**Maternal investment in the offspring of *Pseudodiaptomus*
annandalei under nitrogen deficiency**

Xuejia He^{1,2,*}, Xilu Yang^{1,2}, Bodong Lu^{1,2}, Jungang Zhang^{1,2}

¹ Key Laboratory of Eutrophication and Red Tide Prevention of Guangdong Higher Education

Institutes, Guangzhou, Guangdong, P.R. China 510632

² Research Center of Harmful Algae and Marine Biology, Jinan University, Guangzhou,

Guangdong, P.R. China 510632

*Corresponding author. E-mail: thexuejia@jnu.edu.cn; telephone and fax number: 86-20-85221562

Abstract

Allocation of resources to reproduction varies with food condition experienced by an organism and potentially influences the future growth of the offspring. In this study, adult females of the copepod *Pseudodiaptomus annandalei* were fed diets with different carbon: nitrogen ratios (atomic C:N ratio = 4.8, 20.1, and 28.7 for HN, MN and LN, respectively), and the allocation of elements and fatty acids (FAs) in the offspring, as well as naupliar development of the offspring were measured.

Egg production rate remained constant under the different dietary N conditions (3.2~3.4 eggs day⁻¹). Mother copepod reduced both offspring size and investment of nutrients per progeny under N-deficiency. In NI offspring under N-deficiency, body size and DHA content declined significantly by 19~39% and 52~55%, respectively. There was also a decreasing trend in their N content. Naupliar development was delayed in small-sized offspring originating from maternal MN and LN dietary treatments, with prolonged naupliar duration and shorter body length at Stage CI. Growth recovery occurred only in offspring originating from maternal MN treatment when fed HN food. Generally, DHA content of NI nauplii correlated negatively with the naupliar duration. On the other hand, N content of NI nauplii correlated moderately with the naupliar duration and body length at Stage CI depending on postnatal N condition. These results suggest that performance of offspring was affected by maternal investment of DHA and N. The finding that adult female *P. annandalei* traded offspring quality for fecundity under N-deficiency points to the rule of selfish maternal effect. This is an adaptation to the N-deficiency in respect to maternal fitness, but not offspring fitness. It is implied that the influence of N-deficiency may carry over to the successive generation and impair population development in this species.

1 **Keywords:** copepod, maternal investment, nitrogen deficiency, docosahexaenoic acid, offspring
2 development, selfish maternal effect

3

4

5

6

7

1. Introduction

An organism must allocate resources among somatic growth, maintenance, and reproduction, but such allocation becomes critical when resources are limited. Benefiting both parents and progeny, the optimal strategy of reproductive investment involves a trade-off between the size and number of eggs produced (Smith and Frethwell, 1974; Roff, 2002) and may vary under different environmental conditions. Early studies generally determined egg size as the mass or carbon content (e.g., Guisande et al., 1996; Urabe and Sterner, 2001). This was later extended to nutrient composition, which was regarded as a better predictor of offspring fitness (McIntyre and Gooding, 2000; Giron and Casas, 2003; Lock *et al.*, 2007). When food quantity is low, females of copepod tend to produce fewer, but larger eggs (Cooney and Gehrs, 1980; Guisande et al., 1996; Pond et al., 1996). Recently, production of protein-rich eggs was proposed as a reproductive strategy under food limitation for *Acartia tonsa* to ensure the survival of offspring (Acheampong et al., 2011).

Both the quality of food and the quantity of food fluctuate for copepods in natural waters. Food quality fluctuates with the composition and the nutritional status of phytoplankton assemblages. The nutrient and biochemical composition are among the major factors to determine the nutritional value of prey for aquatic herbivores. Stoichiometry of phytoplankton varies with the concentrations of inorganic macronutrients in the ambient waters, while elemental stoichiometry of herbivorous consumers is generally higher and more stable than that of the prey. Therefore, the growth of the consumers is potentially limited by stoichiometric mismatch between consumers and prey (Hessen, 1992; Siuda and Dam, 2010). Nitrogen is potentially the most limiting macronutrient in brackish and marine ecosystems (Elser and Hassett, 1994; Sterner and Hessen, 1994; Hassett et al., 1997), which can result in herbivore N-deficiency (Koski, 1999). The feeding rate and egg production were linked to N-intake in marine copepods in the study by Kiørboe (1989). More recently, it was reported that a

N-deficient diet alters the reproduction pattern in *A. tonsa* by increasing production of resting eggs (Augustin and Boersma, 2006). In addition to elemental limitation, consumers may be limited by the lack of complex molecules in the diet (Tang and Dam, 1999). These critical components of diet include polyunsaturated fatty acids (PUFAs), in particular, eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). Polyunsaturated fatty acids play important roles in the physiology of animals, such as the maintenance of cell membrane fluidity and the stabilization of neural function in copepods (Scott et al., 2002). Most animals cannot synthesize these components at a rate sufficient to meet the physiological requirements. Therefore, availability of PUFA in the diet is tightly linked with reproduction and hatching rate of egg in copepods (Lacoste et al., 2001; Arendt et al., 2005; Evjemo et al., 2008; Jónasdóttir et al., 2009; Chen et al., 2012). These studies quantified the reproduction rate of copepod in response to food quality, but there is still lack of information about maternal investment to reproduction because potential variation of egg quality has generally been ignored in copepod. Size or nutrient composition of egg, as a trait of reproduction, may actually be as sensitive to maternal food quality as it is to food quantity. Female *Daphnia* produced smaller offspring with lower P or EPA content when exposed to low food quality (Urabe and Sterner, 2001; Boersma and Kreutzer, 2002; Wacker and Martin-Creuzburg, 2007). Nevertheless, the study by Kiørboe (1989) assumed that nutrient composition of egg was independent of dietary N condition.

Maternal effects are mediated by maternal investment of resources in the offspring (Tessier et al., 1983; Cowgill et al., 1984; Goulden et al., 1987). Nevertheless, Marshall and Uller (2007) argued that not all maternal effects are adaptive for offspring fitness. Thus, animals may not always trade fecundity for offspring quality under all adverse environmental conditions. Reproductive strategy of *Daphnia* exposed to low food qualities has been addressed by several studies. Urabe and Sterner (2001) compared the reproduction of *Daphnia* fed on a small quantity of nutrient (N and P)

sufficient food or a large amount of nutrient deficient food and found that the latter produced fewer and smaller eggs. Similarly, a poor diet of cyanobacteria led to a decrease in both fecundity and fatty acid content per neonate in *Daphnia* (Martin-Creuzburg et al., 2005; Wacker and Martin-Creuzburg, 2007). These studies demonstrated that the egg size-egg number trade-off may not be applicable to the circumstances of poor food qualities. Alternatively, the trade-off between somatic and reproductive allocation is complicated by nutrient constraints, which, in turn, affects reproduction pattern. Much less, however, is known about how food qualities modulate reproduction pattern in copepods.

The initial egg size or maternal investment per offspring is of importance because it was assumed that this storage of resources plays important roles in embryonic development and affects the performance of offspring. Nauplii have pre-feeding stages (Stage NI-NII, Landry, 1983; Peterson, 2001), and because of this, maternal investment is most likely crucial for the naupliar development. Lack of critical nutrients can limit offspring growth and development. This could explain the relative insensitivity of naupliar growth in *Calanus glacialis* to food quality (Daase et al., 2011). Therefore, maternal investment per offspring probably contributes to characterization of life history traits of next generations under environmental stress (Räsänen et al., 2005), and has implications for the determination of species dynamics. Despite the clear importance, the naupliar development as a function of initial maternal provisioning has not been addressed in literatures.

