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FEATURE ARTICLE

Different tolerances of jellyfish ephyrae (*Aurelia* sp. 1) and fish larvae (*Paralichthys olivaceus*) to nutrient limitations

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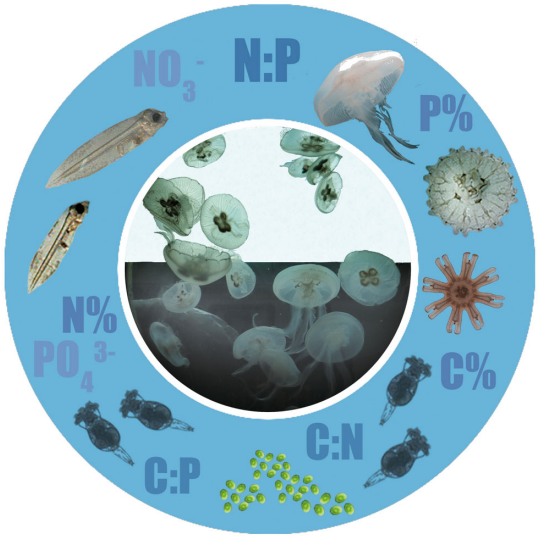
ABSTRACT: Nutrient imbalance — a mismatch in nutrient ratios between the available food supply and the demands of consumers — has the potential to be transported up food chains, exposing higher trophic-level organisms to nutrient limitations. We performed experiments to estimate the tolerance of jellyfish ephyrae (*Aurelia* sp. 1) and fish larvae (*Paralichthys olivaceus*) to nutrient limitations, and analyzed their growth, survival, and elemental homeostasis. As the primary consumer, rotifers *Brachionus plicatilis* exhibited the lowest amino acid content but the highest fatty acid content in a P-limited treatment. Among the secondary consumers, nutrient limitations (especially P limitation) had significantly negative effects on the growth of *P. olivaceus* larvae, but no significantly negative effects on *Aurelia* sp. 1 ephyrae. The 10th percentile mortality time of *Aurelia* sp. 1 ephyrae was much longer than that of *P. olivaceus* larvae. In terms of elemental homeostasis, *Aurelia* sp. 1 ephyrae showed a greater ability to maintain constant chemical composition in their bodies than *P. olivaceus* larvae. Additionally, growth and survival of *P. olivaceus* larvae could be negatively affected by the reduction of amino acid contents (but not fatty acids) in their nutrient-limited food. These results indicate that *Aurelia* sp. 1 ephyrae could be more competitive than *P. olivaceus* larvae with respect to tolerance of nutrient limitations, and, thus, elemental imbalances may favor increases in jellyfish in some eutrophication regions.

KEY WORDS: Ecological stoichiometry · Phosphorus limitation · Nitrogen limitation · Jellyfish ephyrae · Fish larvae



Jellyfish ephyrae could be more competitive than fish larvae under nutrient-limited conditions.

Graphic: Lei Chen and Chaolun Li

INTRODUCTION

Eutrophication, defined as 'an increase in the rate of supply of organic matter to an ecosystem' (Nixon 1995), is a major global pollution problem (Howarth et al. 2002). It is often connected with a reduction in the size of the zooplankton community (Uye 1994), and low dissolved oxygen levels (hypoxia) (e.g. Breitburg et al. 2003), which are thought to be more detrimental to fish than to jellyfish (Purcell & Arai 2001).

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Due to these factors, eutrophication could benefit jellyfish over fish at potentially a regional scale (Purcell et al. 2007). Additionally, eutrophication is also associated with increased nutrient concentrations and altered nutrient ratios (from the Redfield ratio; Nixon 1995, Arai 2001). This can result in nutrient imbalances in planktonic food webs, thus causing a mismatch between nutrient supply and demand in higher trophic-level organisms, including jellyfish and fish. But whether this imbalance would promote jellyfish population increases in eutrophic areas remains unclear.

Ecological stoichiometry offers a new perspective in the study of eutrophication (Sterner & Elser 2002). It is generally accepted that the stoichiometry of primary producers is highly affected by nutrient conditions (Rhee 1978, Goldman et al. 1979, Sterner & Elser 2002, Klausmeier et al. 2004b, Hall et al. 2005). For example, the molar N:P ratio of *Scenedesmus* spp. closely tracks the variations in N:P supply ratios (Rhee 1978). Animals usually maintain relative homeostasis (Elser et al. 2000); however, herbivores that encounter low-quality food sources experience difficulty maintaining strict homeostasis when feeding on food limited in P or N because of the costs of homeostasis, and their nutrient contents change accordingly (Elser et al. 2001, Van Nieuwerburgh et al. 2004, Malzahn et al. 2010). Increasing research has focused on the interface between herbivores and carnivores. A number of studies have shown that the effects of food source quality in the primary producer–herbivore interface could be transported up the food chain, and some carnivores also face the problem of nutritional imbalances, leading to a mismatch between supply and demand (e.g. Malzahn et al. 2007, Schoo et al. 2012, 2014, Lesniowski et al. 2015). Nevertheless, this effect on carnivores varies according to species. In lobster larvae (*Homarus gammarus*), even subtle N and P limitations could cause negative effects on their growth conditions (Schoo et al. 2014), whereas the condition of ctenophores *Pleurobrachia pileus* fed P-limited food sources was better than the condition of ctenophores in a nutrient-sufficient treatment, which was not expected (Schoo et al. 2010). With regard to fish larvae, Malzahn et al. (2007) observed that the food quality of primary producers significantly affected the condition of larval Baltic herring *Clupea harengus*, and a P-limited food chain resulted in significantly worse conditions compared with the N-limited and nutrient-sufficient food chain.

Moreover, it is unclear whether nutrient stoichiometry (e.g. C:N:P ratios) or biochemical compounds (e.g. fatty acids [FA] and amino acids) are better correlated with the life-history traits of organisms (e.g. growth and reproduction) and which factor would be a better predictor of food quality (Andersen et al. 2004, Malzahn et al. 2010, Chen & Li 2014). Obviously, C:N:P ratios have been widely measured (Malzahn et al. 2010) and are not the only factor that determines food quality (Reitan et al. 1997). A number of studies have focused on FA (e.g. Müller-Navarra 1995, Malzahn et al. 2007) since they are biologically important as energy reserves, membrane components, and hormones (Kattner et al. 2007). Meanwhile, amino acids also play an important role in the nutritive aspect of an organism. They are crucial building blocks for the synthesis of proteins; thus, there is high demand for amino acids during growth (Conceição et al. 2003). In addition, amino acids are considered to be important sources in the production of energy during the larval stage (Rønnestad et al. 1999), which contributes to the high demand for dietary amino acids. Although amino acid content may be a good predictor of food quality, to our knowledge, few studies have focused on this aspect of carnivore prey quality under nutrient-limited conditions.

To address these gaps, we performed experiments to test the hypotheses that: (1) compared to fish, jellyfish have higher tolerances to the nutrient imbalances that are potentially caused by eutrophication, and (2) under nutrient-limited conditions, amino acid content is an important factor determining food quality.

