Aquaculture 344-349 (2012) 168–173

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Reproductive performance, growth and development time of a tropical harpacticoid copepod, *Nitocra affinis californica* Lang, 1965 fed with different microalgal diets

Hazel Monica Matias-Peralta [a], Fatimah Md. Yusoff [b,c,*], Mohamed Shariff [b], Suhaila Mohamed [d]

[a] Department of Technology and Heritage, Faculty of Science, Technology and Human, Development, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat, Johor, Malaysia
[b] Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM-Serdang, Selangor, Malaysia
[c] Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM-Serdang, Selangor, Malaysia
[d] Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM-Serdang, Selangor, Malaysia

ARTICLE INFO

Article history:
Received 12 July 2011
Received in revised form 27 February 2012
Accepted 28 February 2012
Available online 19 March 2012

Keywords:
Reproductive performance
Nutritional profile
Microalgal diet
Growth
Harpacticoid copepod
Nitocra affinis

ABSTRACT

This study examines the growth, reproductive performance and survival of a copepod *Nitocra affinis californica* Lang, 1965 fed with three microalgal food items singly (a diatom, *C. calcitrans* ; a green algae, *N. oculata* ; flagellated green algae, *T. tetrathele*) and in combinations (50% *C.calcitrans* + 50% *N. oculata*; 50% *C. calcitrans* + 50% *T. tetrathele*; 50% *N. oculata* + 50% *T.tetrathele*; 50% *C. calcitrans* + 25% *N. oculata* + 25% *T. tetrathele*). The experiments were performed under controlled laboratory condition (30 ppt salinity, temperature of 30 °C, light intensity of 25 μmol m^{-2} s^{-1} and 12 h light:12 h dark cycle). Feeding densities were maintained at 10^6 cells ml^{-1} throughout the study. The highest ($p<0.05$) egg production was achieved by copepods fed with *C. calcitrans* and mixed algal diet (50% *C. calcitrans* + 25% *N. oculata* + 25% *T. tetrathele*) with mean values of 133.6 ± 3.9 and 128.2 ± 2.0 eggs $female^{-1}$, respectively. Similarly, the highest ($p<0.05$) offspring production (132.0 ± 3.8 offsprings $female^{-1}$), survival from nauplii to adult (98.8 ± 0.2) and maximum specific growth rate (K = 0.43 ± 0.0) was achieved with *N. affinis californica* fed *C. calcitrans*. The shortest ($p<0.05$) embryonic development time, time interval between egg sac, development time from nauplii to copepodid and copepodid to adult resulted from feeding with *T. tetrathele*. The longevity of the female *N. affinis* was found to be longest ($p<0.05$) when fed with mixed algal diet (42.2 ± 0.7 days) and combination of *C. calcitrans* + *T. tetrathele* (41.4 ± 0.7 days). The present study revealed that amongst the microalgae offered, *C. calcitrans* was the best food item as indicated by the highest reproductive capacity (eggs $female^{-1}$ and offsprings $female^{-1}$) of the *N. affinis californica*. Significantly higher nutritional values of *C. calcitrans* compared to other microalgae were probably responsible for the best reproductive and growth performance of *N. affinis*.

1. Introduction

Successful growth and development of cultured species depends largely on the nature and content of biochemical constituents in the food provided. These constituents were used in the anabolic process for tissue production, in catalyzing metabolic process and in the creation of energy to power those processes. In general, higher food value is obtained from mixed diets, which are more likely to contain the diversity of biochemicals to satisfy most nutritional requirements for growth (Whyte et al., 1989).

