Grazing and performance of the copepod *Pseudodiaptomus poplesia* on a Chinese strain of *Aureococcus anophagefferens*

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Abstract

Brown tides have recurred in estuary areas globally, but trophic interactions between the causative species *Aureococcus anophagefferens* and planktonic copepods remain poorly understood. In this study, we investigated performance (ingestion, growth, development and reproduction) of the planktonic copepod, *Pseudodiaptomus poplesia*, offered either mono-algal or mixed-algal diets containing a Chinese strain of *A. anophagefferens*. A typical Michaelis-Menten pattern existed between ingestion rate and food level when copepod fed on the mono-algal diet of this species. Nauplii exhibited the highest maximum ingestion rate (I_{max}) than copepodids and adult females. In addition, I_{max} value was higher in nauplii feeding on *A. anophagefferens* than on *Skeletonema costatum*. When fed mixtures of *A. anophagefferens* and *S. costatum*, *P. poplesia* selected against *A. anophagefferens* cells, but less strongly at the naupliar stage. Nauplii did not undergo metamorphosis and died at late naupliar stages feeding on *A. anophagefferens* alone, similar to those under starvation. Furthermore, the presence of *A. anophagefferens* greatly reduced the reproduction rate of females in mixtures but did not influence the growth rate of copepodids. These results suggest that *P. poplesia* nauplii may exert grazing pressure on *A. anophagefferens* population during a brown tide, which, however, may not be persistent because of copepod population decline.

Key words: *A. anophagefferens*, copepod, grazing, growth, development, reproduction

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1 Introduction

Blooms of the eukaryotic microalga *Aureococcus anophagefferens* have recurred in estuary areas in USA and South Africa (Bricelj and Lonsdale, 1997; Probyn et al., 2001; Gobler et al., 2005). Recently, this species was also reported to be responsible for brown tides in estuaries of the Bohai Sea area in eastern China every year since 2009 (Zhang et al., 2012). Trophic interaction with zooplankton is the most direct and widespread biological factor to control phytoplankton population. It has been shown in previous studies that grazing of microzooplankton including protozoans and the nauplii of copepods can potentially regulate *Aureococcus* spp. population during brown tides (Deonarine et al., 2006; Smith et al., 2008).

Size of prey determines efficiency of grazing of copepod and the minimum size spectrum is generally 1–3 μm for copepod. It is expected theoretically that *A. anophagefferens*, with a size of 2–4 μm (Uye et al., 1983; Berggreen et al., 1988), may not be readily grazed by copepods. However, nauplii of *Acartia tonsa* exhibited a high ingestion rate feeding on *A. anophagefferens* alone in the study by Smith et al. (2008). Moreover, copepod at elder stages showed significant consumption on bacterioplankton or phyto-plankton species less than 5 μm in early studies (Boak and Goulder, 1983; Nejstgaard et al., 1997; Broglio et al., 2004). Calbet et al. (2000) reported high consumption of 100%–260% body/d (calculated by carbon) on 2–5 μm nanoplankton by four species of small copepod in a subtropical bay. It appeared that these species flexibly shifted feeding preference according to the size spectrum of the phytoplankton assemblage, indicating opportunistic feeding (Poulet, 1977; Brucet et al., 2008). Thus copepodids or adult copepods may also graze on *A. anophagefferens*, which, however, has been ignored in previous studies.

Field and laboratory evidences have accumulated to show that brown tides bring about detrimental effects on their grazers, which will doubtlessly mitigate the grazing pressure from zooplankton. Bivalve mortality, the retarded growth of planktonic grazers, and the decreased egg production of copepods usually occurred during brown tides (Buskey and Stockwell, 1993; Bricelj and Lonsdale, 1997). Some hypothetical mechanisms may contribute to detrimental effects of *A. anophagefferens*. Some toxic excreta of this species may be involved. Dimethylsulfoniopropionate (DMSP) was found in the culture of *A. anophagefferens* (Keller et al., 1989), and was later proven to be able to inhibit the

grazing of some tintinnids (Carlsson et al., 1990; Buskey and Hyatt, 1995). A dopamine analogue in the outer polysaccharide layer may also account for the inhibition of feeding ability of some bivalves (Gainey and Shumway, 1991). In addition, stickiness of the outer polysaccharide layer might cause feeding cessation in shellfish and feeding reduction in ciliates (Smith et al., 2008). However, the exact mechanism remains obscure so far. It was recently pointed out that the negative effect of this species on copepods could be associated with its nutritional inadequacy because the presence of alternative algal species in the diet mitigated adverse effects (Lonsdale et al., 1996). In most early studies, only survival and reproduction were used as endpoints for estimation of the impact of brown tides on copepods (Buskey and Stockwell, 1993; Lonsdale et al., 1996; Bricelj and Lonsdale, 1997). Recently, the impact of this species also manifested itself as suppression in ingestion and development of nauplii of *A. tonsa* even on mixed diets (Smith et al., 2008). Apperantly, the effect of *A. anophagefferens* on copepods is far from being well understood, and the copedite stages have been neglected in previous studies. Moreover, it is still unknown how these negative effects related to grazing behavior of elder stages of copepod. Finally, the negative effect of *A. anophagefferens*, derived from the morphological and toxicity traits, is probably strain-specific. In the study by Smith et al. (2008), even the different isolates exhibited different toxicities and led to different feeding responses in nauplii. This doubtlessly will complicate evaluation of the impact of *A. anophagefferens* on grazers.