The calanoid copepod species *Pseudodiaptomus annandalei* is distributed widely in coastal, estuarine, and brackish water ecosystems in the subtropical and tropical Indo-Pacific regions and contributes to important fishery with high abundance (Chen et al., 2006; Walter et al., 2006; Hwang et al., 2010). In these areas, herbivorous copepods may experience fluctuating food quality due to varying nutrient conditions in the water. This study aimed to investigate the maternal investment of

nutrients (C, N, P) and fatty acids (FAs) in *P. annandalei* offspring under different N conditions. Additionally, naupliar development of the offspring was studied under different postnatal N conditions.

2. Materials and Methods

2.1. Algae and zooplankton

The copepod *P. annandalei* was originally collected from the coastal waters off southeastern Guangdong Province, China (114°00 E, 22°30 N) and has been maintained in the laboratory for approximately four years. The copepod *P. annandalei* were fed a mixture of *Chaetoceros debilis*, *Skeletonema costatum*, and *Tetraselmis subcordiformis* at saturating food concentration in 0.22 μm -filtered seawater. The algae were grown in f/2 medium prepared with filtered seawater. All the organisms were maintained under a 12:12 h dark:light cycle at a temperature of 20°C with a salinity of 25‰. The alga *T. subcordiformis* was used as the experimental food species. All cultures were started from the identical stock culture, and cells were later assigned to different media for subsequent culture. Cells with a high-N content (HN cells: atomic C:N=4.82±0.22) were grown in the normal f/2 medium, whereas the medium- and the low-N cells (MN cells: atomic C:N=20.05±1.59; LN cells: 28.70±0.32) were grown in the media with reduced concentrations of N (2.76×10^{-5} and 6.89×10^{-6} mol/L, respectively). After at least 7 d of culture, the cells were harvested by centrifugation at 2,000 rpm (8,363 g) for 10 min, and then suspended in MBL artificial seawater (ASW) made from DDW according to Baloun and Morse (1984). The salinity of the artificial seawater was adjusted to 25‰.

2.2. Ingestion rate

Ingestion rate (IR) was determined for the mother *P. annandalei* on *T. subcordiformis* at a food level of 1.87 mg C L⁻¹, which corresponded to 0.45, 0.11, and 0.076 mg N L⁻¹ for the HN, MN and LN treatments, respectively (Table 1). Each treatment included four replicate 50 ml capped tubes. Each capped tube containing 50 ml of ASW received 5 females with the addition of algal cells accordingly. Each treatment had two control tubes without the addition of animals. The caps of the tubes were hand-tightened, and the tubes were placed in a Ferris wheel at 0.8 rpm for 12 h in the darkness at 20°C. At the beginning and at the end of the incubation, a 3 ml aliquot was collected and was fixed with Lugol's iodine to determine the initial and the final cell densities for each tube. Cells were counted with an inverted microscope.

The ingestion rate (IR, µg C ind.⁻¹d⁻¹) was calculated using the following equation (Frost 1972):

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

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

2.3. Reproduction experiment

Twenty-four female and 48 male CV copepodids were randomly assigned into three beakers and were fed with the HN, MN or LN cells for 3 d to acclimate. At the end of the acclimation period, all the copepodids had grown into adults. Each 25-ml beaker, containing 15 ml of ASW, received 1 female without an egg sac and 2 males. Eight beakers were for each treatment. The food level was consistent with that used for the determination of IR. Food was added daily, and the medium was

renewed every other day. Every 6 h, the reproduction of the animals was determined under a stereoscope, and the nauplii were counted, removed and transferred to Petri dishes. Several (3-5) newly born nauplii were fixed with 4% formaldehyde for measurements of nauplii total body lengths. Some of the nauplii produced on or after day 3 were then transferred into another 25-ml beaker for the growth experiment. The reproduction experiment lasted for 13 days and during the experiment, no more than 5% of the adults died.

A total of 1,800 adult females and 900 adult males were distributed into nine 3-L beakers with 2 L of ASW. The beakers were randomly assigned to three treatments of food quality, the HN, MN and LN. Animals were fed daily with the corresponding algal cells at a food level of 1.87 mg C L^{-1} , and the medium was renewed every other day. After the third day, nauplii were collected every 6 h by carefully and gently filtering the waters through a submerged 100- μm sieve. Nauplii were then transferred to a small volume of ASW in a small Petri dish. Nauplii from each replicate beaker were narcotized with a small amount of sodium bicarbonate; the nauplii were counted and then picked out one by one into a small volume of 10% ammonium formate solution in another Petri dish for desalination. Finally, the animals were transferred into 1.5-ml eppendorf tubes, and the extra solution was removed with a Kimwipe. The samples were frozen and stored at -80°C . The incubation was terminated on the 13th day, when the numbers of nauplii collected were sufficient for the measurements of C, N, P, and FA contents (>200 individuals for each measurement). Adult females were collected with the same approach as that used for the nauplii. Approximately 50 adults and 200 nauplii were prepared for FAs analysis for each sample ($n=3$).

2.4. Growth of the offspring

Growth and development of the offspring collected in the reproductive experiment were investigated with different food regimes. Nauplii produced under the maternal MN or LN conditions were divided into two groups with 15 individuals in each group. Nauplii of one group were distributed into five 25-ml beakers that contained 9 ml of ASW and were fed algal cells of the same quality as that used in the maternal diet. Nauplii from the other group were fed the HN cells. The 15 nauplii that were produced under the HN condition were continuously raised under the HN condition as the control. Regardless of the food quality, the food level was kept at 0.94 mg C L⁻¹. Food was changed daily, and the medium was changed every other day. Growth of the nauplii was inspected daily under a stereoscope. Upon reaching copepodite stage, individuals were picked out and were transferred into ASW with 4% formaldehyde for fixation. Body length of the copepodids was measured from the beginning of the cephalothorax to the end of the caudal rami, without including the caudal setae. This experiment was ended when the last nauplius entered the copepodite stage. Somatic growth rates (g) of the nauplii were determined as the increase in body length from Stage NI stage (l_0) to CI (l_t) using:

$$g = \frac{\ln l_t - \ln l_0}{t} \quad (2)$$

2.5. Chemical analyses

Cells of *T. subcordiformis* and animals were collected by filtration onto precombusted (500°C, 4 h) GF/C filters and then rinsed with 1 mL 10% ammonium formate solution for desalination. The algal cells and the animals were dried at 60°C for 2 d for both the dry-weight and the element measurements. Dry weights were determined with a precision balance, and the C and N contents of the algae and the copepods were measured with a CNH analyzer (Series II CHNS/O Analyzer 2400; PerkinElmer Instruments). The P contents were measured with a molybdate blue reduction (Murphy

and Riley, 1962) after hot, acidic oxidative hydrolysis with 5% $K_2S_2O_8$ following the dissolving of the tissues with hydrogen peroxide at 130°C.

