

The effects of temperature on the growth of and ammonia uptake by marine microalgae

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ABSTRACT. Four species of microalgae were evaluated at different temperatures for their ability to remove ammonia from intensive marine fish/shrimp culture systems. Growth rates were highest on the first of three days of culturing for marine microalgae *Nannochloropsis oculata*, *Isochrysis* aff. *galbana*, *Chaetoceros muelleri*, and *Tetraselmis chui*. This rate was used to compare both the growth and the total ammonium nitrogen (TAN) uptake efficiencies of these organisms at different temperatures. Their dry weights (DWs) per cell were 10.3 pg for *Nannochloropsis oculata*, 40.4 pg for *Isochrysis* aff. *galbana*, 39.7 pg for *Chaetoceros muelleri*, and 369 pg for *Tetraselmis chui* at 25°C. Temperatures for their respective optimal growth rates were 26, 28, 33, and 25°C. There were no significant differences in growth rates among these four microalgal species at 25°C. Temperatures for maximal biomass production were 30°C for both *Nannochloropsis oculata* and *Isochrysis* aff. *galbana*, 35°C for *Chaetoceros muelleri*, and 20–30°C for *Tetraselmis chui*. Temperatures for maximal specific TAN uptake were respectively 25, 25, 20–30, and 25°C. Overall, *Tetraselmis chui* was the most efficient at TAN uptake among the four species. Our results suggest that *T. chui* is a good choice for removing ammonia from indoor intensive marine culture systems.

Keywords: Algal production; Ammonia; Growth rate; Marine microalgae.

INTRODUCTION

The growth of microalgae can be affected by many factors, such as nutrients, light intensity, salinity, pH, and temperature. Temperature stress, in particular, influences the growth rate and chemical composition of microalgae and may limit nutrient interactions. Temperature has a major effect on the phase transition of membrane lipids, the kinetics of cellular enzymes, and active transport systems across membranes (Quinn and Williams, 1983; Wheeler, 1983). Microalgal growth rates can be stimulated and cells become smaller at higher temperatures (Rijssel and Gieskes, 2002). Although a high growth temperature was related to a significant decrease in protein content and increases in lipids and carbohydrates in *Spirulina* species (Tomaselli et al., 1988; Oliveira et al., 1999), the response of microalgal chemical compositions to high and low growth temperatures varies from species to species (Thompson et al., 1992a; Renaud et al., 1995).

Ammonia is formed as the principal product of protein metabolism in aquatic organisms. Ammonia exists in two forms that are convertible in water, NH_3 and NH_4^+ , the sum of which is called total ammonia nitrogen (TAN). The ratio of $\text{NH}_3/\text{NH}_4^+$ varies with water pH, ionic strength,

pressure, and temperature. The proportion of un-ionized ammonia (NH_3), the more-toxic form to aquatic organisms, increases with water pH (Randall and Tsui, 2002; Ip and Chew, 2010). Ammonia can be removed by bacterial biofilters (Tseng et al., 1996; Grommen et al., 2002), heterotrophic bacterial biofilters (Avnimelech et al., 1992; Avnimelech, 1999), seaweed biofilters (Cohen and Neroi, 1991; Neori et al., 1996 and 2000), and microalgae (Tseng et al., 1991; Voltolina et al., 2005; de Godos et al., 2010). A bacterial biofilter converts ammonia into nitrate in two steps through the action of ammonium- and nitrite-oxidizing bacteria. However, the end product, nitrate, still remains in the water. Although nitrate is relatively harmless to aquatic organisms (Russo and Thurston, 1991), levels considered toxic to them ($1000\text{--}3000\text{ mg}\cdot\text{L}^{-1}$) should be avoided (Lawson, 1995). Heterotrophic bacteria can absorb ammonia as a precursor for cellular protein synthesis, but the C/N ratio imposes certain limitations on their growth. In addition to ammonia removal, microalgae can also produce biomass which can be used in several ways to improve the economic efficiency of aquaculture systems (Tenore, 1976; Arieli et al., 1993; Muller-Feuga et al., 2003; Lubzens and Zmora, 2003; Plaza et al., 2010; Rodríguez-Meizoso et al., 2010).

