuarine circulation without vertical mixing, indicating that the influence of an extremely high ammonium concentration in the bay should also be discussed. The goal of this study was to clarify the influence of a high concentration of ammonium on phytoplankton growth and its consequences for species determination under eutrophic conditions such as those in Dokai Bay. The effects of ammonium were examined at various concentrations (including those similar to that of Dokai Bay) on the growth of indigenous and non-indigenous phytoplankton species.

2. Materials and methods

2.1 Influence of ammonium on phytoplankton growth

To examine and compare the influence of ammonium on phytoplankton growth under eutrophic conditions such as those in Dokai Bay, cultures of indigenous and non-indigenous species in the bay i.e. *Skeletonema* sp. and *Chaetoceros* sp. (Diatoms), *Heterosigma akashiwo* and *Chattonella antiqua* (Raphidophyceae) and *Karenia mikimotoi* (Dinoflagellates) were chosen for this study. *Skeletonema* sp. was isolated from both Dokai Bay and Harima Nada. Moreover, other phytoplankton species (*Chaetoceros* sp., *H. akashiwo*, *C. antiqua* and *K. mikimotoi*) were isolated from Harima Nada and maintained in the culture collection of the Kagawa University Laboratory.

Seawater (filtrated through Whatman GF/F filters) from Harima Nada was used to prepare an ESM culture medium (OKAICHI *et al.*, 1983) without Tris-hydroxymethyl-aminomethane. The pH of each culture medium was initially adjusted to 8.0. Culture media were then transferred to a 50-ml borosilicate glass test tube with a Teflon-lined cap, and autoclaved at 65℃ for 60 minutes. The resulting medium contain-

ing 1,400 μM of nitrate as the sole N source was used for the following experiments together with various concentrations of ammonium.

Each test species was inoculated into the culture media with a different level of ammonium concentrations by the addition of NH_4Cl and incubated at 21℃ in 100 μmol photons m^{-2} s^{-1} (14:10 hours Light-Dark cycle). Based on the ammonium concentration of the surface water in Dokai Bay, eleven different final ammonium concentrations (0 (no ammonium addition and nitrate as the sole N source), i.e. 10, 50, 100, 150, 200, 500, 700, 1000, 1250 and 1500 μM (with 1,400 μM of nitrate)) were chosen for *Skeletonema* sp., *Chaetoceros* sp. and *H. akashiwo*, while lower levels (0, 5, 10, 25, 50, 100, 150, 200, 300 and 500 μM) were used for *C. antiqua* and *K. mikimotoi*. All treatments were conducted independently in triplicate. Sterile technique was used throughout the study to prevent bacterial contamination. Prior to the experiment, all test species were acclimated by being grown in the desired ammonium concentration for at least six generations. The acclimated exponential phase of test species was inoculated into the new culture medium. Following inoculation, *in vivo* chlorophyll fluorescence was determined at 24-h intervals using a fluorometer (Turner Design 10-AU-005) (BRAND *et al.*, 1981). The experiment was terminated when most replicates began to decline. The growth rate (μ_2, divisions day^{-1}) was estimated from *in vivo* chlorophyll fluorescence data during the exponential phase of growth using the following formula:

$$\mu_2=\frac{(\log_2 F_2-\log_2 F_1)}{(t_2-t_1)},$$

where F_2 and F_1 are the *in vivo* chlorophyll fluorescences in the exponential phase at times 2 (t_2) and 1 (t_1) after incubation, respectively. A minimum of 3 sampling points was included in each calculation. The growth rate and maximum chlorophyll fluorescence of test species in a concentration series of ammonium were compared to the no-ammonium addition treatment (control treatment) using one-way analysis of variance (SPSS® version 10.0 software) to evaluate the influence of ammonium on phytoplankton growth. A post-hoc comparison of means was conducted when a significant difference ($p<0.05$) was observed.

2.2 Relationship between *in vivo* chlorophyll fluorescence and cell density

Another experiment, using the protocol described above, was conducted to assess the correlation between *in vivo* chlorophyll fluorescence and cell density. That correlation was tested on *Skeletonema* sp. and *C. antiqua* at two ammonium concentrations (i.e. no ammonium addition with nitrate (1,400 μM) as the sole N source, and high ammonium concentration with 1,400 μM NO_3^-). That correlation was determined at high ammonium concentrations (500 and 150 μM, respectively) with no ammonium addition. *In vivo* chlorophyll fluorescence was determined using a fluorometer (Turner Design 10-AU-005), and cell density by using a cell and particle counter (Beckman Z2™ Coulter Counter®).

2.3 Influence of pH on growth of *Skeletonema* sp.

An additional experiment using the protocol described above was carried out to assess the influence of pH. The variations of pH during the growth of *Skeletonema* sp. isolated from Dokai Bay under a number of ammonium concentrations (0, 10, 50, 100, 150, 200 and 500 μM) were determined daily using a Shidengen ISFET pH-meter (KS 701).

2.4 Phytoplankton growth and ammonium concentration of Dokai Bay water

The influence of high ammonium on the species composition of the phytoplankton assemblage in Dokai Bay was assessed. To evaluate that influence, the previous data set of ammonium concentrations of surface water obtained from an intensive monitoring program in the bay (SUKSOMJIT *et al.*, 2005) were used. In addition, ammonium concentrations of surface water at 7 stations in the bay from 1996 to 1997 as well as the tolerance data obtained in this study were examined.

3. Results

3.1 Relationship between *in vivo* chlorophyll

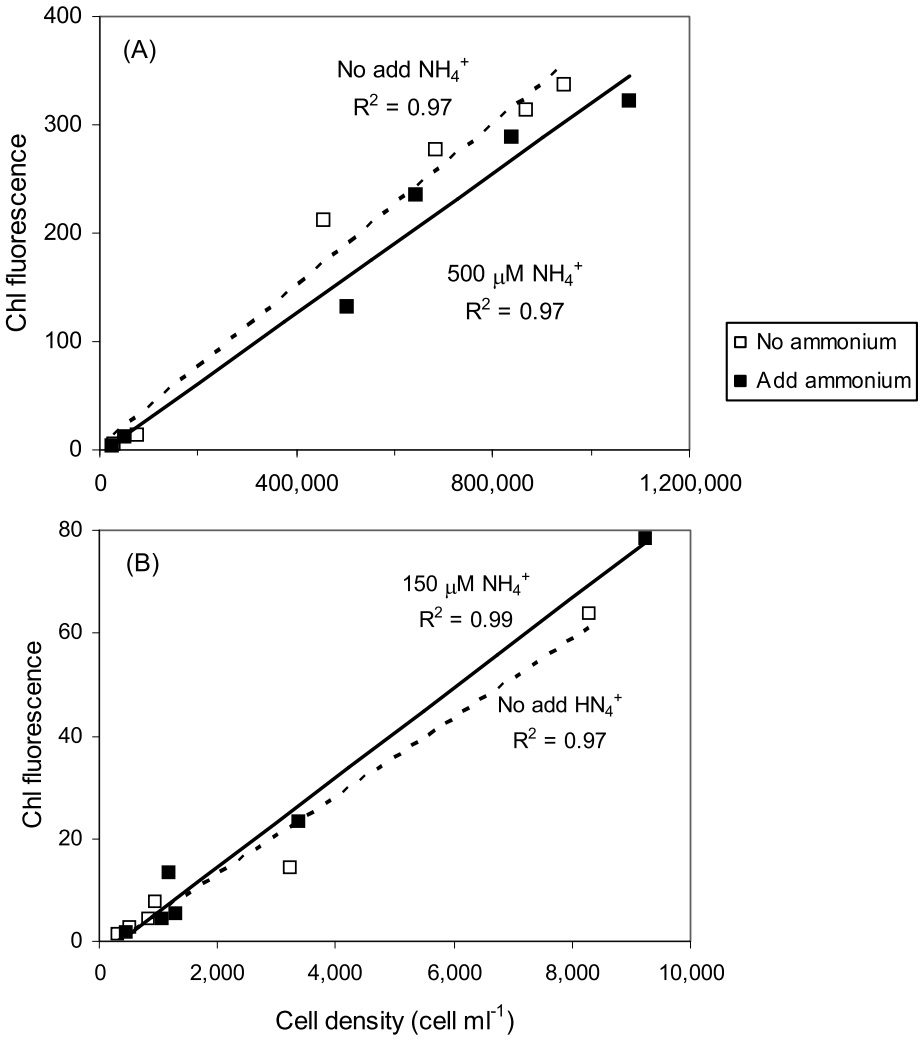

Fig. 1. Correlation between chlorophyll fluorescence and phytoplankton cell density of *Skeletonema* sp. (A) and *Chattonella antiqua* (B) under different ammonium concentrations.

fluorescence and cell density

Figure 1 shows the relationship between the *in vivo* chlorophyll fluorescence and phytoplankton cell density of *Skeletonema* sp. and *C. antiqua* under different ammonium concentrations. Linear regressions of *in vivo* chlorophyll fluorescence versus cell density observed at all ammonium concentrations of both test species and correlation coefficients (R^2) were > 0.97. Moreover, there was no difference (p>0.05) in the correlation between *in vivo* chlorophyll fluorescence and cell density at high ammonium concentration vs. no ammonium addition. This showed that the cells responding to severe conditions of high ammonium concentration based on their cellular chlorophyll contents were not different from those under normal conditions. This result indicated that we could calculate the growth rate using fluorescence instead of cell density, and could discuss our results using data calculated from *in vivo* chlorophyll fluorescence for cell yields.

3.2 Effect of ammonium on growth rates

The growth rates of all tested species and the

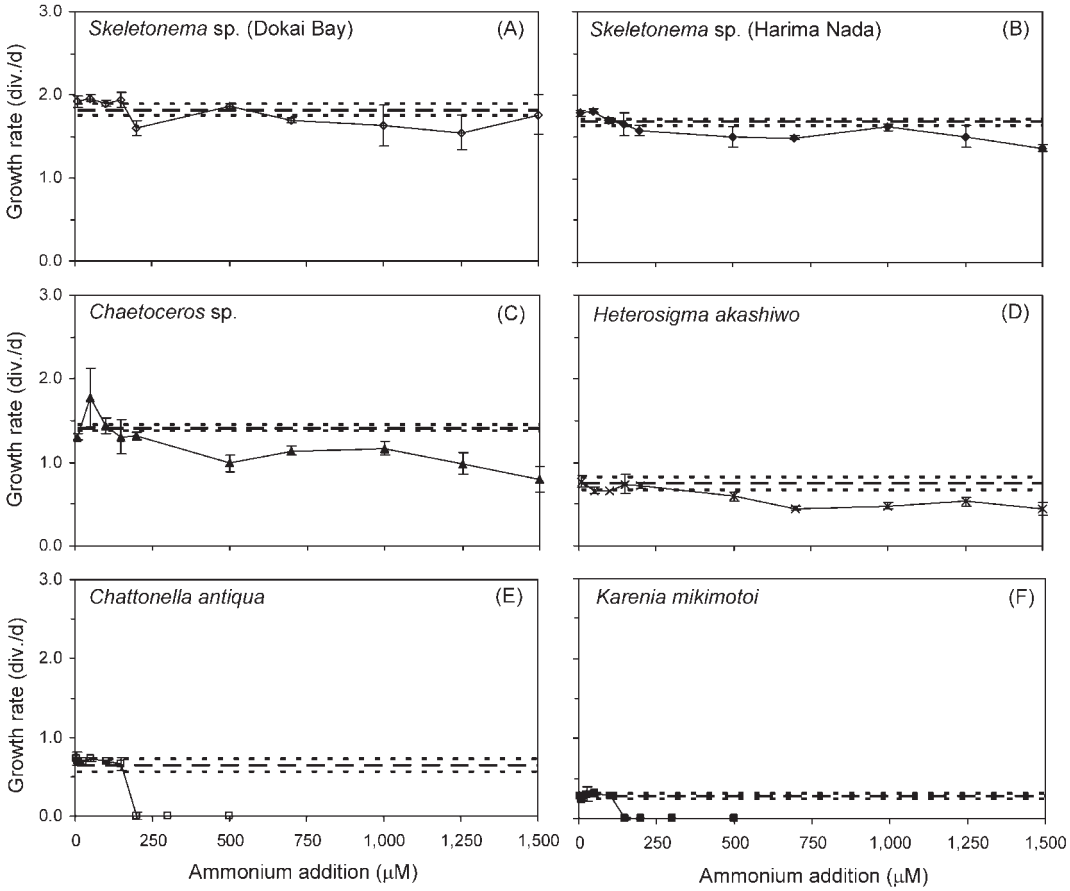

Fig. 2. Growth rates (μ_2, divisions day^{-1}) of *Skeletonema* sp. isolated from Dokai Bay (A) and Harima Nada, Seto Inland Sea (B), *Chaetoceros* sp. (C), *Heterosigma akashiwo* (D), *Chattonella antiqua* (E) and *Karenia mikimotoi* (F) exposed to various ammonium addition. Vertical bars are standard deviations (S.D.) of replicate samples. Longer dashed lines indicate the growth rate of the control treatment (no NH_4^+ addition) and shorter dashed lines indicate ± S.D.

growth rate variations in a series of ammonium concentrations were shown in Fig. 2. Among test species, the growth rates of *Skeletonema* sp. isolated from both Dokai Bay and Harima Nada were slightly higher than those of *Chaetoceros* sp. and markedly higher than those of other test species. The presence of high ammonium exerted a significant impact on the growth rate of indigenous test species, i.e. *Skeletonema* sp., *Chaetoceros* sp. and *H. akashiwo*. Moreover, the lethal effect of such high ammonium concentrations as those found in Dokai Bay became evident in two non-indigenous flagellates, i.e. *C. antiqua* and *K. mikimotoi*.