Samples for lipid extraction were immediately frozen and stored at -80°C until the analysis. Extraction of total lipids and preparation of the fatty acid methyl esters (FAMES) for FA analysis were conducted according to Abdulkadir and Tsuchiya (2008). A 2.5% H_2SO_4 -methanol solution was used as the methylation reagent instead of boron trifluoride-methanol because the BF_3 -methanol can cause artifacts or loss of PUFAs (Eder, 1995). A known amount of Methylnonadecanoate (C19:0, Accustandard Co.) was added as an internal standard for quantification before the extraction. The FAMES that were obtained were analyzed using a Hewlett-Packard 6890N GC equipped with a fused-silica, wall-coated, capillary column (60 m x 0.32 mm i.d., d.f.=0.25; Agilent J and W; Agilent Co., USA), with an Agilent 7683 injector and with flame ionization detection. The carrier gas was hydrogen. The oven thermal gradient increased from an initial 60°C, to 120°C at 15°C min⁻¹ and then to a final temperature of 250°C, with an increase of 2.5°C min⁻¹ with a final 20 min hold. The FAMES were identified by comparison with the retention times of mixture standards (GLC Reference Standard; Nu-Chek Prep, Inc., USA) and were quantified using software chemstation B.01.01CSR1. Quantification of individual FAMES was completed using external standards. The quantification function for each FAME was obtained by linear regression applied to the chromatographic peak areas and the corresponding concentrations of the standards (ranging from 5 to 100 mg l⁻¹).

2.6. Statistical analyses

One-way analysis of variance (ANOVA) was used to compare reproduction rates, lengths of newly born nauplii, and elemental and FA compositions under different maternal dietary N

conditions, which was followed by Tukey's post-hoc tests. The data for development of nauplii and length of copepodids at Stage CI did not meet the assumptions of normality, even after arcsine, log, or square-root transformations. Therefore, a Mann-Whitney U test was used to compare the difference. Two-way analysis of variance (Two-way ANOVA) was used to analyze the effect of life stage, dietary N condition and their interaction on elemental and FA compositions. The level of significance for all tests was $\alpha=0.05$.

3.1 Results

3.1. Effect of food quality on ingestion rate and reproduction of mothers

Ingestion rate of mother copepods under the LN condition was slightly but not significantly lower than that in the MN treatment (0.18 ± 0.03 vs. 0.21 ± 0.01 $\mu\text{g C ind.}^{-1}\text{h}^{-1}$, respectively, $p=0.07$), but both rates were significantly lower than the rate under the HN condition (0.38 ± 0.07 $\mu\text{g C ind.}^{-1}\text{h}^{-1}$; $p=0.041$ and 0.001 , respectively, One-way ANOVA and Tukey's post-hoc tests; Fig. 1). During the 13 days of incubation, reproduction rates were similar and ranged from 3.2 ± 1.3 to 3.6 ± 1.1 eggs day^{-1} regardless of dietary N condition (Fig. 2A). Specific egg production rate for C (0.20 to 0.24 $\mu\text{g C } \mu\text{g C d}^{-1}$) kept constant ($p=0.836$) while, for N, it increased from 0.20 to 0.52 $\mu\text{g N } \mu\text{g N d}^{-1}$ with increasing N-deficiency, but not significantly ($p=0.096$; One-way ANOVA and Tukey post-hoc tests; Fig.2 B,C). Gross egg production efficiency of N or C (GGE_N or GGE_C) could be calculated as reproduction rate divided with ingestion rate. Gross egg production efficiency of C varied in the range of $0.20 \sim 0.40$ day^{-1} , showing an increasing trend with increasing dietary C:N ratios ($p=0.194$, Fig.2D). Gross egg production efficiency of N, however, increased significantly from 0.12 to 1.28 day^{-1} with the gradient of N-deficiency ($p=0.011$, One-way ANOVA and Tukey post-hoc tests, Fig.2D).

Effect of maternal food quality on body size of newly born offspring

The body length and width of the newly born nauplii both varied with maternal food quality. The average body length of the nauplii originating from maternal HN treatment was 13% longer than those of the nauplii originating from MN and LN conditions (200.8 ± 19.8 vs. 177.8 ± 24.4 and 177.9 ± 16.8 μm , respectively; $p < 0.0001$, One-way ANOVA and Tukey's post-hoc tests; Fig. 3A). The nauplii that originated from the maternal LN treatment had the narrowest body width than the others (77.7 ± 12.5 vs. 95.4 ± 10.3 and 92.0 ± 7.9 μm ; both $p < 0.0001$, Mann-Whitney Test, Fig. 2B). The body length and width of nauplii were used to estimate body volume based on the equation $V = L \cdot W^2 \cdot 360$ (Rachor, 1975). Nauplii originating from the maternal HN treatment had larger volume than those originating from maternal N-deficient treatments ($p < 0.0001$, One-way ANOVA and Tukey's post-hoc tests; Fig. 3C).

3.2 Comparison of elemental stoichiometry between mothers and offspring

C content of mothers, in the range of $7.22 \pm 0.16 \sim 7.74 \pm 0.34$ $\mu\text{g ind.}^{-1}$, as well as P content, in the range of $0.32 \pm 0.002 \sim 0.35 \pm 0.006\%$ dry weight, was independent of maternal food quality (both $p > 0.05$, One-way ANOVA and Tukey's post-hoc tests; Figs. 4 A, C). In contrast, N content of mothers in MN and LN treatments was significantly lower than that of those in the HN treatment (2.74 ± 0.45 and 3.56 ± 0.14 vs. $7.76 \pm 0.06\%$ dry weight, respectively; both $p < 0.01$, One-way ANOVA and Tukey's post-hoc tests; Fig. 4 B).

Elemental composition of newly born nauplii was comparable among different maternal food qualities (Fig. 4). The specific N content, in the range of $1.59 \pm 0.03 \sim 2.00 \pm 0.2\%$ dry weight, showed

a decreasing trend with the gradient of N-deficiency, but lack of statistical significance ($p>0.05$; One-way ANOVA, Fig.4B).

There was a difference of up to one order of magnitude in the C content between mother and offspring. Offspring, however, had significantly higher specific P contents than their mothers (Table 2). Notably, the specific N content of mothers was slightly higher than that of NI nauplii under N-sufficient condition; however, under N-deficiency, the trend reversed and the specific N content of nauplii was 1.8~2.2-fold of that of their mothers (Fig.4B).

When the correlation of elemental composition between mothers and offspring was further analyzed, a positive linear relationship was found only for the specific N content between the mothers and offspring ($p=0.036$, $r^2=0.44$; Pearson's correlation; Fig.5A).

3.3 Comparison of FA composition between mothers and offspring

Main FAs of *T. subcordiformis* included 16:0, 18:0, 18:1, and 18:3(n-3) ($\geq 7.65\%$ of total FA), with comparable contributions to total FAs under different N conditions (Table 3 and Fig. 6A). Similarly, 16:0, 18:0 and 18:1 FAs were the main components in the mother copepods ($\geq 4.19\%$ of total FA) but showed slightly less importance (Table 3 and Fig.6B). Docosahexaenoic acid (DHA, 22:6 (n-3)), as an additional main FA, accounted for $8.04\pm 5.03\sim 32.5\pm 7.08\%$ of total FAs in the mothers. Under N-deficiency, the contribution of 18:1 increased significantly ($p>0.05$, One-way ANOVA), whereas there was a decreasing trend in the contribution of 18:0. Concentrations of FAs 16:0 and 18:0 decreased significantly with the gradient of N-deficiency ($p<0.05$, One-way ANOVA and Tukey's post-hoc tests; Fig. 6B). The main FAs of newly born nauplii were identical to those of their mothers ($\geq 2.19\%$); however, differently, most of FAs tended to contribute equally to total FAs (Table 3 and Fig.6C). By contrast, DHA, ranking as the most important FA, accounted for

34.4±4.87~21.91±2.19% to total FAs, which was followed by 16:0. Under maternal N-deficient condition, DHA concentration decreased significantly, so was its percentage (both $p<0.05$, One-way ANOVA; Table 2 and Fig. 6C). Differently, there was an increasing trend in the concentration and percentage of 18:1 with the the gradient of maternal dietary N-deficiency (both $p<0.05$, One-way ANOVA; Table 2 and Fig. 6C).