Uptake rates of nitrate and ammonium were studied as a function of nitrate or ammonium concentrations with cultures of *Chaetoceros gracilis* and *Isochrysis galbana* (Eppley et al., 1969). Although the half-saturation constant

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(the concentration at one-half the maximum uptake rate) for the uptake of nitrate (0.3~0.1 μM) was compared to that of ammonium (0.5~0.3 μM) in *C. gracilis*, ammonium is the preferred nitrogenous nutrient of many marine phytoplankton species (McCarthy, 1980). Since free ammonia is toxic to algae, it does not accumulate in their cells but is stored in the system through an ammonia-binding reaction. Glutamate dehydrogenase and glutamate synthetase activities are higher in green algae, which are peculiar in having a highly effective pathway to detoxify ammonia (Klochenko et al., 2003). Microalgae exhibit an active, and most likely a passive, ammonium uptake. The proportion of ammonia in marine systems can increase to > 10% of TAN. The concentration gradient of ammonia across a cell membrane can be maintained by ammonia protonation within the cell and equilibration between ammonium and ammonia outside the cell (Henderson, 1971). However, nitrate is apparently taken up by an active transport system in algae, and this transport system appears to be ATP-driven rather than directly dependent on an electrochemical gradient (Falkowski, 1975; Stewart, 1980; Wheeler, 1983). Therefore, the uptake rate of ammonium is less temperature-dependent than that of nitrate.

Aquatic organisms in Taiwan widely consume *Nanochloropsis oculata*, *Isochrysis* aff. *galbana*, *C. muelleri*, and *Tetraselmis chui* as live food (Su et al., 1997). The ammonia produced from intensive marine fish/shrimp farms deteriorates water quality but can be removed by these microalgae, which are then consumed by bivalves as food (Forrest et al., 2009). However, information on the relative efficiencies of ammonia uptake among these four species of microalgae is scant. The aim of this study was to evaluate the efficiency of ammonia uptake by these four species of microalgae at various temperatures and finally, to select a candidate for removing ammonia from intensive marine fish/shrimp culture systems.

MATERIAL AND METHODS

Artificial seawater (ASW) preparation and microalgae maintenance

ASW was prepared according to APHA et al. (1995) guidelines, and its composition is given in Table 1. ASW (33 $\text{g}\cdot\text{L}^{-1}$) was freshly prepared and aerated for 3 days. *Tetraselmis chui* (Chlorophyta), *I. aff. Galbana* (Haptophyta), *C. muelleri* (Bacillariophyta), and *N. oculata* (Eustigmatophyceae) were a gift from the Tungkang Biotechnology Research Center, Fisheries Research Institute, Pingtung, Taiwan. They were maintained in Walne's Enriched Seawater (WES) medium (Walne, 1974) as modified by Su (1999) at a continuous light intensity of 10 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and 15°C.

Preparation of inoculated microalgae

Microalgae were acclimated to various temperatures (i.e., 15, 20, 25, 30, and 35°C) for 1 week prior to the experiment. Twenty milliliters of microalgae was inocu-

lated in 250-mL flasks with 180 mL of WES medium at a continuous light intensity of 180 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with shaking (125 rpm, Orbital shaker TS-520) until the microalgae grew to $\text{OD}_{750\text{nm}}$ values of 0.2~0.3. One hundred milliliters of microalgae was transferred to 1-L flasks with 900 mL of WES medium aerated at a level of 3 $\text{L}\cdot\text{min}^{-1}$ and a continuous light intensity of 180 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Preparation was completed after these microalgae had grown to $\text{OD}_{750\text{nm}}$ values of 0.5~0.6.