Since no lethal effect at even the highest ammonium concentrations was found in all indigenous species, the effect of ammonium on the growth rate varied among each species tested. Among the indigenous species, ammonium at a high concentration of even 1,500 μM had no significant effect on the growth rate of *Skeletonema* sp. isolated from Dokai Bay compared to the control, which had no added ammonium and in which nitrate was the sole N source (Fig. 2A). The growth rate of *Skeletonema* sp. varied from 1.60 to 1.96 divisions day^{-1}, and the rates at high ammonium concentrations (700, 1,000, 1,250 and 1,500 μM) were similar to those in with the control

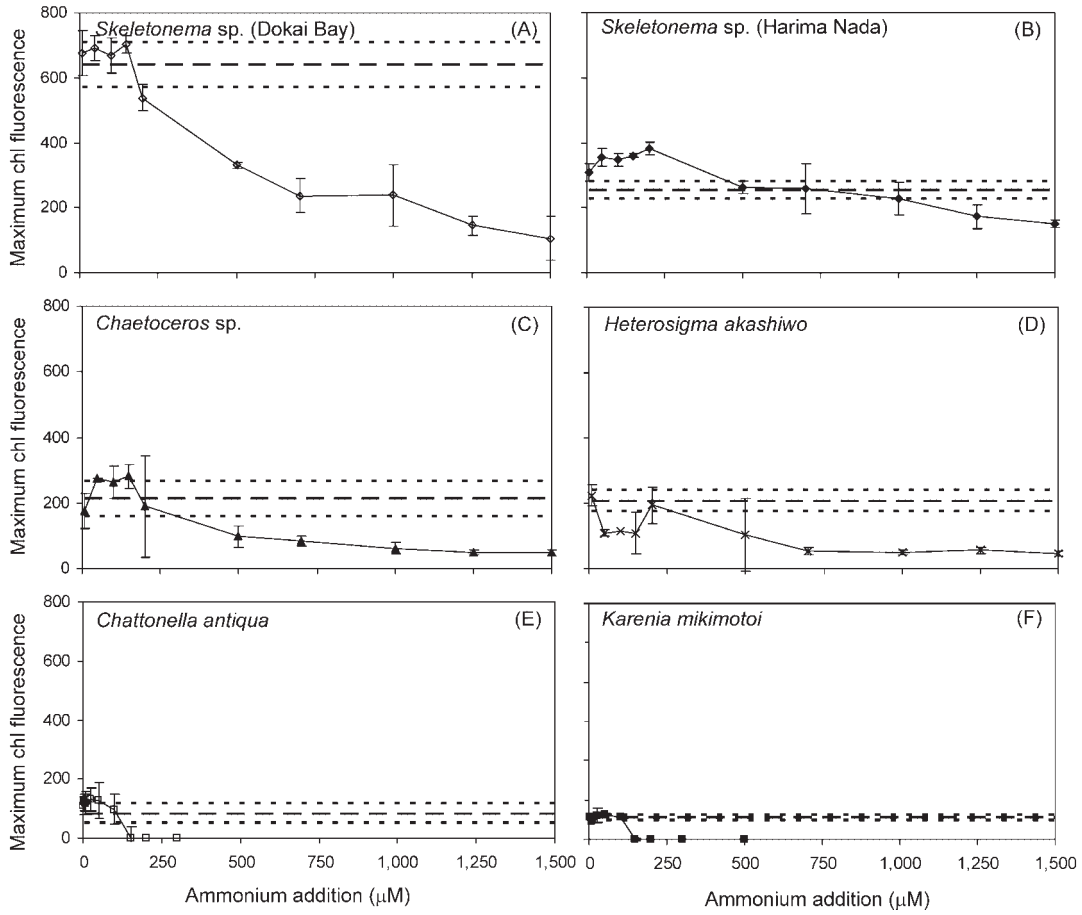

Fig. 3. Maximum chlorophyll fluorescence of *Skeletonema* sp. isolated from Dokai Bay (A) and Harima Nada, Seto Inland Sea (B), *Chaetoceros* sp. (C), *Heterosigma akashiwo* (D), *Chattonella antiqua* (E) and *Karenia mikimotoi* (F) exposed to various ammonium addition. Vertical bars are standard deviations of replicate samples. Longer dashed lines indicate the maximum chlorophyll fluorescence of the control treatment (no NH_4^+ addition) and shorter dashed lines indicate ± S.D.

treatment. Unlike the clone from Dokai Bay, the *Skeletonema* sp. isolated from Harima Nada, *Chaetoceros* sp. and *H. akashiwo*, evidenced a significant reduction in their growth rate at higher ammonium concentrations. The growth rates of *Skeletonema* sp. from Harima Nada and *Chaetoceros* sp. were significantly (p <0.05) reduced at ammonium concentrations of 500, 700, 1,250 and 1,500 μM but not at 1,000 μM compared to the control treatment (Fig. 2B and 2C). Those rates also varied from 1.37 to 1.51, and from 0.80 to 1.14 divisions $d-a y^{-1}$, whereas the growth rate of the control treatment was 1.68 and 1.41 divisions day^{-1}. In addition, *H. akashiwo* growth rates were significantly lower than the control treatment when ammonium concentrations reached 500 μM or higher (Fig. 2D). The growth rate at those concentrations varied from 0.44 to 0.59 divisions day^{-1}, whereas that in the control treatment was 0.75 divisions day^{-1}.

The lethal effect of high ammonium concentrations was shown in both non-indigenous flagellates, *C. antiqua* and *K. mikimotoi*. These test species were unable to grow when the ammonium concentrations reached 200 and 150 μM, respectively (Figs. 2E and 2F), whereas below those concentrations, they could have survived and grown at the same rate as the control treatment.

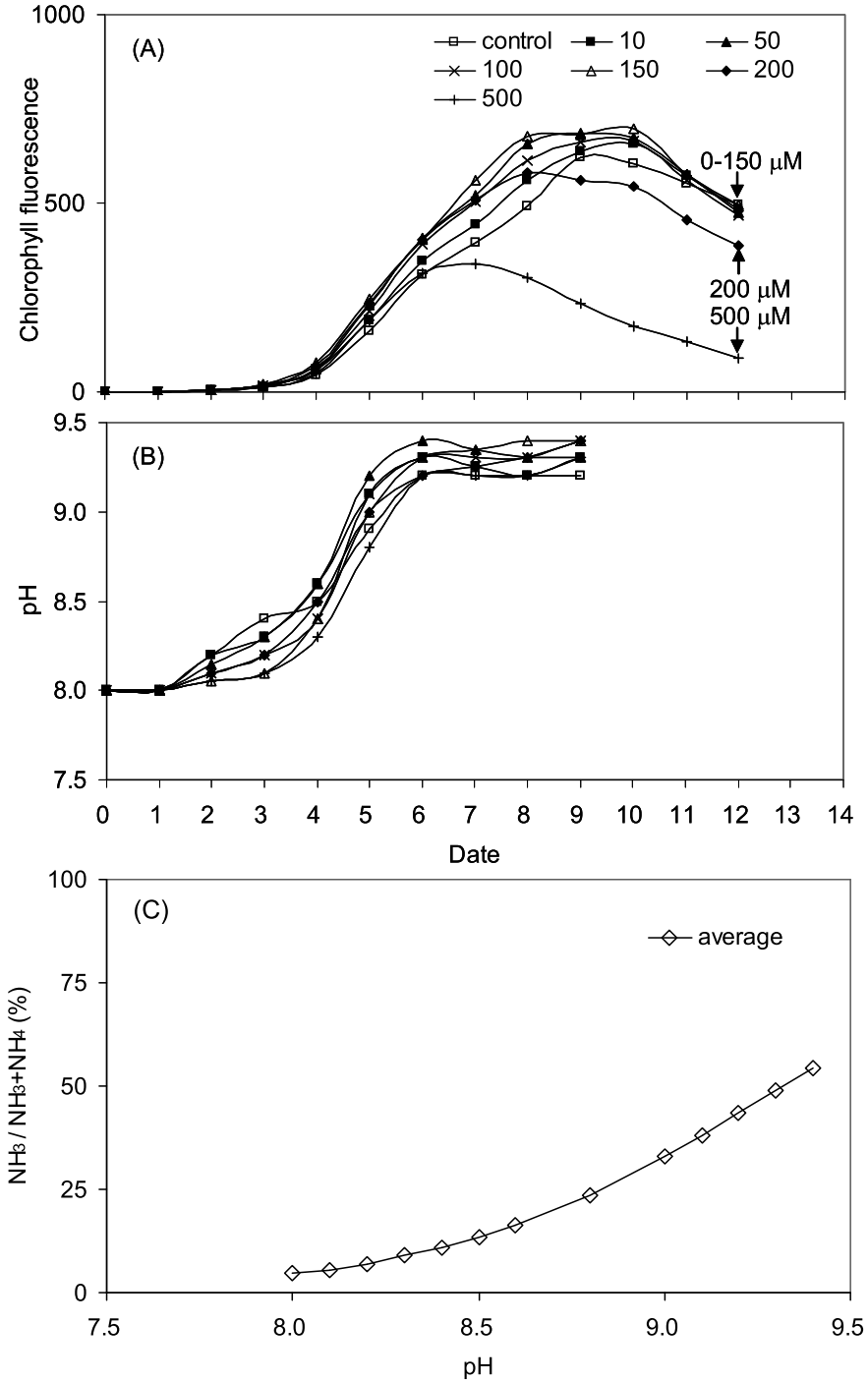

Fig. 4. Influence of pH on toxicity of ammonium. (A) indicates the variation of chlorophyll fluorescence of *Skeletonema* sp. (Dokai Bay) and (B) indicates pH variation at various ammonium concentrations. Average relative percentage of NH_3 in NH_3 NH_4 due to increase of pH at various ammonium concentrations is shown in (C).

In this study, an acceleration of growth rates at lower ammonium concentrations was observed. The growth rate of *Chaetoceros* sp. at 50 μM was significantly higher ($p<0.05$) than that of the control treatment as well as other ammonium concentrations. However, there was no acceleration effect on growth rates of other indigenous and non-indigenous species at lower ammonium concentrations.

3.3 Effect of ammonium on maximum chlorophyll fluorescence

The maximum chlorophyll fluorescence of all species tested in a series of ammonium concentrations was shown in Fig. 3. Maximum chlorophyll fluorescence of all test species gradually decreased with the increase in ammonium concentration. However, the level of ammonium that adversely affected the maximum chlorophyll fluorescence varied from species to species.

In Fig. 3A, the maximum chlorophyll fluorescence of *Skeletonema* sp. isolated from Dokai Bay did not differ from that in the control treatment at low ammonium concentrations from 10 to 150 μM. That maximum level rapidly decreased when the ammonium concentration reached 200 μM. From 200 to 1,500 μM, the maximum chlorophyll fluorescence decreased gradually as a function of ammonium concentration, reaching a significantly lower level ($p<0.05$) compared to that in the control treatment. In the case of the clone from Harima Nada (Fig. 3B), the maximum chlorophyll fluorescence was significantly higher ($p<0.05$) than that in the control treatment at low ammonium concentrations from 10 to 200 μM. That maximum decreased when the ammonium reached over 500 μM. However, the maximum chlorophyll fluorescences at 1,250 and 1,500 μM were significantly lower ($p<0.05$) than those in the control treatment. As for *Chaetoceros* sp., its maximum chlorophyll fluorescences did not differ from those of the control treatment at low ammonium concentrations and then decreased when ammonium exceeded 200 μM (Fig. 3C). The *Chaetoceros* sp. maximum was significantly lower ($p<0.05$) than that of the control treatment when ammonium concentrations ranged between 500 and 1,500 μM. Similar to the other indigenous species, the adverse effect on the maximum chlorophyll fluorescence of *H. akashiwo* emerged from an ammonium concentration of 25 μM up to the highest concentration (Fig. 3D). The maximum chlorophyll fluorescence of this test species at 500 to 1,500 μM was significantly lower ($p<0.05$) than the one in the control-treatment maximum.

Although those two non-indigenous species, *C. antiqua* and *K. mikimotoi*, were unable to grow at ammonium concentrations of 200 and 150 μM, respectively, no adverse effect on their maximum chlorophyll fluorescence at lower ammonium concentrations was observed. In Figs. 3E and 3F, the maximum chlorophyll fluorescence of *C. antiqua* and *K. mikimotoi* varied over a small range and did not differ from the control-treatment maximum.

3.4 Variation of pH

During the growth of *Skeletonema* sp. isolated from Dokai Bay under various ammonium concentrations (Fig. 4A), the pH in the culture medium of all treatments increased slightly between days 1 and day 3, but then increased rapidly, reaching 9.0 on day 5. This phenomenon was also observed in the control treatment. On day 6, the pH of all treatments varied over 9.0 (Fig. 4B).

4. Discussion

4.1 Inhibition effect of high ammonium on phytoplankton growth

In this study, the presence of high ammonium caused significant effects on the growth of phytoplankton. At a high ammonium concentration, suppression of the growth rates and maximum chlorophyll fluorescence of all indigenous species or a total growth inhibition in the case of *C. antiqua* and *K. mikimotoi* were observed. Suppressions of the growth rates found in the present study also corresponded with those in previous studies (Admiraal, 1977; Abeliovich and Azov, 1976; Bates *et al.*, 1993; Källqvist and Svenson, 2003; Livingston *et al.*, 2002). Admiraal (1977) reported that ammonia at >500 μM reduced the growth of benthic diatoms. Källqvist and Svenson (2003) found that ammonium at 224

Table. 1. Summary of negative ammonium effects and ranges on several phytoplankton species

Species	Effect	NH_4^+ conc. (μM)	Remark	Sources
Thalassiosira pseudonana	reduced NO_3^- uptake	3	6-min incubation	Yin *et al.* (1998)
Scenedesmus obliquus, Phaeodactylum tricornutum				
Dunaliella tertidecta	reduced photosynthesis	>2,000	90-min incubation	Azov and Goldman (1982)
D. tertidecta				
	reduced photosynthesis	15	15-min incubation	Turpin (1983)
Alexandrium tamarense	increased growth	6-50	−	Leong *et al.* (2004)
	reduced growth	100		
Navicular arenaria, N. c.f. dissipata				
N. dubiformis,Amphiprora c.f. paludosa	reduced growth	>500	−	Admiraal (1977)
Stauroneis constricta, Gyrosigma spencerii, N. sigma				
		95-166	(pH 7-8)	
Nephroselmis pyriformis	inhibited growth rate	21-70.7	(pH>8)	Källqvist and Svenson (2003)
			24-h exposure time	
Nitzschia pungens	reduced cell yield	>110		
	prevented growth	>880	−	Bates *et al.* (1993)
Skeletonema costatum	no effect	>880		
Pseudonitxchia pungens f. multiseries Hasle	reduced growth rate	>20		
			−	Hillbrand and Sommer (1996)
S. costatum	increased growth	4.2	−	
	reduced chlorophyll *a*	32.8		Livingston *et al.* (2002)

μM reduced the growth rate of *Nephroselmis pyriformis* (Chlorophyta) within 24h. LIVINGSTON *et al.* (2002) showed that the chlorophyll *a* concentration was significantly decreased at ammonium concentrations from 0.11 to 0.24 mg l^{-1} and even much lower at over 0.46 mg l^{-1}. HILLEBRAND and SOMMER (1996) reported that ammonium inhibited or at least slowed down the nitrate uptake at a high ammonium/nitrate ratio, leading to a lower growth rate of *Pseudo-nitzschia pungen* f. *multiseries* Hasle. A summary of the negative ammonium effects and ranges on several phytoplankton species was shown in Table 1. Moreover, the concentration of ammonium that caused the inhibition of growth rates in test species was about the same across several species. BATES *et al.* (1993) reported little suppression of the *Skeletonema costatum* cell yield at 880 μM (the highest test concentration). FUKAZAWA (1980) found that the growth rate of *Gymnodinium* sp. decreased at 50 μM of NH_3. NAKAMURA and WATANABE (1983) indicated that ammonium concentrations higher than 150 μM caused a severe inhibition of *C. antiqua* growth, and that the species could not survive at 300 μM.