The brackish-water calanoid copepod *Pseudodiaptomus poplesia* distributes in many temperate and subtropical estuaries in East Asia and is also the common species in the area with occurrence of brown tides in China. The objective of this work was to explore grazer-prey interaction between all developmental stages of *P. poplesia* and *A. anophagefferens*. A Chinese strain of *A. anophagefferens* and a common species *Skeletonema costatum* in Chinese coastal waters were chosen as main preys. We firstly examined feeding behavior of *P. poplesia* on *A. anophagefferens* in mono-algal or mixed-algal diets at juvenile and adult stages. Then, somatic growth, the development of juveniles, survival, and reproduction were investigated with and without the presence of alternative food particles to understand the effect of *A. anophagefferens* on the performance of *P. poplesia*.

2 Materials and methods

2.1 *Zooplankton and algae*

The copepod *P. poplesia* were originally collected in the Daya Bay, the South China Sea (21°C, salinity of 25) and then main-

tained in our laboratory for half a year. They were fed a mixture of *Isochrysis galbana* and *Thalassiosira weissflogii* in 0.22-μm filtered seawater (salinity of 25). The Chinese strain of *A. anophagefferens* was firstly isolated from the Bohai Sea, China. The alga *I. galbana* originated from the Key Laboratory of Tropical Marine Bio-resources and Ecology, Chinese Academy of Sciences. These two species had been maintained in our laboratory for one and a half years, whereas *T. weissflogii* and *S. costatum* more than five years. The f/2 medium for culture of *I. galbana* and *S. costatum* was made from MBL artificial sea water (ASW; Cavanaugh, 1956) according to Guillard and Ryther (1962). *Aureococcus anophagefferens* was grown in L_1 medium made also from ASW (Guillard and Hargraves, 1993). Algal cells were harvested at the exponential phase with centrifugation (3 000 r/min, 5 000 *g*) for 10 min at 4°C. The algal cells were re-suspended with ASW to prepare for the following feeding and performance experiments. All organisms were grown at 20°C under a 12 h:12 h light: dark cycle. *Aureococcus anophagefferens* was grown at an irradiance of 200 μE/(m²·s), whereas *T. weissflogii*, *I. galbana*, and *S. costatum* at an irradiance of 100 μE/(m²·s).

2.2 *Grazing experiments*

Grazing experiments were performed with nauplii, copepodids and adult females of *P. poplesia* on a mono-specific diet of *A. anophagefferens* or *S. costatum*. Food concentrations were 0.1, 0.25, 0.5, 0.75, 1.0, and 2 mg/L, equal to 6.9×10⁴, 1.7×10⁵, 3.4×10⁵, 5.2×10⁵, 6.9×10⁵, and 1.4×10⁶ cells/mL for *A. anophagefferens* and 6.7×10³, 1.6×10⁴, 3.3×10⁴, 5.0×10⁴, 6.6×10⁴, and 1.3×10⁵ cells/mL for *S. costatum*, respectively (Table 1). One hundred and eighty nauplii at Stage III (NIII), 180 copepodids at Stage IV (CIV) or 90 adult females from the same cohort were distributed equally into six 200-mL beakers holding 150 mL ASW and fed at corresponding food levels for acclimation for 1 d. Then 30 nauplii, 30 copepodids or 15 adult females of each beaker were further randomly distributed into three 50-mL tubes containing 50 mL medium at the corresponding food level using open-mouth pipettes. Each treatment had three control tubes containing only medium and algal cells. The tubes were capped and then placed in a Ferris wheel, which rotated at 0.8 r/min for 12 h in darkness at 20°C. At the beginning and end of the incubation, 3 mL of aliquots were sampled from each tube and fixed with Lugol's iodine for determination of the initial and final cell densities. When the experiment ended, animals were counted for mortality estimation. During the experiment, the mortality was less than 5%. The cell densities were counted under inverted microscopy (Olympus IMT-2) using a Neubauer hemocytometer and plankton-counting chamber. Ingestion rate (*IR*, μg/(ind.·d)) was calculated us-

Table 1. Summary of experiments: nine experiments were run comparing three stages of *P. poplesia* on *A. anophagefferens* (*Aa*), *S. costatum* (*SKE*), and *I. galbana* (*ISO*) in mono-algal or mixed-algal diets

Exp.	Set	Life stage of copepod	Treatment	Food concentration/mg·L⁻¹
Grazing	1	nauplii (NIII)	100% *Aa*	0.1, 0.25, 0.5, 0.75, 1.0, 2.0
		copepodid (CIV)		
		adult females		
	2	nauplii (NIII)	100% *SKE*	0.1, 0.25, 0.5, 0.75, 1.0, 2.0
		copepodid (CIV)		
		adult females		
Grazing selectivity	3	nauplii (NIII)	20:80, 50:50, 80:20 *Aa*: *SKE*	1.0
		copepodid (IV)		
		adult females		
Development	4	nauplii (NI)	starvation, 100% *Aa*, 100% *ISO*, 100% *SKE*,	1.0
Reproduction	5	adult females	50:50 *Aa*: *ISO*, 50:50 *SKE*: *ISO*	1.0

ing the following equation (Frost, 1972):

$$IR = \frac{V(\ln C_t - \ln C_{tf})(C_{tf} - C_0)}{N(\ln C_{tf} - \ln C_0)t}, \qquad (1)$$

where V is the volume of seawater in the experimental beakers, N is the number of copepods, C_0 and C_{tf} are the concentration of algal cells before and after feeding, respectively, C_t is the final algal concentration in each control tube, and t is the feeding time.