The availability of food resources is a critical factor in reproduction of the meiofauna and it was reported that maximum breeding occurs during times when food availability is at the highest level (Huys et al., 1986). Likewise, reproduction specifically egg production, is determined metabolically by interaction between the amount and the nutritional characteristics of the food that is assimilated (Mitra and Flynn, 2005). According to Abdullahi (1992), the availability of food and food type influenced copepod reproduction and modified the rate of development, longevity and fecundity. In addition, the development time, size and reproductive output of adult copepods varied according to diet or food supply (Hansen and Santer, 1995; Smyly, 1970). Furthermore, food is an important factor that determines the rate of development and it is considered a contributing factor in controlling the copepod size (Harris and Peffenhöfer, 1976). According to Mullin and Brooks (1971), low food concentration results in smaller sized maturing females, which in turn produced fewer eggs. Thus, it is of great importance to have ample supply of food during this stage of the copepod life cycle.

One of the most important information in sustainable copepod culture is the kind and amount of food needed for optimum growth. Previous studies suggested that many harpacticoids are highly specific in their choice of food organisms either for survival or as a required stimulus for growth and reproduction (Giere, 1993; Hicks and Coull, 1983; Huys et al., 1986, 1996; Marcotte, 1984). As a potential species for aquaculture, it is important to determine the nutritional content

* Corresponding author at: Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia. Tel.: +60 3 89472236; fax: +60 3 89472191.
E-mail addresses: fatimah@ibs.upm.edu.my, fatimahyus@gmail.com (F.M. Yusoff).

0044-8486/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2012.02.026

and reproductive capacity of *Nitocra affinis californica* Lang, 1965. Therefore the objective of this study was to determine the effect of different algal food types on the growth and development time of this species. The nutritional values of each food type were also evaluated in this study.

2. Materials and methods

2.1. Species isolation

Prior to isolation, copepod samples were collected from the coastal waters off Port Dickson, Malaysia (Latitude 2°27′ N and Longitude 101º51′ E) using a Schindler Patalas trap. The copepods caught in the trap were transferred to 1-L beaker containing filtered (0.20 μm filter) seawater and provided with aeration. Thereafter, copepods carrying egg sacs were transferred separately to a new beaker containing filtered (0.20 μm filter) seawater and mixed algae as food. To ensure a monospecies stock, gravid females were transferred to several beakers (putting only one gravid female in each beaker), until the eggs were hatched and grow to adult. Approximately 10 adult copepods from each of the beakers were collected, preserved with 5% buffered formalin, and subjected to identification procedure (dissection, mounting and drawing) to confirm the species. After the species was confirmed as *Nitocra affinis californica* Lang, 1965 (UPMC-Z0005), all the beakers containing the same species of copepod were combined into several containers of 2 L capacity and were grown continuously in the laboratory for several generations. The copepods were then mass produced in plastic basins with filtered seawater under room temperature of 28–30 °C, and fed with mixed algae containing diatom *Chaetoceros calcitrans*, a unicellular green algae *Nannochloropsis oculata* and *Tetraselmis tetrathele* (Matias-Peralta et al., 2011).

2.2. Algae culture

Axenic monocultures of microalgae, *Chaetoceros calcitrans* (UPMAAHU10), *Nannochloropsis oculata* (UPMAAHU20), and *Tetraselmis tetrathele* (UPMC-A0007) were used for this study. The isolates where supplied by the Aquatic Animal Health Unit of the Faculty of Veterinary Science and Medicine at the Universiti Putra Malaysia, Serdang, Malaysia. Each species of algae was subsequently cultured in Conway medium (Tompkins et al., 1995) at a salinity of 30 psu, and at 24 ± 2 °C under 12-h light:12-h dark cycle. All algal food used in this experiment were harvested at the exponential growth phase. At harvest the algae were washed, using a centrifugation RCF of 423, for four times with sterile synthetic seawater. The numbers of cells to feed were determined by cell counts made using a haemocytometer.