Fig. 4C showed the relative percentage of NH_3 in NH_3 plus NH_4^+, which was calculated by the equation presented in KÖRNER *et al.* (2001). In the present study, a lower yield of maximum chlorophyll fluorescence at higher ammonium concentrations with almost a similar growth rate was observed. The onset of premature senescence (Fig. 4A) seemed to be triggered by the increase in ammonia (NH_3) due to the pH increase initially from 8.0 to over 9.0 on day 6 (Fig. 4B). We considered that the lower yield of maximum chlorophyll fluorescence of phytoplankton test species at the higher ammonium concentration shown in Fig. 3 was due to the increase in ammonia concentration following the elevation of pH. This suggested that the inhibition effect on phytoplankton growth occurred when the proportion of NH_3 increased due to pH elevation. KÄLLQVIST and SVENSON (2003) also suggested that the toxicity of total ammonia (the sum of NH_4^+ and NH_3) strongly depended on pH and NH_3 is the main toxic form of total ammonia.

4.2 Tolerances among phytoplankton species

The tolerances of test phytoplankton species to ammonium varied with each phytoplankton species showing different growth rates under various ammonium concentrations. Comparing the growth rates of all test species, we found that *Skeletonema* sp. isolated from Dokai Bay showed similar growth rates even at 1,500 μM, whereas the growth rates of *Skeletonema* sp. isolated from Harima Nada, *Chaetoceros* sp. and *H. akashiwo*, gradually diminished with the increase in ammonium concentrations over 500 μM. Moreover, *C. antiqua* and *K. mikimotoi* were unable to grow with ammonium concentrations of 200 and 150 μM, respectively. An unchanged growth rate despite high ammonium concentrations indicated that *Skeletonema* sp. isolated from Dokai Bay was more tolerant than *Chaetoceros* sp. and *H. akashiwo*. Moreover, the lethal effect on *C. antiqua* and *K. mikimotoi* also suggested that, compared to indigenous species, non-indigenous species in Dokai Bay were less tolerant. Superior tolerance for the ammonium of diatoms, particularly *Skeletonema* sp., than that shown by other phytoplankton species was reported in several previous studies. FUKAZAWA (1980) found that 50 μM of NH_3 failed to inhibit the growth rate of *S. costatum,* whereas those of *H. akashiwo* and *Gymnodinium* sp. were inhibited at that concentration. BATES *et al.* (1993) concluded that *Skeletonema* was the species most tolerant of high ammonium. Levels higher than 110 μM were found to significantly reduce the photosynthetic rate of *Nitzschia pungens*, while having no effect on that of *S. costatum*. In addition, we could assume that *Skeletonema* sp. isolated from Dokai Bay was more tolerant of ammonium than *Skeletonema* sp. isolated from Harima Nada. The differences in ammonium tolerance among phytoplankton species isolated from different locations have also been reported in a previous study. HILLEBRAND and SOMMER (1996) discovered major differences in ammonium tolerances among *Pseudo-nitzschia* clones isolated from locations under a variety of original conditions.

Therefore, the result from this study simulated conditions of high ammonium

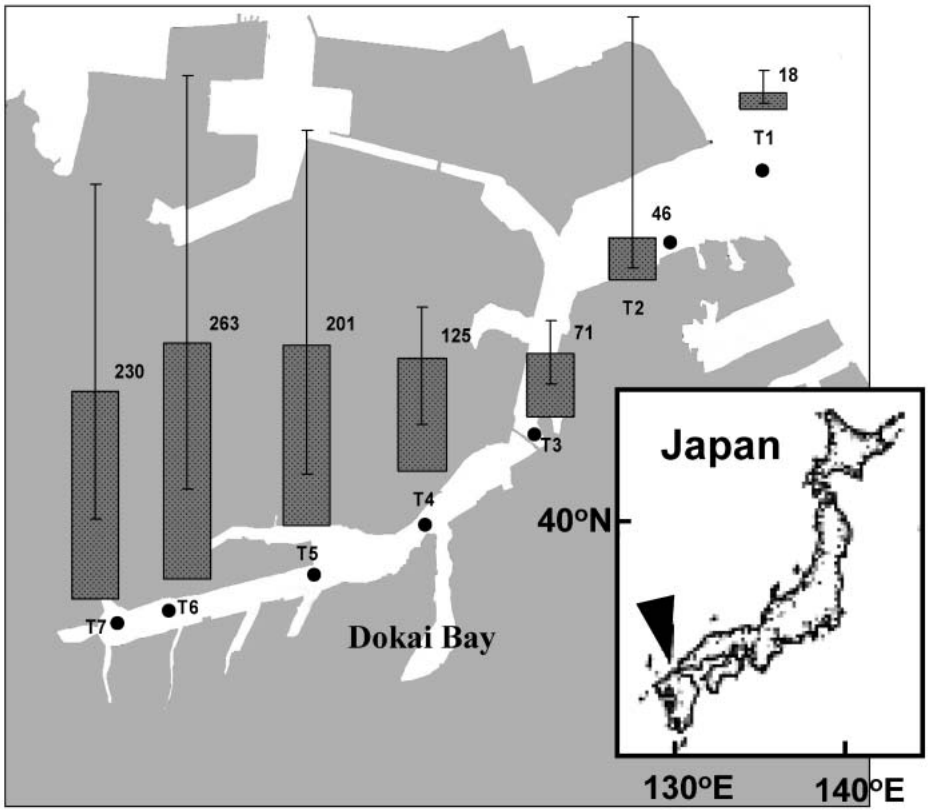

Fig. 5. Map of Dokai Bay, in northern Kyushu Island, Japan. Average ammonium concentration (μM) of surface water since 1996–1997 from Station T1 (bay mouth) to T7 (inner part). Bar graphs represent maximum and minimum ammonium concentrations and black dot (●) indicates sampling stations. Vertical bars show range (minimum to maximum) of ammonium concentration at each sampling station.

concentrations and sufficient nutrients in Dokai Bay, demonstrated that the tolerance of ammonium was also one factor that determined the species composition of the phytoplankton assemblage in that bay.

4.3 Field implications

It is widely acknowledged that phytoplankton growth in natural environments is controlled by three key factors, i.e. physical (e.g. water circulation, light intensity, water temperature, etc.), chemical (e.g. nutrients, trace elements, etc.) and biological (e.g. taxonomic variations, phytoplankton origins, zooplankton grazing, etc.). Under suitable conditions, phytoplankton grow actively and often produce blooms. TADA *et al.* (2001 and 2004) concluded that the nutrient concentration in Dokai Bay was sufficient for phytoplankton growth during the entire year, and that phytoplankton blooms were controlled by strong water circulation. They suggested that only phytoplankton species having a higher growth rate than the flash-out speed of the surface water mass could be dominant and would subsequently produce a bloom. However, the results from this study indicated that high ammonium had the potential to function as a selective factor in determining the species composition and the dominant species of phytoplankton blooms in Dokai Bay.

YAMADA and KAJIWARA (2004) reported that the frequency of *Skeletonema* red tides was highest in Dokai Bay. They reported 51 red tides occurring during the observation period, 36 of which were attributed to *Skeletonema*

species, which 20 times were sole and 16 were a mixture with other diatoms or flagellates; however, a few *Chaetoceros* sp. and *H. akashiwo* blooms were also observed. Furthermore, there were no reports on blooms of *C. antiqua* or *K. mikimotoi*. Data from an intensive monitoring program from 1996 to 1997 (SUKSOMJIT *et al.*, 2005) indicated that a high ammonium concentration was always found in this bay throughout the year. The average ammonium concentrations of surface water between 1996 and 1997 plotted from the bay mouth (Station T1) to the inner part (Station T7) were shown in Fig. 5. In this bay, phytoplankton blooms were usually observed at the inner part, with phytoplankton biomass decreasing gradually from there to the bay mouth (YANAGI *et al.*, 1997). Moreover, at Stations T5, T6 and T7, important areas for bloom development (YANAGI *et al.*, 1997), average ammonium concentrations were at their highest, often exceeding 200 μM. As a rule, concentrations decreased gradually from the inner part to the bay mouth (Station T1). Thus, blooms of *Skeletonema* spp., *Chaetoceros* sp. and *H. akashiwo* but none of *C. antiqua* or *K. mikimotoi* were compatible with each species tolerance of the existing level of ammonium concentration. Although a level of 200 μM had a lethal effect on *C. antiqua* and *K. mikimotoi* growth, no inhibitory effect on *Skeletonema* sp., *Chaetoceros* sp. or *H. akashiwo* could be observed at the existing level of ammonium concentration. This would explain why these highly tolerant species (i.e. *Skeletonema* sp., *Chaetoceros* sp. and *H. akashiwo*) could be readily observed in this area, whereas *C. antiqua* and *K. mikimotoi* could not. Moreover, superior tolerance for the ammonium of the *Skeletonema* sp. isolated from Dokai Bay should be observed more frequently than species with lower tolerance. This finding coincided with the observations of *Skeletonema* sp. all year long (TADA *et al.*, 2004). This influence of high ammonium on the determination of species composition was reported in several previous studies. ADMIRAAL (1977), for example, revealed that the distribution of benthic diatoms in an estuary would be affected by the occurrence of ammonia, and that *Navicula salinarum*, with its extreme tolerance of high ammonium, was the dominant species in that polluted mudflat. LIVINGSTON *et al.* (2002) found that phytoplankton abundance and species richness were significantly lower in the Amelia River-Estuary, which received wastewater from a nearby pulp mill. HÜRLIMANN and SCHANZ (1993) reported that the addition of 364 μM or more resulted in a decrease in the biomass and triggered drastic changes in species composition; after a 42-day enrichment period, diatoms (which are known to be tolerant of organic pollution) were found.

5. Conclusion

In summary, we have concluded that ammonium at high concentrations functions as an important factor in regulating phytoplankton growth and dominant phytoplankton species via the different ammonium tolerance levels of each species. TADA *et al.* (2001, 2004) had already shown that the growth rates of phytoplankton were an important factor in determining species composition because of the sudden advections of the surface water mass by a strong estuarine circulation without vertical mixing. However, we considered that the results of this current study provide additional evidence for why *Skeletonema* sp. was such a dominant species in Dokai Bay, producing blooms more frequently than *Chaetoceros* sp. or *H. akashiwo*. These results also provided convincing evidence why some flagellates, (i.e. *C. antiqua* and *K. mikimotoi*) were not observed in this bay.

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Ammonium accelerates the growth rate of *Skeletonema* spp. in the phytoplankton assemblage in a heavily eutrophic embayment, Dokai Bay, Japan

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Abstract: High ammonium loading in Dokai Bay produces large diatom blooms and therefore laboratory experiments was conducted to determine if ammonium stimulated the occurrence of the observed massive phytoplankton blooms. The presence of ammonium as a nitrogen source significantly increased the growth rate of *Skeletonema* spp. compared to nitrate. Growth rate (μ_{chl}) on ammonium was significantly higher (~13–15%) than on nitrate. However, the effect of ammonium on growth rate acceleration was species specific, because the effect was not observed when the field assemblage was a mixture of *Skeletonema* spp. and other diatoms. In addition, the magnitude of the growth acceleration effect varied, depending on the irradiance level. The largest significant increase in growth rate on ammonium compared to nitrate occurred when irradiance was at irradiance around 200 μmol photons m^{-2} s^{-1}. Our results suggested that the growth of *Skeletonema* spp. which was dominant species in Dokai Bay was accelerated by ammonium and particularly under an irradiance which occurs in the mixed surface layer of this bay in summer. This bay may act like a selective growth incubator for certain diatoms such as *Skeletonema* that are subsequently exported to nearby coastal waters.

Keywords: *High ammonium, growth rate stimulation, irradiance, Dokai Bay, Skeletonema, nitrogen.*

1. Introduction

In most coastal environments, nitrogen is the important macronutrient for the growth of phytoplankton. A deficit of nitrogen reduces primary productivity and an excess of nitrogen can stimulate excessive algal blooms. Among the various forms of nitrogen, ammonium and nitrate are traditionally considered to be the most important nitrogen sources for a natural phytoplankton assemblage (e.g. SYRETT, 1981; LEVASSEUR *et al.*, 1993; HERNDON and COCHLAN, 2007).