Ingestion rate data for *P. poplesia* were fitted to the Michaelis-Menten equation:

$$IR = \frac{I_{max}(C - C_0)}{K_{IR} + (C - C_0)}, \qquad (2)$$

where I_{max} is the maximum ingestion rate (μg/(ind.·d), calculated by carbon) or represented as the percentage of copepod body carbon ingested with unit of %/d, C is the prey concentration (mg/L), C_0 is the concentration at which ingestion ceases, and K_{IR} is the prey concentration sustaining 1/2 I_{max}.

2.3 *Feeding selectivity*

Feeding selectivity of *P. poplesia* was studied using a similar approach described for grazing experiments except for that mixed diets were used. Three mixed diets comprised 20:80, 50:50, or 80:20 *A. anophagefferens*:*S. costatum* (in carbon content) at a food level of 1 mg/L (Table 1). The experiment was conducted in triplicate in a Ferris wheel at 0.8 r/min for 12 h. Each replicate tube containing 50 mL medium had 10 nauplii at Stage III, 10 copepodids at Stage IV, or 5 adult females with the mixed algae. Each treatment had three control tubes containing only food particles. Based on *IR*s calculated following grazing experiments, three electivity indices were calculated and compared to determine whether *P. poplesia* displayed feeding preference for algal species in mixtures of algal cells: (1) Ivlev's original index, (2) forage ratio, and (3) Jacob's Index (a variation of Ivlev's), which accounts for the relative abundance of the prey items (Ivlev, 1961; Jacobs, 1974). Ivlev and Jacob indices range from –1 (selective avoidance) to 1 (preference), and a value of zero indicates no selective behavior. FR index ranges from 0 to 1 (negative selection) and 1 to +∞ (positive selection), whereas a value of 1 indicates no selective behavior.

2.4 *Development of juveniles*

Copepods were fed 100% *A. anophagefferens* (*Aa*), 100% *I. galbana* (*ISO*), 100% *S. costatum* (*SKE*), 50:50 *Aa:SKE*, or 50:50 *SKE:ISO* or starved from naupliar stage to investigate the effect of *A. anophagefferens* on the growth and development of juveniles. Nauplii were cultured individually in Petri dishes each containing 10 mL medium with an initial food level of 1 mg/L except for the starved group. Food was added daily, and the medium was renewed every other day. There were 60 Petri dishes for each food regime. Every day, animals were inspected under a microscope, and development and survival were recorded. Body length was measured at the beginning of the incubation, at copedite Stage I, and Stage VI for each alive individual. The body length of copepodids and adults was measured immediately from the beginning of the cephalothorax to the end of the caudal rami without accounting for the caudal setae. For nauplii the total body length was measured. Specific growth rates (SGR) were calculated using the following equation:

$$\mu = \frac{\ln l_t - \ln l_0}{t}, \qquad (3)$$

where l_0 and l_t are the mean body length of *P. poplesia* at the beginning and end of a time interval t (d). The experiment lasted 20 d.

2.5 *Reproduction experiment*

Reproduction of matured adults in the development experiment was studied. One female without eggs and one male were transferred into a Petri dish containing 20 mL medium to produce eggs. There were 10 to 18 replicates for each treatment. During the four days of incubation, animals were fed with the same food regime as in the development experiment as medium was changed daily. Every day, the adults were gently transferred to new medium using open mouth pipettes, and the nauplii were counted under a stereo microscope. At the end of incubation, eggs in egg sacs were also counted. Reproduction rate was then calculated as the sum of nauplii and eggs divided by the number of female and the duration of incubation (4 d). Because the nauplii died in the treatments of starvation and *A. anophagefferens* alone, no reproduction was recorded for these two treatments.

2.6 *Chemical measurement and data analysis*

One hundred juveniles (Stages NIII or CIV) or 40 adult females were filtered gently on pre-combusted (480°C, 2 h) Whatman GF/C filters and then rinsed with 1 mL 2.5% ammonium formate solution for desalination. The samples were dried at 60°C for 48 h and weighed with a Sartorius microbalance to the nearest μg. Then, the particulate carbon and nitrogen were measured with a CHN analyzer (Series II CHNS/O Analyzer, PerkinElmer Instruments).

Differences in grazing, growth, reproduction, or survival rate of animals among treatments were compared by One-way ANOVA, and Turkey post-hoc tests were used to analyze differences between two treatments. The contribution of life stage and the interaction between algal species and life stage to I_{max} were analyzed by Two-way ANOVA. The Wilcoxin signed-rank test was applied to determine the significance of grazing preference. All statistical analyses were performed using SPSS 19.0 statistical software. The level of significance for all critical ranges was set at $P<0.05$.