Aurelia sp. 1 ephyrae and *Paralichthys olivaceus* larvae were selected as the subjects of our study, because *Aurelia* sp. 1 is one of the main blooming jellyfish species in coastal waters, and *P. olivaceus* is an economically important species of fish. In addition, *Aurelia* sp. 1 ephyrae and *P. olivaceus* larvae could be observed during the same period in the same area (e.g. Jiaozhou Bay, China; from April to June) (Wu et al. 1987, Wan & Zhang 2012). Moreover, jellyfish ephyrae derived from strobilating polyps could be considered as a critical life stage (Ishii et al. 2004), just like newly hatched larvae in fish. In this study, the effects of food of varying quality (nutrient-sufficient, P-limited, and N-limited) on the condition of *Aurelia* sp. 1 ephyrae and *P. olivaceus* larvae were investigated. Our main objectives were to (1) estimate the tolerance of *Aurelia* sp. 1 ephyrae and *P. olivaceus* larvae to nutrient limitations based on their growth, survival, and elemental homeostasis, and (2) explore whether FA content or amino acid content was a better predictor of food quality under nutrient-limited conditions.

MATERIALS AND METHODS

Primary producer

Stock cultures of the green algae *Chlorella vulgaris* were cultivated in *C. vulgaris* culture medium (625 µM NH_4NO_3, 28.7 µM $K_2HPO_4^{3-}$, and 2041.3 µM ferric citrate). The seawater used in the experiment was collected from Huiquan Bay in Qingdao, China. Prior to the experiment, the seawater was filtered through a sterile 0.2 µm filter, heated to a boil, cooled, and stored until use. This sterile seawater consisted of 9.39 µM NO_3^-, 0.38 µM PO_4^{3-}, 0.53 µM NO_2^-, and 2.72 µM NH_4^+. All of the algal treatments were maintained at 20 ± 0.5°C, under a 12 h light: 12 h dark cycle and light intensity of 4000 lux.

The nutrient-sufficient treatment ('Full' or '–F') consisted of seawater that was culture-enriched with 740.4 µM NO_3^--N, 661.1 µM NH_4^+-N, and 29.3 µM PO_4^{3-}-P (N:P ratio = 48). The N-limited treatment (or '–N') was obtained by reducing the concentrations of NO_3^--N and NH_4^+-N to 115.4 µM and 36.1 µM (N:P = 5), respectively. In the P-limited treatment (or '–P'), the addition of $K_2HPO_4^{3-}$ was omitted and the concentration of PO_4^{3-}-P was reduced to 0.6 µM (N:P = 2336). This high value of the N:P ratio (2336) was intended to create nutrient gradients in the rotifers in our study similar to those in natural zooplankton populations (Kütter et al. 2014), as the rotifers were able to maintain their elementary homeostasis to some extent. The ionic strength in the nutrient-limited treatments was maintained by the addition of KCl. Therefore, the algae of the 2 limitation treatments could only utilize the natural P or N sources present in the seawater, with the other nutrient added in excess, ensuring that the desired nutrient was limiting (Boersma et al. 2009, Malzahn et al. 2010, Schoo et al. 2014). Preliminary tests showed that nutrient composition (% of dry mass [DM]: %C, %N, and %P) and molar ratios (C:N, C:P, and N:P) of *C. vulgaris* (Table 1) changed significantly (all $p < 0.05$; Table 1) under the experimental conditions after a growth period of 5 d. Algae grown under the N-limited condition showed a lower value of N:P than the Full treatment (11.49 for –N and 27.54 for –F; Table 1), whereas the P-limited algae showed a higher value of N:P (71.58 for –P; Table 1). These conditions allowed *Brachionus plicatilis* to feed on grown algae having different nutritional content. To ensure the quality and constant supply of algae, new cultures of the 3 treatments were inoculated every other day and harvested after 5 d.

Primary consumer

B. plicatilis were hatched from dormant eggs in shallow dishes containing sterile seawater. All of the treatments were maintained at 20 ± 0.5°C under a 12 h light:12 h dark cycle and light intensity of 4000 lux.

Newly born *B. plicatilis* (up to 2 h old) were divided into 9 groups and transferred to 1 l glass beakers at a concentration of 5 individuals ml^{-1}. Three treatments (–F, –N, and –P) × 3 replicates were performed. To prevent food-quantity effects, *B. plicatilis* were fed the same concentration of algae (3 µm C ml^{-1}), and algal food was refreshed every other day. After 6 d of cultivation and 12 h of starvation, rotifers were filtered on GF/F filters (precombusted and acid-washed) and then analyzed for nutrient composition (C, N, and P) and nutritional composition (FA and amino acids).

Because of the low biomass of *B. plicatilis*, large numbers were needed to feed the secondary consumers. However, the dormant eggs of the rotifers could not meet the consumers' needs. To ensure a sufficient food supply, dormant eggs were hatched and cultivated in a 15 l container under the –F condition, and approximately 75 000 rotifers were filtered out every other day. After the rotifers were starved for 24 h, they were transferred to 3 glass beakers (5 l size) and cultivated under the 3 nutrient conditions. After a period of approximately 8 d, rotifers were harvested as food for the secondary consumers.

Table 1. Nutrient composition (% of dry mass: %C, %N, and %P) and molar ratios (C:N, C:P, and N:P) of *Chlorella vulgaris* grown under nutrient-sufficient ('Full'), N-limited, and P-limited conditions after 5 d. Values represent the means of 3 replicate samples and are listed as mean ± SD. Significant differences (Tukey's HSD test, $p < 0.05$) among the 3 treatments are indicated by different superscripted lowercase letters

	%C	%N	%P	C:N	C:P	N:P
Full	13.12 ± 1.51^{a}	2.36 ± 0.36^{a}	0.19 ± 0.03^{a}	6.50 ± 0.22^{a}	178.05 ± 40.40^{a}	27.54 ± 6.95^{a}
N-limited	18.04 ± 2.01^{b}	1.57 ± 0.19^{b}	0.30 ± 0.05^{b}	13.64 ± 2.98^{b}	157.33 ± 38.74^{a}	11.49 ± 0.81^{b}
P-limited	20.81 ± 1.51^{b}	1.83 ± 0.04^{ab}	0.06 ± 0.01^{c}	13.25 ± 1.04^{b}	946.90 ± 238.32^{b}	71.58 ± 17.67^{c}

Secondary consumers

For fish larvae, *Paralichthys olivaceus* eggs from the same parents were obtained from a commercial hatchery. Eggs were incubated at 15°C. During the 2 d incubation, water temperature was raised by 1.5°C d^{-1} to the experimental temperature of 18°C. All of the treatments were subjected to a 12 h light: 12 h dark cycle and light intensity of 4000 lux. Terramycin (3 ppm the first time, thereafter 5 ppm) was added to all of the treatments every other day to control epizootics (Fukuda et al. 1996). Swimming larvae without yolk sacs were divided into 9 groups (3 treatments × 3 replications) and transferred to containers filled with 4 l filtered seawater at a concentration of 5 fish l^{-1}. The larvae of the 3 treatments were fed the same concentration of rotifers (10 individuals ml^{-1} except for 7 individuals ml^{-1} on the first day) grown under 3 nutritional conditions every other day. Before feeding, any remaining rotifers were removed and the water was refreshed.

