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# Effect of diet on the development, survival, and reproduction of the calanoid copepod *Pseudodiaptomus dubia**

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**Abstract** *Pseudodiaptomus dubia* is a calanoid copepod abundant in the mariculture ponds of southern China. However, our understanding of the population dynamics of *P. dubia* in aquaculture ponds is limited. In this study, groups of larval *P. dubia* were each fed a different microalgal species, and the effects of these different diets on development, survival, and reproduction were assessed. The five microalgae used were species common in aquaculture farms in China, and included two chlorophytes (*Chlorella saccharophila* and *Platymonas subcordiformis*), one golden microalga (*Isochrysis zhanjiangensis*), and two diatoms (*Chaetoceros muelleri* and *Cyclotella meneghiniana*). Our results indicated that *C. saccharophila* was not a suitable food for larval *P. dubia*, as all larvae fed this microalga died at stage III (as copepodites). The survival rates of *P. dubia* larvae fed *C. muelleri*, *I. zhanjiangensis*, and *P. subcordiformis* were significantly higher than that of larvae fed *C. meneghiniana*. In the adult stage, copepods fed *C. muelleri*, *I. zhanjiangensis*, and *C. meneghiniana* produced more nauplii (430–566 nauplii/female), had higher intrinsic growth rates (0.2–0.253/d), and better longevity (59–60 days) than those fed *P. subcordiformis*. Our results therefore suggest that *P. dubia* has different nutritional needs and food preferences at different life stages. For example, *P. subcordiformis* was suitable for developing larvae but not for breeding adults, while *C. meneghiniana* was suitable for breeding adults but not for developing larvae. Both *C. muelleri* and *I. zhanjiangensis* were excellent foods for *P. dubia* throughout the entire life cycle.

**Keyword**: copepods; development; survival; reproduction; *Pseudodiaptomus dubia*; diet

## 1 INTRODUCTION

*Pseudodiaptomus dubia* (formerly *Schmackeria dubia*), is a calanoid copepod widely distributed in the estuarine-coastal waters of Asia; this species is the most abundant copepod in the mariculture ponds of southern China (Li et al., 2008a, 2009). *P. dubia* not only serves as an excellent food source for aquatic animals such as fish, shrimp, and shellfish, but is also the main consumer of microalgae in breeding ponds (Luo et al., 2008). This species, therefore, plays an extremely important role in the regulation of aquaculture water quality (Li et al., 2008a).

To date, most studies of *P. dubia* have focused on the adults, and only rarely on the larvae (Huang and Luo, 1980; Shang et al., 2005; Li et al., 2008a). However, the behavior of the latter is of great significance when studying copepod population dynamics, especially for the establishment of dynamic population models (Li et al., 2008a). Previously, Li et al. (2009) studied the effect of temperature on the entire life cycle of *P. dubia*, and showed that higher temperatures increased egg production and growth rate, while shortening the reproductive cycle and hatching time. However, diet also strongly influences the growth and reproduction of copepods (Ban, 1994; Koski and Kuosa, 1999; Carotenuto et al., 2002; Murray and Marcus, 2002; Yu et al., 2017), as food

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**Table 1 Details of the microalgal species used in this study, showing the concentration supplied to correspond to 1.5 mg C/L**

| Microalga | Diameter (μm) | Volume (μm$^3$) | Concentration (cells/mL) |
|---|---|---|---|
| *Chlorella saccharophila* | 1.8±0.00 | 3±0.00 | 140×10$^4$ |
| *Isochrysis zhanjiangensis* | 3±0.00 | 14±0.00 | 34×10$^4$ |
| *Chaetoceros muelleri* | 3±0.01 | 14±0.00 | 30×10$^4$ |
| *Platymonas subcordiformis* | 11±0.1 | 180±0.00 | 2.3×10$^4$ |
| *Cyclotella meneghiniana* | 7.5±0.01 | 154±0.01 | 2.7×10$^4$ |

quality directly affects development, survival, and reproduction (Harrison, 1990; Taipale et al., 2014).

We therefore aimed to investigate the effects of different foods on the entire life cycle of *P. dubia*, including development, survival, reproduction, and longevity. We aimed to use various microalgal species commonly present in the mariculture ponds of southern China as food for *P. dubia*: *Chlorella saccharophila* and *Platymonas subcordiformis* (phylum Chlorophyta); *Isochrysis zhanjiangensis* (phylum Chrysophyta); and *Chaetoceros muelleri* and *Cyclotella meneghiniana* (phylum Bacillariophyta). We then aimed to compare the intrinsic growth rate, net reproductive rate, and generation time among groups of *P. dubia* fed different diets. With these results, we aimed to provide a framework for the mass culture of *P. dubia*, and improve our understanding of *P. dubia* population dynamics in aquaculture ecosystems.

## 2 MATERIAL AND METHOD

### 2.1 Larval production and microalgae used

All seawater used in this study was first filtered through a plankton net (mesh size: 39 μm), and then boiled to kill any remaining living organisms. *P. dubia* were collected from several shrimp ponds in Zhanjiang, China. We acclimated the copepods to laboratory conditions (temperature: 28°C; salinity: 27; irradiance: 700–1 200 lux) for three months. During the acclimation period, copepods were fed diatoms (*C. muelleri*).

After three months, 250 healthy and energetic females carrying mature egg sacs were identified under a microscope (Nikon, Japan). Each egg-carrying female was carefully transferred with a pipette dropper to a different 20 mL glass tube containing 10 mL clean seawater. Every 2 h, newly hatched nauplii were collected in a 1 000-mL glass beaker filled with clean seawater. All nauplii collected at the same time were considered a cohort and were collected in the same beaker. At the end of the hatching period, the largest cohort was used for all experiments.