Anthropogenic nutrient pollution is considered to cause one of the most pervasive changes in lower trophic levels in the coastal environment. The input of inorganic nitrogen, particularly ammonium from sewage, agricultural runoff, or untreated wastewater from industry, is steadily increasing and causes the deterioration of water quality. In Japan, for example, untreated wastewater from

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industries along the coast of Dokai Bay, Kyushu Island, caused the deterioration of water quality since 1960. High ammonium concentrations were observed throughout the year particularly in the inner bay (TADA *et al.*, 2001). High concentrations of ammonium are increasingly found in several discharge areas (e.g. LIVINGSTON *et al.*, 2002).

The responses of phytoplankton to an increase of ammonium concentration have been reviewed previously (e.g. SYRETT, 1981; FLYNN, 1991; FLYNN *et al.*, 1997). The adverse effect of high (toxic) ammonium on phytoplankton growth has been reported in several studies (e.g. NATARAJAN (1970); AZOV and GOLDMAN (1982); LIVINGSTON *et al.* (2002)). On the other hand, the stimulation of growth rate of phytoplankton by ammonium has also been reported for laboratory cultures (THOMPSON *et al.,* 1989; LEVASSEUR *et al.*, 1993; HERNDON and COCHLAN, 2007).

Early studies in Dokai Bay, suggested that strong estuarine circulation was the explanation for the occurrence of phytoplankton blooms during only summer (YANAGI and YAMADA, 2000; TADA *et al.*, 2001 and 2004). YAMADA and KAJIWARA (2004) reported that 51 phytoplankton blooms were observed in this bay between 1980 and 1995, and 20 phytoplankton blooms were dominated by *Skeletonema costatum* and *S. tropicum* and it was also a co-dominant 16 times in blooms with other species. However, *S. costatum* has long been considered one of the most conspicuous and widespread members of the coastal marine phytoplankton. Recently, it was discovered that a number of distinct species were included under this name (SARNO *et al.*, 2005) and they are difficult or impossible to distinguish based on morphological characters. So, we expressed the species *Skeletonema* spp. in this paper. In contrast, *Chaetoceros* sp. and *Heterosigma akashiwo* blooms were rarely observed. TADA *et al.* (2001) reported that the phytoplankton assemblage in Dokai Bay was dominated by *S. tropicum* in summer season and *S. costatum* in other seasons. However, the occurrences of phytoplankton blooms in this bay appear to be associated with the high ammonium concentrations. This hypothesis is derived from the observation of *Skeletonema* spp. as a dominant species throughout the year and *Skeletonema* spp. usually formed the major component of these phytoplankton blooms. Recently, it was determined that the high ammonium level that was found in the inner bay was an important factor regulating phytoplankton growth and dominant species through differences in ammonium tolerance efficiency (SUKSOMJIT *et al.*, in press).

It was not clear whether the presence of high ammonium enhanced the occurrence of very frequent *Skeletonema* spp. blooms in this bay with strong estuarine circulation, although the growth stimulation of phytoplankton by ammonium has been previously reported in the laboratory (THOMPSON *et al.*, 1989; LEVASSEUR *et al.*, 1993). In addition, there is a need to clarify the effect of irradiance on the growth rate of *Skeletonema* spp. under different nitrogen sources, because the previous studies revealed that the influence of ammonium acceleration of growth rate depends on light irradiance (e.g. HERNDON and COCHLAN, 2007; WOOD and FLYNN, 1995). The goal of this study was to clarify the effect of ammonium on the acceleration of phytoplankton growth rate in Dokai Bay, with a focus on *Skeletonema* since it was always the dominant bloom forming species.

2. Materials and methods

2.1 Effect of ammonium on phytoplankton growth

Natural phytoplankton assemblages and natural seawater were collected from the surface of the middle and outer part of Dokai Bay (station DK1 and DK2, Fig. 1) on June 20 and July 31, 2007. The samples were pre-filtered through a 300 μm mesh net in order to remove large zooplankton and brought back to the laboratory.

The natural phytoplankton assemblage collected from station DK1 was diluted 50-fold with filtered (pre-combusted GF/F) natural seawater from station DK2 and transferred into six 1-L Erlenmeyer flasks (initial chlorophyll fluorescence ca. 2). Either 100 μM ammonium (1^{st} treatment) or nitrate (2^{nd} treatment) was added along with phosphate (29 μM) and silicic acid (88 μM) in triplicate.

These flasks were incubated at 20℃ under 100 μmol photons m^{-2} s^{-1} (14 L:10 D cycle) using cool white fluorescence lamps with gentle stirring. After nitrogen in the culture medium of each treatment was depleted, both the NH_4^+ and NO_3^- grown cultures were transferred into new medium, but the nitrogen source was switched (i.e. from NH_4^+ to NO_3^- and vise versa). According to this incubation experiment, we tested the effect of either ammonium or nitrate individually, because of the following reasons. In Dokai Bay, the DIN concentration often exceeded 100 μM of which 40–70% was ammonium. Namely, both ammonium and nitrate concentration were high in this bay. TADA *et al.* (in press) conducted the incubation experiment of surface water and they showed that natural phytoplankton assemblage of mainly diatoms took up ammonia instead of nitrate. Moreover, nitrate was only taken up after ammonium was almost depleted, although ammonium was never depleted in the natural seawater in this bay.

Samples were taken daily and chlorophyll fluorescence was determined using a fluorometer (Turner Design 10-AU-005) according to BRAND *et al.* (1981). Samples were preserved in formaldehyde (final concentration 4%) and phytoplankton species and cell density was determined using an inverted microscope and a Sedgewick-Rafter counting chamber. Part of the daily sample was filtered through a 25 mm pre-combusted GF/F filter (450℃, 2 h) and frozen at −30℃ for measuring NH_4^+, NO_3^- $+NO_2^-$, PO_4^{3-} and Si $(OH)_4$ using an autoanalyzer (Bran+Luebbe, TRACCS 2000) according to STRICKLAND and PARSONS (1972). To determine the approximate time when nitrogen was depleted, NH_4^+ and NO_3^- were determined daily using fluorometric and optical methods according to HOLMES *et al.* (1999) and COLLOS *et al.* (1999), respectively.

Specific growth rate (μ, d^{-1}) was determined during exponential growth phase and was calculated by a least-squares linear fit to the logarithmically transformed chlorophyll fluorescence (μ_{Chl}) or cell density ($\mu_{Cell\ density}$) using the following formula:

$$\mu = \frac{\ln C_2 - \ln C_1}{d_2 - d_1}$$

where C_2 and C_1 are the chlorophyll fluorescence, or cell density (cell ml^{-1}) in the exponential phase at time 2 (d_2) and 1 (d_1), respectively. For each treatment, a paired t-test was used to determine the difference between the growth rates on ammonium and nitrate using Microsoft® Excel software.

2.2 Influence of irradiance on Skeletonema japonicum growth

Additional laboratory experiments were carried out to assess the influence of irradiance on the growth of the dominant phytoplankton of Dokai Bay, *S. japonicum* under two nitrogen sources. *S. japonicum* was isolated from a germination cyst from Dokai Bay sediment which collected in 2005, and maintained under 20℃ in the culture collection at Kagawa University. The culture was grown in 30 ml of enriched artificial seawater (modified from HARRISON *et al.,* 1980) containing either 100 μM ammonium or nitrate in 50 ml borosilicate glass test tubes. Cultures were incubated under cool white fluorescence lamps with four different irradiances of 58, 197, 260 and 450 μmol photons m^{-2} s^{-1} using black color nylon screen. The irradiance levels were measured inside the tube of each irradiance condition using a QSL-2101 irradiance meter (Biospherical Instruments Inc.). Cultures were grown at 20℃ under a 14 L:10 D cycle and each treatment was also conducted independently in three replicates. Prior to the experiment, all cultures were acclimatized to each irradiance for at least six generations. Acclimated exponential phase cultures were inoculated into the new medium and determined *in vivo* chlorophyll fluorescence at 24 h intervals using a fluorometer (Turner Design 10-AU-005). Specific growth rate (μ, d^{-1}) was determined using the protocol described above. For each irradiance condition, a paired t-test was used to determine the difference between the growth rates on ammonium and nitrate.

2.3 Role of ammonium and irradiance in Dokai Bay

The influence of high ammonium and

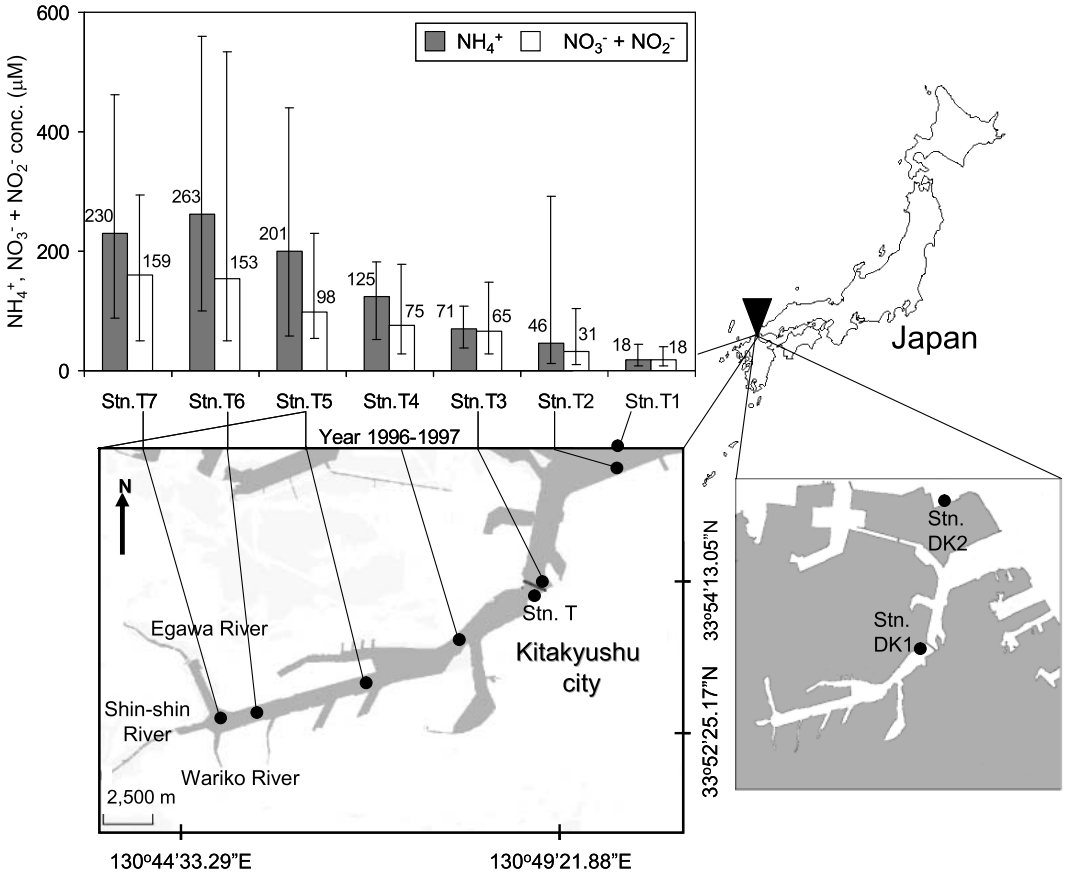

Fig. 1. Map of Dokai Bay in northern Kyushu Island, Japan and the 8 sampling stations. DK1 and DK2 represent sampling stations for the collection of the natural phytoplankton assemblages and natural seawater. The average ammonium and nitrate + nitrite concentration (μM) of surface water during 1996-1997 for station T1 (bay mouth) to T7 (inner bay) is given above the bar. Vertical bars show range (minimum to maximum) at each sampling station.

irradiance on the determination of species composition of phytoplankton assemblage in Dokai Bay was assessed. The previous data set of ammonium concentrations of surface water obtained from an intensive monitoring program in this bay (SUKSOMJIT *et al.*, 2005) was used. A vertical profile of irradiance in Dokai Bay from the inner part to mouth of the bay (Station T1 to T7 and Station T of central part in the bay, Fig. 1) determined in mid-summer of 2000, using a quantum irradiance meter (LI-205 Light meter, LI-COR) was used. Moreover, species composition of phytoplankton in Doaki Bay was determined in August, 1995 and 1998. Phytoplankton cell density and species along a transect from station T1 to T7 were counted and identified under an inverted microscope.

3. Results

3.1 Effect of ammonium on the growth of natural phytoplankton assemblages

In the first experiment using the sample collected on June 20, the dominant species in the natural phytoplankton assemblage was *Skeletonema* spp. All additional nutrients i.e. ammonium, nitrate, phosphate and silicic acid were assimilated for phytoplankton growth in the both treatments. In Fig. 2A and 3A, the concentration of all nitrogen sources decreased rapidly during the growth of phytoplankton.

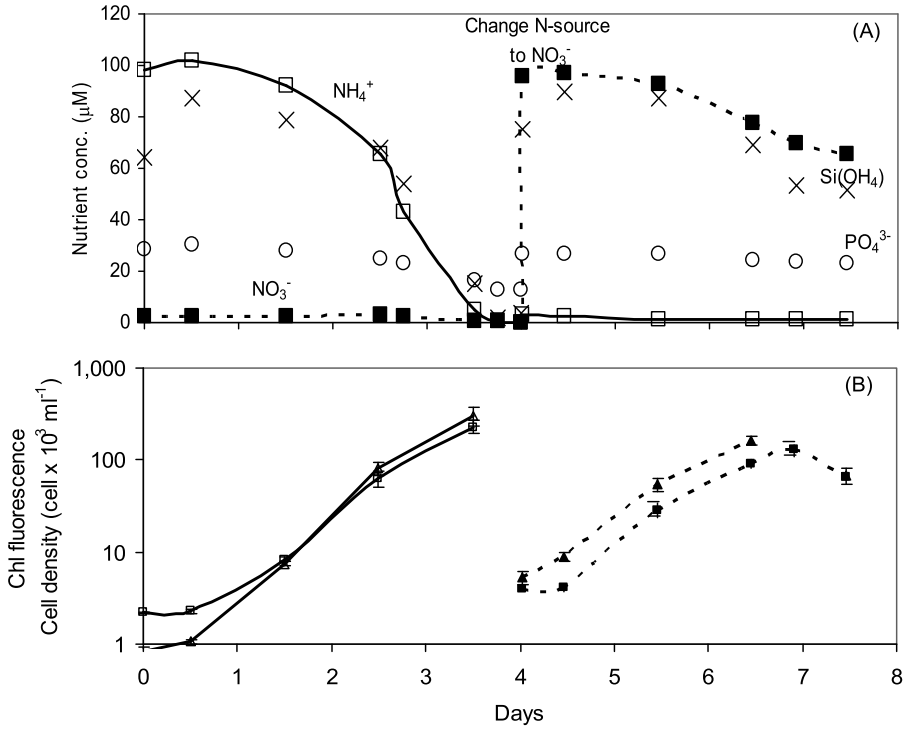

Fig. 2. Time series for the natural assemblage grown in the laboratory: (A) nutrients, NH_4^+ = □, NO_3^- = ■, Si $(OH)_4$ = X and O = PO_4^{3-} and (B) cell density (□) and chlorophyll fluorescence (Δ) of an NH_4^+-grown culture (solid lines and open symbols, n = 3) and then switched to growth on NO_3^- on day 4 (dashed lines and fill symbols, n = 2). Error bars show +/- 1 standard deviation (S.D.) of replicate samples.