3 Results

3.1 *Ingestion rate*

Generally, *IR* of *P. poplesia* increased with food level up to a feeding plateau. Ingestion rate as a function of food level was closely fitted to Michaelis-Menten kinetics in all cases ($P<0.05$, Fig. 1 and Table 2). Copepodids and adults feeding on *A. anophagefferens* had comparable I_{max} values, which were 40%–48% higher than that of nauplii ($P<0.01$, One-way ANOVA and Tukey post-hoc tests). Accordingly, mass-specific I_{max} dropped remarkably from (89.5±7.2) to (43.0±3.4)%/d with increasing age, showing a typical allometric trend ($P<0.000\ 1$, One-way ANOVA; $P<0.013$, Tukey post-hoc tests). With *S. costatum* as the prey, I_{max} value increased continuously from (1.03±0.12) to (2.95±0.15) μg/(ind.·d) with developmental stage (Table 2; $P<0.01$, One-way ANOVA and Tukey post-hoc tests), but no ontogenic variation in mass-specific I_{max} was present (all $P>0.05$, One-way ANOVA and Tukey post-hoc tests). The interaction between prey species and life stage on *IR* was analyzed. Both I_{max} and K_{IR} values were strongly influenced by life stage ($P<0.005$) but only moderately by algal species ($0.01<P<0.05$). Furthermore, a strong interaction ex-
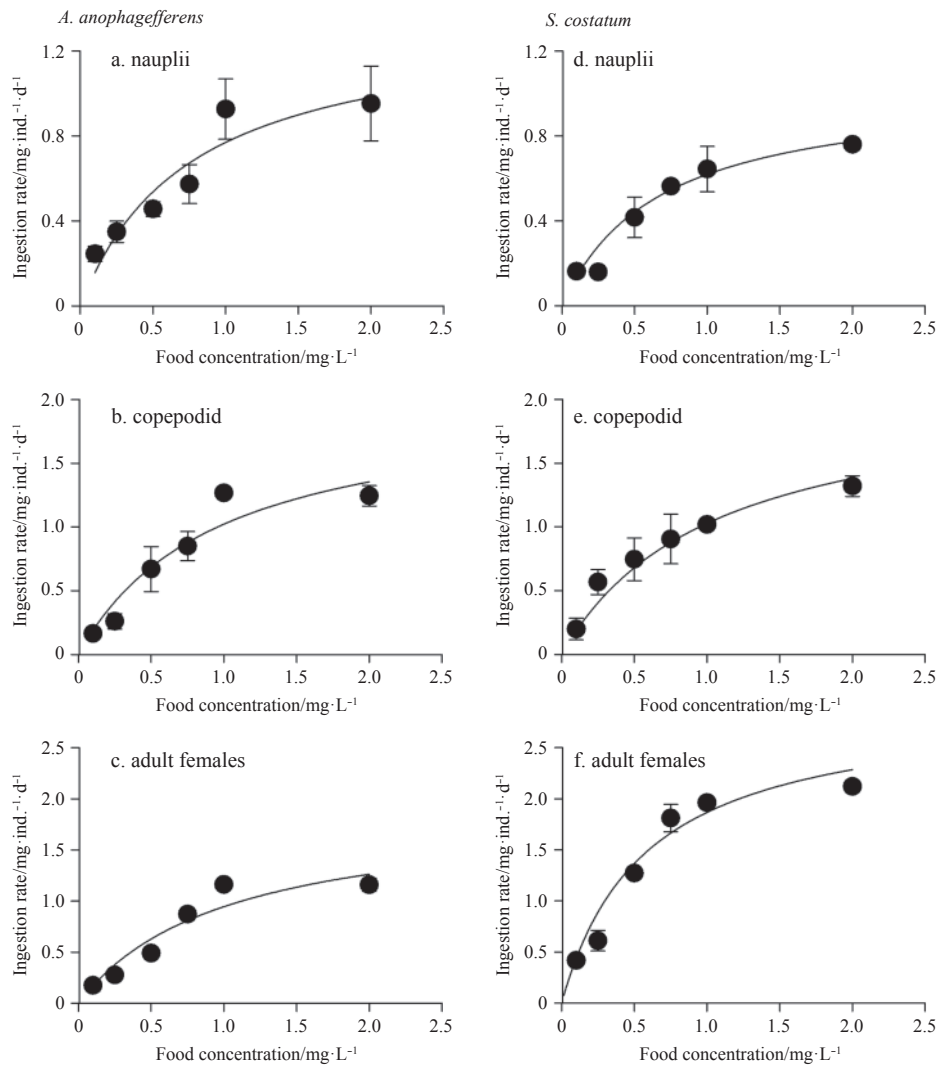
Fig. 1. Functional feeding responses of nauplii (NIII), copepodids (CIV), and adult females of *P. poplesia* on *A. anophagefferens* and *S. costatum*. Ingestion rates (mg/(ind.· d)) are presented as a function of food concentration (mg/L) (means±SD, *n*=3). Parameters of the fitted model are provided in Table 2.

Table 2. The curve fitting of Michaelis-Menten equation in different feeding experimental groups

Treatments		$IR = \dfrac{I_{max}(C - C_0)}{K_{IR} + (C - C_0)}$				
Algae	Life stage	I_{max}/μg· ind.$^{-1}$·d^{-1}	I_{max}/%·d^{-1}	K_{IR}/mg·L^{-1}	r^2	P
A. anophagefferens	nauplii	1.36±0.11	89.5±7.2	0.76±0.07	0.89	0.011*
	copepodid	2.01±0.15	61.3±4.6	0.97±0.14	0.91	0.018*
	adult females	1.90±0.15	43.0±3.4	1.01±0.18	0.90	0.023*
S. costatum	nauplii	1.03±0.12	67.8±7.9	0.67±0.06	0.99	0.000 1*
	copepodid	2.11±0.36	64.3±11.0	1.05±0.15	0.93	0.014*
	adult females	2.95±0.15	66.7±3.4	0.58±0.13	0.96	0.001*

Note: I_{max} represents the maximum ingestion rates when the food concentration reaches saturation, K_{IR} the food concentration while the IR being half as I_{max} (IR, μg/(ind.·d); C or C_0, mg/L), r^2 coefficient of determination; and P Monte Carlo P-value. *Significance of the curve fitting.

isted between the two factors (*P*<0.05, Two-way ANOVA). In detail, nauplii had a higher I_{max} value on *A. anophagefferens* than on *S. costatum* and the opposite trend occurred in female adults.