For jellyfish ephyrae, *Aurelia* sp. 1 ephyrae were released from stock-cultured sessile polyps in the laboratory at the Institute of Oceanology, Chinese Academy of Sciences in Qingdao, China. Polyps were fed with newly hatched *Artemia* nauplii but were not fed for 24 h before the collection of ephyrae. Every 50 newly released ephyrae (<24 h, bell diameter was approximately 3 mm) were placed in a container with 5 l filtered seawater. Nine containers were assigned to 3 treatments (–F, –N, and –P) and replicated 3 times. The experiments were conducted at 18°C. Ephyrae in the 3 treatments were fed rotifers (10 individuals ml^{-1}) grown under the 3 nutritional conditions. Every other day, any remaining rotifers were removed and the water was refreshed. Then newly cultured rotifers under 3 nutritional conditions were added to the containers.

Length and bell diameter were used as measurements of body size. For *P. olivaceus* larvae, length was measured from the tip of the snout to the tip of the longer lobe of the caudal fin (total length), using a calibrated Vernier caliper every other day. For *Aurelia* sp. 1 ephyrae, bell diameter was the distance between the edges of the 2 opposite lappets, and was measured using the ocular micrometer of a dissecting microscope (ZEISS Stemi SV 11) every 3 d. The 10th percentile mortality time (MT10) was defined as the time span at which 10% of the larvae died or the ephyrae deformed under 3 nutritional conditions. Larvae that showed no movement after several stirrings of seawater using a pipette were judged to be dead. Ephyrae that were reduced to a rod-like oral core were judged to be deformed. Meanwhile, the morphological changes of the secondary consumers and swim speed of the *P. olivaceus* larvae were also observed during the experiment.

Once the secondary consumers in the 2 limitation treatments reached MT10, the organisms in all 3 treatments were harvested. Ten to 15 individuals of *Aurelia* sp. 1 ephyrae and 4 to 5 individuals of *P. olivaceus* larvae were collected from each container to analyze C and N content, and P content, respectively. Additionally, the newly released *P. olivaceus* larvae (without yolk sacs) and the *Aurelia* sp. 1 ephyrae at t_0 (start of the experiment) in each container were collected to determine C and N content and P content, respectively. All organisms were collected by filtration through Whatman GF/F filters (pre-combusted, washed, and dried), stored at –80°C, freeze-dried, weighed, and kept in a desiccator until further analysis.

Analytical procedures

For the analysis of C and N contents, samples were coated by tin boats and measured using an elemental analyzer (Elementar Company). To analyze P content, samples were weighed and digested with 5 ml nitric acid and 1 ml perchloric acid. The temperature of heating plate was increased from 175°C to 220°C to dry out the acid solution. Nitric acid (1:1) was added to the sample after the solution was cooled to room temperature. The samples were then rinsed with Milli-Q deionized water and then transferred to 25 ml specimen tubes. The blank sample was made in the same way so that the P content of Whatman GF/F filters we used (pre-combusted and washed) could be measured. Then all samples with a series of standard solution (0, 1, 5, 10, 20, 50 µg l^{-1}) and 20 µg l^{-1} Rh internal standard solution were analyzed using an ICP plasma emission spectrometer (Thermo Fisher Scientific, iCAP-Qc).

To analyze amino acid content, approximately 20 mg of the samples was placed in an ampoule, 10 ml 6 mol l^{-1} HCl and N_2 were added, and then the ampoule was sealed and placed in a 110°C drying oven for 24 h of hydrolyzation. Subsequently, 0.02 mol l^{-1} of HCl was added to a volume of 10 ml. The amino acid content was detected by HPLC using phenylisothiocyanate as the reagent. An Agilent 1100 liquid chromatograph with a Venusil-AA (4.6 × 250 mm, 5 µm) column at a temperature of 40°C, detection wavelength of 254 nm, mobile phase A of 0.1 M sodium acetate (7% acetonitrile), mobile phase B of 80% acetonitrile, and gradient elution of 1 ml

min^{-1} was used. FA were measured as fatty acid methyl esters (FAMEs) after the method described by Liu et al. (2011).

Statistical analysis

To estimate the ability of the secondary consumers to maintain homeostasis, the homeostatic regulation coefficient (H) was calculated according to the following equation (Sterner & Elser 2002):

$$y = cx^{\frac{1}{H}} \quad (1)$$

H was calculated by linearizing the equation above using logarithms (Sterner & Elser 2002):

$$\log(y) = \log(c) + \frac{\log(x)}{H} \quad (2)$$

where y is the stoichiometry of *Aurelia* sp. 1 ephyrae or *P. olivaceus* larvae, x is the stoichiometry of their food source (rotifers), and c is a constant. Stoichiometry here means the ratio of the elements or element content (% of DM) (e.g. N:P, %P, etc.). Linear regressions were fitted, and the parameters of these regressions were used to assess the homeostatic responses of the secondary consumers.

The nutrient composition (% of DM: %C, %N, and %P) and molar ratios (C:N, C:P, and N:P) of *C. vulgaris*, *B. plicatilis*, *P. olivaceus*, and *Aurelia* sp. 1 as well as the nutritional composition (FA, amino acids) of *B. plicatilis* were statistically analyzed using a 1-way ANOVA, with treatment used as the independent variable. Tukey's HSD was used as the post hoc test. Kruskal-Wallis H test was used if the data after being $\ln(x + 1)$-transformed did not approach normality (Kolmogorov-Smirnov test) or homoscedasticity of variance (Levene's test). A repeated-measures ANOVA was used to test the effects of nutrient conditions on the changes in length of *P. olivaceus* and bell diameter of *Aurelia* sp. 1, with treatment and time as the variables. Fisher's HSD was performed as the post hoc test. All of the statistical tests were conducted using the software SPSS 19.0 and Origin 9.0.

RESULTS

Primary consumer

Rotifers (Fig. 1) reared on P-limited algae showed the lowest content of P (0.51%; Fig. 1c), and the lowest contents of C and N (30.50% C and 3.86% N; Fig. 1a,b) relative to the other treatments. Contrary to our expectations, the N content of consumers in the −N treatment was not the lowest, and it was greater than that of the −P treatment (6.74% N; Fig. 1b). The same pattern was found for the C and P contents (36.69% C and 0.59% P; Fig. 1a,c). The −F treatment provided the highest contents of C, N, and P (43.91% C, 8.45% N, and 0.73% P; Fig. 1a–c).

The same pattern was observed for total amino acid content. The −P treatment showed the significantly lowest value (1-way ANOVA, $F_{2,6} = 46.76$, $p < 0.01$; Table 2, Fig. 2). However, the FA content exhibited opposite results. The −P treatment showed the highest concentration of total FA (μg mg DM^{-1}), highest concentration and proportion (% total FA content) of total unsaturated FA, lowest proportion of total saturated FA, highest proportion of ω3FA, and the highest value of the ω3FA:ω6FA ratio (all $p < 0.05$; Table 3, Fig. 2).

In summary, the −P treatment provided the highest content in terms of FA content, although these results were the opposite with regard to the nutrient composition and total amino acids. A comparison of the different effects according to the physiological indicators of secondary consumers (growth, MT10, and elemental homeostasis) was useful for determining the factors that have more important effects on food quality.

Secondary consumers

Growth

Significant differences were observed in the length of the *Paralichthys olivaceus* larvae among the 3 treatments during the experimental period (2-way repeated-measures ANOVA, $F_{3,40} = 526.43$, $p < 0.01$; Fig. 3a). The −F treatment presented the longest larvae, followed by the −N treatment and the −P treatment, which had much smaller values. The morphological status on the last day of the experiment showed obvious differences among the 3 treatments (Fig. 4). The larvae in the −F treatment qualitatively swam faster and had more developed digestive systems (Fig. 4) than those in the other treatments, whereas the −P treatment produced the thinnest and weakest larvae (Fig. 4), which qualitatively swam the slowest.