Although the depletion of silicic acid (<1.8 μM for NH_4^+ and <8.0 μM for NO_3^-grown culture) occurred at the end of the incubation period on day 4 of the both treatments (Fig. 2A and 3A), it did not affect to the calculation because specific growth rate was determined during the exponential growth phase from day 1 to day 3, when silicic acid was >50 μM. In this experiment, *Skeletonema* spp. were always dominated and its contribution varied from 82 to 100% with the mean value 96% in both treatments at before and after nitrogen source was changed.

In the first treatment, the cell density of NH_4^+-grown culture reached a maximum (3.0×10^5 cells ml^{-1}) and the chlorophyll fluorescence increased to 229 after 4 days (Fig. 2B). When the nitrogen source was switched to nitrate, the highest cell density was 1.6×10^5 cells ml^{-1} and the fluorescence was only 128 on day 7.

For the second treatment, the phytoplankton cell density and chlorophyll fluorescence of the NO_3^-grown culture reached a maximum of 2.1×10^5 cells ml^{-1} and 182, respectively after 4 days. After NO_3^- was depleted and the nitrogen source was switched to ammonium, the highest cell density was 1.8×10^5 cells ml^{-1} and chlorophyll fluorescence reached 160 on the day 7 (Fig. 3B).

In this experiment, the specific growth rates (μ_{Chl} and $\mu_{Cell\ density}$) on ammonium were significantly higher ($p < 0.05$) compared to those on nitrate (Table 1). In the first treatment, the growth rate (μ_{Chl}) on ammonium was significantly higher ($1.59 \pm 0.05\ d^{-1}$, $p = 0.015$) than the growth rate ($1.40 \pm 0.01\ d^{-1}$) after the culture was switched to nitrate. Similarly, the growth rate (μ_{Chl}) of the natural phytoplankton assemblage of the NO_3^-grown culture in the second treatment was only $1.47 \pm 0.01\ day^{-1}$ and increased significantly to $1.69 \pm 0.01\ d^{-1}$ ($p < 0.01$) after the nitrogen source was switched to ammonium. For $\mu_{Cell\ density}$, a

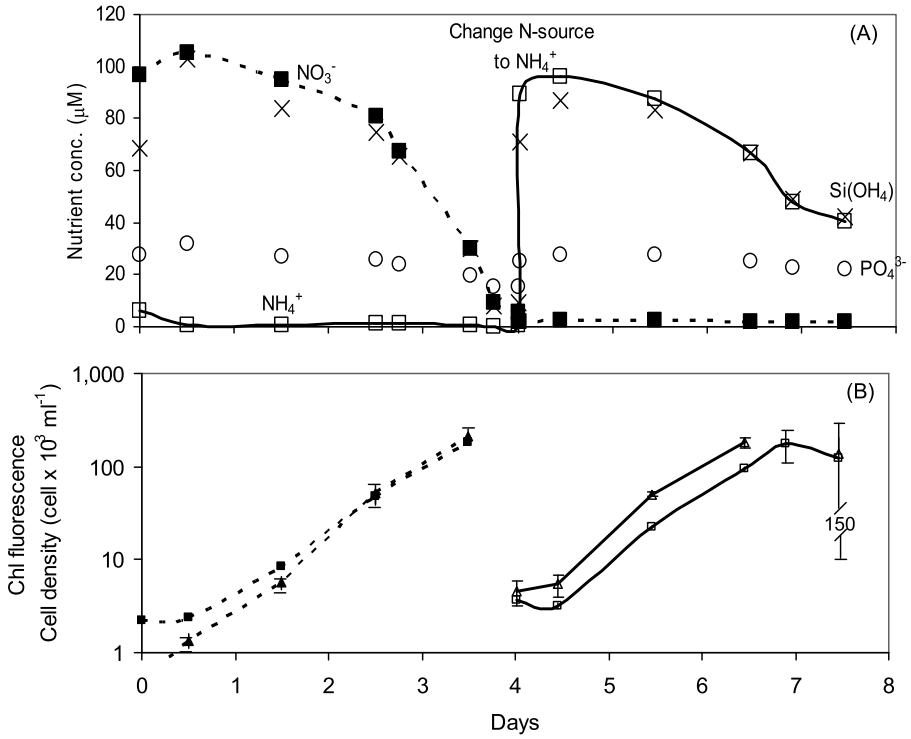

Fig. 3. Time series for the natural assemblage grown in the laboratory: (A) nutrients, NH_4^+ = □, NO_3^- = ■, Si $(OH)_4$ = X and O = PO_4^{3-} and (B) cell density (□) and chlorophyll fluorescence (Δ) of an NO_3^-grown culture (dashed lines and fill symbols, n = 3) and then switched to growth on NH_4^+ on day 4 (solid lines and open symbols, n = 2). Error bars show +/- 1 S.D. of replicate samples.

Table 1. Summary of average growth rates of the natural phytoplankton assemblage grown on ammonium and nitrate during the first experiment. The nitrogen source of each treatment is shown in parentheses. The difference between the two nitrogen sources is significant at $p < 0.05$ level (*) ; $p < 0.01$ level (**) ; n.s. = non-significant difference.

Growth rate (μ_{Chl}, d^{-1})	NH_4^+-sufficient treatment		p
	(NH_4^+) 1.59±0.05 (n=3)	(NO_3^-) 1.40±0.01 (n=2)	0.0152*
	NO_3^-sufficient treatment		
	(NO_3^-) 1.47±0.01 (n=3)	(NH_4^+) 1.69±0.01 (n=2)	0.0008**
Growth rate ($\mu_{Cell\ density}$, d^{-1})	NH_4^+-sufficient treatment		p
	(NH_4^+) 1.94±0.06 (n=3)	(NO_3^-) 1.46±0.01 (n=2)	0.046*
	NO_3^-sufficient treatment		
	(NO_3^-) 1.74±0.09 (n=3)	(NH_4^+) 1.80±0.01 (n=2)	n.s.

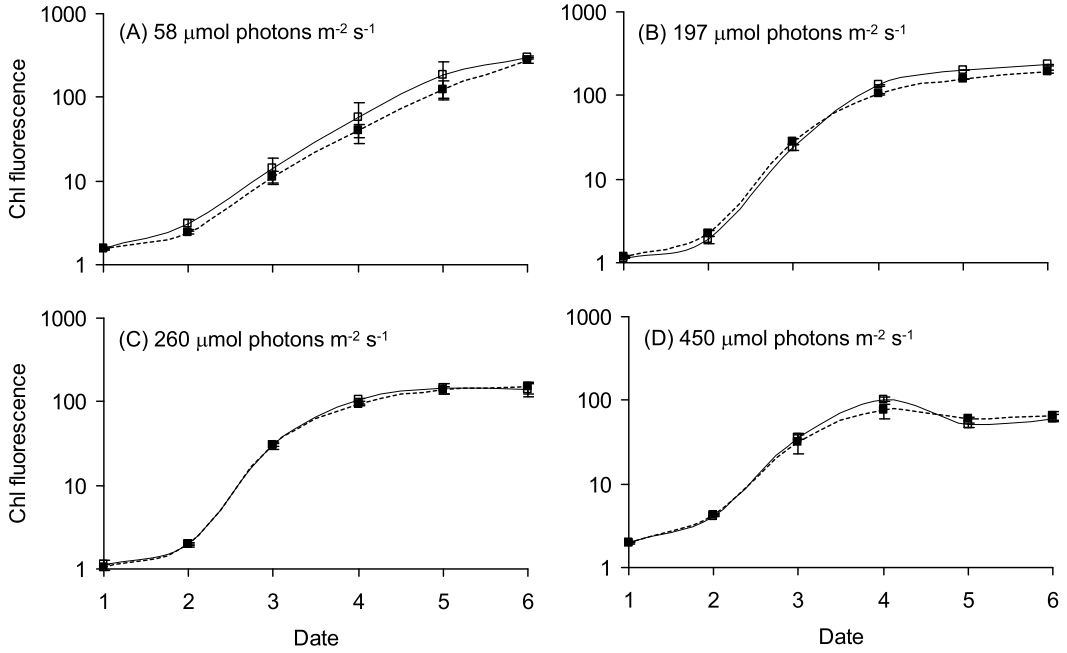

Fig. 4. Growth of *Skeletonema japonicum* at 58 (A), 197 (B), 260 (C) and 450 (D) μmol photons m^{-2} s^{-1} using NH_4^+ (solid line and open block) or NO_3^- (dash line and fill block). Error bars show +/- 1 S.D. of triplicate samples.

similar response of an increase in growth rate on ammonium compared to nitrate was also observed. The growth rate ($\mu_{Cell\ density}$) on ammonium was significantly higher ($1.94 \pm 0.06\ d^{-1}$, $p < 0.05$) than the growth rate ($1.46 \pm 0.01\ d^{-1}$) after the culture was switched to nitrate. μ_{Chl} of the NO_3^-grown culture in the second treatment was $1.74 \pm 0.09\ day^{-1}$ and increased to $1.80 \pm 0.08\ d^{-1}$ after the nitrogen source was switched.

In the second experiment using the sample collected from Dokai Bay on July 31, 2007, there was no difference in the growth rate of natural phytoplankton assemblage on either ammonium or nitrate The specific growth rate (μ_{Chl} and $\mu_{Cell\ density}$) of NH_4^+-growing cell was not significantly different ($p > 0.05$) from nitrate (data not shown). In this second experiment, the dominant species of natural phytoplankton assemblages collected on July 31 were mixed diatoms, i.e. *Skeletonema* spp., *Chaetoceros* spp., *Nitzschia* spp. and *Pseudo-nitzschia* spp. The proportion of *Skeletonema* spp. at the initial was 56.8%. However, the proportion of *Chaetoceros* spp. was 27.5%, while *Nitzschia* spp. and *Pseudo-nitzschia* spp. was 16%. Moreover, the proportion of *Skeletonema* spp. also decreased gradually and showed the lowest at the end of this experiment. Average proportion of *Skeletonema* spp. and *Chaetoceros* spp. in this experiment was 30.1% and 12.8%, respectively, while *Nitzschia* spp. and *Pseudo-nitzschia* spp. was 57.1%. This evidence was differed from the first experiment where *Skeletonema* spp. was almost completely dominant.

3.2 Growth of Skeletonema under different four irradiances

Variations of chlorophyll fluorescence of *S. japonicum* at various irradiances under two nitrogen sources were shown in Fig. 4. The photoadapted response allowed *S. japonicum* to grow well in a wide range of irradiances. At any irradiance (i.e. 58, 197, 260 and 450 μmol photons m^{-2} s^{-1}), all cultures grew by using either ammonium or nitrate. In Fig. 5, the growth rate of *S. japonicum* on either ammonium or nitrate increased with the irradiance up to 197 and 260 μmol photons m^{-2} s^{-1},

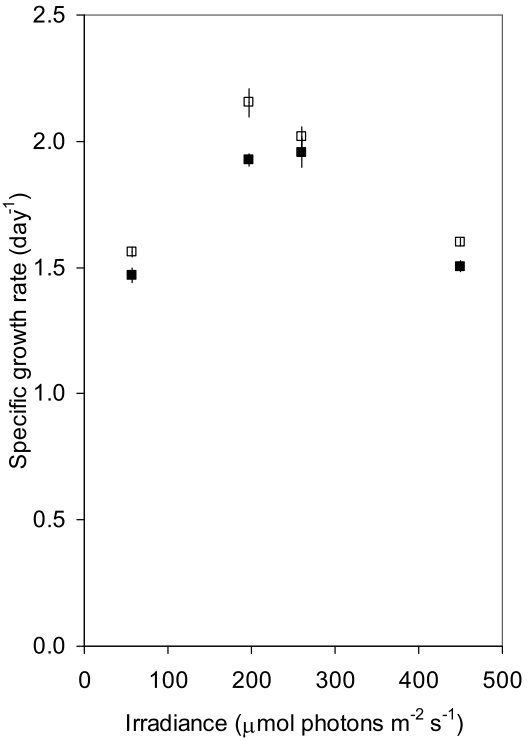

Fig. 5. Relationship between growth rate and irradiance for *Skeletonema japonicum* grown on either ammonium (□) or nitrate (■).The error bars show +/- 1 S.D. of triplicate samples.

respectively. Higher irradiance resulted in the inhibition of growth rate of the both nitrogen sources. However, the growth rates of *S. japonicum* on ammonium at all irradiances particularly at 197, 260 and 450 μmol photons m^{-2} s^{-1} were significant (p <0.05) higher than on nitrate. Individual growth rates on ammonium and nitrate at the above three irradiances were 2.15 $\pm$ 0.06 and 1.93 $\pm$ 0.02, 2.02 $\pm$ 0.04 and 1.95 $\pm$ 0.06, 1.60 $\pm$ 0.01 and 1.51 $\pm$ 0.02 d^{-1}, respectively. Moreover, the largest difference on the growth rate between the two nitrogen sources occurred at 197 μmol photons m^{-2} s^{-1} (p = 0.01). At 58 μmol photons m^{-2} s^{-1}, the growth rate of ammonium-grown cells was 1.56 $\pm$ 0.02 d-1 and not different from nitrate (1.47 $\pm$ 0.03 d^{-1}).