3.2 *Grazing selectivity*

　　Ivlev, Jacob and FR indices all indicated that *P. poplesia* selected against *A. anophagefferens* at Stages NIII, CIV and CVI regardless of proportion of *A. anophagefferens* and *S. costatum* in

mixed diets (*P*<0.05, Wilcoxin signed-rank test) (Table 3). However, it seemed that nauplii were less selective than copepodids and adults because most of selectivity indices were close to the neutral values in nauplii (zero for Ivlev's and Jacob's indices and 1 for the FR index). Ingestion rate ratio (IR_{Aa}: IR_{SKE}) was correlated significantly with Aa:SKE ratio in mixed-algal diets (Fig. 2). Slopes of regression were in the range of 0.17±0.04 to 0.36± 0.05 and nauplii had the highest value, demonstrating a tend-

Table 3. Electivity indices for nauplii (NIII), copepodid (CIV), adult females of *P. poplesia* grazing on mixed-algal diets of *A. anophagefferens* (*Aa*) and *S. costatum* (*SKE*)

Growth stage	Experimental group	Ratio of food	Algae type	Ivlev's index	Jacob's index	*FR* (forage ratio)	+/–
Nauplii (NIII)	1	2/8	*Aa*	–0.379 8	–0.433 6	0.449 5	–
		2/8	*SKE*	0.064 4	0.433 6	1.137 6	+
	2	5/5	*Aa*	–0.080 1	–0.148 3	0.851 7	–
		5/5	*SKE*	0.069 0	0.148 3	1.148 3	+
	3	8/2	*Aa*	–0.134 3	–0.436 8	0.763 2	–
		8/2	*SKE*	0.321 4	0.436 8	1.947 1	+
Copepodid (CIV)	1	2/8	*Aa*	–0.378 4	–0.432 1	0.451 0	–
		2/8	*SKE*	0.064 2	0.432 1	1.137 3	+
	2	5/5	*Aa*	–0.221 0	–0.362 0	0.638 2	–
		5/5	*SKE*	0.153 3	0.362 0	1.362 0	+
	3	8/2	*Aa*	–0.284 3	–0.665 1	0.557 3	–
		8/2	*SKE*	0.469 6	0.665 1	2.770 8	+
Adult females	1	2/8	*Aa*	–0.467 4	–0.523 1	0.363 0	–
		2/8	*SKE*	0.073 8	0.523 1	1.159 3	+
	2	5/5	*Aa*	–0.444 1	–0.615 0	0.385 0	–
		5/5	*SKE*	0.235 2	0.615 0	1.615 0	+
	3	8/2	*Aa*	–0.293 4	–0.675 0	0.546 3	–
		8/2	*SKE*	0.475 8	0.675 0	2.815 0	+

Note: The Ivlev's, Jacob's indices and forage ratio all showed that the three stages of *P. poplesia* significantly select against *A. anophagefference* in mixture ($P<0.05$, Wilcoxin signed-rank test).
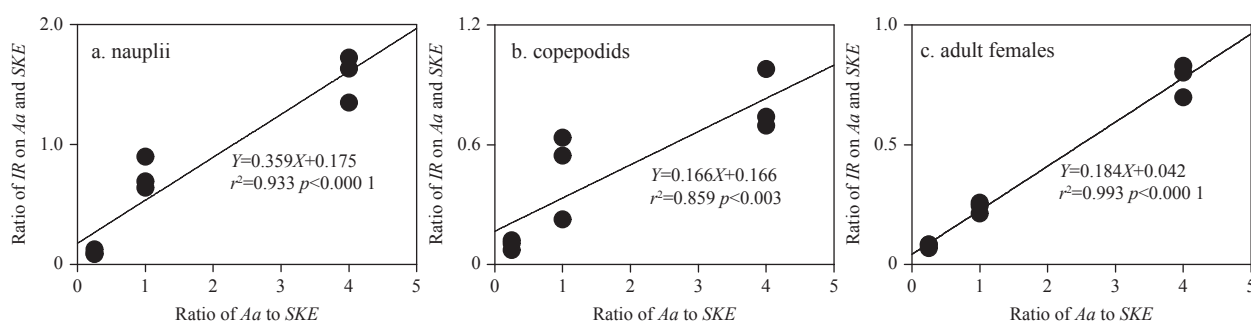

Fig. 2. The regression of ingestion rate ratio (*Aa*: *SKE*) of nauplii (NIII), copepodids (CIV) and adult females of *P. poplesia* with the ratio of *Aa* to *SKE* in the mixted-algal diets. *Aa* represents *A. anophagefferens* and *SKE S. costatum* (means±SD, *n*=3).

ency of opportunistic feeding.