2.3. Rearing experiment

Seven different treatments, using three algal food items singly (a diatom, *C. calcitrans* ; a green algae, *N. oculata* ; flagellated green algae, *T. tetrathele*) and in combinations (50% *C. calcitrans* +50% *N. oculata*; 50% *C. calcitrans* + 50% *T. tetrathele*; 50% *N. oculata* + 50% *T. tetrathele*; 50% *C. calcitrans* + 25% *N. oculata* + 25% *T. tetrathele*) were assigned. Each treatment was replicated five times. Algal cell densities were maintained at 1.0×10^6 cells ml^{-1}. The experiment was performed using glass Petri plates (50 mm × 15 mm) containing 15 ml filtered autoclaved seawater at 30 ppt salinity maintained inside an environmental test chamber (Sanyo Versatile Environmental Test Chamber) under a constant temperature of 28°C and 12 h light:12 h dark cycle. Gravid females from first copulation (before first brood appears) were selected for this study. All the dishes were checked daily (at 4 and 6 h intervals) to ascertain the time of egg release. Once the female completed hatching and egg release, it was transferred to a new plate with fresh culture medium. The number of eggs produced and total number of offspring (nauplii, copepodid and adult copepods) were counted and checked for survival everyday. The experiment continued until the nauplii were hatched from the last clutch. The nauplii from the first three clutches (from each food types) were reared in the culture media until the female hatched the first clutch to determine the generation time, while the original females were maintained until they died to determine longevity. The maximum specific growth rate (K) of *N. affinis californica* was calculated at the end of the experiment. In addition, the following parameters were measured: (a) time between the appearance of the egg sac and its hatching (embryonic development time); (b) time between hatching of the first egg sac and the appearance of the next (interval time between egg sacs); (c) development time from nauplii I (NI) to copepodid I (CI) and from CI to adult; (d) time interval between hatching of nauplii to the time this nauplii hatch its own eggs (generation time; hatch to hatch) and (e) the longevity of female. The daily routine consisted of counting each individual copepod under Carl Zeiss dissecting microscope; removing fecal materials, dead animals, and molted exoskeleton; and replenishing fresh seawater and food.

2.4. Proximate analysis

Protein analysis of each algal diet followed the procedure described by Rausch (1981) and measured by the sensitive microburette method spectrophotometrically (Itzhaki and Gill, 1964). Lipid analysis followed the method described by Rausch (1981) and Zöllner & Kirsch (1962 in Meyer and Walther, 1988) where lipid in the cells are oxidized to short fragments (build in the sulpho-phospho-vanilline reaction which gives a red colored complex) with the aid of boiling concentrated sulfuric acid. The determination of carbohydrates was accomplished according to the phenol-sulfuric acid method (Herbert et al., 1971) with glucose as standard for the calibration curve.

2.5. Fatty acid analysis

Fatty acid methyl esters (FAME) of each algal diet were prepared according to the direct methylation techniques (Divakaran and Otrowski, 1989). Freeze dried algal samples were refluxed at 100 °C for 10 min with 10 ml of 2% methanolic NaOH. The samples were further refluxed with 6.25 ml 14% Boron Triflouride and Heptane. Then 2 ml of saturated NaCl was added to make the FAME float, and 1 g of anhydrous Na_2SO_4 was added to absorb the remaining water contained in the FAME for best recovery. The FAME of the samples was analyzed with a gas liquid chromatograph (Shimadzu GC-8A) equipped with a FID and BPX-70 (SGE) or Supelco 2330 capillary column. Individual peak of FAME was identified by comparison with retention times of known standards obtained from Sigma Chemicals Company and using cod liver oil as a secondary standard. A Chromatopac (SHIMADZU C-R3A) quantified the magnitude of the peaks of each chromatographic reading.

2.6. Data Analysis

The growth of copepod was described by the maximum specific growth rate (K) as defined by Omori and Ikeda (1984): $K = \ln(x2) - \ln(x1)/t2–t1$ where: x1 is the number of copepods at the initial of selected time interval; *x2* is the number of copepods at the final of selected time interval; t2–t1 is the selected time (in days) for determination of number of copepods. The collected data were analyzed using one-way analysis of variance (ANOVA). Significant differences among individual treatment effects were determined using Tukey's honestly significant different test (T-HSD) at 0.05 level of probability. The data for maximum specific growth rate, survival and all other data which were expressed in percentages were arcsine-