3.3 Irradiance and species composition of phytoplankton in Dokai Bay

Vertical profiles of irradiance on a sunny day in mid-summer (August 23, 2000) as an example of the light condition in the Dokai Bay at 8 stations when diatom blooms could form are shown in Fig. 6. The irradiance levels in the surface water (1 m depth) ranged between 183 to 959 μmol photons m^{-2} s^{-1} (average irradiance was 577 μmol photons m^{-2} s^{-1}). However, the irradiance showed a rapid decrease with water depth to about 200 μmol photons m^{-2} s^{-1} at 2 to 3 m depth) through the bay. Actually, the irradiance level at 2 to 3 m ranged from 71 to 675 (mean 267 $\pm$ 181) and from 32 to 284 (mean 102 $\pm$ 79) μmol photons m^{-2} s^{-1}, respectively.

The species composition of phytoplankton in Doaki Bay in mid-summer on August 31, 1995 and August 20, 1998 are showed in Fig. 7. In the both years, *Skeletonema* spp. was clearly dominant over other species from the inner to the bay mouth. The proportion of *Skeletonema* spp. ranged from 61–92% of the other species (except station T1 in 1995).

4. Discussion

4.1 Accelerated growth rate of natural phytoplankton assemblages

The growth of the natural phytoplankton assemblage collected from Dokai Bay was increased due to a 100 μM ammonium addition under laboratory conditions. The significantly higher phytoplankton growth rate on ammonium (~13 to 15%) compared to nitrate agreed with the previous studies summarized in Table 2. LEVASSEUR *et al.* (1993) found that the growth rate of *Thalassiosira pseudonana* on ammonium was 12% higher compared to nitrate. HERNDON and COCHLAN (2007) reported that the addition of ammonium at 50 μM increased the growth rate of *Heterosigma akashiwo* and growth rates on ammonium were 9 to 24% faster than those on urea and nitrate respectively. WOOD and FLYNN (1995) reported that during exponential growth of *H. carterae*, ammonium-grown cells attained higher growth rates by at least 20%.

The higher growth rates of ammonium-grown cells in this study might be caused by the extra energy cost for growth on nitrate. A higher energy cost of nitrate utilization over ammonium was reported in several previous studies (e.g. SYRETT, 1981 and reference

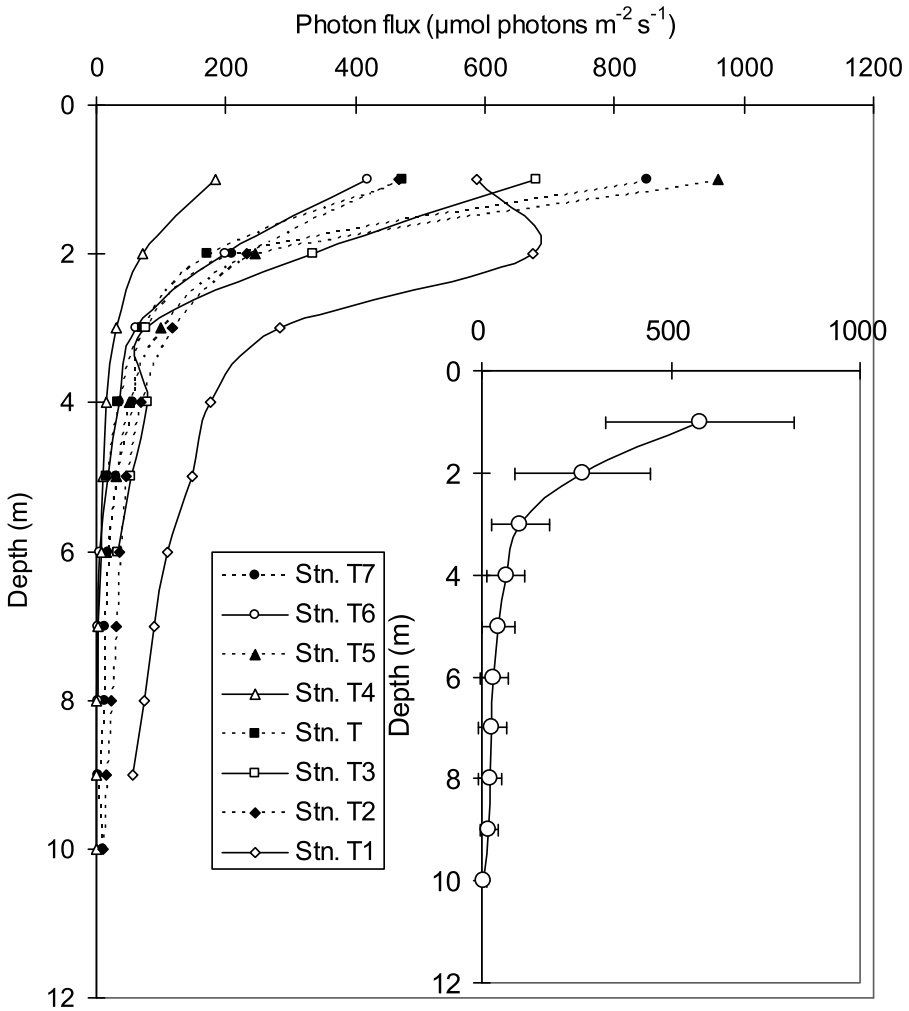

Fig. 6. Vertical profiles of photon flux in Dokai Bay from Station T1 (bay mouth) to Station T7 (inner bay), including Station T (middle of the bay), in mid-summer, 2000. See map in Fig. 1 for station locations. Small vertical profiles show the average photon flux of all stations and error bars show +/- 1 S.D. of replicate samples.

therein; FLYNN, 1991; FLYNN *et al.*, 1997; LEVASSEUR *et al.*, 1993; WASER *et al.*, 1998). LEVASSEUR *et al.* (1993) and THOMPSON *et al.* (1989) concluded that the higher energy requirement for nitrate reduction might be the reason of the lower growth rate of nitrate-growing cell, although there are several strategies that may be used to compensate for the higher energy requirement. WOOD and FLYNN (1995) demonstrated that nitrate-grown *H. carterae* decreased its growth rate possibly to compensate for the higher energy requirement of nitrate reduction.

Although ammonium had the potential to accelerate the growth rate of the natural phytoplankton assemblage in the first experiment when the dominant species was *Skeletonema* spp., this effect was not observed for other dominant species. In the second experiment, there was no significant difference for the growth of natural phytoplankton assemblages on ammonium and nitrate when the dominant species were a mixture of diatoms (i.e. *Skeletonema* spp., *Chaetoceros* spp.,

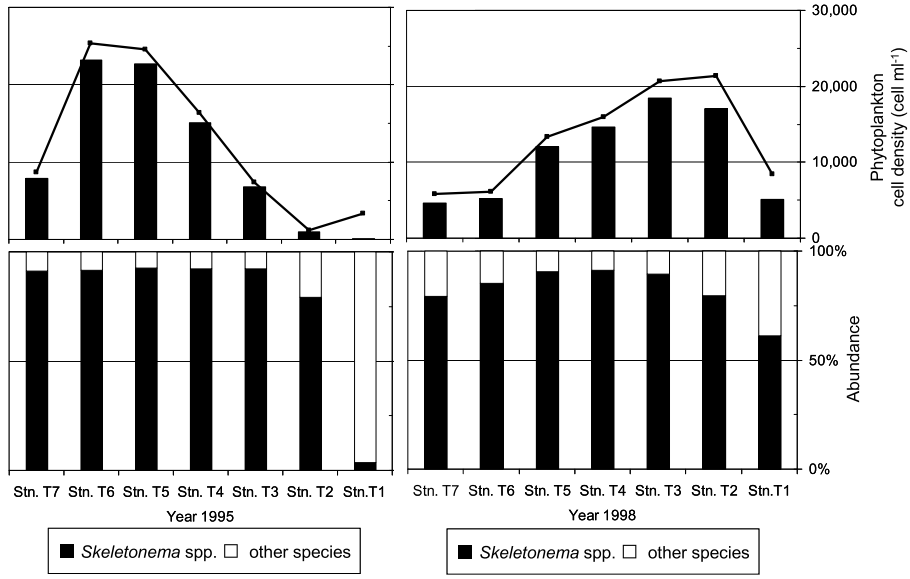

Fig. 7. Distribution of total phytoplankton abundance and proportion (%) between *Skeletonema* spp. and other species along a transect from station T1 (bay mouth) to station T7 (inner part) in mid-summer, 1995 (left) and 1998 (right). Solid lines indicate total phytoplankton cell density in the surface water and fill bars indicate the cell density of *Skeletonema* spp.

Nitzschia spp. and *Pseudo-nitzschia* spp). These results indicated that the effect of ammonium on growth acceleration was species specific and this agreed with previous studies. LEVASSEUR *et al.* (1993) reported that growth rate of *Thalassiosira pseudonana* on ammonium was higher compared to nitrate. In contrast, *Chaetoceros gracilis* grew significantly faster on nitrate than ammonium at similar irradiances. However, *Dunaliella tertiolecta* and *Gymnodinium sanguineum* showed very little influence of nitrogen source on growth rate. Moreover, species specificity on the growth acceleration effect of high ammonium had also been observed in higher plants. TYLOVA-MUNZAROVA *et al.* (2005) found that the growth rate of *Glyceria maxima* was 16% higher on ammonium than on nitrate, but the growth rate of *Phragmites australis* was not affected by the different forms of nitrogen.

4.2 The influence of irradiance on the growth rate of Skeletonema japonicum

The growth rates of *S. japonicum* on ammonium at all irradiances of 58, 197, 260 and 450 μmol photons m^{-2} s^{-1} were significantly higher than on nitrate. However, the largest difference in growth rate between the two nitrogen sources was observed at 197 μmol photons m^{-2} s^{-1}. This effect of irradiance level on the growth of *S. japonicum* between the two nitrogen sources in our study agreed with previous reports (HERNDON and COCHLAN, 2007; WOOD and FLYNN, 1995). WOOD and FLYNN (1995) reported that a significant difference between cell specific growth rates of *H. carterae* on ammonium and nitrate was observed at mid irradiances (200 μmol photons m^{-2} s^{-1}). In contrast, at low and high irradiances (50 or 350 μmol photons m^{-2} s^{-1}), they found that there was no significant difference between ammonium and nitrate.

In our study, the largest significant difference in growth rate at 197 μmol photons m^{-2} s^{-1} and smaller at high irradiances (260 and 450 μmol photons m^{-2} s^{-1}) between ammonium and nitrate might be explained by the following reason. At 197 μmol photons m^{-2} s^{-1}, the irradiance was optimal for growth. However, at 260 and 450 μmol photons m^{-2} s^{-1}, the growth rate was photoinhibited on either nitrogen sources. In contrast, light was limited

Table 2. Summary of the acceleration of growth rate of ammonium for several phytoplankton species.

Species	Concentration (μM)	Irradiance (μmol photons m^{-2} s^{-1})	Growth rate (d^{-1})	Reference
Thalassiosira pseudonana	100	170	1.48 (NH_4^+) 1.30 (NO_3^-)	LEVASSEUR *et al.* (1998)
Chaetoceros gracilis			1.56 (NH_4^+) 1.84 (NO_3^-)	
Dunaliella tertiolecta			1.42 (NH_4^+) 1.41 (NO_3^-)	
Gymnodinium sanguineum			0.43 (NH_4^+) 0.41 (NO_3^-)	
Heterosigma akashiwo	50	40	0.57 (NH_4^+) 0.46 (NO_3^-)	HERNDON and COCHLAN (2007)
		110	0.89 (NH_4^+) 0.82 (NO_3^-)	
Thalassiosira pseudonana	75	$\leq$29 $\geq$61	$NH_4^+ = NO_3^-$ $NH_4^+ > NO_3^-$	THOMPSON *et al.* (1989)
Heterosigma carterae	100	50 200 350	$NH_4^+ > NO_3^-$ $\sim$ 20%	WOOD and FLYNN (1995)
Skeletonema sp.	100	58	1.56 (NH_4^+) 1.47 (NO_3^-)	This study
		197	2.15 (NH_4^+) 1.93 (NO_3^-)	
		260	2.02 (NH_4^+) 1.95 (NO_3^-)	
		450	1.60 (NH_4^+) 1.51 (NO_3^-)	

at 58 μmol photons m^{-2} s^{-1} for cells using either nitrogen source. The equal growth rates of ammonium and nitrate-grown cells at 58 μmol photons m^{-2} s^{-1} agreed with the previous studies (THOMPSON *et al.*, 1989; WOOD and FLYNN, 1995). Our study clearly showed that the growth acceleration of *Skeletonema* spp. in this bay was particularly significant at an irradiance level of about 200 μmol photons m^{-2} s^{-1}.

4.3 Field implications

Generally, it is well known that phytoplankton growth in the natural environment is controlled by physical (e.g. water circulation, irradiance, water temperature, etc.), chemical (e.g. nutrient, trace element, etc.) and biological (e.g. taxonomic variation, origin of phytoplankton, zooplankton grazing, etc.) factors. YANAGI and YAMADA (2000) and TADA *et al.* (2001 and 2004) concluded that phytoplankton blooms in Dokai Bay were mainly controlled by the fast flushing time of the bay because nutrient concentrations in this bay were sufficient for phytoplankton growth during the entire year. They suggested that phytoplankton species that have a higher growth rate than their loss rate due to the high flushing rate of the surface water in the bay, could bloom and become dominant. However, evidence from two laboratory experiments helps to explain why *Skeletonema* spp. is the dominant species in the phytoplankton assemblage throughout the year in Dokai Bay.