Total ingestion rates (*TIR*s) of copepodids and adult females *P. poplesia* feeding on 20:80 (*Aa*:*SKE*) mixture was (0.27±0.06) and (0.47±0.05) μg/(ind. ·d), higher than on 50:50 and 80:20 mixtures ((0.29±0.08) to (0.40±0.07) μg/(ind.·d), Fig. 3). Differently, nauplii had comparable *TIR*s on all mixed-algal diets (Fig. 3). Generally, *TIR*s on mixed-algal diets were lower than on mono-algal diets in the study ($P<0.05$, One-way ANOVA and Tukey post-hoc tests).

3.3 *Growth and development*

The naupliar stage lasted (5.6±0.9) and (5.3±0.5) d in 100% *ISO* and 50:50 *SKE*: *ISO* treatments ($P>0.05$) but a significantly longer period of (6.6±0.9) and (7.1±0.6) d in 100% *SKE* and 50:50 *Aa*:*ISO* treatments ($P≤0.001$, One-way ANOVA and Tukey post-hoc tests; Table 4). In the contrast, the nauplii under starvation or feeding on 100% *Aa* diet died at Stage V on Day 9 or Day 11 (Fig. 4). On the other hand, copepodite duration was (9.8±1.3) and (10.1±0.9) d in 100% *SKE* and 50:50 *SKE*: *ISO* treatments ($P>0.05$), significantly shorter than that in 100% *ISO* and 50:50 *Aa*: *ISO* treatments ((11.6±0.9) and (12.7±0.5) d; $P<0.05$, One-way ANOVA and Tukey post-hoc tests; Table 4). Nevertheless, no obvious difference was shown with regard to body length at Stages CI
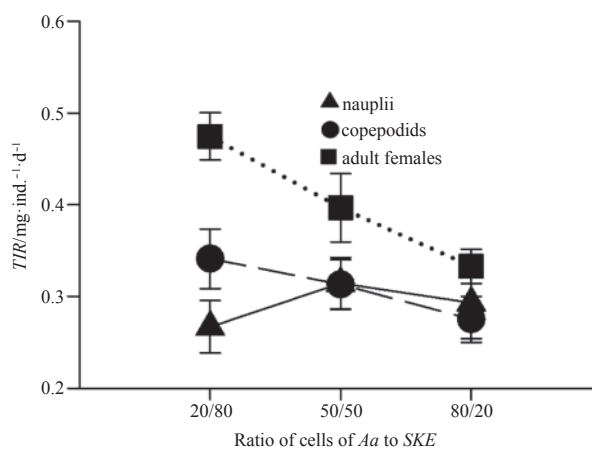
Fig. 3. Total ingestion rate (*TIR*) of nauplii (NIII), copepodids (CIV), and adult females of *P. poplesia* on mixed-algal diets containing different proportions of *A. anophagefferens* (*Aa*) and *S. costatum* (*SKE*) (means±SD, *n*=3).

and CVI among treatments ((500±0) and (522±34) μm vs. (1 236±20) and (1 289±87) μm; both $P>0.05$, One-way ANOVA and Tukey post-

Table 4. Duration, growth rate of *P. poplesia* at different life stages (Naup represents naupliar stage and Cop copepodite stage) and reproduction rate feeding different diets (*Aa* represents *A. anophagefferens*, *SKE S. costatum*, *ISO I. galbana*, mixture with different proportions of each alga) with total carbon concentration equal to 1 mg/L

Treatments	Duration/d		Growth rate/d^{-1}		Reproduction rate /eggs·ind.$^{-1}$·d^{-1}
	Naup	Cop	Naup	Cop	
Starved	–	–	0.077±0.010[a]	–	–
100% *Aa*	–	–	0.080±0.005[a]	–	–
100% *ISO*	5.6±0.9[a]	12.7±0.5[a]	0.172±0.030[b, d]	0.080±0.013[f]	5.1±0.4[b]
100% *SKE*	6.6±1.1[b]	9.8±1.3[b]	0.092±0.021[a]	0.142±0.015[e]	5.2±1.3[b]
50:50 *Aa:ISO*	7.1±0.6[b]	11.6±0.9[a]	0.126±0.026[c, b]	0.085±0.017[f]	3.1±0.8[a]
50:50 *SKE:ISO*	5.3±0.5[a]	10.1±0.9[b]	0.168±0.034[b]	0.108±0.007[e, f]	6.3±0.5[b]

Note: Data were presented as means±SD ($n \geqslant 10$). Growth of nauplii *P. poplesia* under starvation and treated with 100% *Aa* ceased before entering the copedite stage. Different letters indicate significant difference ($P<0.05$) between treatments by the Tukey post-hoc tests.

hoc tests). When growth rate was concerned, nauplii exhibited comparable growth rates in 100% *Aa* or 100% *SKE* or starvation treatments, significantly slower than those in the treatments with *ISO*. Moreover, among three treatments with *ISO*, nauplii had the slowest growth rate with the presence of *Aa* (Table 4; both $P<0.05$, one-way ANOVA and Tukey post-hoc tests). Differently, the copepodids had the highest and lowest growth rates in 100% *SKE* and 50:50 *Aa:ISO* treatments ($P<0.05$), whereas those feeding on 50:50 *SKE:ISO* diet had medium ($P>0.05$, One-way AN-OVA and Tukey post-hoc tests; Table 4).