Growth rate of microalgae and their TAN uptake at different temperatures

In order to apply this approach in the field, only ammonia and phosphate were added in the experiment on microalgal growth and TAN uptake. Fifty milliliters of microalgae was inoculated in 1-L flasks with 950 mL of ASW containing 13 $\text{mg}\cdot\text{L}^{-1}$ TAN-N and 4 $\text{mg}\cdot\text{L}^{-1}$ $\text{PO}_4^{3-}\text{-P}$ aerated at 5 $\text{L}\cdot\text{min}^{-1}$ under a continuous light intensity of 180 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The cell densities of *T. chui*, *I. aff. galbana*, *C. muelleri*, and *N. oculata* at $\text{OD}_{750\text{nm}}$ values of 0.5~0.6 were approximately 1.2×10^6 , 6×10^6 , 7×10^6 , and 1.9×10^7 cells/mL, respectively. Therefore, their initial respective concentrations were 6×10^4 , 2.9×10^5 , 3.4×10^5 , and 9.3×10^5 cells $\cdot\text{mL}^{-1}$. After 1 day of culture, the microalgae were sampled to determine their cell densities and dry weights, as well as the concentration of TAN remaining in the culture. The experiments were performed in duplicate at 15, 20, 25, 30, and 35°C.

Analytical methods

Growth rate and cell density. After the microalgae were sampled, 0.5% povidone iodine was added to immobilize them. The cell density of the microalgae was quantified with a 0.1-mm-deep Neubauer chamber in duplicate. The growth rate was calculated as:

$$\mu = \{\log_2(N_2) - \log_2(N_1)\} / (t - t_0);$$

where μ is the growth rate (divisions day^{-1}), and N_1 and N_2 represent initial (t_0) and at t time cell densities (cells $\cdot\text{mL}^{-1}$) in the medium, respectively.

Table 1. Composition of artificial seawater (36 $\text{g}\cdot\text{L}^{-1}$).

Electrolyte	Concentration ($\text{g}\cdot\text{L}^{-1}$)
NaCl	24.53
Na_2SO_4	4.09
KCl	0.70
NaHCO_3	0.20
KBr	0.10
H_3BO_3	0.03
NaF	0.003
MgCl_2	5.20
CaCl_2	1.16
SrCl_2	0.03

Table 2. Dry weight (pg) per cell of marine microalgae at various temperatures. Data represent the mean \pm the standard deviation. Different letters indicate significant differences in a specific species of microalgae among different temperatures ($p < 0.05$).

Species	Temperature ($^{\circ}\text{C}$)				
	15	20	25	30	35
	(Dry weight (pg) cell $^{-1}$)				
<i>Nannochloropsis oculata</i>	11.9 \pm 0.91 ^a	9.8 \pm 0.42 ^b	10.3 \pm 0.51 ^b	13.0 \pm 0.84 ^a	--
<i>Isochrysis</i> aff. <i>galbana</i>	46.2 \pm 2.0	46.7 \pm 4.0	40.4 \pm 1.4	51.8 \pm 3.0	--
<i>Chaetoceros muelleri</i>	35.4 \pm 1.6 ^b	41.7 \pm 4.2 ^b	39.7 \pm 2.2 ^b	39.4 \pm 3.4 ^b	81.4 \pm 3.7 ^a
<i>Tetraselmis chui</i>	441 \pm 14 ^b	424 \pm 8.7 ^b	369 \pm 29 ^{bc}	539 \pm 15 ^a	--

Dry weight. This assay was based on a method described by D'Souza and Kelly (2000). The dry weight of the microalgae was determined by filtering 100 mL of microalgae onto precombusted glass-fiber filters (Whatman GF/C for *T. chui*, *I. aff. galbana*, and *C. muelleri*; GF/F for *N. oculata*, 47 mm in diameter) and washing with 0.5 M ammonia formate to remove residual salts from the culture. The filters were then dried at 60 $^{\circ}\text{C}$ until a constant weight was reached.

TAN remaining and specific uptake. Twenty milliliters of microalgal medium was sampled and centrifuged at 14,000 $\times g$ for 15 min at 4 $^{\circ}\text{C}$. After centrifugation, the supernatant was removed and used to determine the TAN concentration. TAN determination was according to the phenol hypochlorite method in APHA et al. (1995). Specific TAN uptake (q_t) was calculated by using the equation below:

$$q_t = \frac{(C_o - C_t) * V}{M}$$

where C_o and C_t ($\text{mg}\cdot\text{L}^{-1}$) represent the TAN concentration initial and at t time in the medium. V (L) is the volume of the medium, M (g) is the biomass (g).