Ontogenetic variation in FA composition was further analyzed. Offspring had significantly higher concentrations of 18:1 and DHA than their mothers under N-deficient condition; and the opposite pattern was shown for the offspring originating from maternal HN treatment ($p=0.035$ and <0.001 , respectively; Two-way ANOVA; Fig.6B,C). Docosahexaenoic acid concentration of the offspring was approximately 2.6~4.6-fold of that of mothers and the difference increased with the gradient of N-deficiency. Notably, DHA concentration in the offspring was positively correlated with that of their mothers ($p=0.0017$, $r^2=0.78$; Pearson's correlation; Fig.7).

3.4 Effect of maternal food quality on naupliar development of the offspring

The offspring originating from the maternal LN condition has a shorter body length at Stage CI than almost all the other offspring ($332\pm8\ \mu\text{m}$ vs. $350\pm15\sim370\pm27\ \mu\text{m}$; $p<0.05$, Mann-Whitney test; Fig. 8A). Furthermore, no difference was detected between the offspring in the control and in the recovery treatments ($p<0.05$, Mann-Whitney test; Fig. 8A).

The nauplii treated with maternal HN condition, as the control, developed into Stage CI in 4.0 ± 0.0 d, whereas those treated with maternal MN or LN conditions required a longer period of 7.2 ± 2.5 or 7.4 ± 2.1 d, respectively ($p=0.016$ and 0.016 , respectively; Mann-Whitney test; Fig. 8B). Recovery in growth was only found in the nauplii that originated from the maternal MN treatment and had a naupliar duration of 4.6 ± 0.5 d, similar to that of the control nauplii ($p>0.05$, Mann-

Whitney test; Fig. 8B). The nauplii treated with maternal MN and LN conditions had significantly lower growth rates than that of the control nauplii ($0.096 \pm 0.036 \sim 0.10 \pm 0.031$ vs. 0.15 ± 0.019 d⁻¹; both $p < 0.05$, One-way ANOVA and Tukey's post-hoc tests). Recovery nauplii originating from the maternal MN treatment had the exact same growth rate to that of control nauplii (0.15 ± 0.029 vs. 0.15 ± 0.019 d⁻¹), whereas recovery nauplii from the LN treatment continued to have a slower growth rate (0.12 ± 0.018 d⁻¹; $p < 0.05$, One-way ANOVA and Tukey's post-hoc tests). In summary, nauplii from the maternal N-deficient condition showed delayed development, and their growth recovery depended on the extent of maternal dietary N-deficiency when fed HN diet.

3.5 Correlation between development and nutrient composition in offspring

The relationship between naupliar development and elemental or FA composition of the NI nauplii was analyzed separately for the two groups of offspring, i.e., the one with consistent maternal and postnatal N conditions and the other with postnatal N-sufficient condition. A significant positive correlation was found between the specific N content of NI nauplii and body length at Stage CI, with the recovery treatments excluded ($r^2 = 0.41$, $p < 0.05$; Fig. 9A). By contrast, the duration of the naupliar stage was negatively related to the specific N content of NI nauplii under the postnatal N-sufficient condition ($r^2 = 0.53$, $p < 0.05$; Pearson's correlation; Figs. 9B). Docosahexaenoic acid concentration of NI nauplii was negatively related with the duration of the naupliar stage in both groups ($r^2 = 0.907$ and 0.713 , respectively; both $p < 0.05$; Fig. 10).

4. Discussion

Dietary N condition influences copepod reproductive traits and several studies indicated that N-depleted food altered their reproduction rate or pattern (Kiørboe, 1989; Augustin and Boersma,

2006). Kiørboe (1989) reported that specific egg production in *A. tonsa* declined hyperbolically from
 0.4 to $<0.1 \mu\text{g C } \mu\text{g C d}^{-1}$ or 0.2 to $0.03 \mu\text{g N } \mu\text{g N d}^{-1}$ with increasing food C:N ratio in the range of
 0~30. Augustin and Boersma (2006) studied egg production of *A. tonsa* and *A. clausii* under dietary
 C:N molar ratio 4.5~9.1 and found that egg production rate increased dramatically when fed N-
 depleted algae. Instead, the higher algal C:N ratio (9.1) in the study cannot cause N deficiency of
 copepod reproduction rate. In this present study, copepod *P. annadalei* were fed algae with C:N ratio
 in a range of 4.8 ~28.7 and exhibited different pattern from previous studies. Fecundity remained
 constant under different N conditions ($3.2 \sim 3.6 \text{ eggs ind.}^{-1} \text{ day}^{-1}$). Specific egg production rate for C
 ($0.20 \text{ to } 0.24 \mu\text{g C } \mu\text{g C day}^{-1}$) kept constant while those for N exhibited an increasing trend from
 0.20 to $0.52 \mu\text{g N } \mu\text{g N d}^{-1}$ with increasing N-deficiency. Gross egg production efficiency of N was
 comparable ($0.20 \sim 0.40 \text{ day}^{-1}$) while GGE_C increased from 0.12 to 1.28 with increasing dietary C:N
 ratio. In contrast, *A.tonsa* had consistent GGE_N (means=0.385) and decreased GGE_C with the
 gradient of N-deficiency in the study by Kiørboe (1989). In that study, calculation of reproduction
 rate and GGE for *A.tonsa* was based on the assumption of stable C and N contents of eggs and mass
 density of adult copepod, which was later proved to be incorrect. Adult *A.tonsa* decreased their body
 N content by 20-30% indeed when fed on N-limited diet (Miller and Roman, 2008). Likewise, the
 present study demonstrated that N-limitation decreased body N content in adult *P. annadalei* by
 52~65% but only slightly in NI nauplii. Therefore, the study by Kiørboe (1989) reported only a
 reduction in fecundity but does not reflect actual reproduction allocation of *A.tonsa* under N-
 deficiency. In the present study, absolute allocation of C and N remained comparable among
 different treatments although there was a decreasing trend in terms of N under N-deficiency (Table
 4). Adult females, however, significantly increased utilization efficiency of N in egg production
 when experiencing N-deficiency. A sharp decrease (7.8 to 2.7 %) was observed in body N content of

adult females feeding on MN and LN diets. When this gap between requirement and acquirement of N enlarged, adult females exhibited extremely high $GGE_N (>1)$. It was speculated that internal body reserves of N were utilized in reproduction in addition to maintenance of basic metabolism under this circumstance.