All microalgae used in this study were provided by the Algal Culture Research Laboratory of Guangdong Ocean University (Zhanjiang, China). Five species of microalgae were tested as food for *P. dubia*: *C. saccharophila* and *P. subcordiformis* (phylum Chlorophyta); *I. zhanjiangensis* (phylum Chrysophyta); and *C. muelleri* and *C. meneghiniana* (phylum Bacillariophyta). All microalgae were fed to *P. dubia* such that the amount of carbon provided was equivalent (1.5 mg C/L; see Table 1 for the details of the individual microalgal species); all food carbon concentrations were estimated based on the photometric light extinction (682 nm) and the carbon-extinction regressions determined in preliminary experiments. The microalgae were cultured in 1-L Erlenmeyer flasks using Zhanshui 107 medium (Chen, 1995), with silicates added for diatoms. Cultures were maintained at 28°C, with irradiance of 1 000 lux and a 12-h light:12-h dark photoperiod. Microalgae were maintained in the logarithmic growth phase by diluting each culture two-fold with fresh medium every 10 days. All cultures were in the exponential growth stage when fed to copepods.

### 2.2 Experimental set up

The single nauplii cohort was divided into 75 125-mL glass bottles, each bottle containing 100 mL of microalgal suspension and 10 nauplii. Fifteen bottles were used per microalgae species; these 15 bottles were divided into five groups (A–E), each group comprised of three replicate bottles. In group A bottles, we measured the effects of diet on nauplius and copepodite development. We used groups B and C to determine the effects of diet on the survival of nauplii and copepodites, respectively. We used groups D and E to investigate the effects of diet on reproduction. For the purposes of our reproduction experiment, and to ensure a sufficient reproductive population, groups D and E were treated identically and considered a single group. All glass bottles were kept in an incubator (PGX-280A-3H; Lai Fu, China) at 28°C, with light intensity of 1 200 lux, and a 12-h light:12-h dark photoperiod. Seawater in each bottle was refreshed every 2 days. All the feeding experiments were conducted simultaneously, and all experimental animals were considered a single cohort.

### 2.3 Effect of diet on nauplius and copepodite development

The life cycle of *P. dubia* consists of 12 stages: six nauplius stages (NI, NII, NIII, NIV, NV, and NVI), five copepodite stages (CI, CII, CIII, CIV, and CV), and the adult stage (Li et al., 2009). Because *P. dubia* is an egg-carrying invertebrate, eggs develop into NI within the egg sac, before the sac detaches from the female and the NI nauplii hatch, and NI nauplii transition to the NII stage occurs within 2–4 min of hatching (Li et al., 2009). Therefore, our observations of larval development began at stage NII.

Larval development in group A was monitored every 6 h by randomly selecting three to four larvae, quickly photographing them using a microscope (Nikon, Japan) equipped with an ocular micrometer (NE1, China), and immediately returning them to the group A bottle. We measured the prosome length of all collected larvae, and determined the developmental stage based on Li et al. (2009). Each stage was considered complete when 50% of the cohort had moulted. The weight (in μg carbon) of each *P. dubia* nauplius and copepodite was calculated as

$\log C=2.00\log CL-5.67$ and

$\log C=2.81\log L-8.03$,

respectively, where CL was the prosome length (in μm) of the nauplius, and $L$ was the prosome lengths (in μm) of the copepodite (Uye et al., 1983; Li et al., 2009). We then calculated the average larval growth rate $G$ (/d) as

$$G=(\ln C_i-\ln C_1)/T_i,$$

where $C_i$ was the weight (in μg carbon) of the larvae at stage $i$, $C_1$ was the weight (in μg carbon) at the beginning of the experiment, and $T_i$ was the duration (in days) of stage $i$ (Martin-Creuzburg et al., 2005).

### 2.4 Effect of diet on nauplius and copepodite survival

As soon as the larvae in group A reached the nauplius stage NVI, the live larvae in Group B were fixed with Lugol's iodine. As soon as the larvae in group A reached the copepodite stage CV, we fixed all remaining live larvae in Group C. We counted these larvae under a stereomicroscope (SZMN645-B4, China), and calculated separate survival rates (SR) for the nauplii and copepodites as follows:

$$SR=(a/10)\times 100\%,$$

where $a$ was the number of live larvae at stage NVI in group B and at stage CV in group C.

### 2.5 Effect of diet on *P. dubia* reproduction

As soon as the larvae in groups D and E reached the adult stage and the females were observed to carry eggs, females and males at a ratio of 1:2 were incubated in ten 20 mL glass tubes, each containing 10 mL of a different microalgal suspension. Nauplius production was recorded, and microalgal suspensions were renewed every 1–2 days. The experiment was not terminated until all adult females had died. We then calculated several life-history parameters, including net reproductive rate ($R_0$), generation time ($T$), intrinsic rate of population growth ($r_m$), finite rate of growth ($\lambda$), and population doubling time ($t$) as

$$R_0=\Sigma l_x m_x,$$

$$T=\Sigma x l_x m_x/R_0,$$

$$r_m=\ln R_0/T,$$

$$\lambda=e^{rm}, \text{ and}$$

$$t=(\ln 2)/r_m,$$

where $x$ was age or time interval, $l_x$ was survival rate at time $x$, and $m_x$ was the birth rate at time $x$ (Meyer et al., 1986; Shen and Shi, 2002; Tang et al., 2005; Li et al., 2009).

### 2.6 Data analyses

All statistics were calculated in SPSS v17.0 (IBM, USA). All significant results were analyzed for homogeneity of variance with Levene's test and then with Duncan's multiple range tests. Results were considered significant at $P$=0.05.

## 3 RESULT

### 3.1 Effect of diet on *P. dubia* larval development time and body length

The larvae fed different microalgae had different development times (Table 2). Nauplii developed most quickly when fed *C. muelleri*, taking only 60 h to progress from stage NII to stage NVI; nauplii fed *C. saccharophila*, *I. zhanjiangensis*, and *P. subcordiformis* took 72 h, and nauplii fed *C. meneghiniana* took 96 h (Table 2). Similarly, copepodites developed most quickly when fed *C. muelleri*, taking only 108 h to progress from stage CI to stage CV; copepodites fed *P. subcordiformis* and *C. meneghiniana* took 126 h, and copepodites fed *I. zhanjiangensis* took 138 h. All copepodites fed *C. saccharophila* died during stage CIII. Therefore, *C. saccharophila* as food was clearly detrimental to copepodite development. The species of microalgae