The *Aurelia* sp. 1 ephyrae bell diameter presented a different pattern from that of the larval length

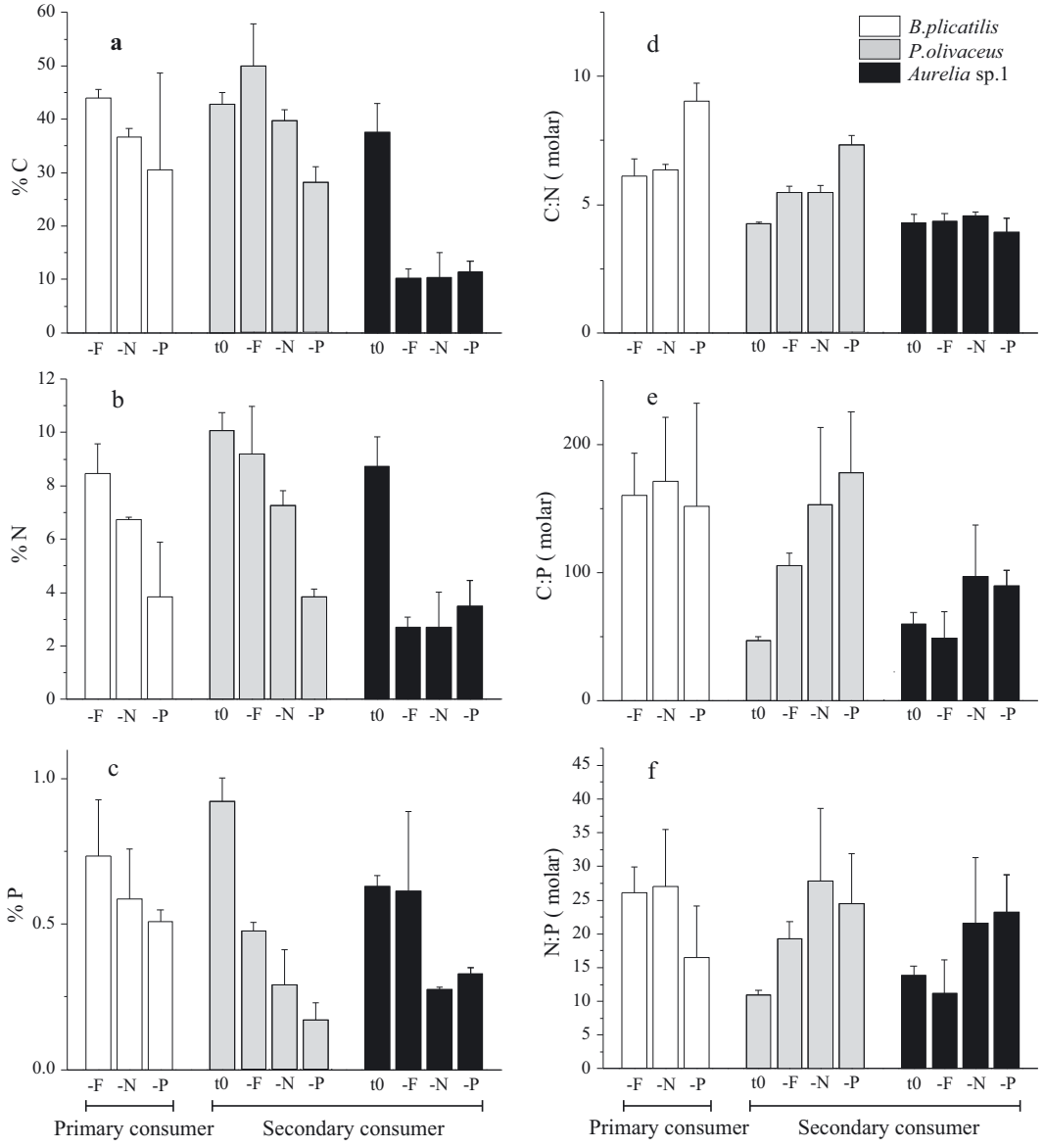

Fig. 1. (a–c) Nutrient composition (% of dry mass: %C, %N, and %P) and (d–f) molar ratios (C:N, C:P, and N:P) of primary (*Brachionus plicatilis*) and secondary consumers (*Paralichthys olivaceus* larvae and *Aurelia* sp. 1 ephyrae) at t_0 (start of the experiment) and when fed food of different nutrient conditions (Full or '–F', N-limited or '–N', P-limited or '–P'). Error bars: SD. Note different *y*-axis scales

(Fig. 3b). Significant differences were not detected between the –F treatment and the –P treatment (HSD, $p > 0.05$), and the –N treatment always produced a greater bell diameter than the other treatments (HSD, $p < 0.01$; Fig. 3b). In terms of the morphological status, obvious differences were not observed among the treatments during the experimental period, although the ephyrae under the –N condition showed more rapid morphological changes, with flat circular bells and 4 conspicuous central gastric pouches observed on the 17th day (Fig. 4).

MT10

The death rate of *P. olivaceus* larvae and the deformity rate of *Aurelia* sp. 1 ephyrae both showed the highest values in the –P treatment and the lowest values under the –F condition (Fig. 5a,b). In the –P treatment, MT10 was estimated at 5.6 d for larvae and 12 d for ephyrae. Under the –N condition, the MT10 estimates were 7 d for larvae and 16.1 d for ephyrae. These results implied that the MT10 of *Aurelia* sp. 1 ephyrae was much longer than that of *P. olivaceus* larvae in both limitation treatments (Fig. 5a,b).

Table 2. Amino acid composition of *Brachionus plicatilis* reared on phytoplankton *Chlorella vulgaris* grown under 3 different nutrient conditions. Values represent the means of 3 replicate samples and are listed in mg per g dry mass (DM) (mean ± SD) and % of total amino acid content (mean ± SD). Significant differences (Tukey's HSD test or Kruskal-Wallis *H* test, $p < 0.05$) among the 3 treatments indicated by different superscripted lowercase (for DM) or uppercase letters (for % of total amino acid content)