Reproduction allocation accounted for 89~99% of available DHA in mother *P. annadalei* (Table 4) independent of dietary N condition. It was shown in previous studies that reproduction rate or hatching rate of eggs is dependent on availability of DHA in the copepods *Temora longicornis* and *A. erythraea*, highlighting the critical role of DHA in reproduction (Jónasdóttir et al., 2009; Chen et al., 2012). The present study demonstrated that most available DHA ended up in offspring, where it may act directly on embryonic development. This probably explained the close link between hatching success and availability of DHA in those studies. Docosahexaenoic acid for reproduction may originate from dietary provisioning or maternal storage and the formal source was emphasized in those previous studies mentioned. Mother copepods clearly reduced the DHA amount allocated to reproduction by 59~60% under N-deficient conditions. It is further speculated that DHA synthesis was constrained under N-deficiency. Decrease in ingestion rate led to the reduced accumulation of precursors. Enzyme for bioconversion of DHA may also decrease with the decrease in body N content of mothers. Future research is needed to clarify regulation of PUFA synthesis by nutrient condition in zooplankton. The allocation pattern of DHA in this study, however, may not necessarily apply for other cases, e.g. the copepod species living in temperate or polar waters may also need DHA to adapt to the low temperature condition.

Numerous studies support that it is generally beneficial for an organism to produce large-sized offspring under adverse food conditions (Smith and Frethwell, 1974; Roff, 2002). Nevertheless, in this study, small-sized offspring with less investment of nutrients were produced by mother *P.*

annadalei under N-deficiency. Body volume of newly born NI nauplii declined by 19~39% under N-deficiency. Because NI nauplii were separated within 6 h after hatching, size and nutrient contents of NI nauplii reflected hatching size and initial maternal investment. Very little information is available regarding to response of egg size of copepod to food quality in the literature. Only Augustin and Boersma (2006) reported that egg size decreased slightly in *A. clausi* but increased in *A. tonsa* when fed on N-stressed algal food. The copepods themselves, however, may not have been N-stressed in their study. Studies on other taxa reported similar findings to the present study. Urabe and Sterner (2001) compared the reproduction of *Daphnia* fed on a small quantity of nutrient (N and P) sufficient food or a large amount of nutrient deficient food and found that the latter produced smaller-sized eggs. This study directly pointed out clear differences in reproduction mode under low food quality and low food quantity. These studies, in combination with this present study, suggest that producing small-sized offspring may be a general response for some animals facing low nutrient conditions. Moreover, the provisioning per offspring under varying food quality in copepod was addressed in this present study for the first time. There was a significant reduction of 52~55% in DHA content and a non-significant decrease of 5~9% in N content for individual *P. annadalei* nauplius. Likewise, a poor diet of cyanobacteria led to a decline in individual fatty acid content of neonates in *Daphnia* (Martin-Creuzburg et al., 2005; Wacker and Martin-Creuzburg, 2007). Some studies actually showed that nutrient composition is the better indicator of quality of propagule than size (McIntyre and Gooding, 2000; Giron and Casas, 2003; Lock *et al.*, 2007).

Integrating fecundity and reproduction allocation of DHA and N in mother *P. annadalei*, it was shown that mothers maintained the fecundity but reduced maternal investment of DHA and N per offspring under N-deficiency. Such a reproduction pattern appears beneficial to fitness of mother

copepod if the extent of reduction in nutrient provisioning per offspring will not result into failure to hatch. Nevertheless, less investment of crucial nutrients may impair offspring performance.

Traits of naupliar development of *P. annandalei* were linked to nutrient contents of NI nauplii, supporting the argument that initial propagule size is an important determinant of offspring performance (Smith and Frethwell, 1974; Hendry et al., 2001). When the postnatal and maternal food condition were the same, small-sized nauplii originating from maternal MN and LN treatments showed slower naupliar development and growth rate compared to large-sized offspring originating from the maternal HN treatment. As we know, nauplii of copepod rely on internal storage of materials for growth until Stage NIII, when they can begin to feed (Landry, 1983; Peterson, 2001).

There is increasing understanding of the functions of DHA in organisms. Not limited to the maintenance of cell membrane fluidity and the stabilization of neural function, DHA also may be involved in the molting and morphogenesis of crustacean (Scott et al., 2002; Supreyudi et al., 2004). Supreyudi et al. (2004) observed prolonged inter-molt period in mud crab larvae feeding on low quality diets with less or no DHA. This probably explains the close correlation between DHA and naupliar duration. N content of NI nauplii correlated moderately with both body length of CI copepodid and naupliar duration. Maternal provisioning of N, as an indicator of protein content, doubtlessly is importance for the growth of nauplii. It remains unresolved in the literatures if nauplii are sensitive to food quality. Rey et al. (2001) reported that naupliar development of *Calanus helgolandicus* differed when fed on different algal species. In the same species, naupliar development was independent of food quality in terms of elemental composition and PUFA concentration (Daase et al., 2011). Nevertheless, growth recovery occurred when *P. annadalei* nauplii from maternal MN condition were fed the HN diet. This present study demonstrates that the variation of naupliar duration can be attributed to maternal investment of DHA when nauplii fed on

1 N-deficient diet. This is probably because nauplii possess insufficient bioconversional capability.

2 Alternatively, DHA synthesis may be limited in N-deficient nauplii.

3 Marshall and Uller (2007) argued that maternal fitness should be the first consideration when
4 organisms face selection pressure and proposed the concept of selfish maternal effects (SME's). In
5 this present study, adult female *P.annandalei* reduced maternal allocation of DHA and N while
6 maintaining fecundity under N-deficiency. Nauplii originating from deficient mothers with
7 insufficient provisioning of nutrient exhibited poor performance. Even though this study lasted 16
8 days, a relatively short period of time compared with the whole life time of copepod, the trade-off
9 between offspring quality and quantity conformed to SME's. This reproductive strategy will
10 facilitate copepod to cope with N-deficiency. Producing high-quality offspring was costly to mother
11 *P. annandalei*, and they tended to reallocate the somatic N to reproduction under severe N
12 deficiency in this study. In addition, copepod produced offspring repetitively. Therefore, maternal
13 fitness can be maximized by reducing maternal investment per offspring in the current reproductive
14 bouts. If N-deficiency lasted beyond the point of time when minimal nutrient provisioning per
15 offspring cannot be offered for successful embryonic development or alteration of maternal body
16 stoichiometry cannot secure maternal survival, mother copepod may have to decrease fecundity. It is
17 speculated that copepod may adjust reproduction strategy dynamically according to nutritious status
18 and environmental food quality. Fecundity of *A.tonsa* decreased under N-deficiency in the study by
19 Kiørboe (1989). The calanoid copepod *A.tonsa*, belonging to broadcast spawners, is characterized
20 with high egg reproduction and probably investing less nutrients per egg (reviewed by Niehoff,
21 2007). It seems that it may be more beneficial for them to reduce egg production than to reduce per
22 egg investment, which may increase the risk of failure in hatching. In addition, reduction of egg
23 production may not necessarily lead to low fecundity because per egg investment may be increased

under this circumstance. The copepod *P.annandalei*, in contrast, is an ovigerous species and has low egg production. It may be less costly for them to maintain stable egg reproduction by down-regulating investment per egg. It, however, remains to be clarified whether the reproduction pattern of *P. annandalei* under nutrient limitation is representative of other ovigerous copepod, and more comparative studies should be performed to find out whether reproduction mode will modulate reproduction in response to food condition.

5. Conclusions

This study showed that adult female *P. annadalei* maintained fecundity but produced small-sized offspring with reduced maternal investment of DHA and N per offspring feeding on MN and LN diets. Offspring originating from maternal MN and LN conditions had slower naupliar development compared to offspring from maternal HN condition. Recovery in growth only occurred in offspring originating from maternal MN condition in postnatal HN treatment. It was suggested that this reproduction strategy is adaptive for maternal fitness of *P. annadalei*. On the other hand, as the effect of N-deficiency may carry over into the successive generation, population development would be impaired even after ambient resource quality improved.