**Table 2 Development time (h) for larval *Pseudodiaptomus dubia* fed different species of microalgae**

| Larval stage | *Chlorella saccharophila* | *Isochrysis zhanjiangensis* | *Chaetoceros muelleri* | *Platymonas subcordiformis* | *Cyclotella meneghiniana* |
|---|---|---|---|---|---|
| Nauplii II | 12 | 12 | 12 | 12 | 12 |
| Nauplii III | 12 | 12 | 12 | 12 | 18 |
| Nauplii IV | 12 | 12 | 12 | 12 | 18 |
| Nauplii V | 18 | 18 | 12 | 18 | 24 |
| Nauplii VI | 18 | 18 | 12 | 18 | 24 |
| Nauplii II–VI | 72 | 72 | 60 | 72 | 96 |
| Copepodite I | 42 | 18 | 18 | 18 | 18 |
| Copepodite II | 48 | 24 | 18 | 18 | 18 |
| Copepodite III | 48 | 24 | 18 | 24 | 24 |
| Copepodite IV | n/a | 30 | 24 | 30 | 30 |
| Copepodite V | n/a | 42 | 30 | 36 | 36 |
| Copepodite I–V | n/a | 138 | 108 | 126 | 126 |
| Nauplii II to Copepodite V | n/a | 210 | 168 | 198 | 222 |

**Table 3 Body length (μm) for larval of *Pseudodiaptomus dubia* fed different species of microalgae**

| Larval stage | *Chlorella saccharophila* | *Isochrysis zhanjiangensis* | *Chaetoceros muelleri* | *Platymonas subcordiformis* | *Cyclotella meneghiniana* |
|---|---|---|---|---|---|
| Nauplii II | 168±2 | 170±0 | 165±3 | 166±1 | 169±0 |
| Nauplii III | 213±3 | 212±8 | 220±5 | 216±4 | 212±2 |
| Nauplii IV | 227.5±3 | 232.5±3 | 225±5 | 245±1 | 248±5 |
| Nauplii V | 267±8 | 290±5 | 270±13 | 260±0 | 270±5 |
| Nauplii VI | 308±7[a] | 305±2[a] | 310±5[a] | 320±9[a] | 305±5[a] |
| Copepodite I | 380±0 | 395±0 | 397.5±2 | 425±5 | 365±5 |
| Copepodite II | 475±0 | 479.7±10 | 478.8±8 | 483.75±11 | 467.4±2 |
| Copepodite III | 590±0 | 594±3 | 594.5±13 | 586.3±6 | 557.6±5 |
| Copepodite IV | n/a | 701.1±8 | 686.5±10 | 685±0 | 676.5±6 |
| Copepodite V | n/a | 861±5[a] | 848.7±12[a] | 861±2[a] | 845.3±5[a] |

consumed had no obvious effects on body length (Table 3).

### 3.2 Effect of diet on *P. dubia* larval growth rate

The species of microalgae consumed significantly affected the growth rate of *P. dubia* larvae (ANOVA followed by Duncan's multiple range tests, $P<0.05$; Fig.1). At stage NVI, the growth rate of nauplii fed *C. muelleri* or *P. subcordiformis* was >0.44/d, significantly greater than that of nauplii fed *I. zhanjiangensis* (0.389/d; $P<0.05$), *C. saccharophila* (0.405/d; $P<0.05$), and *C. meneghiniana* (0.295/d; $P<0.05$; Fig.1). There were no significant differences in growth rates between nauplii fed *C. saccharophila* and *I. zhanjiangensis* (Fig.1), but the growth rate of nauplii fed *C. meneghiniana* was significantly lower than that of all other microalgal diets ($P<0.05$ for all comparisons; Fig.1).

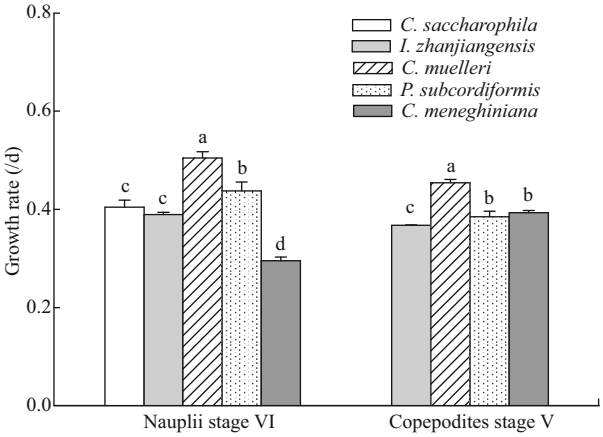


**Fig.1 Growth rate of larval *Pseudodiaptomus dubia* when fed different species of microalgae**

Bars represent means±standard deviation of three replicates per microalgal species. Bars labeled different lowercase letters are significantly different (analysis of variance (ANOVA) and Duncan's multiple range tests; $P<0.05$). For details of larval growth stages, please see the text.

At stage CV, the growth rate of copepodites fed *C. muelleri* (0.454/d) was significantly higher than that of copepodites fed all other algal species (0.368–0.393/d; $P<0.05$; Fig.1). Indeed, copepodites fed *C. muelleri* grew 15%–23% faster than those fed *I. zhanjiangensis*, *P. subcordiformis*, or *C. meneghiniana*. The growth rate of copepodites fed *P. subcordiformis* was significantly greater than that of copepodites fed *I. zhanjiangensis*, but there were no significant differences in growth rate between copepodites fed *C. meneghiniana* and *P. subcordiformis* (Fig.1).

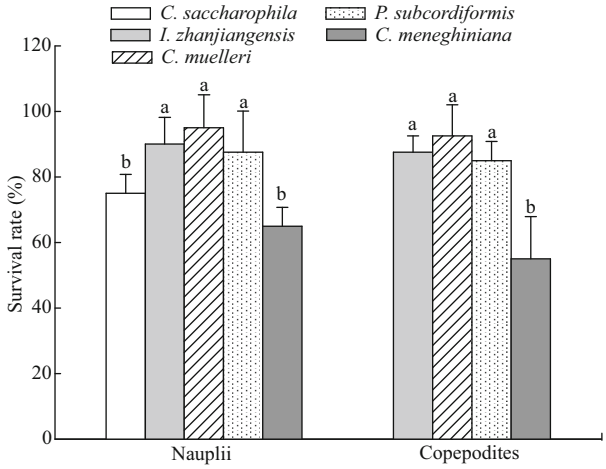


**Fig.2 Survival rate of larval *Pseudodiaptomus dubia* when fed different species of microalgae**

Bars represent means±standard deviation of three replicates per microalgae. Bars labeled different lowercase letters are significantly different (analysis of variance (ANOVA) and Duncan's multiple range tests; $P<0.05$).