Data analysis

The mean values of growth rates, and of TAN removal at studied conditions were compared by variance analysis (ANOVA) using SigmaStat statistical software from SPSS (SPSS, Chicago, IL, USA), ver. 10. Duncan's test for pairwise comparisons was used at the 5% significance level.

RESULTS

Dry weights per cell of marine microalgae

Dry weights per cell of *I. aff. galbana* and *C. muelleri* were temperature-independent at temperatures of $\leq 30^{\circ}\text{C}$ (Table 2). Among these four microalgal species, only *C. muelleri* could grow at 35 $^{\circ}\text{C}$. The dry weight of *C. muelleri* at 35 $^{\circ}\text{C}$ was much higher than those at $\leq 30^{\circ}\text{C}$. The dry weight of *N. oculata* at 20, and 25 $^{\circ}\text{C}$ was significantly lower than those at 15, and 30 $^{\circ}\text{C}$. However, the dry weight of *T. chui* at 30 $^{\circ}\text{C}$ was significantly higher than those at $\leq 25^{\circ}\text{C}$.

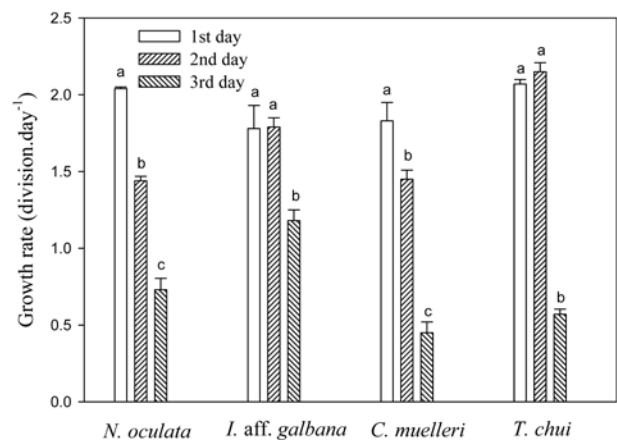


Figure 1. Growth rates of four microalgae species on the first, second, and third days of culture at 25 $^{\circ}\text{C}$. Each bar represents the mean value with the standard deviation. Different letters indicate a significant difference in a specific species of microalgae among different days culture ($p < 0.05$).

Temperature effect on the growth rates (μ) and biomass production

Microalgae in log-phase were inoculated into ASW only with 13 $\text{mg}\cdot\text{L}^{-1}$ TAN-N and 4 $\text{mg}\cdot\text{L}^{-1}$ $\text{PO}_4^{3-}\text{-P}$ at 25 $^{\circ}\text{C}$. The 3-day microalgal culture growth rates are given in Figure 1. First-day growth rates for *N. oculata* and *C. muelleri* were significantly higher than those for the second and third days. First-day growth rates of *I. aff. galbana* and *T. chui* were comparable to those for the second day but significantly higher than those for the third day. Therefore, first-day growth rates for the four microalgal species could be used to evaluate their growth rates and their TAN uptake rates at different temperatures.

Figure 2 shows the microalgal growth rates at different temperature levels. The μ values for *N. oculata* and *T. chui* were significantly greater than those for *I. aff. galbana* and *C. muelleri* at 20 $^{\circ}\text{C}$, while values of the latter were significantly greater than the former at 30 $^{\circ}\text{C}$. Based on polynomial regression data, respective optimal temperatures for *N. oculata*, *I. aff. galbana*, *C. muelleri*, and *T. chui* growth were 26, 28, 33, and 25 $^{\circ}\text{C}$.

Figure 3 shows the biomass production in dry weight (DW, $\text{mg}\cdot\text{L}^{-1}$) per liter of microalgae at various temperatures. Biomass values of *T. chui*, *I. aff. galbana*, *C. muelleri*, and *N. oculata* were 85, 43, 39, and 33 DW $\text{mg}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ at 25°C, respectively. The *N. oculata* and *I. aff. galbana* biomass production levels at 30°C were significantly higher than those at 15–25°C. *T. chui* biomass production at 15°C was significantly lower than it was between 20 and 30°C, where there was no significant difference. The biomass production of *C. muelleri* at 35°C was much higher than levels at 15–30°C.