Amino acid	Full		N-limited		P-limited	
	mg g DM^{-1}	% of total	mg g DM^{-1}	% of total	mg g DM^{-1}	% of total
Essential						
Threonine	2.13 ± 0.12^{a}	4.30 ± 0.15^{A}	1.77 ± 0.12^{b}	4.09 ± 0.17^{AB}	0.95 ± 0.07^{c}	3.86 ± 0.05^{B}
Valine	2.87 ± 0.15^{a}	5.78 ± 0.29^{A}	2.70 ± 0.10^{a}	6.25 ± 0.03^{AB}	1.70 ± 0.28^{b}	6.89 ± 0.56^{B}
Methionine	0.53 ± 0.32	1.05 ± 0.57	0.47 ± 0.15	1.09 ± 0.37	0.30 ± 0.00	1.22 ± 0.11
Isoleucine	2.40 ± 0.26^{a}	4.82 ± 0.17	2.10 ± 0.10^{a}	4.86 ± 0.05	1.10 ± 0.14^{b}	4.46 ± 0.19
Leucine	3.67 ± 0.32^{a}	7.37 ± 0.14^{A}	3.23 ± 0.15^{a}	7.48 ± 0.12^{A}	1.70 ± 0.14^{b}	6.91 ± 0.02^{B}
Phenylalanine	2.20 ± 0.17^{a}	4.42 ± 0.12	2.03 ± 0.12^{a}	4.71 ± 0.13	1.15 ± 0.07^{b}	4.68 ± 0.12
Histidine	2.70 ± 0.20^{a}	5.43 ± 0.08^{A}	2.43 ± 0.06^{a}	5.64 ± 0.13^{A}	1.90 ± 0.14^{b}	7.73 ± 0.09^{B}
Lysine	4.13 ± 0.38^{a}	8.31 ± 0.19^{A}	3.47 ± 0.21^{a}	8.02 ± 0.23^{A}	1.80 ± 0.14^{b}	7.32 ± 0.06^{B}
Arginine	2.73 ± 0.21^{a}	5.50 ± 0.05^{A}	2.30 ± 0.17^{a}	5.32 ± 0.26^{A}	1.10 ± 0.14^{b}	4.46 ± 0.19^{B}
Total essential	23.37 ± 1.96^{a}	46.78 ± 0.32	20.50 ± 0.85^{a}	47.45 ± 0.44	11.70 ± 1.13^{b}	47.54 ± 0.50
Non-essential						
Aspartic acid	5.10 ± 0.46^{a}	10.25 ± 0.10	4.20 ± 0.20^{b}	9.72 ± 0.11	2.25 ± 0.21^{c}	9.14 ± 0.07
Serine	2.60 ± 0.17^{a}	5.23 ± 0.15	2.37 ± 0.12^{a}	5.48 ± 0.16	1.30 ± 0.14^{b}	5.28 ± 0.12
Glutamic acid	7.50 ± 0.62^{a}	15.08 ± 0.15^{A}	6.17 ± 0.21^{b}	14.28 ± 0.20^{B}	3.50 ± 0.42^{c}	14.21 ± 0.5^{B}
Proline	2.33 ± 0.23^{a}	4.69 ± 0.29	1.83 ± 0.06^{b}	4.25 ± 0.10	1.00 ± 0.14^{c}	4.06 ± 0.23
Glycine	2.20 ± 0.20^{a}	4.42 ± 0.07^{A}	1.87 ± 0.12^{a}	4.32 ± 0.16^{A}	0.95 ± 0.07^{b}	3.86 ± 0.05^{B}
Alanine	2.43 ± 0.21^{a}	4.89 ± 0.05^{A}	2.00 ± 0.10^{b}	4.63 ± 0.06^{B}	1.05 ± 0.07^{c}	4.27 ± 0.08^{C}
Cysteine	2.40 ± 0.10	4.84 ± 0.39^{A}	2.70 ± 0.44	6.26 ± 1.02^{AB}	2.05 ± 0.21	8.40 ± 1.59^{B}
Tyrosine	1.80 ± 0.20^{a}	3.61 ± 0.12	1.57 ± 0.06^{a}	3.63 ± 0.05	0.80 ± 0.14^{b}	3.24 ± 0.30
Total non-essential	26.37 ± 2.06^{a}	53.02 ± 0.32	22.70 ± 0.79^{a}	52.55 ± 0.44	12.90 ± 0.99^{b}	52.46 ± 0.50
Total	49.73 ± 4.33^{a}		43.20 ± 2.47^{a}		24.60 ± 2.55^{b}	

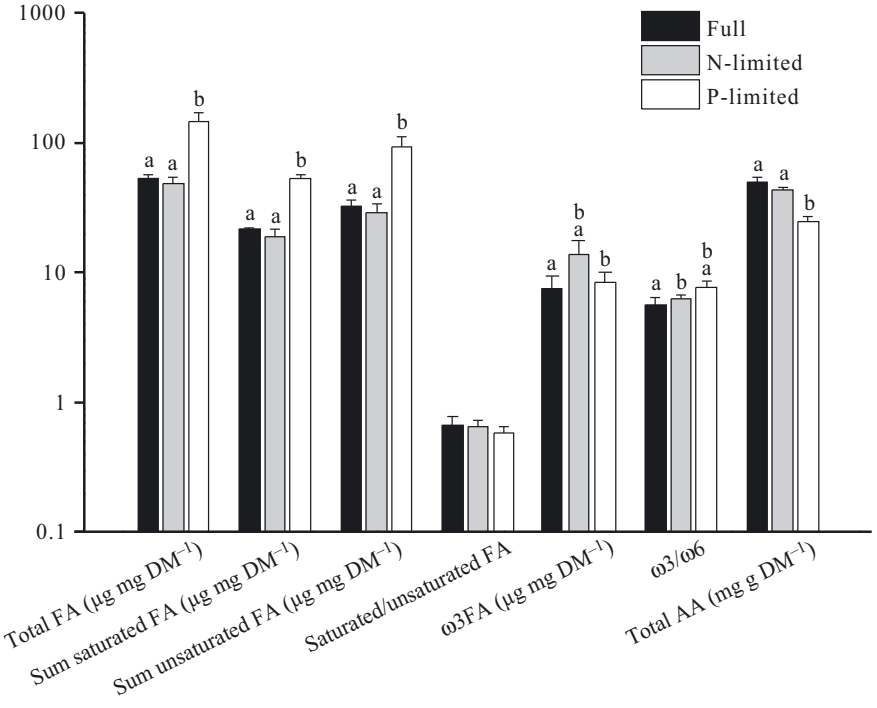

Fig. 2. Summary of several aggregated indicators related to fatty acid (FA) and amino acid (AA) content of *Brachionus plicatilis* reared on phytoplankton *Chlorella vulgaris* grown under 3 different nutrient conditions. Error bars: SD. Different letters above columns indicate significant difference among the 3 treatments (Tukey's HSD test, $p < 0.05$). DM: dry mass. Note the *y*-axis log scale

Table 3. Fatty acid (FA) composition (mean ± SD) of *Brachionus plicatilis* reared on phytoplankton *Chlorella vulgaris* grown under 3 different nutrient conditions. Values represent the means of 3 replicate samples and are listed in µg per mg dry mass (DM) and % of total FA content. Significant differences (Tukey's HSD test or Kruskal-Wallis *H* test, $p < 0.05$) among the 3 treatments indicated by different superscripted lowercase (for DM) or uppercase letters (for % of total FA content)