### 3.3 Effect of diet on survival of *P. dubia*

The microalgal species consumed had a significant effect on the survival rate of larval *P. dubia* (ANOVA followed by Duncan's multiple range tests, $P<0.05$; Fig.2). Significantly more nauplii and copepodites survived when fed *C. muelleri*, *I. zhanjiangensis*, or *P. subcordiformis* (range: 85%–95%), as compared to those fed *C. saccharophila* or *C. meneghiniana* (<75%; Fig.2).

The survival rates of *P. dubia* varied tremendously depending on the microalgal species consumed (Fig.3). Throughout the entire lifecycle, copepods fed *C. muelleri* had the highest survival rate, remaining above 87% for 33 days; 30% of the copepods fed *C. muelleri* remained alive after 57 days (Fig.3). The survival curve of the copepods fed *I. zhanjiangensis* was similar, but with a lower rate of survival throughout (Fig.3). Copepods fed *C. meneghiniana* had a low survival rate at the larval stage (<10 days), but had little mortality as adults until ~50 days, when the survival rate dropped sharply (Fig.3). Copepods fed either *P. subcordiformis* or *C. saccharophila* had

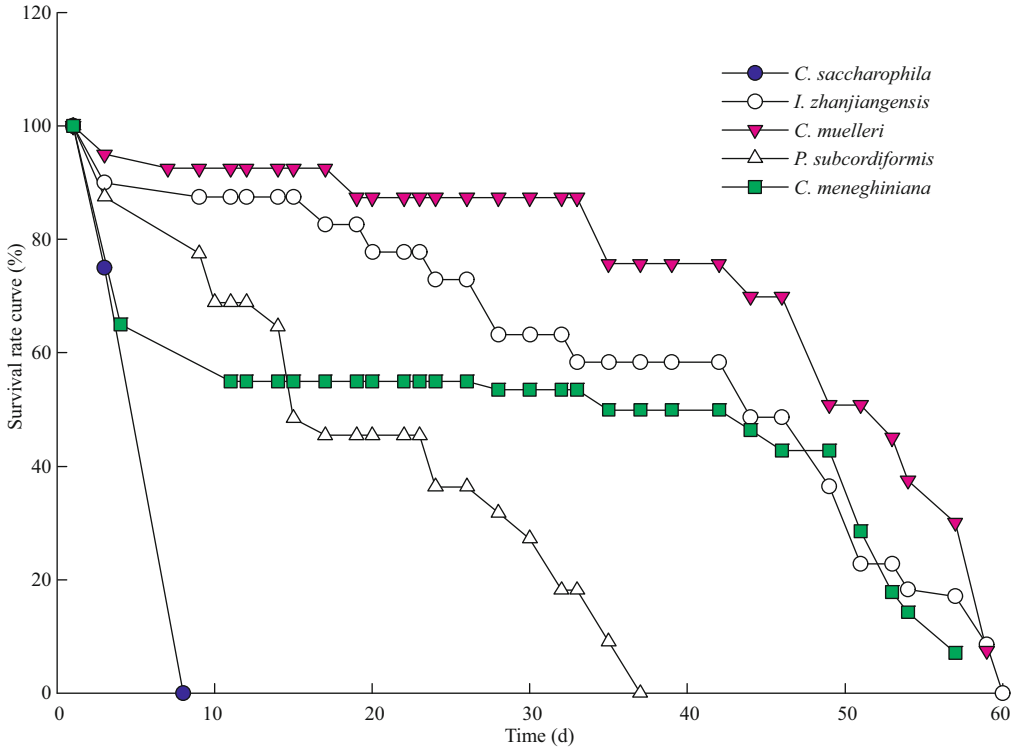


**Fig.3 Survival curve for different developmental stages of *Pseudodiaptomus dubia* fed different microalgal species**

**Table 4 Growth and reproduction indices for *Pseudodiaptomus dubia* fed different microalgae**

| Index | Microalgal species | | | |
|---|---|---|---|---|
| | *Isochrysis zhanjiangensis* | *Chaetoceros muelleri* | *Platymonas subcordiformis* | *Cyclotella meneghiniana* |
| Days to hatch (d) | 8.75 | 7 | 8.25 | 9.25 |
| Days to reproduce (d) | 35±9.9[b] | 46.2±6.6[a] | 19.1±3.5[c] | 33±5.9[b] |
| Nauplii produced per female[1] | 430±173.6[a] | 566.6±117.7[a] | 57.6±15.6[b] | 440.1±71.7[a] |
| Reproductive frequency (times) | 26±5.7 | 29±4.3 | 12±2.1 | 28±5.3 |
| Average clutch size | 16.5 | 19.5 | 4.8 | 15.7 |
| Longevity (d) | 36–60 | 49–59 | 28–37 | 42–59 |

[1] mean±standard deviation of 10 replicates. Different lowercase letters within a row indicate significant differences among means (analysis of variance (ANOVA) followed by Duncan's multiple range tests; $P<0.05$).

poor survival rates curves, with all individuals dead by day 37 and day 8, respectively (Fig.3).

The average longevity of copepod adults fed *C. muelleri*, *I. zhanjiangensis*, or *C. meneghiniana* was 47–55 d (Fig.4), significantly longer than the longevity of copepods fed *P. subcordiformis* (32 d; $P<0.05$; Fig.4). There were no significant differences in average longevity between copepods fed *I. zhanjiangensis* and those fed *C. meneghiniana*, but the average longevity of adults fed *I. zhanjiangensis* was significantly lower than that of copepods fed *C. muelleri* ($P<0.05$; Fig.4).