Due to the strong estuarine circulation in Dokai Bay, phytoplankton in the inner bay would be flushed out of the bay within 2.5 days. Only phytoplankton species that are able to obtain a high density due to their high growth rate, could become dominant and produce a bloom in this bay (YANAGI and YAMADA, 2000; TADA *et al.*, 2001 and 2004). Data from the intensive monitoring program in 1996 to 1997 (SUKSOMJIT *et al.*, 2005) indicated that an

ammonium concentration of 100 μM occurred in the middle part of Dokai Bay (station T4 and T3, Fig. 1.), while a higher ammonium concentration >200 μM occurred at the inner bay. In addition, the variation of nitrate + nitrite in this bay also showed a similar pattern as ammonium. The highest concentration was observed at the inner bay and decreased gradually to the bay mouth. The ammonium concentration of 100 μM that was found in the middle part of this bay, was in the range of ammonium that caused the acceleration effect of phytoplankton growth. This suggests that the growth of natural phytoplankton assemblages in Dokai Bay would be accelerated by this ammonium concentration during the approximate 2.5 days flushing time out of the bay. This conclusion agreed with the observation that *Skeletonema* spp. was the dominant species in Dokai Bay in 1995 and 1998 (Fig. 7). The proportion of *Skeletonema* spp. of the total phytoplankton assemblage was 90% in both years throughout the bay. We therefore concluded that high ammonium was one reason to regulate the phytoplankton composition in Dokai Bay through the acceleration on growth rate of *Skeletonema* spp., although there were many factors regulating the phytoplankton composition, such as phytoplankton ability of adaptation to eutrophication, sinking rate, cyst formation characteristic and the dependences of temperature on growth and this might also explain the dominance of *Skeletonema* spp. in Dokai Bay over other species.

However, the effect of ammonium on the acceleration of growth rate was influenced by the irradiance level. The growth of *S. japonicum* was significantly accelerated by ammonium at an irradiance level of about 200 μmol photons m^{-2} s^{-1}. Although irradiance at the surface water on a sunny day in summer was higher and might inhibit the growth of phytoplankton, the acceleration in growth rate could occur in the surface (2 or 3 m) mixed layer, or on cloudy days. In Dokai Bay, an irradiance level around 200 μmol photons m^{-2} s^{-1} was observed in sub-surface waters (2 and 3 m depth) through the bay as showed in Fig. 6. This observation was consistent with a previous report in this bay. TADA *et al.* (2001) also reported that the transparency in Dokai Bay was low and irradiance was strongly limited in the water column. In the field, high irradiance would be observed only for a few hours around noon, while the irradiance of 200 μmol photons m^{-2} s^{-1} in laboratory was maintained over a 14 h period. Hence, we conclude that the present results showed the possibility of growth acceleration of phytoplankton community in Dokai Bay and the importance of the irradiance level on the growth of phytoplankton in this bay.

The high dominance of *Skeletonema* spp. over other species at most stations in the bay and the high flushing rate of ～2.5 days, indicates that this bay exports large amount of *Skeletonema* spp. to coastal waters. Hence, Dokai Bay acts like a selective growth incubator for certain diatoms such as *Skeletonema* spp. whose growth rate is greater that the loss rate as a result of flushing. During the 2.5 day flushing period, 7.8 doublings would occur (assuming growth rate = 2.15 d^{-1}) on ammonium, while 7.0 doublings would occur (assuming a growth rate of 1.95 d^{-1}) if *Skeletonema* spp. used only nitrate. This nearly one extra doubling during the 2.5 day transient out of the bay for growth on ammonium at optimum light, may only partially explain Skeletonema's dominance and other factors such as grazing should be explored.

Acknowledgements

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資　料

第 47 巻第 3 号掲載欧文論文の和文要旨

Nguyen Khac Bat*, **, *, Chu Tien Vinh*, Arild Folkvord**, Arne Johannessen ** 土屋光太郎***, 瀬川進***：ベトナム・トンキン湾産ヒラケンサキイカ *Photololigo chinensis* の平衡石微細構造に基づく成長解析**

トンキン湾沖合海域から採集されたヒラケンサキイカ254個体について平衡石輪紋から推定された日齢に基づき成長解析を行った。最長齢の個体は夏秋季に採集された194日齢のものであった。日齢から推定された春夏季および秋冬季発生群の齢–外套長の関係はそれぞれ高い有意性を示した。本種は外套の成長において性的二型を示し，体重量の成長が類似するのに対し，雄の外套長の成長は雌にくらべ優位に速いことが示された。ヤリイカ類の成長には季節的な要因が強く影響し，成長率は寒冷期にくらべ温暖期で明らかに高かった。

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Marut Suksomjit[1)]・多田邦尚[1)]・一見和彦[2)]・門谷茂[3)]：富栄養海域（北九州市・洞海湾）における植物プランクトンの高アンモニア濃度に対する耐性

洞海湾の植物プランクトンのアンモニア耐性を，高アンモニア濃度の培地を用いて検討した。6 種の植物プランクトン，即ち，3 種の珪藻類（*Skeletonema* sp., *Chaetoceros* sp.）と 3 種の鞭毛藻類（*Heterosigma akashiwo*, *Chattonella antiqua*, *Karenia mikimotoi*）を用いて様々なアンモニア濃度条件下で培養試験を行った。高アンモニア濃度は植物プランクトンの増殖に阻害効果を示した。洞海湾に生息しない種である*C. antiqua*と*K. mikimotoi* はそれぞれ，200 μM，150 μMでは増殖できなかった。また，播磨灘で単離された *Skeletonama* sp. と，*Chaetoceros* sp.と *H.akashiwo* は高アンモニア濃度下で対照区に対して増殖速度の減少が認められた。しかしながら，洞海湾で単離された *Skeletonama* sp. は1500 μMの高濃度のアンモニア下でもその増殖速度の阻害は認められなかた。さらに，試験藻の最大クロロフィル蛍光値は培地中のアンモニア濃度の増加に伴って減少した。本研究で認められた高濃度のアンモニア下での植物プランクトンの増殖阻害の結果は，洞海湾に出現する植物プランクトンの種組成を支持するものであった。本研究の結果は，洞海湾の高いアンモニア濃度がこの湾内の植物プランクトンの種組成を決定する重要な要因のひとつであることを示している。

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Marut Suksomjit[1)]・一見和彦[2)]・濱田健一郎[3)]・山田真知子[4)]・多田邦尚[1)]：・Paul J. Harrison[5)]：富栄養海域（北九州市・洞海湾）の植物プランクトン*Skeletonema* spp. のアンモニアによる増殖促進

洞海湾ではアンモニアが大量に負荷されており，珪藻類の大増殖が観察される。そこで，高濃度のアンモニアが植物プランクトンのブルームを促進するかどうかを明らかにするために，室内実験を行った。*Skeletonema* spp. が優占していた洞海湾の表層水を室内培養したところ，窒素源がアンモニアのときには硝酸のときよりもその増殖が促進された。即ち，アンモニアを窒素源としたときの植物プランクトンの増殖速度は，硝酸を窒素源としたときよりも13〜15%高かった。しかしながら，このアンモニアによる増殖促進は種特異性があり，現場の植物プランクトン群集が *Skeletonema* spp. と他の種が混在している場合には見られなかった。さらに，洞海湾から分離された *Skeletonema* spp. のアンモニアによる増殖速度は光条件により異なり，光量子量が 200 μmol phtons m^{-2} s^{-1}レベルで最も促進された。本研究の結果は，洞海湾の優占種である *Skeletonema* spp. はアンモニアによりその増殖が促進され，特に夏季の亜表層の光条件下でその増殖が促進されることを示していた。

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Société franco-japonaise d'océanographie, Tokyo

学 会 記 事

1. 2009年 6 月20日（土）日仏会館会議室において，平成21年度学術研究発表会が開かれた。発表題目と発表者は次のとおり。

期日：2009年 6 月20日（土）

場所：日仏会館501会議室（東京都渋谷区恵比寿3–9–25 Tel. 03–5421–7641）

午前（10：00－11：00）　座長　野村　英明（東大海洋研）

① 河口域底質間隙水中の溶存有機物・栄養塩類の挙動
安井沙織・神田穣太（海洋大）・碓井敏宏・小川浩史（東大海洋研）

② 東京湾における二酸化炭素分圧の分布と変動
久保篤史・前田洋作*・神田穣太（海洋大，*現：海洋研究開発機構）

③ 褐藻類アラメ遊走子の基質着生への堆積粒子の影響とその粒径による相違
荒川久幸・伊東未来（海洋大）

午後（14：00－15：20）　座長　長島　秀樹

④ 東京湾における河川からのダイオキシン類負荷による海底への堆積分布の予測
今野 聡・中村 倫明・和田 明（日大総合科学）

⑤ 相模湾大島東水道深層の流況変動に関する研究
王 琦・根本 雅生・吉田 次郎・北出 裕二郎（海洋大）

⑥ 南極大陸ケープダンレー沖大陸斜面近傍における乱流場の特徴
平野大輔・北出裕二郎（海洋大）

⑦ Applying wavelet de-noising on turbulent patch identification: study case in Lombok Strait, Indonesia
Yuli Naulita・Yujiro Kitade（海洋大）

2. 2009年 6 月20日（土）日仏会館会議室において，評議員会に続き，平成21年度（第50回）総会，学会賞・論文賞授賞式および学会賞受賞記念講演が行なわれた。

a）2009年度（第50回）日仏海洋学会総会議事録（案）

日　時：2009年 6 月20日（土）15：30〜16：30

場　所：日仏会館会議室（501室）東京都渋谷区恵比寿3–9–25

出席者：15名，委任状：43名

議　題：

1）2008年度事業報告

① 会員異動状況について報告された。

	2008年 4 月	入会	退会	逝去	資格変更	2009年 3 月
名誉会員	8	－	－	－	－	8
正会員	264	4	▲8	▲1	－	259
学生会員	5	1	▲4	－	－	2
賛助会員	6	1	－	－	－	7

② 活動状況について報告された。
評議員会　1 回（2008/6/28　日仏会館）
幹 事 会　4 回（2008/4/7，6/23，12/2，2009/2/14　日仏会館）
総　会　1 回（2008/6/28　日仏会館）
学術研究発表会　1 回（2008/6/28　日仏会館）
学会賞 1 件，論文賞 2 件の授与
バックナンバーDVD作成（La mer 46巻 4 号に添付予定）
第13回日仏海洋学シンポジウム「Marseille＋Paris」共催。（2008/9/8–12）
日仏交流150周年記念日仏関連諸学会総合シンポジウム参加・ACTの作成（2008/9/26–28）

③ 編集関係
学会誌「La mer」45巻 3 号，4 号，46巻 1－2 号発刊した事を報告。

④ その他：水産教育推進委員会派遣委員報告（4 回）

2）2008年度収支決算報告が行なわれ，監事から監査報告がなされた。（資料 1 ）

3）2009年度事業案が審議・承認された。

① 総会（1 回），学術研究発表会（1 回），評議員会（1 回），幹事会（3－4 回）開催

② 学会誌「La mer」発刊46巻 3 号，4 号，47巻 1－2 号，47巻 3 号

③ 学会賞，論文賞の授与

④ 2010–2011年度評議員選挙，学会賞委員選挙（半数改選）

⑤ 創立50周年記念事業準備｛「50周年の歩み」（仮称）出版，第14回日仏海洋学シンポジウム（「Techno-Ocean 2010」（2010年10月14–16日，神戸）の一環として）｝。なお，2010年には，定例の総会，評

議員会，研究発表会を，東京ではなく神戸で行う予定。

⑥ ホームページの充実

4）2009年度予算案が審議・承認された。（資料２）

5）2009年度評議員（追加），賞委員（半数改選）が承認された。

日仏海洋学会　役員・評議員（2008－2009年度）

会　長：今脇資郎

副会長：八木宏樹　森永　勤

幹　事：（庶務）河野　博　　荒川久幸
（会計）神田穣太　　山崎秀勝
（編集）田中祐志　　北出裕二郎
（研究）石丸　隆　　和泉　充
（渉外）小松輝久　　小池康之
（広報）野村英明

監　事：長島秀樹　小池　隆

編集委員長：吉田次郎

評議員：荒川久幸　有元貴文　石丸　隆　和泉　充　磯田　豊　今脇資郎　神田穣太　北出裕二郎　小池康之　小池勲夫　河野　博　小松輝久　斉藤誠一　関根義彦　千手智晴　田中祐志　寺崎　誠　中田英昭　長島秀樹　野村英明　森永　勤　門谷　茂　柳　哲雄　八木宏樹　山口征矢　山崎秀勝　吉田次郎

以上27名

賞委員：

2009年度：土屋光太郎　野村英明　山口征矢　前田昌調　小松輝久　荒川久幸　河野博　和泉充　門谷茂

6）日仏海洋学会会則の一部改正案が審議・承認された。ただし，第15条の解散規定については，「解散」の後に「と資産の処分」を追加することとした。（資料３）

7）その他：学会のロゴマークについて今後検討することとした。

b）2009年度日仏海洋学会賞および論文賞の受賞者は以下の通り。

学会賞　河野　博　会員（東京海洋大学海洋科学部　教授）
研究課題：『仔稚魚の分類と生態に関する研究』

論文賞

① Kuroda, H. Y. Isoda, S. Honda, H. Takeoka, and M. Simizu（2008）：「Diurnal Tidal Current on the Eastern Shelf of Hidaka Bay -Can juvenile walleye pollock, Theragra chalcogoramma, move southeastward with the diurnal tidal current?, La mer, 46 37–47」

② Izawa, M. and M. Kobayashi（2006）：「Estimation of the sediment flux from the cultured Japanese oyster in Ofunato Estuary and its annual variation -Calculation by incorporating the monthly mean environmental data for ten years, La mer, 43 117–128」

3. 新入会員

氏名	所属	紹介者
黒田　寛（正）	水産総合センター中央水産研究所	磯田　豊
伊沢　瑞夫（正）	水産大学校海洋機械工学科	小林雅人
松本　陽（学生）	東京海洋大学大学院	荒川久幸
王　　歓（学生）	東京海洋大学大学院	荒川久幸

4. 所属および所在地の変更

氏名	新所属および所在地
土井　航（正会員）	（独）水産総合研究センター遠洋水産研究所 〒424-0901　静岡県静岡市清水区折戸5-7-1　Tel:054-336-6000
（財）海洋生物環境研究所（賛助）	〒162-0801　東京都新宿区山吹町347番地　藤和江戸川橋ビル　7階

5. 寄贈図書および資料

農工研ニュース（農村工学研究所）；No.61，62

農村工学研究所研究成果情報（農村工学研究所）；H20年度

FRAN　NEWS（水産総合研究センター）；Vol.19

Ship & Ocean Newsletter（海洋政策研究財団）；No. 212–217

なつしま（JAMSTEC）；第63号通巻280–282

ぶらりねっとＣＨＩＢＡ（ちば国際コンベンションビューロー）；Vol.113&114

ＣＩＣ　ＮＥＷＳＬＥＴＴＥＲ（東京大学）；No. 7

Ｊ－ＳＴＡＧＥ　ＮＥＷＳ（独立行政法人科学技術振興機構）；Ｎｏ．20

海洋産業研究会会報（社団法人　海洋産業研究会）；通巻第344号Vol. 40, No.2

ニュースレター（東京大学海洋研究所）；No.18

「海－自然と文化」（東海大学海洋学部）；Vol. 7, No.1

Techno-ocean News（テクノオーシャンネットワーク）；No.34

中国海洋大学学報（中国海洋大学）；Vol.39, No.167–168.