	Full		N-limited		P-limited	
	µg mg DM^{-1}	% of total	µg mg DM^{-1}	% of total	µg mg DM^{-1}	% of total
C12:0	0.23 ± 0.08^{ab}	0.42 ± 0.16	0.12 ± 0.03^{a}	0.25 ± 0.04	0.33 ± 0.04^{b}	0.22 ± 0.01
C14:0	3.14 ± 0.18^{a}	5.86 ± 0.48^{A}	2.03 ± 0.32^{a}	4.21 ± 0.23^{B}	6.97 ± 0.86^{b}	4.78 ± 0.21^{B}
C15:0	0.29 ± 0.02^{a}	0.55 ± 0.05^{A}	0.11 ± 0.05^{b}	0.22 ± 0.09^{B}	0.27 ± 0.04^{a}	0.18 ± 0.00^{B}
C16:0	14.19 ± 0.7^{a}	26.53 ± 2.62	12.72 ± 1.94^{a}	26.41 ± 2.3	34.55 ± 2.57^{b}	23.79 ± 2.17
C16:1	19.39 ± 1.35^{a}	36.11 ± 0.53^{A}	15.68 ± 2.55^{a}	32.46 ± 0.78^{B}	58.08 ± 10.81^{b}	39.62 ± 0.84^{C}
C18:0	2.42 ± 0.28^{a}	4.54 ± 0.76	2.34 ± 0.44^{a}	4.88 ± 0.93	6.76 ± 0.14^{b}	4.69 ± 0.87
C18:1	3.76 ± 0.28^{a}	7.00 ± 0.30^{A}	3.72 ± 0.61^{a}	7.69 ± 0.19^{B}	17.37 ± 2.92^{b}	11.87 ± 0.04^{C}
C18:2ω6	1.13 ± 0.14^{a}	2.10 ± 0.13^{A}	0.73 ± 0.11^{a}	1.51 ± 0.04^{B}	1.80 ± 0.29^{b}	1.23 ± 0.00^{C}
C20:0	0.18 ± 0.12^{a}	0.35 ± 0.23	0.29 ± 0.34^{a}	0.66 ± 0.84	2.30 ± 0.31^{b}	1.57 ± 0.05
C20:3ω6	0.18 ± 0.08	0.33 ± 0.13^{A}	0.36 ± 0.06	0.74 ± 0.05^{B}	0.40 ± 0.20	0.27 ± 0.09^{A}
C20:3ω3	1.61 ± 0.43	2.97 ± 0.65^{AB}	1.99 ± 0.39	4.12 ± 0.46^{A}	2.46 ± 0.63	1.67 ± 0.16^{B}
C20:5ω3	5.90 ± 1.61	10.90 ± 2.40	6.45 ± 1.33	13.38 ± 1.83	11.46 ± 3.31	7.75 ± 0.98
C22:1ω9	0.28 ± 0.20	0.52 ± 0.38	0.21 ± 0.05	0.43 ± 0.06	1.53 ± 1.59	0.97 ± 0.93
C24:0	0.99 ± 0.31	1.82 ± 0.48^{A}	1.47 ± 0.31	3.05 ± 0.44^{B}	2.08 ± 0.75	1.40 ± 0.28^{A}
Total FA	53.70 ± 3.45^{a}		48.20 ± 6.71^{a}		146.36 ± 24.17^{b}	
Sum saturated	21.45 ± 1.00^{a}	40.07 ± 3.69	19.07 ± 2.39^{a}	39.67 ± 2.78	53.25 ± 4.42^{b}	36.63 ± 3.03
Sum unsaturated	32.25 ± 3.86^{a}	59.93 ± 3.69	29.13 ± 4.85^{a}	60.33 ± 2.78	93.11 ± 19.75^{b}	63.37 ± 3.03
Saturated: unsaturated ratio	0.67 ± 0.11	0.67 ± 0.11	0.66 ± 0.08	0.66 ± 0.08	0.58 ± 0.08	0.58 ± 0.08
ω3	7.51 ± 2.04	13.87 ± 3.04^{AB}	8.44 ± 1.72	17.50 ± 2.29^{A}	13.92 ± 3.94	9.42 ± 1.14^{B}
ω6	1.31 ± 0.21^{a}	2.43 ± 0.24^{A}	1.08 ± 0.17^{a}	2.25 ± 0.08^{A}	2.20 ± 0.49^{b}	1.50 ± 0.09^{B}
ω3:ω6 ratio	5.66 ± 0.85^{a}	5.66 ± 0.85^{A}	7.77 ± 0.76^{b}	7. 77 ± 0.76^{B}	6.27 ± 0.40^{ab}	6.27 ± 0.40^{AB}

Elemental homeostasis

Among the 3 treatments, the nutrient composition and molar ratios of the fish larvae showed similar patterns to that of the rotifers (Fig. 1). For example, larvae in the –F treatment showed the highest value of %C, followed by the –N treatment, and the –P treatment, which had the lowest value (50.02 for –F, 39.66 for –N, and 28.20 for –P; Fig. 1a). For the rotifers, the %C showed the same pattern as that of the larvae (43.91 for –F, 36.69 for –N, and 30.50 for –P; Fig. 1a). However, compared with fish larvae, the stoichiometry of the jellyfish ephyrae showed patterns that were inconsistent with that of the rotifers.

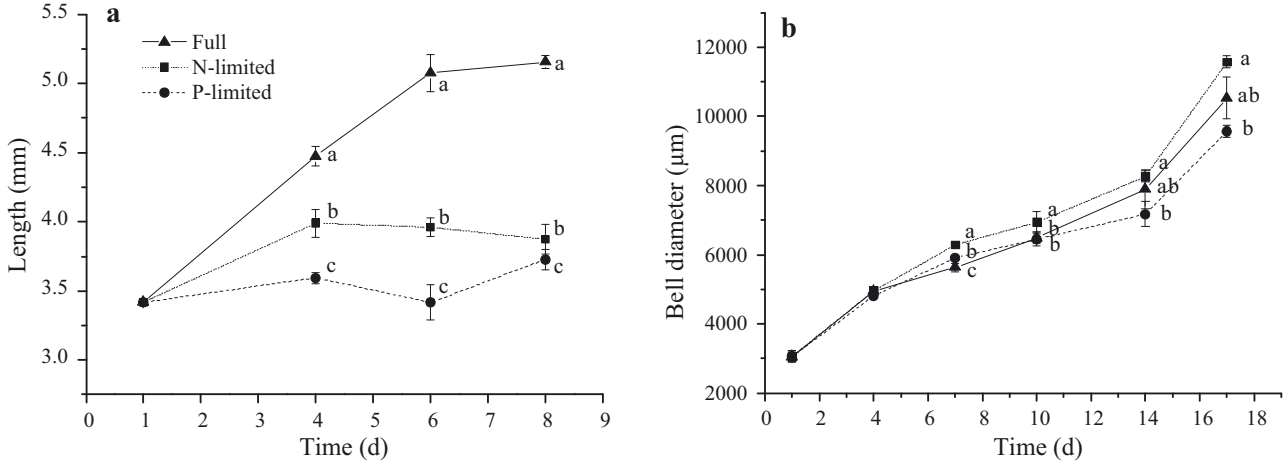

Fig. 3. Changes in (a) length of *Paralichthys olivaceus* larvae and (b) bell diameter of *Aurelia* sp. 1 ephyrae fed *Brachionus plicatilis* reared under nutrient-sufficient (Full), N-limited, and P-limited conditions. Error bars: SD. Significant differences (Fisher's HSD test, $p < 0.05$) among the 3 treatments indicated by different lowercase letters