### 3.4 Effect of diet on *P. dubia* reproduction and population growth

As all copepods fed *C. saccharophila* died before reaching reproductive maturity, we were only able to measure the reproduction of *P. dubia* fed the other four microalgal species. Copepods fed *C. muelleri*, *I. zhanjiangensis*, and *C. meneghiniana* had long reproductive periods (averaging 46.2, 35, and 33 days, respectively), while those fed *P. subcordiformis* reproduced for an average of only 19.1 days (Table 4). Nauplii production did not differ significantly among copepods fed *C. muelleri*, *I. zhanjiangensis*, and *C. meneghiniana*: all females produced >430 nauplii each (Table 4). However, the production of nauplii by females fed *P. subcordiformis* was significantly lower: approximately 58 nauplii/female. Copepods fed *C. muelleri*, *I. zhanjiangensis*, and *C. meneghiniana* had similar levels of reproductive frequency (~26–29 times throughout the experiment) and average clutch size (15.7–19.5 eggs); these metrics were noticeably lower in copepods fed *P. subcordiformis* (reproduction frequency: ~12 times; average clutch size: 4.8 eggs; Table 4).

*Pseudodiaptomus dubia* fed *C. muelleri* had the highest net reproductive rate (491.5; Tables 5 & 6),

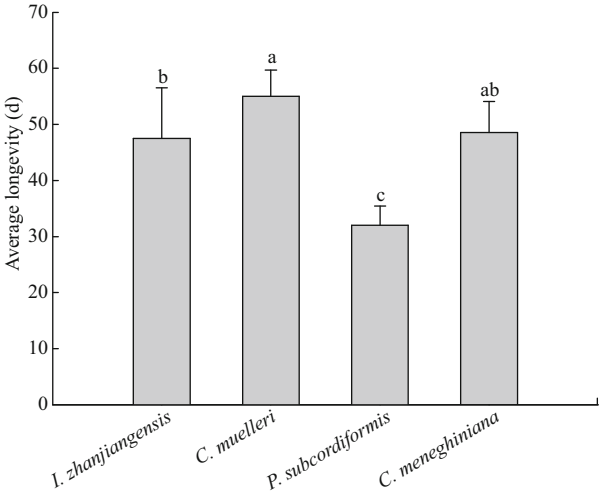


**Fig.4 Average longevity of *Pseudodiaptomus dubia* fed different microalgal species**

Bars represent means±standard deviation of six replicates per microalgal species. Bars labeled different lowercase letters are significantly different (analysis of variance (ANOVA) and Duncan's multiple range tests; $P<0.05$).

finite growth rate (1.28), and intrinsic growth rate (0.253/d), as well as the fastest population doubling time (2.74 d; Table 6). In contrast, *P. dubia* fed *P. subcordiformis* had the lowest net reproductive rate (28.7), finite growth rate (1.2), and intrinsic growth rate (0.185/d), as well as the slowest population doubling time (3.75 d; Table 6). The life-history parameters for *P. dubia* fed *C. meneghiniana* and *I. zhanjiangensis* were intermediate between these two extremes (Table 6).

## 4 DISCUSSION

### 4.1 Effect of diet on larval development and survival

Diet strongly affects growth and reproduction in copepods (Carotenuto et al., 2002; Murray and Marcus, 2002; Yu et al., 2017). Appropriate foods

