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

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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 d^{-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 11–39% 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. Introduction

An organism must allocate resources among somatic growth, maintenance, and reproduction, but such allocation becomes critical when resources are limited. Benefiting both parent and progeny, the optimal strategy of reproductive investment involves a trade-off between the size and number of eggs produced (Smith and Fretwell, 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,

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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 days of culture, the cells were harvested by centrifugation at 2000 rpm (8363*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 $\text{ind.}^{-1}\ d^{-1}$) was calculated using the following equation (Frost, 1972):

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

where *V* is the volume of seawater in the experimental beakers, *N* is the number of copepods, C_0 and C_{tf} are the 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 days 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