Acknowledgments

We thank Hans G. Dam and two anonymous referees for valuable comments on the manuscript, and Matthew Sasaki for language editing of the manuscript. The Nature Science Foundation of China (No. 41276153), the Joint Project of the Nature Science Foundation of China-Guangdong (U1301235), and the China NSF (No. 41176087) supported this study financially.

Reference

- Abdulkadir, S., Tsuchiya, M., 2008. One-step method for quantitative and qualitative analysis of fatty acids in marine animal samples. *J. Exp. Mar. Biol. Ecol.* 354,1-8.
- Acheampong, E., Campbell, R.W., Diekmann, A.B.S., St John, M.A., 2011. Food availability effects on reproductive strategy: the case of *Acartia tonsa* (Copepoda: Calanoida). *Mar. Ecol. Prog. Ser.* 428,151-159.
- Arendt, K.E., Jónasdóttir, S.H., Hansen, P.J., Gärtner, S., 2005. Effects of dietary fatty acids on the reproductive success of the calanoid copepod *Temora longicornis*. *Mar. Biol.* 146, 513-530.
- Augustin, C.B., Boersma, M., 2006. Effects of nitrogen stressed algae on different *Acartia* species. *J. Plankton. Res.* 28,429-436.
- Baloun, A.J., Morse, D.E., 1984. Ionic control of settlement and metamorphosis in larval *Haliotis rufescens* (Gastropoda). *Biol. Bull.* 167, 124-138.
- Boersma, M., Kreutzer, C.K., 2002. Life at the edge: Is food quality really of minor importance at low quantities? *Ecology* 83, 2552-2561.
- Chen, M., Liu, H.B., Chen, B.Z., 2012. Effects of dietary essential fatty acids on reproduction rates of a subtropical calanoid copepod, *Acartia erythraea*. *Mar. Ecol. Prog. Ser.* 455, 95-110.
- Chen, Q., Sheng, J., Lin, Q., Gao, Y., Lv, J., 2006. Effect of salinity on reproduction and survival of the copepod *Pseudodiaptomus annandalei* Sewell, 1919. *Aquaculture* 258, 575-582.
- Cooney, J.D., Gehrs, C.W., 1980. Effects of varying food concentrations on reproduction *Diaptomus clavipes* Schace. *Am. Mid. Nat.* 40, 63-69.
- Cowgill, U.M., Williams, D.W., Esquivel, J.B., 1984. Effects of maternal nutrition on fat content and longevity of neonates of *Daphnia magna*. *J. Crust. Biol.* 4,173-190.

- 1 Daase, M., Søreide, J.E., Martynova, D., 2011. Effects of food quality on naupliar development
2 in *Calanus glacialis* at subzero temperatures. Mar. Ecol. Prog. Ser. 429, 111–124.
- 3 Eder, K., 1995. Gas chromatographic analysis of fatty acid methyl esters. J. Chromatogr. 671,
4 113-131.
- 5 Elser, J.J., Hassett, R.P., 1994. A stoichiometric analysis of the zooplankton-Phytoplankton
6 interactions in marine and freshwater ecosystems. Nature 370, 211-213.
- 7 Evjemo, J.O., Tokle, N., Vadstein, O., Olsen, Y., 2008. Effect of essential dietary fatty acids on egg
8 production and hatching success of the marine copepod *Temora longicornis*. J. Exp. Mar. Biol.
9 Ecol. 365, 31-37.
- 10 Frost, B., 1972. Effects of Size and Concentration of Food Particles on the Feeding Behavior of
11 the Marine Planktonic Copepod *Calanus pacificus*. Limnol. Oceanogr. 17, 805-815.
- 12 Giron, D., Casas, J., 2003. Mothers reduce egg provisioning with age. Ecol. Lett. 6, 273-277.
- 13 Guisande, C., Sanchez, J., Maneiro, I., Miranda, A., 1996. Trade-off between offspring number
14 and offspring size in the marine copepod *Euterpina acutifrons* at different food
15 concentrations. Mar. Ecol. Prog. Ser. 143, 37-44.
- 16 Goulden, C.E., Henry, L., Berrigan, D., 1987. Egg size, postembryonic yolk, and survival ability.
17 Oecologia 72, 28-31.
- 18 Hendry, A.P., Day, T., Cooper, A.B., 2001. Optimal size and number of propagules: allowance
19 for discrete stages and effects of maternal size on reproductive output and offspring fitness.
20 Am. Nat. 157, 387 – 407.
- 21 Hassett, R.P., Cardinale, B., Stabler, L.B., Elser, J.J., 1997. Ecological stoichiometry of N and P in
22 pelagic ecosystems: comparison of lakes and oceans and emphasis on zooplankton-
23 phytoplankton interactions. Limnol. Oceanogr. 42, 648-662.

- 1 Hessen, D.O., 1992. Nutrient element limitation of zooplankton production. *Am. Nat.* 140,799-814.
- 2 Hwang, J.S., Kumar, R., Hsieh, C.W., Kuo, A.Y., Souissi, S., Hsu, M.H., Wu J. T., Liu, W.C.,
3 Wang, C.F., Chen Q.-C. 2010. Patterns of zooplankton distribution along the marine,
4 estuarine, and riverine portions of the Danshuei ecosystem in northern Taiwan. *Zool. Stud.*
5 49, 335-352.
- 6 Jónasdóttir, S.H., Visser, A., Jespersen, C., 2009. Assessing the role of food quality in the
7 production and hatching of *Temora longicornis* eggs. *Mar. Ecol. Prog. Ser.* 382,139–150.
- 8 Kiørboe, T., 1989. Phytoplankton growth rate and nitrogen content: implications for feeding and
9 fecundity in a herbivorous copepod. *Mar. Ecol. Prog. Ser.* 55, 229-234.
- 10 Koski, M., 1999. Carbon:nitrogen ratios of Baltic Sea copepods indication of mineral limitation? *J.*
11 *Plankton. Res.* 21, 1565-1573.
- 12 Lacoste, A., Poulet, S.A., Cueff, A., Kattner, G., Ianora, A., Laabir, M., 2001. New evidence of the
13 copepod maternal food effects on reproduction. *J. Exp. Mar. Biol. Ecol.* 259, 85-107.
- 14 Landry, M.R., 1983. The development of marine calanoid copepods with comment on the
15 isochronal rule. *Limnol. Oceanogr.* 28, 614–624.
- 16 Lock, J. E., Smiseth, P. T., Moore, P. J. & Moore, A. J. 2007: Coadaption of prenatal and
17 postnatal maternal effects. *Am. Nat.* 170, 709-718.
- 18 Marshall, D. J., Uller, T., 2007. When is a maternal effect adaptive? *Oikos* 116, 1957-1963.
- 19 Martin-Creuzburg, D., Wacker, A., Von Elert, E., 2005. Life history consequences of sterol
20 availability in the aquatic keystone species *Daphnia*. *Oecologia* 144, 362-372.
- 21 McIntyre, G.S., Gooding, R.H., 2000. Egg size, contents, and quality: maternal-age and -size
22 effects on house fly eggs. *Can. J. Zool.* 78, 1544–1551.
- 23 Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of
24 phosphate in natural waters. *Anal. Chim. Acta.* 27, 31-36.