**Table 5 Fecundity life table for *Pseudodiaptomus dubia* fed different microalgae**

| Age/$x$ (days) | Survival rate $l_x$ | | | | Birth rate $m_x$ | | | | $l_xm_x$ | | | | $xl_xm_x$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Food type | | | | Food type | | | | Food type | | | | Food type | | | |
| | *I* | *Cha* | *Pl* | *Cy* | *I* | *Cha* | *Pl* | *Cy* | *I* | *Cha* | *Pl* | *Cy* | *I* | *Cha* | *Pl* | *Cy* |
| 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0.9 | 0.95 | 0.88 | 0.65 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0.88 | 0.93 | 0.78 | 0.55 | 0 | 36.7 | 0 | 0 | 0 | 33.9 | 0 | 0 | 0 | 237.4 | 0 | 0 |
| 9 | 0.88 | 0.93 | 0.78 | 0.55 | 16.7 | 4 | 0 | 4.57 | 14.6 | 3.7 | 0 | 2.51 | 131.6 | 33.3 | 0 | 27.66 |
| 11 | 0.88 | 0.93 | 0.69 | 0.55 | 6.86 | 18.5 | 3.57 | 18.9 | 6 | 17.1 | 2.46 | 10.4 | 66 | 188.2 | 24.6 | 124.5 |
| 12 | 0.88 | 0.93 | 0.69 | 0.55 | 26 | 22.8 | 0 | 32 | 22.8 | 21.1 | 0 | 17.6 | 273 | 253.5 | 0 | 246.4 |
| 14 | 0.88 | 0.93 | 0.69 | 0.55 | 31 | 36.5 | 5.57 | 8.71 | 27.1 | 33.8 | 3.84 | 4.79 | 379.8 | 472.7 | 46.06 | 71.89 |
| 15 | 0.88 | 0.93 | 0.65 | 0.55 | 18.3 | 23.3 | 4.14 | 8.43 | 16 | 21.6 | 2.68 | 4.64 | 240 | 323.8 | 37.46 | 78.81 |
| 17 | 0.83 | 0.93 | 0.48 | 0.55 | 25.3 | 35.7 | 8.43 | 41.4 | 20.9 | 33 | 4.08 | 22.8 | 355.2 | 560.9 | 61.24 | 432.9 |
| 19 | 0.83 | 0.87 | 0.45 | 0.55 | 39.9 | 36.3 | 5.71 | 17.6 | 32.9 | 31.7 | 2.6 | 9.66 | 625.8 | 603.1 | 44.13 | 193.3 |
| 20 | 0.78 | 0.87 | 0.45 | 0.55 | 13.4 | 22.5 | 6 | 14.3 | 10.4 | 19.7 | 2.73 | 7.86 | 208.9 | 393.1 | 51.79 | 172.9 |
| 22 | 0.78 | 0.87 | 0.45 | 0.55 | 24 | 25.8 | 2.57 | 13.4 | 18.7 | 22.6 | 1.17 | 7.39 | 410.7 | 496.5 | 23.36 | 169.9 |
| 23 | 0.78 | 0.87 | 0.45 | 0.55 | 19.6 | 15.2 | 3.86 | 14.6 | 15.2 | 13.2 | 1.75 | 8.01 | 350.1 | 304.7 | 38.55 | 192.3 |
| 24 | 0.73 | 0.87 | 0.45 | 0.55 | 10.9 | 14 | 6.14 | 37.6 | 7.92 | 12.2 | 2.79 | 20.7 | 190 | 293.5 | 64.18 | 537.3 |
| 26 | 0.73 | 0.87 | 0.36 | 0.54 | 39.3 | 28 | 1.43 | 27.4 | 28.7 | 24.5 | 0.52 | 14.7 | 745.7 | 636 | 12.46 | 410.9 |
| 28 | 0.63 | 0.87 | 0.36 | 0.54 | 21.8 | 34.8 | 0.71 | 41 | 13.8 | 30.4 | 0.26 | 21.9 | 386.3 | 851.2 | 6.749 | 658.1 |
| 30 | 0.63 | 0.87 | 0.32 | 0.54 | 21.8 | 36.6 | 8 | 34.3 | 13.8 | 29.9 | 2.54 | 18.3 | 413.9 | 959.2 | 71.23 | 587 |
| 32 | 0.63 | 0.87 | 0.27 | 0.54 | 26.7 | 25 | 3.4 | 19 | 16.9 | 21.8 | 0.93 | 10.2 | 539.3 | 698.9 | 27.8 | 335.4 |
| 33 | 0.58 | 0.87 | 0.18 | 0.5 | 2.8 | 22.2 | 1.8 | 31.9 | 1.63 | 19.4 | 0.33 | 15.9 | 53.9 | 640 | 10.47 | 556.8 |
| 35 | 0.58 | 0.76 | 0.18 | 0.5 | 19.4 | 28.6 | 0 | 34.6 | 11.3 | 18.6 | 0 | 17.3 | 396.1 | 757.9 | 0 | 638.7 |
| 37 | 0.58 | 0.76 | 0.09 | 0.5 | 15.8 | 32.8 | 0 | 17 | 9.22 | 24.8 | 0 | 8.49 | 341 | 918.9 | 0 | 331.1 |
| 39 | 0.58 | 0.76 | 0 | 0.5 | 12.2 | 21.6 | 0 | 12.3 | 7.12 | 17.4 | 0 | 6.16 | 277.6 | 637.8 | 0 | 258.7 |
| 42 | 0.58 | 0.76 | | 0.46 | 22.6 | 32.4 | | 9.2 | 13.2 | 13.2 | | 4.27 | 553.7 | 1030 | | 187.7 |
| 44 | 0.49 | 0.7 | | 0.43 | 9.2 | 11 | | 1.33 | 4.47 | 6.01 | | 0.57 | 196.8 | 338.3 | | 26.25 |
| 46 | 0.49 | 0.7 | | 0.43 | 1.33 | 18 | | 0 | 0.65 | 6.15 | | 0 | 29.81 | 578.7 | | 0 |
| 49 | 0.36 | 0.51 | | 0.29 | 6 | 21.7 | | 0 | 2.19 | 8.3 | | 0 | 107.2 | 539.6 | | 0 |
| 51 | 0.23 | 0.51 | | 0.18 | 8 | 9.67 | | 37 | 1.82 | 1.52 | | 6.6 | 92.97 | 250.6 | | 349.7 |
| 53 | 0.23 | 0.45 | | 0.14 | 23 | 10.7 | | 4 | 5.24 | 2.7 | | 0.57 | 277.8 | 254.5 | | 30.82 |
| 54 | 0.18 | 0.38 | | 0.07 | 4 | 2.33 | | 0 | 0.73 | 0.88 | | 0 | 39.38 | 47.28 | | 0 |
| 57 | 0.17 | 0.3 | | | 6 | 7.67 | | | 1.03 | 2.3 | | | 58.48 | 131.2 | | |
| 59 | 0.09 | 0.08 | | | 3 | 0 | | | 0.26 | 0 | | | 15.22 | 0 | | |
| 60 | 0 | | | | 0 | | | | 0 | | | | 0 | | | |
| Net reproduction rate ($R_0$) | | | | | | | | | 324.6 | 491.5 | 28.7 | 241.2 | | | | |

Note: *I* represents *Isochrysis zhanjiangensis*; *Cha* represents *Chaetoceros muelleri*; *Pl* represents *Platymonas subcordiformis*; *Cy* represents *Cyclotella meneghiniana*.

**Table 6 Population growth indices for *Pseudodiaptomus dubia* fed different microalgae**

| Index | Microalgal species | | | |
|---|---|---|---|---|
| | *Isochrysis zhanjiangensis* | *Chaetoceros muelleri* | *Platymonas subcordiformis* | *Cyclotella meneghiniana* |
| Net reproductive rate ($R_0$) | 324.6 | 491.5 | 28.7 | 241.2 |
| Generation time d ($T$) | 23.90 | 24.5 | 18.14 | 27.44 |
| Intrinsic growth rate/d ($r_m$) | 0.242 | 0.253 | 0.185 | 0.2 |
| Population doubling time d ($t$) | 2.86 | 2.74 | 3.75 | 3.47 |
| Finite growth rate ($\lambda$) | 1.27 | 1.28 | 1.2 | 1.22 |

improve larval survival and increase the speed of larval development, while inappropriate foods may lead to larval death or retard larval development. Inappropriate foods include those that provide inadequate nutrition (Jones and Flynn, 2005), are inappropriately sized (Li et al., 2008b), contain biotoxins (Yu et al., 2017) or produce special chemical compounds (Wolfe et al., 1997; Pohnert et al., 2007; Yu et al., 2017).