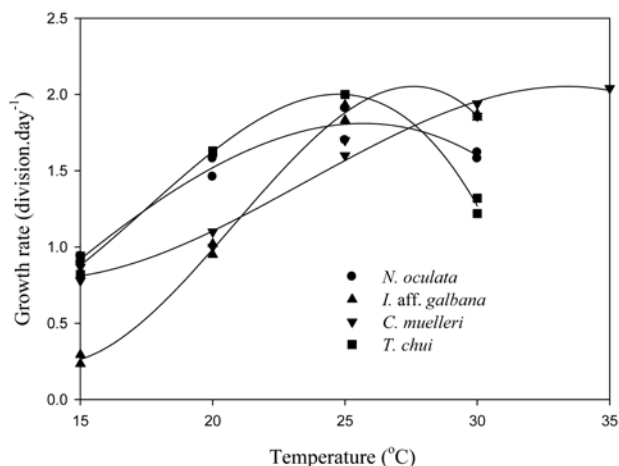


Figure 2. Effects of temperature on the growth rate of microalgae. The polynomial regressions of the growth rate (μ) against temperature (T) between 15 and 35°C were: *Nannochloropsis oculata*: $\mu = -1.02 + 0.066T + 0.0077T^2 - 0.0002T^3$, $R^2 = 0.96$; *Isochrysis aff. galbana*: $\mu = 10.03 - 1.683T + 0.091T^2 - 0.0015T^3$, $R^2 = 0.99$; *Chaetoceros muelleri*: $\mu = 3.364 - 0.44T + 0.023T^2 - 0.0003T^3$, $R^2 = 0.98$; and *Tetraselmis chui*: $\mu = 3.78 - 0.74T + 0.05T^2 - 0.001T^3$, $R^2 = 0.99$.

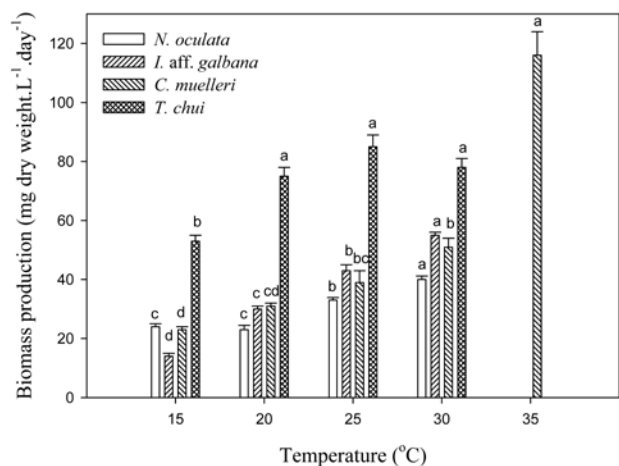


Figure 3. Effects of temperature on the daily algal biomass production per liter of microalgae. Each bar represents the mean value with the standard deviation. Different letters indicate significant differences in a specific species of microalgae among different temperatures ($p < 0.05$).

Effect of temperature on microalgal TAN uptake

The specific TAN uptake rates of microalgae at different temperatures are given in Figure 4. The optimal temperature for specific TAN uptake by *N. oculata*, *I. aff. galbana*, and *T. chui* was 25°C. However, there was no significant change in the specific TAN uptake rates by *C. muelleri* between 20 and 30°C. The specific TAN uptake rates by *N. oculata* and *T. chui* were higher than those for *I. aff. galbana* and *C. muelleri* at 25°C. Figure 5 shows the efficiency of the daily TAN uptake per liter for the four microalgal species at various temperatures. The daily TAN uptake rate for *T. chui* was much greater than for the other three microalgal species at all temperatures tested. The temperatures for optimal TAN uptake by *T. chui*, *I. aff. galbana*, and *C. muelleri* were 25, 25, and 35°C. However, TAN uptake by *N. oculata* at 25°C was comparable to that at 30°C. TAN uptake by *T. chui* at 25°C was roughly 2-fold that at other temperatures and 3-fold that of other microalgal species. Although the specific TAN uptake rate ($111 \text{ mg TAN}\cdot\text{N}\cdot\text{g}^{-1} \text{ dry weight}\cdot\text{day}^{-1}$) of *T. chui* was smaller than that ($130 \text{ mg TAN}\cdot\text{N}\cdot\text{g}^{-1} \text{ dry weight}\cdot\text{day}^{-1}$) for *N. oculata* at 25°C, the biomass produced by the former ($85 \text{ mg dry weight}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$) was much greater than for the latter ($33 \text{ mg dry weight}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$). *T. chui* was thus more efficient at daily TAN uptake per liter due to this higher biomass production.