- 1 Niehoff, B. 2007. Life history strategies in zooplankton communities: The significance of female
2 gonad morphology and maturation types for the reproductive biology of marine calanoid
3 copepods. *Progress in Oceanography* 74 (2007) 1 – 47.
- 4 Peterson, W.T., 2001. Patterns in stage duration and development among marine and freshwater
5 calanoid and cyclopoid copepods: a review of rules, physiological constraints, and
6 evolutionary significance. *Hydrobiologia*, 453-454:91-105.
- 7 Pond, D., Harris, R., Head, R., Harbour, D., 1996. Environmental and nutritional factors
8 determining seasonal variability in the fecundity and egg viability of *Calanus helgolandicus*
9 in coastal waters off Plymouth, UK. *Mar. Ecol. Prog. Ser.* 143,45-63.
- 10 Rachor, E., 1975. Quantitative Untersuchungen über das Meiobenthos der nordostatlantischen
11 Tiefsee, "Meteor" Forschungsergebn. Reihe D. 21, 1-10.
- 12 Räsänen, K., Laurila, A., Merila, J., 2005. Maternal investment in egg size: environment- and
13 population-specific effects on offspring performance. *Oecologia* 142: 546-553.
- 14 Rey, C., Harris, R., Irigoien, X., Head, R., Carlotti, F., 2001. Influence of algal diet on growth
15 and ingestion of *Calanus helgolandicus* nauplii. *Mar. Ecol. Prog. Ser.* 216, 151-165.
- 16 Roff, D.A., 2002. Life History Evolution. Sinauer Associates Inc., Sunderland
- 17 Scott, C.L., Kwasniewski, S., Falk-Petersen, S., Sargent, J.R., 2002. Species differences, origins
18 and functions of fatty alcohols and fatty acids in the wax esters and phospholipids of
19 *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus* from Arctic waters. *Mar. Ecol. Prog.*
20 *Ser.* 235, 127–134.
- 21 Siuda, A.N.S., Dam, H.G., 2010. Effects of omnivory and predator-prey elemental stoichiometry
22 on planktonic trophic interactions. *Limnol. Oceanogr.* 55, 2107-2116.
- 23 Smith, C.C., Fretwell, S.D., 1974. The optimal balance between size and number of offspring.

1 Am. Nat. 108, 499-506.

2 Sterner, R.W., Hessen, D.O., 1994. Algal nutrient limitation and the nutrition of aquatic herbivores.

3 Ann. Rev. Ecol. Syst. 25, 1-29.

4 Supreyudi, M. A., Takeuchi, T., Hamasaki, K., 2004. Effects of Artemia enriched with

5 eicosapentaenoic and docosahexaenoic acid on survival and occurrence of molting failure in

6 megalop larvae of the mud crab *Scylla serrata*. Fisheries Sci., 70, 650–658.

7 Tang, K.W., H.G. Dam. 1999. Limitation of zooplankton production: beyond stoichiometry.

8 Oikos, 84, 537-542.

9 Tessier, A.J., Henry, L.L., Goulden, C.E., Durand, M.W. 1983. Starvation in *Daphnia*: Energy

10 reserves and reproductive allocation. Limnol. Oceanogr. 28, 667-676.

11 Urabe, J., Sterner, R.W., 2001. Contrasting effects of different types of resource depletion on

12 life-history traits in *Daphnia*. Funct. Ecol. 15, 165-174.

13 Wacker, A., Martin-Creuzburg, D., 2007. Allocation of essential lipids in *Daphnia magna* during

14 exposure to poor food quality. Funct. Ecol. 21, 738-747.

15 Walter, T.C., Ohtsuka, S., Castillo, L.V., 2006. A new species of *Pseudodiaptomus*

16 (Crustacea:Copepoda: Calanoida) from the Philippines, with a key to pseudodiaptomids from

17 the Philippines and comments on the status of the genus Schmackeria. Proc. Biol. Soc. Wash.

18 119, 202-221.

19

Figure legends

Fig. 1. Ingestion rate of mother *P. annandalei* on *T. subcordiformis* with high N quality (HN, atomic C:N = 4.8), medium N quality (atomic C:N = 20), or low N quality (atomic C:N = 29). Data are mean $\pm$ SD ($n=4$).

Fig. 2. Reproduction rate (RR, A), specific reproduction rate in terms of C (RR_N, B) and N (RR_C, C) and gross egg reproduction efficiency (GGR_C and GGR_N, D) of mother *P. annandalei* under different dietary N conditions. Data are mean $\pm$ SD ($n=18$).

Fig. 3. Body length (A), width (B), and volume (C) of NI nauplii *P. annandalei* under different maternal dietary N conditions. Data are mean $\pm$ SD ($n=10$).

Fig. 4. Carbon (A), nitrogen (B) and phosphorus (C) contents of mother and NI nauplii *P. annandalei* under different maternal dietary N conditions. Data are mean $\pm$ SD ($n=2$ or 4).

Fig. 5. Relationship between nauplii and mothers N content in *P. annandalei*.

Fig. 6. Contents of main fatty acids in *T. subcordiformis* (A), mothers (B), and NI nauplii *P. annandalei* (C) under different N conditions. Different letters above bars indicate significant differences between treatments. Data are Mean $\pm$ SD ($n=3$).

Fig. 7. Relationship between NI nauplii and mothers DHA content in *P. annandalei*.

Fig. 8. Body length of copepodid at CI stage (A) and duration of naupliar stage (B) for offspring of *P. annandalei* originating from different maternal N conditions. The full bars represent the nauplii cultured with postnatal N-sufficient diet, and the grey bars represent those treated with maternal N conditions. Data are mean $\pm$ SD ($n=8$).

- 1 **Fig. 9.** Relationship between N content and development parameters including body length of CI
2 copepodid (A) and duration of naupliar stage (B) for offspring *P. annandalei* . Open circles represent
3 offspring treated with maternal N conditions and full circles represent those treated with HN diet.
- 4 **Fig.10.** Relationship between DHA concentration of NI nauplii and duration of naupliar stage for
5 offspring of *P. annandalei*. Open circles represent offspring treated with maternal N conditions and
6 full circles represent those treated with HN diet.

Table's and Figure's

1 Table 1 Elemental compositions of *T. subcordiformis* under different N conditions. (HN: high
2 nitrogen, MN: medium nitrogen, LN: low nitrogen). (*n*=3)

3

Treatment	C content (x10 ⁻⁷ mg cell ⁻¹)	N content (x10 ⁻⁸ mg cell ⁻¹)	P content (x10 ⁻⁶ ug cell ⁻¹)	Atomic C:P	Atomic N:P	Atomic C:N
HN	1.87±0.26	4.52±0.63	5.51±0.95	88±10	17.0±2.0	4.8±0.6
MN	1.46±0.16	0.86±0.16	3.33±0.22	114±20	5.4±1.4	20±2.0
LN	2.02±0.06	0.82±0.02	11.9±3.70	46±13	1.5±0.4	29±0.3

4
5
6

1 Table 2. Two-way ANOVA testing for difference between stage (female or newly born nauplii)
2 and dietary N condition (high, medium and low) by elemental composition of *P. annandalei*.