It has been suggested that diatoms have deleterious effects on nauplius development (Carotenuto et al., 2002; Ianora et al., 2004; Koski et al., 2008). For example, larvae of the copepod *Temora stylifera* had high mortality rates and did not develop into adults when fed diatoms such as *Thalassiosira*, *Skeletonema costatum*, and *Phaeodactylum tricornutum*, possibly because these diatoms produce aldehydes that block copepod development (Carotenuto et al., 2002). The development of a closely related species, *T. longicornis*, was also retarded, with high larval mortality, when fed diatoms (Koski et al., 2008). However, other studies have shown that copepods favored diatoms, and that diatoms sustain copepod development form hatching to adulthood (Vidal, 1980; Koski, 2007). The nutritional status of a given diatom species affects its utility as copepod prey (Jones and Flynn, 2005). Here, *P. dubia* larvae thrived when fed the diatom species *C. muelleri* and, to a lesser extent, *C. meneghiniana*. The rates of larval development, growth, and survival were all significantly greater in larvae fed *C. muelleri*, as compared to larvae fed chlorophytes (*C. saccharophila* and *P. subcordiformis*) or chrysophyte (*I. zhanjiangensis*). Lora-Vilchis et al. (2004) found that juveniles of the mollusc *Atrina maura* fed *C. muelleri* had a higher growth rate than these fed *Isochrysis* sp., even though *Isochrysis* sp. was higher in proteins, carbohydrates, and lipids. It may be that the metabolites (e.g., polyunsaturated fatty acids and cholesterol) from the diatom *C. muelleri* increase the ingestion and growth rates of various mollusk species (Ward et al., 1992; Lora-Vilchis et al., 2004; Chen et al., 2013). Polyunsaturated fatty acids and cholesterol also play a critical role in zooplankton growth (Harrison, 1990; Martin-Creuzburg et al., 2009; Taipale et al., 2014). Thus, our results indicated that *C. muelleri* was a high-quality diet that improved the growth rate of *P. dubia* larval. Interestingly, a *C. meneghiniana* diet had different effects on *P. dubia* larvae at different developmental stages. During the nauplii stage, larvae fed *C. meneghiniana* had a significantly lower growth rate than the larvae fed other microalgae ($P<0.05$). However, copepodites fed *C. meneghiniana* had significantly greater growth rates than larvae fed *I. zhanjiangensis* and *C. saccharophila*, similar to the growth rates of larvae fed *P. subcordiformis*. This suggested that *C. meneghiniana* was more suitable as food for copepodites rather than for nauplii, probably because *C. meneghiniana* has a hard siliceous shell that is difficult for nauplii to digest and absorb. Therefore, the effects of diatom prey on copepod growth may vary greatly due to species-specific differences.

*Platymonas subcordiformis* is a suitable food for *Acartia bifilosa* larvae (Li et al., 2008b) as well as for adult *P. dubia* (Luo et al., 2008). *I. zhanjiangensis* is also considered an excellent food for zooplankton (Zhou et al., 2007; Huang et al., 2008), as this species has no cell walls and is easily digestible (Chen et al., 2013). Consistent with these studies, diets of *P. subcordiformis* and *I. zhanjiangensis* led to high survival rates for *P. dubia* larvae.

Copepod fed on *C. saccharophila* did not exhibit good growth performance; indeed all larvae fed *C. saccharophila* died before reaching adulthood. *Chlorella* sp. have low total lipids (Chen et al., 2013), as well thick fibrous cell walls that make this species difficult to digest (Deng et al., 2016). In addition, several researchers have suggested that copepods favor large motile prey (Hargrave and Geen, 1970; Frost, 1977; Calbet et al., 2007). The nauplii of small copepods can ingest foods with a minimum particle size of 3–5 μm (Hargrave and Geen, 1970; Li et al., 2008b). However, the average size of *C. saccharophila*, as measured here, was 1.8 μm (Table 1). Thus, *C. saccharophila* particles were too small to be retained by the copepodite of even small copepods. Indeed, the copepodite survival rate decreased dramatically because *C. saccharophila* particles were too small to be retained by the larger larvae, leading to starvation and death. Similarly, when the microalga *Nannochloropsis oculata* (maximum particle size: 3.1 μm) were supplied as food to larval *Acartia bifilosa*, all larvae died upon reaching stage CI, due to the small size of the microalgal particles (Li et al., 2008b). The mass mortality of copepods fed *C. saccharophila*, may also have been caused by the chemical compounds produced by these microalgae. Unicellular marine algae produce a variety of chemicals as defenses against predators (Wolfe and Steinke, 1996; Wolfe et al., 1997), including toxins

and/or intracellular inhibitors such as dimethylsulfoniopropionate (DMSP) (Wolfe and Steinke, 1996; Wolfe et al., 1997). DMSP, which is segregated within microalgal cells and which is only activated during microzooplankton grazing (Wolfe and Steinke, 1996), has deleterious effects on zooplankton ingestion and survival (Wolfe et al., 1997). Li et al., (2010) found that *Chllorella* (Chlorophyta) produced DMSP, and that DMSP concentrations peaked on days 6–10 of the culture period. This was consistent with our results, as all larvae fed *C. saccharophila* died on days 8–9 of our feeding experiment (Table 1). Therefore, it is clear that a diet of *C. saccharophila* is deleterious to the copepod growth probably because this microalga provides inadequate nutrition, is difficult to digest, is too small to retain, and produces biotoxins.

### 4.2 Effect of diet on reproduction

The amount of polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), in food directly influences the reproduction, development, and survival of copepods (Harrison, 1990; Taipale et al., 2014). Foods containing high levels of PUFAs may increase copepod fertility (Jónasdóttir, 1994; Dam and Lopes, 2003; Yu et al., 2017). Microalgae are one of the few taxa that synthesize EPA and DHA (Brett and Müller-Navarra, 1997; Brett et al., 2009). As different species of microalgae contain different types and levels of nutrients, the fertility, larval development rate, and survival rate of a single species of copepod can vary significantly depending on the microalgal species upon which it feeds (Murray and Marcus, 2002). Here, *P. dubia* adults fed *C. muelleri*, *I. zhanjiangensis*, and *C. meneghiniana* had significantly higher survival rates, increased nauplii production per female, and longer reproductive periods, as well as more frequent breeding periods as compared to those fed *P. subcordiformis*. This difference was likely due to the differences in nutritional composition among the microalgal species.