Progress in Fishery Sciences（中国水産学会）；Vol.30, No.2–4.

Oceanologia et Limnologia Sinica（中国科学院海洋研究所）；Vol.40, No.2–3.

資料 1

平成20年度収支決算

収入の部

費　目	予算額	決算額	増　減	備　考
正会員会費	1,160,000	1,072,000	▲88,000	134名×8,000円（クレジットカード払い外国会員含む)
特別会員（65歳以上）	72,000	42,000	▲30,000	7名×6,000円
学生会員会費	20,000	12,000	▲8,000	3名×4,000円
賛助会員会費	120,000	110,000	▲10,000	11口×10,000円(7社)
学会誌売上金	60,000	285,400	225,400	
広告料	50,000	40,000	▲10,000	
別刷り印刷費	240,000	303,000	63,000	
掲載料，超過頁印刷費	800,000	830,000	30,000	
雑収入	100,000	103,013	3,013	研究発表会，学術著作権使用料，発表会DVD他
寄付金	0	0	0	
小　計	2,622,000	2,797,413	175,413	
前年度繰越金	661,079	661,079	0	
合　計	3,283,079	3,458,492	175,413	

支出の部

費　目	予算額	決算額	増　減	備　考
学会誌印刷費	2,120,000	1,362,450	▲757,550	45(3)，45(4)，46(1-2)　合併号各350部
送料・通信費	100,000	77,575	▲22,425	
事務費	700,000	622,418	▲77,582	人件費，事務用品，封筒他
交通費	20,000	31,880	11,880	
会議費	5,000	8,507	3,507	
学会賞経費	50,000	14,238	▲35,762	メダル，賞状他
雑費	25,000	58,265	33,265	郵便・銀行振込手数料
予備費	263,079	564,637	301,558	バックナンバーDVD製作費他
小　計	3,283,079	2,739,970	▲543,109	
次年度繰越金	0	718,522	718,522	
合　計	3,283,079	3,458,492	175,413	

貸借対照表（案）

流動資産（A）	
著者負担印刷費未入金	0
普通預金	718,522
流動負債（B）	
学会誌印刷費未払い金	0

次期繰り越し差額（A）－（B）	718,522

資料 2

平成21年度予算（案）

収入の部

費　目	21年度予算	20年度予算	増　減	備　考
正会員会費	1,160,000	1,160,000	0	145名×8,000円
特別会員（65歳以上）	72,000	72,000	0	12名×6,000円
学生会員会費	20,000	20,000	0	5名×4,000円
賛助会員会費	110,000	120,000	▲10,000	11口×10,000円（7社）
学会誌売上金	100,000	60,000	40,000	
広告料	40,000	50,000	▲10,000	
別刷り印刷費	240,000	240,000	0	
掲載料，超過頁印刷費	800,000	800,000	0	16編×50,000円
雑収入	100,000	100,000	0	学術著作権使用料，DVD他
小　計	2,642,000	2,622,000	20,000	
前年度繰越金	718,522	661,079	57,443	
合　計	3,360,522	3,283,079	77,443	

支出の部

費　目	21年度予算	20年度予算	増　減	備　考
学会誌印刷費	2,200,000	2,120,000	80,000	46(3)，46(4)，47(1－2)合併号，47(3)　各350部
送料・通信費	100,000	100,000	0	
事務費	700,000	700,000	0	人件費，事務用品，封筒他
交通費	20,000	20,000	0	
会議費	5,000	5,000	0	
学会賞経費	50,000	50,000	0	メダル，賞状他
雑費	25,000	25,000	0	郵便・銀行振込手数料
予備費	260,522	263,079	▲2,557	
小　計	3,360,522	3,283,079	77,443	
次年度繰越金	0	0	0	
合　計	3,360,522	3,283,079	77,443	

資料 3

改定案	現　行
第 7 条　本会会員は本会の目的に賛成し，所定の会費を納めるものとする。会員は正会員，特別会員（年度初めに満65歳以上で申告のあった者），学生会員および賛助会員とする。会費（年額）は，正会員8,000円，特別会員6,000円，学生会員4,000円，賛助会員一口10,000円とする。	**第 7 条**　本会会員は……ものとする。 会員は正会員，学生会員,および賛助会員とする。
第 8 条　会員は，退会，死亡，または除名によって，資格を喪失する。 (1)　会員で退会しようとするものは，理由を付して退会届を会長に提出しなければならない。この場合，未納会費があるときはこれを全納しなければならない。 (2)　会員が本会の名誉を毀損または会費を3年以上滞納したとき，評議員会の承認によってこれを除名することができる。	**第 8 条**　正会員会費は年額8000円，学生会員会費は年額4000円，賛助会員会費は一口年額10000円とする。
評議委員会の定足数が未規定 **第 9 条**　本会は評議員会によって運営される。……評議員の任期は 2 年とする。ただし，再任を妨げない。 評議員会は評議員総数の 3 分の 1 以上の出席がなければ成立しない。ただし，出席できない評議員で，委任状により他の出席評議員または議長に決議を委任した者は，出席者とみなす。評議員会の議決は出席者の過半数でなされる。	**第 9 条**　本会は評議員会によって運営される。……評議員の任期は 2 年とする。ただし，重任を妨げない。
総会の議決規定がない **第13条**　通常総会は……会の重要問題を審議する。総会は正会員，特別会員および学生会員の 6 分の 1 以上の出席がなければ成立しない。ただし，出席できない会員で，委任状により他の出席会員または議長に決議を委任した者は，出席者とみなす。 総会の議決は出席者の過半数でなされる。	**第13条**　通常総会は……会の重要問題を審議する。総会は正会員および学生会員の 6 分の 1 以上の出席がなければ成立しない。ただし，出席できない正会員および学生会員は委任状により他の出席会員または議長に決議を委任し，出席会員とみなすことが出来る。
解散規定の新設 **第15条**　本会の解散は総会における，出席者の 3 分の 2 以上の議決を経なければならない。	
	注：このほかにも，会則中にある「正会員および学生会員」の表現は，「正会員，特別会員および学生会員」と全て読み替えるものとする。日仏海洋学会会則第 9 条，日仏海洋学会評議員•役員選出規定 2 .

日仏海洋学会会則

昭和35年４月７日　制定
昭和60年４月27日　改正
平成４年６月１日　改正
平成19年６月９日　改正
平成21年６月20日　改正

第１条　本会は日仏海洋学会と称する。

第２条　本会の目的は日仏海洋および水産学者の連絡を密にし，両国のこの分野の科学の協力を促進するものとする。

第３条　上記の目的を実現するため本会は次の事業を行なう。

⑴　海洋および水産に関する研究会および講演会の開催

⑵　定期刊行物，学術上の刊行物の発行

⑶　学会賞の授与

⑷　日仏両国を主とする海洋および水産に関する共同研究成果の発表，ならびに，技術開発成果の導入および普及

⑸　両国の海洋・水産関係者の交流促進および親睦をはかること

⑹　その他本会の目的を達成するために必要な事業

第４条　本会には，海洋，水産学の分野に応じて分科会を設けることができる。

分科会は評議員会の決議によって作るものとする。

第５条　本会の事務所は日仏会館（〒150–0013　東京都渋谷区恵比寿３丁目９番25号）に置く。

第６条　本会に地方支部を置くことができる。

第７条　本会会員は本会の目的に賛成し，所定の会費を納めるものとする。会員は正会員，特別会員（年度初めに満65歳以上で申告のあった者），学生会員および賛助会員とする。会費（年額）は，正会員8,000円，特別会員6,000円，学生会員4,000円，賛助会員一口10,000円とする。

第８条　会員は，退会，死亡，または除名によって，資格を喪失する。

⑴　会員で退会しようとするものは，理由を付して退会届を会長に提出しなければならない。この場合，未納会費があるときはこれを全納しなければならない。

⑵　会員が本会の名誉を毀損または会費を３年以上滞納したとき，評議員会の承認によってこれを除名することができる。

第９条　本会は評議員会によって運営される。評議員の定数は28名以内とし，24名は正会員，特別会員および学生会員の投票によって選出される。

会長は評議員会の同意を得て４名以内の正会員，特別会員および学生会員を評議員に委嘱することができる。評議員の任期は２年とする。ただし，再任を妨げない。

評議員会は評議員総数の３分の１以上の出席がなければ成立しない。ただし，出席できない評議員で，委任状により他の出席評議員または議長に決議を委任した者は，出席者とみなす。評議員会の議決は出席者の過半数でなされる。

第10条　評議員はその内より次の役員を選ぶ。ただし，監事は評議員以外からも選ぶことができる。

会長　１名，副会長　２名，

幹事　10名以上12名以内，監事　２名

役員の任期は２年とする。ただし，再任を妨げない。

第11条　本会に名誉会長，顧問および名誉会員を置くことができる。名誉会長，顧問および名誉会員は評議員会の決議により会長がこれを委嘱または推薦する。

日仏会館フランス人学長を本会の名誉会長に推薦する。

第12条　会長は本会を代表し，総会および評議員会の議長となる。会長に事故あるときは副会長がこれに代わる。

会長，副会長および幹事は幹事会を構成し，本会の庶務，会計，編集，研究発表，渉外などの会務を行う。

監事は本会の会計を監督する。

第13条　通常総会は毎年１回会長が招集する。会長は必要に応じて評議員会の決議を経て臨時総会を招集することができる。総会では評議員会の報告に基づいて，会の重要問題を審議する。
総会は正会員，特別会員および学生会員の６分の１以上の出席がなければ成立しない。ただし，出席できない会員で，委任状により他の出席会員または議長に決議を委任した者は，出席者とみなす。総会の議決は出席者の過半数でなされる。

第14条　本会則の変更は総会の決議による。

第15条　本会の解散と資産の処分は総会における，出席者の３分の２以上の議決を経なければならない。

日仏海洋学会評議員・役員選出規定

１．本規定は日仏海洋学会会則第９条および第10条に基づき本会の評議員および役員の選出方法について規定するものである。

２．評議員の選出は正会員，特別会員および学生会員の24名連記無記名投票による。
評議員の選挙事務は庶務幹事が行なう。ただし，開票にあたっては本会役員以外の会員２名に立会人を委嘱するものとする。

３．会長は評議員の単記無記名投票により選出する。会長選挙の事務は庶務幹事が行なう。ただし，開票にあたっては本会役員以外の会員２名に立会人を委嘱するものとする。

４．副会長，幹事，および監事は，会長の推薦に基づき評議員会で決定する。

５．本規定の改正は評議員会の議を経て行なう。

日仏海洋学会賞規定

１．日仏海洋学会賞（以下「学会賞」という）および日仏海洋学会論文賞（以下「論文賞」という）を本学会に設ける。学会賞は本学会員で，海洋学および水産学において顕著な学術業績を挙げた者のなかから，以下に述べる選考を経て選ばれた者に授ける。論文賞は若手研究者や大学院生を筆頭著者とする論文を対象とする。原則として選考年度を含む３年（暦年）の間に，本学会誌に発表された論文のなかから，優秀な論文２編以内を選び，その著者（共著者を含む）に以下に述べる選考を経て授ける。

２．学会賞および論文賞候補者を選考するため学会賞および論文賞受賞候補者推薦委員会（以下「委員会」という）を設ける。

３．委員会の委員は９名とする。委員は毎年春の評議員会で選出し，委員長は委員の互選により定める。委員の任期は２年とし，隔年に４名および５名を交代する。会長は委員会が必要と認めた場合，評議員の同意を得て２名まで委員を追加委嘱することが出来る。ただし，追加委嘱された委員の任期はその年度限りとする。

４．委員会は学会賞受賞候補者１件および論文賞受賞候補者２件以内を選び，12月末までに選考理由書をつけて会長に報告する。

５．会長は委員会が推薦した各候補者につき無記名投票の形式により評議員会にはかる。投票数は評議員総数の３分の２以上を必要とし，有効投票のうち４分の３以上の賛成がある場合，これらを各賞受賞者として決定する。

６．授賞式は翌年春の総会において行い，学会賞受賞者には賞状およびメダルを，論文賞受賞者には賞状をそれぞれ贈呈する。

７．本規定の改正は評議員会の議を経て行なう。

覚書

１．委員は各専門分野から選出されるように十分配慮すること。

２．受賞者は原則として順次各専門分野にわたるよう十分配慮すること。

３．平成14年度より適用する。

賛 助 会 員

ＪＦＥアレック株式会社	神戸市西区井吹台東町７－２－３
株式会社 イーエムエス	神戸市中央区東川崎町１－３－３ 神戸ハーバーランドセンタービル　13F
有限会社 英和出版印刷社	北区中里２－14－８シャンボール駒込101
財団法人 海洋生物環境研究所	千代田区神田神保町３－29　帝国書院ビル５Ｆ
ケー・エンジニアリング株式会社	台東区浅草橋５－14－10
いであ株式会社	世田谷区駒沢３－15－１
テラ株式会社	文京区湯島４－１－13－402
八洲商事株式会社	静岡市清水区宍原630－５