3.4 *Eggs production and survival*

The females feeding on 100% *SKE*, 100% *ISO*, and 50:50 *SKE*:*ISO* diets showed comparable egg production rates ((5.10±0.36) and (6.31±0.50) eggs/(ind. ·d); all $P>0.05$, one-way ANOVA and Tukey post-hoc tests), higher than those feeding on 50:50 *Aa*: *ISO* diets (3.10±0.84 eggs/(ind.·d); $P<0.01$, One-way ANOVA; Table 4).

None of the nauplii under starvation or feeding on 100% *Aa* diet lived longer than 9 d or 12 d (Fig. 4). At the end of incubation, the animals feeding on 100% *ISO* and 50:50 *SKE*: *ISO* diets had higher survival rate (80.0%±5.77% and 83.3%±8.82%), whereas lower survival rates appeared in 100% *SKE* and 50:50 *Aa*: *ISO* treatments (76.7%±8.82% and 72.2%±1.92%, Fig. 4; all $P<0.01$, One-way ANOVA and Tukey post-hoc tests).

4 Discussion

4.1 *Grazing behavior of copepods on A. anophagefferens*

Significant grazing of *A. anophagefferens* by *P. poplesia* at Stages NIII, CIV and CIV was clearly shown in our study. *Pseudo-diaptomus poplesia* exhibited Type II functional ingestion response to the mono-specific diet of *A. anophagefferens* because *IR* as a function of food level conformed to the Michaelis-Menten model regardless of developmental stage. The mass specific I_{max} showed a typical allometric trend and ranged from 43%/d to 90%/d. In previous studies dealing with the size spectrum of copepods, the lowest size limit was proposed to be located in the range of 1–3 μm for copepods, which suggests that negligible consumption was expected near the size limit (Uye et al., 1983; Berggreen et al., 1988). Following this assertion, *A. anophagefferens* individuals with a size of 2–4 μm, appeared to be unsuitable for grazing by copepods. However, several previous studies revealed that some species of copepod including *Oithona simplex*, *O. Nana*, *Acrocalanus inermis*, *Parvocalanus crassirostris*, *Calanipeda aquaedulcis* can exploit significantly small particles with cell size equal to or even smaller than 2 μm (Boak and Goulder, 1983; Calbet et al., 2000; Brucet et al., 2008), in agreement with this present study. For example, nauplii or adults of *Eurytemora*
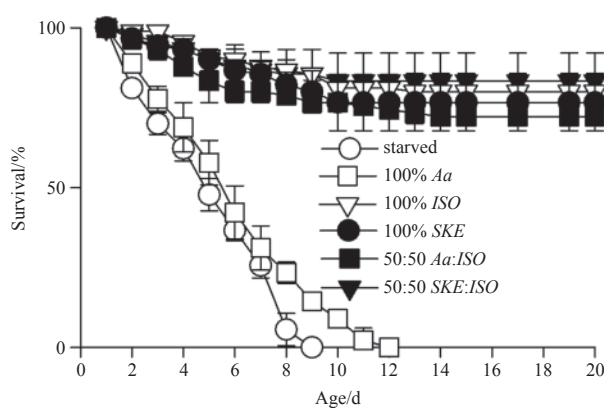

Fig. 4. Survival rate of *P. poplesia* on different diets with varying proportions of *Aa*, *SKE*, and *ISO*. Total food concentration was 1 mg/L (*Aa* represents *A. anophagefferens*, *SKE S. costatum*, and *ISO I. galbana*) (means±SD, n=3).

sp. grazed on free-living bacteria (Boak and Goulder, 1983). Thus, these species of copepod including *P. poplesia* behave as opportunistic feeders and can flexibly consume particles near the lower end of the size spectrum under the circumstance with a dominance of small particles. It was speculated that behavioral adaptation might be involved in feeding flexibility on small particles during the short-term feeding in this study, but this still needs to be verified in future studies.

Copepod exhibited ontogenic difference in grazing *A. anophagefferens* and this alga was readily grazed by nauplii in our study. Nauplii at Stage III had lower K_{IR} value of the Michaelis-Menten function than copepodids and adults, indicating the weakening tendency of grazing for *A. anophagefferens* with developmental stage. In addition, nauplii had a 30% higher value of I_{max} feeding on *A. anophagefferens* than on *S. costatum*. Likewise, nauplii of *A. tonsa* showed a two-fold higher *IR* on *A. anophagefferens* than on *I. galbana* (Smith et al., 2008). There was disagreement on capability of different stages feeding on smaller-sized prey in literatures. Berggreen et al. (1988) found that nauplii of *A. tonsa* capture small prey more efficiently than adults. There was an obvious ontogenic trend in preference of prey size spectrum in *Calanipeda aqueadulcis* and nauplii consumed preferentially picoplankton (Brucet et al., 2008). In contrast, it was argued that nauplii of *Eucalanus* sp. were not able to capture small prey as efficiently as later stages because of immature feeding apendages (Paffenhöfer and Lewis, 1989). It remains unclear whether interspecific difference exists in these findings.

On mixtures of *A. anophagefferens* and *S. costatum*, *P. pop-

lesia exhibited selective grazing against *A. anophageferens*, as indicated by Ivlev's, Jacob's and FR indices. Our study demonstrated that the degree of selectivity against *A. anophageferens* differed in different ontogenic stages. Elective indices values were high in most cases for copepodids and adults but close to neutral values in nauplii, pointing to less avoidance of *A. anophageferens* in nauplii. It was speculated that copepod discriminated food particles in mixtures based on prey size rather than on other traits in this study since copepods did not show a clear rejection of *A. anophageferens* in mono-algal diet. *Aureococcus anophagefferens* is smaller than *S. costatum* (2–4 μm vs. 5–8 μm), and this should be avoided by copepodids and adults in the mixture of both.