DISCUSSION

Effects of temperature on the dry weight per cell of microalgae

It is generally recognized that microalgal cell sizes are inversely proportional to their growth rate, which increases with temperature in a certain range (Atkinson et al., 2003; Sayegh and Montagnes, 2011). Therefore, cell

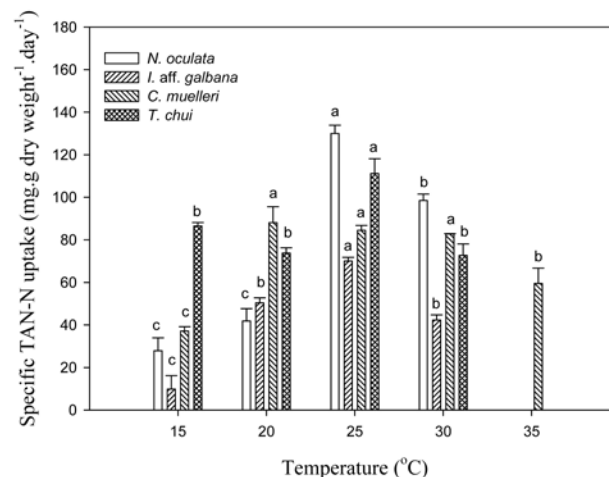


Figure 4. Effects of temperature on specific total ammonia nitrogen (TAN) uptake of microalgae. Each bar represents the mean value with the standard deviation. Different letters indicate significant differences in a specific species of microalgae among different temperatures ($p < 0.05$).

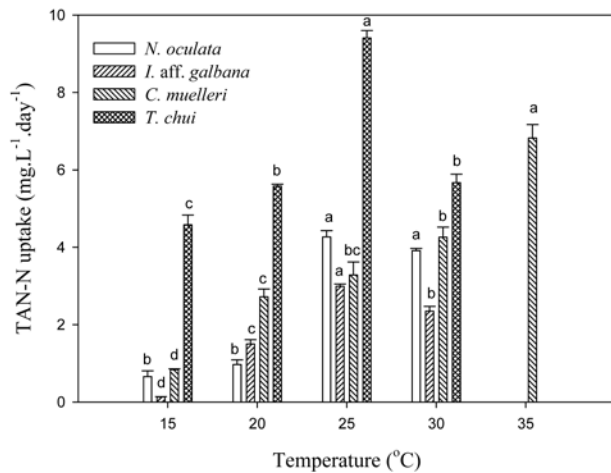


Figure 5. Effects of temperature on daily total ammonia nitrogen (TAN) uptake per liter by microalgae. Each bar represents the mean value with the standard deviation. Different letters indicate significant differences in a specific species of microalgae among different temperatures ($p < 0.05$).

sizes are larger at low temperatures or above the optimal temperature. *N. oculata* showed a higher dry weight per cell at 15°C, however, the effect of temperature on dry weights per cell of *T. chui*, *I. aff. galbana*, and *C. muelleri* was not significant. Nitrogen sources from ammonium can probably explain this unexpected phenomenon, since the ammonium uptake rate is less temperature-dependent than that of nitrate, which is the sole nitrogen nutrient in many medium formulas.