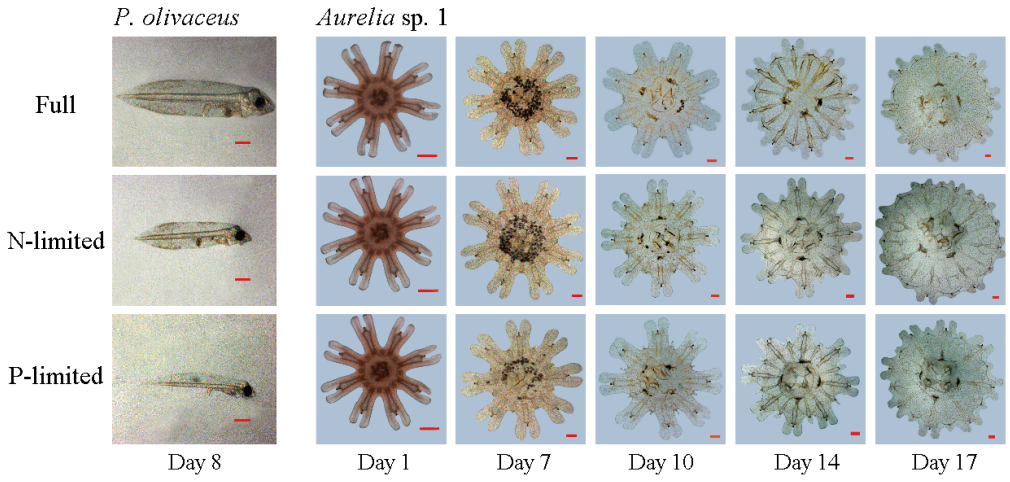

Fig. 4. Morphological status of *Paralichthys olivaceus* larvae on the last day and of *Aurelia* sp. 1 ephyrae during the experimental period when fed food with different nutrient conditions. All scale bars = 500 µm

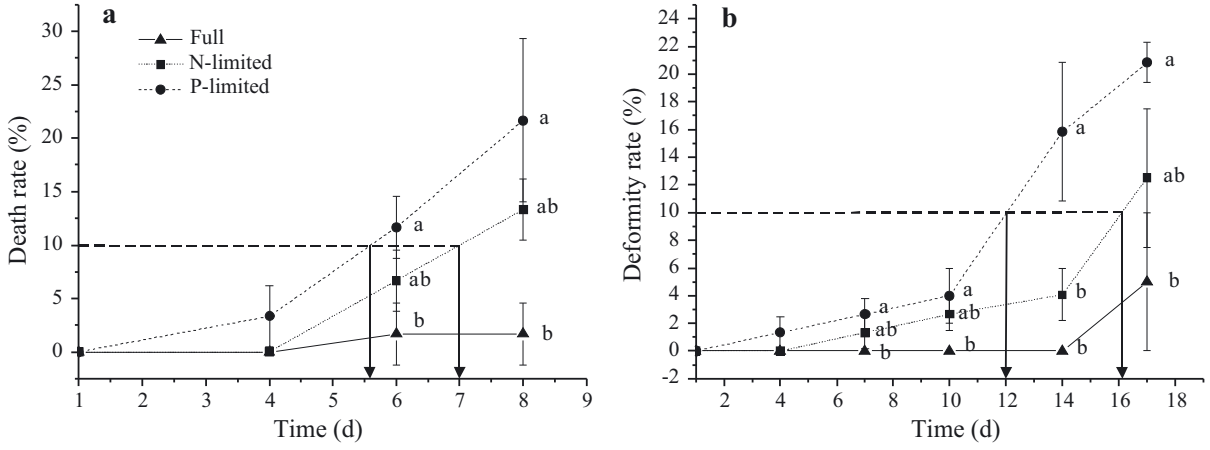

Fig. 5. (a) Death rate of *Paralichthys olivaceus* larvae and (b) deformity rate of *Aurelia* sp. 1 ephyrae when fed food of different nutrient conditions. Error bars: SD. Significant differences (Fisher's HSD test, $p < 0.05$) among the 3 treatments indicated by different lowercase letters. The 10th percentile mortality time (MT10, arrows) is the time span at which 10% (dashed line) of the larvae died or ephyrae deformed

Table 4. Homeostatic regulation coefficient (H) (see Eqs. 1 & 2) and parameters calculated from linear regression for each stoichiometric composition (e.g. %P, N:P, etc.) of secondary consumers (*Paralichthys olivaceus* larvae and *Aurelia* sp. 1 ephyrae). Results with $R^2 < 0$ were excluded

	Stoichiometry	Slope ($1/H$)	log(c)	R^2	H
P. olivaceus	%C	1.226	−0.319	0.999	0.846
	%P	2.945	0.100	0.975	0.340
	%N	1.001	0.030	0.999	0.999
	C:N	0.763	0.135	0.981	1.311
	C:P	−3.174	9.017	0.101	−0.315
Aurelia sp. 1	%C	−0.27	1.44	0.803	−3.76
	%P	−1.30	−0.88	0.114	−0.77
	%N	−0.27	0.68	0.758	−3.70
	C:N	−0.35	0.93	0.171	−2.86
	N:P	−0.87	2.40	0.079	−1.14

With regards to the homeostatic regulation coefficient H, for each stoichiometric component (e.g. N:P, %P, etc.), the absolute value of the *Aurelia* sp. 1 ephyrae H was higher than that of the *P. olivaceus* larvae, and the $1/H$ (slope) of the *Aurelia* sp. 1 ephyrae was much closer to zero than that of the *P. olivaceus* larvae (Table 4). For example, the $1/H_{\%C}$ value of the fish larvae differed from 0 by 1.23, whereas that of the *Aurelia* sp. 1 ephyrae differed by only 0.27. The *P. olivaceus* larvae $1/H_{C:N}$ value was 0.76, and the absolute $1/H_{C:N}$ value of the *Aurelia* sp. 1 ephyrae was only 0.35 (Table 4).

DISCUSSION

Different responses of fish larvae and jellyfish ephyrae

In our study, nutrient limitations, especially P limitation, showed strong negative effects on the growth conditions of *Paralichthys olivaceus* larvae (Figs. 1, 4 & 5). These observations could be attributed to the characteristics of fish larvae, which have a high demand for nutrients and low tolerance to nutrient limitations compared to jellyfish. The P content of the fish body (P of DM) increased sharply during the larval and early juvenile stages, which was mostly because of bone formation (Pilati & Vanni 2007). In addition to the ossification of bones, the growth of muscle and development of fin rays in the larval stage also promoted an increase in P contents (Malzahn et al. 2007). This ontogenetic increase in P in fish larvae resulted in a high demand for nutrients, thus leading to the low tolerance to nutrient limitations. Therefore, under a diet with lower P content (0.51% in the –P treatment), the growth conditions of *P. olivaceus* larvae were poor, whereas the growth conditions were improved under a diet with a higher P content (0.73% in the Full treatment) (Figs. 1 & 3–5). This finding is consistent with that of Vielma et al. (2002), who demonstrated that when the dietary P content increased from 0.50% to 0.75%, the specific growth rate (% d^{-1}) of European whitefish fingerlings (*Coregonus lavaretus* L.) rapidly increased.

However, nutrient limitations had little negative effect on the *Aurelia* sp. 1 ephyrae, since no significant differences were detected between the Full treatment and the P-limited treatment, and the N-limited treatment always produced a greater bell diameter than the other 2 treatments (Fig. 3b). Schoo et al. (2010) also found that P-limited copepods represented a food source of higher quality for ctenophores *Pleurobrachia pileus*, and Malzahn et al. (2010) demonstrated that nutrient limitations did not affect the growth conditions of limnomedusae *Gonionemus vertens*. These studies and our results imply that jellyfish may not be negatively affected by nutrient limitations as compared to *P. olivaceus* larvae.