Copepods carry oil sacs that are rich in unsaturated fatty acids; these fatty acids primarily originate from its diet (Brett and Müller-Navarra, 1997; Graeve et al., 2005). The types of foods consumed have been shown to affect lipid accumulation in the copepod *Calanus sinicus* (Zhou and Sun, 2016). During larval development, we observed that the larvae fed different microalgae had oil sacs of different sizes. Indeed, copepodites fed *I. zhanjiangensis*, *C. muelleri* and *C. meneghiniana* had large oil sacs, while those fed *P. subcordiformis* and *C. saccharophila* had no oil sacs. This may have been due to differences in nutritional composition among the microalgae. Several studies have shown that diatoms contain EPA and sterol (Renaud et al., 1995; Lin and Li, 1999; Jiang and Zheng, 2003; Chen et al., 2013), up to 21.45% of the total fatty acids (Lu and Lin, 2001). In addition, *I. zhanjiangensis* contains high levels of DHA (Chai et al., 2009). However, *P. subcordiformis* and *C. saccharophila* contain little or no EPA and DHA (Lu and Lin, 2001; Jiang and Zheng, 2003; Chai et al., 2009; He et al., 2014). This is consistent with our results, as larvae fed *C. muelleri*, *C. meneghiniana* and *I. zhanjiangensis* had good longevity and produced many nauplii, while larvae fed *C. saccharophila* died at stage CIII stage and larvae fed *P. subcordiformis* treatment had a relatively low nauplii production per female and poor longevity. Thus, the good performance of *P. dubia* larvae fed *C. muelleri*, *C. meneghiniana*, and *I. zhanjiangensis* might have been due in part to the high levels of EPA or DHA contained in these microalgae. These results further indicated that *C. muelleri*, *C. meneghiniana*, and *I. zhanjiangensis* were suitable foods for *P. dubia*.

Copepods have different nutritional needs and restrictions at different life stages (Li et al., 2008b; Yu et al., 2017). Copepod larvae mainly require proteins and carbohydrates for physical development, while female adults need more lipids during the reproductive process (Murray and Marcus, 2002). Indeed, some foods suitable for copepod development are not necessarily suitable for copepod reproduction, while those suitable for copepod reproduction might not be suitable for copepod development and survival (Murray and Marcus, 2002). In addition, the food species which induce the shortest development times do not necessarily result in the highest nauplii production rates (Bonnet and Carlotti, 2001). Consistent with this, although larvae fed *P. subcordiformis* developed more quickly with a better survival rate at larval stage (Figs.1, 2), nauplii production was low and longevity was short in adults (Fig.3; Tables 3 & 4). This indicated that *P. subcordiformis* was only suitable as feed for developing larvae, and not for reproducing adults. It is also noteworthy that the development and survival rates of larvae fed *C. meneghiniana* were significantly lower than those of larvae fed other microalgae, but nauplii production by adult females fed

*C. meneghiniana* (440 hatched nauplii/female) was not significantly different from nauplii production of adult females fed the more high-quality foods *C. muelleri* (566.6 hatched nauplii/female) and *I. zhanjiangensis* (430 hatched nauplii/female). In addition, a diet of *C. meneghiniana* led to a long period of vigorous breeding and a high adult survival rate. Thus, *C. meneghiniana* was a better food for reproductively-active adult *P. dubia* than for larvae. Some previous studies have shown that mixtures of foods were more favorable for copepod development and reproduction than single foods (Bonnet and Carlotti, 2001; Colin and Dam, 2002). Therefore, a mixture of *P. subcordiformis* and *C. meneghiniana* might benefit *P. dubia*, as these microalgae are nutritionally complementary.

Providing an appropriate diet at each stage of copepod development can significantly improves copepod development, survival, and reproduction (Murray and Marcus, 2002). Our results suggested that foods with a larger particle size should be given at the copepodite stage, such as *C. meneghiniana*, *P. subcordiformis*, *C. muelleri* and *I. zhanjiangensis*. At the reproductive stage, *P. dubia* should be fed nutritious foods containing high levels of EPA and DHA, such as *C. muelleri*, *I. zhanjiangensis*, and *C. meneghiniana*. *C. muelleri* and *I. zhanjiangensis* were excellent foods for *P. dubia* throughout its entire lifecycle.

### 4.3 Effects of diet on population growth

The parameters of population growth (including intrinsic growth rate, net reproduction rate, and finite growth rate) calculated for *P. dubia* were most optimal in copepods fed *C. muelleri*. Therefore, *C. muelleri* was the best food for the support of *P. dubia* population growth, followed by *I. zhanjiangensis* and *C. meneghiniana*. A diet of *P. subcordiformis* led to slow copepod population growth.

With adequate food, the copepod *Euterpina acutifrons* had a net reproductive rate of 70.89 and an intrinsic growth rate of 0.161/d (Zurlini et al., 1978). Here, the net reproductive rate of *P. dubia* fed *C. muelleri* was 491.5 and the intrinsic growth rate was 0.253/d. Both of these factors were substantially higher in *P. dubia*, suggesting that *P. dubia* had a better capacity for population growth than *E. acutifrons*. Indeed, excluding external factors, populations of *P. dubia* fed *C. muelleri* would be expected to double every 2.74 days (Table 6).

## 5 CONCLUSION

Our results provided a framework for the optimal feeding of *P. dubia*, based on the five microalgal species most commonly available as live food for *P. dubia* in the aquaculture ponds of southern China. Our results indicated that *C. saccharophila* was not a suitable food for *P. dubia* larvae, as all larvae fed this microalga died at copepodite stage III. Both *C. muelleri* and *I. zhanjiangensis* were excellent foods for *P. dubia* throughout its entire life cycle. *P. subcordiformis* was suitable for developing larvae but not for breeding adults, while *C. meneghiniana* was suitable for breeding adults but not for developing larvae. Therefore, our results suggested that *P. dubia* has different nutritional needs and food preferences at different life stages.

## 6 DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed in this study are available from the corresponding author upon reasonable request.

## 7 ACKNOWLEDGMENT

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