Based on dry weight per cell, the order of the four microalgal species was *T. chui* > *I. aff. galbana* > *C. muelleri* > *N. oculata* at 25°C. The value of 40–51 pg dry weight·cell⁻¹ for *I. aff. galbana* obtained in this study was comparable to data (36–46 pg dry weight·cell⁻¹) reported by Brown (1991) and by Brown et al. (1998). The cell dry weight of *Isochrysis* sp. (clone T-ISO) was affected by the irradiance, with 30 and 62 pg dry weight·cell⁻¹ at 50 and 500 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively (Brown et al., 1993a). Brown et al. (1993b) reported a 10 pg·cell⁻¹ dry weight for *N. oculata*, which is in the 9.8–13 pg·cell⁻¹ range in this study. No significant difference in the dry weights (35.4–41.7 pg·cell⁻¹) for *C. muelleri* cultured at 15–30°C was observed in this study. The dry weight of *C. muelleri* cultured at 35°C, however, sharply increased to 81.4 pg·cell⁻¹. Among these four microalgal species, only *C. muelleri* could grow at 35°C. High-temperature tolerance by *Chaetoceros* species was also reported by Renaud et al. (2002), who observed that its dry weight increased from 25 pg·cell⁻¹ at 25°C to 81.7 pg·cell⁻¹ at 35°C. A significantly lower dry weight (369 pg·cell⁻¹) for *T. chui* at 25°C than (539 pg·cell⁻¹) at 30°C may have been due to a much-higher growth rate (2.0) at 25°C than (1.27) at 30°C. However, Wikfors et al. (1996) determined a 104–135 pg·cell⁻¹ dry weight for *T. chui* harvested in a nitrogen-deficient stationary phase by semi-continuous, E-medium

cultures. These smaller sizes may be due to nitrogen nutrient limitation. Lourenco and Barbarino (1998) reported that the amount of total inorganic nitrogen *T. gracilis* accumulated at the mid-log phase was 2.5-fold that of its stationary phase. The discrepancy in *T. chui* dry weight may have been due to the microalgae being in different phases.

Temperature effects on the growth rate (μ) and biomass production

As with many rate processes, the microalgal growth rate is expected to increase with temperature, and rapidly decline above the optimal temperature. Low temperatures usually reduce enzymatic activity, which causes a decrease in the growth rate (Davison, 1991). Minimal growth rates were found at 15°C for all four microalgal species. The temperature tolerance of *C. muelleri* was the highest among these microalgae. Renaud et al. (2002) found a similar pattern in *Chaetoceros* sp., which grew well at 33 and 35°C. However, *Isochrysis* sp. and a commercial *Isochrysis* sp. (clone T-ISO) grew very slowly at 35°C (Renaud et al., 1995). Thompson et al. (1992b) also claimed that the microalgal growth response to temperature is species-specific.

The growth rate (0.99 day⁻¹) of *I. aff. galbana* grown at 20°C in this study was higher than the average (0.65 day⁻¹) of that during 7 days of culture (Valenzuela-Espinoza et al., 1999) and was comparable to that (0.84 day⁻¹) in the comparative study on growth performance and biochemical composition of mixed culture of *Isochrysis galbana* and *Chaetoceros calcitrans* with monocultures (Phatarpekar et al., 2000). The *T. chui* growth rate (1.63 day⁻¹) at 180 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensity at 20°C in this study was much higher (0.64 day⁻¹) than for *T. chui* grown at 110 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensity at 18°C (Meseck et al., 2005). Meseck et al. (2005) cultured *T. chui* with Guillard's f/2-enriched medium, which contains 13 μM ethylenediaminetetraacetic acid (EDTA) (Guillard, 1975). Although EDTA can alleviate the toxic effects of some metals (e.g., Cd, Cu, and Mn) and increase the bioavailability of others (e.g., Fe) by preventing precipitation, it suppressed the growth of oceanic phytoplankton (Muggli and Harrison, 1996; Okauchi and Kawamura, 1997). In fact, we found that the growth rate for *T. chui* after 1 day of culture in medium containing only ammonium and phosphate was higher than for WES medium (data not shown).