Limited studies are available on the effect of nutrient limitations on marine jellyfish (e.g. Malzahn et al. 2010, Schoo et al. 2010, Lesniowski et al. 2015), and thus, we can only speculate on the physiological processes underlying the above results. Ontogenetic changes in the elemental composition of *Aurelia* sp. 1 and *P. olivaceus* implied that they had different nutrient demands. From our results, the N content of newly released *Aurelia* sp. 1 ephyrae (fed with newly hatched *Artemia* nauplii) was 8.72% DM and the C content was 37.46% DM (Fig. 1b). Meanwhile, Ikeda (2014) reported much lower values for *Aurelia aurita* (the same genus as *Aurelia* sp. 1) medusae with an N content of approximately 1.30% DM and a C content of approximately 4.30% DM, indicating that the C and N content decreased considerably with ontogenesis. However, Pilati & Vanni (2007) found that the body P content in fish (both *Dorosoma cepedianum* and *Danio rerio*) increased considerably during the life cycle. These differences in how stoichiometry changes with ontogeny imply that there might be a lower nutrient demand in *Aurelia* sp. 1 than in fish. This could be attributed to the jellyfish body plan. The jellyfish body primarily contains mesoglea, an extracellular matrix consisting of water, salts, and collagen fibers (Verde & McCloskey 1998), which decreases the concentration of N and P. In addition to predation, jellyfish could acquire nutrients through the uptake of dissolved sources (Pitt et al. 2009) which are abundant in coastal regions. Although it could account for only <10% of the metabolic requirements of non-zooxanthellate jellyfish (Ferguson 1982), the uptake of limiting elements from dissolved sources could provide an advantage to alleviate the nutrient imbalance of the food. In general, the C:nutrient values of the prey under the condition of altered nutrient ratios were higher than those under the Redfield ratio, implying an excess of C in the prey (Sterner & Elser 2002). For jellyfish medusae, the excess C could be excreted as mucus and dissolved organic matter (Condon et al. 2011, Pitt et al. 2013), and this strategy might mitigate the nutrient imbalance of the food resources as well. As limited studies are available and the above statements are mostly speculations, more research is needed to explore the physiological processes underlying the high tolerance of *Aurelia* sp. 1 to nutrient limitation. Meanwhile, research on coastal and open-ocean species is also needed to determine whether the high tolerance of *Aurelia* sp. 1 is consistent with the gelatinous body plan.

In terms of the homeostasis of the organisms, our results showed that for each stoichiometric composition (e.g. N:P, %P, etc.), the $1/H$ (slope) of the *Aurelia* sp. 1 ephyrae was much closer to zero than that of the *P. olivaceus* larvae (Fig. 1, Table 4). Because a slope ($1/H$) of zero represents homeostasis (Sterner & Elser 2002), the above results imply that *Aurelia* sp. 1 ephyrae had a greater ability to maintain stable chemical compositions within their bodies compared with the *P. olivaceus* larvae. Thus, compared with *P. olivaceus* larvae, the chemical compositions of the *Aurelia* sp. 1 ephyrae were less likely to be affected

by nutrient limitations. Because elemental homeostasis plays an important role for jellyfish and weaker homeostasis would have negative consequences (e.g. lower growth rate) (Sterner & Elser 2002), *Aurelia* sp. 1 ephyrae could be more competitive than *P. olivaceus* larvae in terms of homeostasis.

Biochemical compositions and food quality

Nutrient limitation is known to be reflected by alterations of the biochemical composition (e.g. FA content) (Sterner & Elser 2002). In our experiments, the rotifers in the P-limited treatment produced significantly higher FA contents than those in the other treatments, and similar phenomena have been reported in other organisms under nutrient-limited conditions, exhibiting higher FA content (Boersma 2000, Klausmeier et al. 2004a, Malzahn et al. 2007, Boersma et al. 2009). Siron et al. (1989) stated that the enhanced FA contents exhibited in P-limited treatments could be attributed to C storage in response to an imbalanced nutrient supply (suboptimal growth condition). Because the FA saturation threshold may be low for most zooplankton species (Becker & Boersma 2005), the growth of *P. olivaceus* larvae may not be negatively affected by the lowest FA contents among the 3 treatments or the highest FA content of the P-limited treatment. As eicosapentaenoic acid (EPA) (C20:5ω3) and ω3FA are beneficial to growth, reproduction, and other factors (Harrison 1990, Lee et al. 2000), they have typically been used to assess food quality. However, in our experiments, the Full treatment yielded the best growth condition with the highest growth rate and the highest MT10 value (Figs. 3–5) but the lowest EPA content (C20: 5ω3) and ω3FA, and the lowest ω3:ω6 ratio (Fig. 2, Table 3). Therefore, FA contents may not provide a reasonable explanation for the lower food quality of organisms raised under the P-limited treatment.

With regard to amino acids, our results indicated that rotifers in the P-limited treatment provided the lowest content (Table 3, Fig. 2). This result had the same pattern as the growth conditions of *P. olivaceus* larvae among the 3 treatments (lowest growth rate and MT10 value in the P-limited treatment; Figs. 3–5). These results suggest that under nutrient-limited conditions, the growth conditions of *P. olivaceus* larvae could be tightly coupled to the amino acid contents of rotifers (*P. olivaceus* larvae food).

Many studies have examined the importance of amino acid contents to the growth of fish larvae. A reduction in amino acid contents affects fish larvae, especially at the start of exogenous feeding (Rønnestad et al. 1999, Park et al. 2000, Wright & Fyhn 2001, Alam et al. 2002, Kim et al. 2003, Aragão et al. 2004). In the early stages, the larval digestive system is not fully developed, and complex proteins are not easily digested (Rønnestad et al. 1999, Aragão et al. 2004, Naz & Türkmen 2009). Because the free amino acid (FAA) content could be absorbed by fish larvae rapidly and efficiently (Alam et al. 2001, Applebaum & Rønnestad 2004, Ohkubo et al. 2008), the FAAs were essential for fish larvae. In addition, the FAA content provided important building blocks for protein synthesis (Rønnestad et al. 2003), and higher contents of amino acids in the food could increase the amount of trypsin secretion, thus contributing to protein digestion (Cahu & Infante 1995, Jobgen et al. 2006, Kim et al. 2007). In addition, amino acids were identified as one of the major energy sources for first-feeding larvae (Wright & Fyhn 2001, Rønnestad et al. 2003, Brown et al. 2005). In addition to FAA, the soluble proteins, which constituted approximately 50% of the total proteins (Srivastava et al. 2006), were expected to be more available for uptake by pinocytosis and digestion by intestinal proteases relative to the total proteins (Carvalho et al. 2004). Once the soluble proteins were digested, their amino acids could be readily absorbed by fish larvae. These physiological processes resulted in the constant consumption and high demand of amino acids from the food. In our experiment, rotifers are the food source of *P. olivaceus* larvae, and rotifers in the P-limited treatments offered significantly lower contents of amino acids than those in the Full treatment (Table 3, Fig. 2). This result implied that the amino acid content of rotifers under P-limited conditions did not meet the needs of *P. olivaceus* larvae, which negatively affected the growth of *P. olivaceus* larvae. Therefore, the content of amino acids could be a good predictor of food quality.

CONCLUSIONS

Our experiments indicated that nutrient limitations had stronger negative effects on *Paralichthys olivaceus* larvae than they did on *Aurelia* sp. 1 ephyrae, based on growth, survival, and elemental homeostasis of the organisms, suggesting that jellyfish ephyrae could be more competitive than fish larvae under nutrient-limited conditions. The reduction in amino acid content had negative effects on growth and survival of *P. olivaceus* larvae, which implied that this parameter may be a good predictor of food quality in nutrient-limited environments.

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