Parameter	Source	SS	df	MS	F	P
C content	N cond.	0.142	2	0.071	2.808	0.138
	Stage	145.520	1	145.520	5750.876	0.000
	N cond. x Stage	0.137	2	0.068	2.701	0.146
	Error	0.152	6	0.025		
	Total	145.950	11			
Specific N content	N cond.	0.400	2	0.200	206.765	0.000
	Stage	1.520	1	1.520	1570.074	0.000
	N cond. x Stage	00.383	2	0.192	197.904	0.000
	Error	0.006	6	0.001		
	Total	2.310	11			
Specific P content	N cond.	0.00000457	2	0.00000229	0.499	0.630
	Stage	0.004	1	0.004	921.143	0.000
	N cond. x Stage	0.00000781	2	0.00000391	0.853	0.472
	Error	0.000000275	6	0.00000458		
	Total	0.004	11			

Table 3 The fatty acid composition in proportion (%) of *T. subcordiformis*, females and newly born nauplii of *P. annandalei* under different N conditions (HN: high nitrogen, MN: medium nitrogen, LN: low nitrogen).($n=3$)

	<i>T. subcordiformis</i>			<i>P. annandalei</i>					
				Females			Newly born nauplii		
	HN	MN	L N	HN	MN	LN	HN	MN	LN
14:0	0.64 ±0.05	0.71 ±0.04	0.65 ±0.05	1.12 ±0.15	1.74 ±0.42	0.55 ±0.18	1.55 ±0.35	1.79 ±0.26	3.68 ±1.10
16:0	49.2 ±3.53	47.6 ±2.66	47.84 ±2.95	32.8±11	20.3 ±10.0	28.94 ±11.04	15.9 ±11.3	30.85 ±3.74	8.5 ±0.67
16:1	0.54 ±0.26	0.62 ±0.06	0.20 ±0.07	0.14 ±0.06	1.76 ±0.79	0.71 ±0.20	2.05 ±0.43	1.93 ±0.36	5.30 ±1.13
17:0	0.22 ±0.01	0.24 ±0.05	0.21 ±0.03	0.65 ±0.03	1.72 ±0.88	0.69 ±0.18	0.89 ±1.25	1.13 ±0.04	3.89 ±0.72
18:0	27.66± 2.60	26.20 ±0.13	25.05 ±1.24	17.8±5.15 ^a	9.64 ±3.34 ^b	11.63 ±3.00 ^b	9.11 ±4.85 ^a	18.0 ±1.01 ^b	7.26 ±1.49 ^a
18:1	8.69 ±5.38	7.82 ±0.24	13.1 ±0.85	4.90 ±1.18 ^a	12.8± 4.78 ^b	22.11 ±6.17 ^b	2.19 ±1.09 ^a	6.9 ±1.63 ^{ab}	13.5 ±4.06 ^b
18:2	1.68 ±0.94	1.68 ±0.14	1.06 ±0.38	7.99 ±0.24	3.16 ±1.30	4.36 ±1.11	1.46 ±0.66	1.87 ±0.07	5.50 ±1.60
18:3(n-3)	7.65 ±2.25	10.23 ±2.21	8.19 ±4.04	2.94 ±0.98	5.55 ±1.41	3.66 ±1.43	1.95 ±0.76	2.50 ±0.09	6.18 ±3.80
18:3(n-6)	0.26 ±0.03	0.20 ±0.17	0.14 ±0.00	1.14 ±0.04	2.51 ±2.05	1.14 ±0.61	1.46 ±0.17	1.87 ±0.07	6.16 ±1.28
20:0	0.86 ±0.11	0.91 ±0.01	1.01 ±0.08	1.31 ±0.08	3.29 ±0.81	2.88 ±0.58	1.20 ±0.69	1.71 ±0.88	3.79 ±1.74
20:2	0.02± 0.03	0.08 ±0.08	0.04 ±0.07	0.35 ±0.13	4.92 ±1.48	1.10 ±0.45	5.00 ±0.77	3.84 ±0.59	8.4 ±2.83
20:3(n-6)	2.52 ±0.43	2.70 ±0.49	2.18 ±0.03	3.48 ±0.84	3.78 ±1.03	2.35 ±0.11	5.27 ±2.52	4.85 ±1.40	4.11 ±0.96
22:1	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	1.12 ±0.39	4.05 ±1.42	4.12 ±0.68	6.53 ±2.97
22:4	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	17.6 ±10.4	0.09 ±0.03	0.05 ±0.01	13.5 ±2.78	1.99 ±0.59	0.22 ±0.14
22:5	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00±0.00
22:6(n-3)	0.08 ±0.05	1.12 ±0.99	0.41 ±0.28	8.04 ±5.03	32.5 ±7.08	18.3 ±10.98	34.4 ±4.87 ^a	21.91 ±2.19 ^b	25.7 ±4.3 ^b

Different letters indicate significant differences between treatments by the Tukey post hoc tests.

Table 4 Estimation of investment of nutrients (C: $\mu\text{g ind.}^{-1} \text{d}^{-1}$, N: $\mu\text{g ind.}^{-1} \text{d}^{-1}$, P: $\text{ng ind.}^{-1} \text{d}^{-1}$, DHA: $\text{pg ind.}^{-1} \text{d}^{-1}$) as proportion of available nutrients (ingested+maternal nutrients) in reproduction in mother *P. annandalei* under different N conditions (HN: high nitrogen, MN: medium nitrogen, LN: low nitrogen) ($n=3$).

	Treatment	C	N	P	DHA
Daily nutrient requirement for reproduction	HN	1.80 $\pm$ 0.27	0.26 $\pm$ 0.06	66.60 $\pm$ 15.10	257.42 $\pm$ 12.87
	MN	1.60 $\pm$ 0.33	0.22 $\pm$ 0.05	57.54 $\pm$ 12.08	101.86 $\pm$ 18.50
	LN	1.76 $\pm$ 0.32	0.23 $\pm$ 0.04	79.26 $\pm$ 14.40	104.56 $\pm$ 55.14
Daily ingestion	HN	9.13 $\pm$ 1.71	2.21 $\pm$ 0.41	269.11 $\pm$ 50.28	0.99 $\pm$ 0.19
	MN	5.10 $\pm$ 0.19	0.30 $\pm$ 0.01	116.42 $\pm$ 4.31	7.63 $\pm$ 1.80
	LN	4.42 $\pm$ 0.71	0.18 $\pm$ 0.03	260.24 $\pm$ 41.66	2.07 $\pm$ 2.08
Available nutrients	HN	16.59 $\pm$ 2.90	3.49 $\pm$ 0.42	324.11 $\pm$ 51.28	247.92 $\pm$ 5.12
	MN	12.84 $\pm$ 0.53	0.91 $\pm$ 0.04	172.82 $\pm$ 4.67	176.31 $\pm$ 72.35
	LN	11.63 $\pm$ 0.87	0.62 $\pm$ 0.10	317.94 $\pm$ 42.63	111.13 $\pm$ 67.30
Proportion of available nutrient invested in reproduction	HN	10.85 $\pm$ 1.70	7.44 $\pm$ 1.80	20.55 $\pm$ 3.11	94.69 $\pm$ 12.93
	MN	12.46 $\pm$ 2.86	24.45 $\pm$ 6.47	33.29 $\pm$ 6.40	89.29 $\pm$ 48.94
	LN	15.10 $\pm$ 2.74	37.64 $\pm$ 3.73	24.93 $\pm$ 4.53	99.45 $\pm$ 26.55

Fig.1

Fig. 2

Fig. 3

Fig. 4

Fig.5

Fig.6

Fig.7

Fig. 8

Fig. 9

Fig. 10