TAN-N uptake by microalgae

Although nitrogen is available to microalgae in various forms, nitrate, ammonium ions, and urea are the dominant forms (Syrett, 1981). Among these, ammonium ions are preferentially taken up, followed by nitrate and urea (Levasseur et al., 1990; Levasseur et al., 1993). Less energy is required to take up ammonium, and its more-reduced state may be the reason it is preferred over nitrate (Dortch, 1990). Ammonia is generally thought to directly assimilate into the amino acid, glutamine (Flynn, 1991). However, some species, e.g., *Hillea* sp. and *Prorocentrum minimum*,

failed to grow with ammonium-N because of ammonia's toxic effects in high concentrations (Lourenco et al., 2002). Similarly, *I. galbana* grown with ammonium-N had a lower growth rate (Lourenco et al., 2002). Valenzuela-Espinoza et al. (1999) reported that 2.16 mg ammonium-N·L⁻¹ was taken up by *I. galbana* after the first day of incubation. This value is very close to the 2.06 mg TAN-N·L⁻¹ in our data. A similar phenomenon was observed with *N. oculata*. The amount of ammonium-N (3.73 mg ammonium-N·L⁻¹) taken up by microalgae reported by Su et al. (1997) was comparable to the 4.27 mg TAN-N·L⁻¹ in our study. A rate of 9.4 mg TAN-N·L⁻¹·day⁻¹ taken up by *T. chui* at 25°C can be approximately expressed as 40 µg ammonium-N·10⁶ cells·day⁻¹ which is 2-fold that taken up by *Chlorella vulgaris* grown in 20 mg ammonium-N·L⁻¹ (Tam and Wong, 1996). The ammonium uptake efficiency of *T. chui* was comparable to that of *Scenedesmus obliquus*, which took up 9.27 mg TAN-N·L⁻¹·day⁻¹ in a semi-continuous culture with a 30% water exchange rate (Voltolina et al., 2005).

Less than 30% and 21% of the supplied nitrogen is respectively recovered as harvested fish and shrimp by aquaculture (Porter et al., 1987; Hall et al., 1992; Briggs and Funge-Smith, 1994). Most nutrients are released into the environment and contribute to water pollution. In addition to environmental impacts, discharging nutrients causes economic losses and reduces farm profitability. The discharged nutrients could be taken up by microalgae which could then be used as aquaculture feed. Although *T. chui* is more efficient at TAN removal, its growth is greatly limited above 30°C (data not published). Therefore, *T. chui* is not a practical solution to the problem of TAN removal from outdoor ponds in the summer in Taiwan. Tseng et al. (2006) reported that the water quality of the shrimp ponds was not well controlled by the addition of *T. chui*. It is very important to maintain the dominant microalgal species when removing TAN from aquacultural systems. Those microalgal species dominant in batch reactors are more stable than those found in continuous-flow reactors (Tseng et al., 1991). In our previous work, we used batch reactors in a recirculating aquaculture system for indoor tilapia culture (Chen et al., 2010). In that study, *T. chui* took ammonia up so that TAN levels in the tilapia tank water remained at < 3 mg·L⁻¹. Therefore, *T. chui* is a good candidate for removing ammonia from intensive culture systems.

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溫度對海洋微藻生長和吸收氨氮能力的影響

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擬球藻 (*Nannochloropsis oculata*)、等鞭金藻 (*Isochrysis aff. galbana*)、牟氏角毛藻 (*Chaetoceros muelleri*) 和周氏扁藻 (*Tetraselmis chui*) 在三天的培養中以第一天的生長速率最快。將四種微藻培養在不同的溫度，以第一天的生長速率比較這些微藻的成長和吸收總氨氮 (TAN) 的效率。它們在 25°C 時，每個細胞的乾重分別為 10.3、40.4、39.7 和 369 pg。它們的最適成長溫度分別為 26、28、33 和 25°C。在 25°C 時，四種微藻的生長速率沒有顯著的差異。四種微藻最大生產量的溫度分別為 30、30、35、和 20~30°C，四種微藻最大比總氨氮吸收效率的溫度分別為 25、25、20~30 和 25°C。在所有測試的溫度中，周氏扁藻總氨氮吸收的效率在四種微藻中是最高的。而且周氏扁藻在 25°C 的總氨氮吸收效率高於其它溫度。本研究的結果建議利用周氏扁藻去除室內集約海水養殖系統的氨氮是最好的選擇。

關鍵詞：氨；海洋微藻；生長速率；藻類產量。