The edibility of *A. anophagefferens* appears to differ with strains and culture conditions when we compared the feeding behavior of copepod on different strains of *A. anophagefferens* in limited literatures. In the study by Smith et al. (2008), one strain, of which toxicity decreased after a long period (five years) of acclimation in the laboratory, was consumed by nauplii of *A. tonsa* but the other with a shorter period (two years) of laboratory maintenance was not. On the contrary, no inhibition of grazing was found when *P. poplesia* was fed the Chinese strain, which had been isolated less than two years. Therefore, it was speculated that the Chinese strain may not be as toxic as the American strain. It remains unclear whether any morphological or physiological difference exists between two strains.

4.2 *Development, somatic growth and reproduction in copepod*

Effects of *A. anophagefferens* on the performance of *P. poplesia* were examined continuously along different life stages in this present study. Nauplii did not complete metamorphosis or even survive on *A. anophagefferens* alone while the reproduction rate of females declined severely by 39%–50% with the presence of alternative algal species. Most of earlier studies usually used survival and reproduction rates to assess impacts of *A. anophagefferens* on copepod populations in field investigations (Buskey and Stockwell, 1993; Lonsdale et al., 1996; Bricelj and Lonsdale, 1997). These studies revealed that reproduction rates decreased greatly during brown tides, consistent to our laboratory work. Recently, Smith et al. (2008) for the first time reported retarded development of *A. tonsa* nauplii feeding on *A. anophagefferens*. The presence of alternative food particles mitigated the negative effect of *A. anophagefferens* on naupliar development of *P. poplesia* but not fully, which also occurred in the study by Smith et al. (2008) for *A. tonsa*. Nevertheless, it seems that development of copepodid was not affected in the presence of *A. anophagefferens*, probably since *I. galbana* itself is not good food for copepodids and masked the effect of *A. anophagefferens*.

Negative effects of the Chinese strain of *A. anophagefferens* on *P. poplesia* could be potentially caused by cellular toxicity or inadequate nutrition. As the correlation between *IR* and food level complied to Michaelis-Menten function with mono-specific diet of *A. anophagefferens*, possibility of cellular toxicity can be ruled out. Therefore, nutritional inadequacy may account for its detrimental effects, in agreement with the American strain (Bricelj and Lonsdale, 1997; Smith et al., 2008). Eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) are regarded as the most important components of food particles for zooplankton and accounted for 4.8% and 14.6% of total fatty acids in the Chinese strain cells (personal communication), comparable to 3.5%–5.1% and 11.8%–14.0% in the American strain determined by Bricelj and Fisher (1989) and also close to those of a broad spectrum of algae. In recent years, researchers have become increasingly interested in nutritional sterol requirement of copepod. Giner and Boyer (1998) quantified the sterol composition of *A. anophagefferens* and found this species is rich in Δ^5 components, which are suitable for copepod to utilize (Martin-Creuzburg and Von Elert, 2009). Therefore, it seems that lipid composition may not explain the detrimental effects of *A. anophagefferens* to copepod and other mechanisms should be involved for detrimental effects of the Chinese strain. Alternatively, *P. poplesia* always exhibited lower ingestion rates when feeding on mixed-algal diets of *A. anophagefferens* than on the mono-algal diet at the same food level, and actually, Smith et al. (2008) reported a similar phenomenon in *A. tonsa*. It appeared that copepods may suffer low food consumption. Yet, this should be verified in future quantitative studies.

In the Bohai Sea, *S. costatum* usually co-occurs with *A. anophagefferens* in the phytoplankton assemblage. It was inferred from our findings on grazing behavior that age class distribution of *P. poplesia* population determines grazing pressure on *A. anophagefferens*. Nauplii may contribute to control the *A. anophagefferens* population while copepodids and adults may contribute to development of the bloom by removing the alternative algal cells. Nutritional inadequacy of *A. anophagefferens* as food means that its negative effect is density-dependant. When a brown tide develops and reachs the climax, the detrimental effect of *A. anophagefferens* will appear gradually because copepods at elder stages than NII may consume a large amount of *Aureococcus* cells. Combining effects of *A. anophagefferens* on somatic growth in juveniles and reproduction in female adults, *P. poplesia* population will not maintain its development over a long term, which will constrain grazing pressure on *A. anophagefferens* population.

5 Conclusions

Ingestion rates of *P. poplesia* at Stages NIII, CIV, and CVI complied with the Michaelis-Menten function on a mono-algal diet of Chinese strain of *A. anophagefferens*. There existed an ontogenic trend in preference to *A. anophagefferens*. In mixtures with *S. costatum*, *A. anophagefferens* was avoided strongly by copepodids and adult females, but weakly by nauplii, which suggested that naupliar *P. poplesia* may potentially exert grazing pressure on *A. Anophagefferens* population during a brown tide. Nevertheless, the somatic growth and metamorphosis of nauplii were greatly suppressed by the presence of *A. anophagefferens*, as was the reproduction of adult females. It is suggested that the overall robust development of the copepod population may not occur during a brown tide, which will constrain the role of copepods in the control of brown tides over the long term.

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