



Egg production, egg hatching success and population increase of the tropical paracalanid copepod, *Bestiolina similis* (Calanoida: Paracalanidae) fed different microalgal diets

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ABSTRACT

A series of experiments was conducted to evaluate the suitability of ten microalgal diets, including 4 monoalgal, 5 binary and 1 tri-algal diet, for the culture of the tropical paracalanid copepod *Bestiolina similis*. The four monoalgal diets were the Tahitian strain of *Isochrysis* sp. (T-Iso), *Pavlova* 50 (Pav), *Tetraselmis chuii* (Tet) and the diatom *Chaetoceros muelleri* (Chaet), the 5 binary diets were Tet + T-Iso, Tet + Pav, T-Iso + Pav, Chaet + Tet and Chaet + Pav while the tri-algal diet was T-Iso + Tet + Pav.

After feeding *B. similis* with 10 algal diets for 3 days, 24 h egg production rate (EPR, eggs female⁻¹ day⁻¹) was obtained for each diet treatment by averaging the egg output of 6 individual females (replaced daily) for 4 consecutive days. The tri-algal diet T-Iso + Tet + Pav produced the highest EPR (44.1 eggs female⁻¹ day⁻¹), which was significantly higher than any other diets tested ($p < 0.05$). Highest 48 h and 96 h egg hatching rates (EHR) were also found from the eggs produced by *B. similis* fed with T-Iso + Tet + Pav (48 and 96 h EHR = 91.0% and 96.3% respectively) and significant differences in 48 h EHR were detected for 5 out of the 9 other diets tested, while for 96 h EHR, only for 2 diets. Population increase was determined over a 12 day culture period for 10 initial *B. similis* adults (7 females and 3 males) and the result showed that the tri-algal diet T-Iso + Tet + Pav produced significantly higher population number by the end of 12 days than any of the mono-algal or binary diets tested ($p < 0.05$). When all developmental stages were included (including eggs), the tri-algal diet produced a population increase from 10 to 887 over the 12 days. The binary diet Tet + T-Iso was the second most productive diet, providing a total population increase from 10 to 541, which was significantly higher than the rest of the diets, except that of Pav + T-Iso (479), T-Iso (334) and Chaet + Pav (285) ($p > 0.05$).

Based on the current results, it is suggested that among the diets tested, the tri-algal diet of T-Iso + Tet + Pav was the best for the culture of *B. similis*.

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1. Introduction

Copepods are probably the most numerous metazoan sub-class on the planet and usually dominate the mesozooplankton, constituting about 80% of its biomass (Verity and Smetacek, 1996). Copepods, particularly their nauplii, constitute an important part of marine fish larval diets in the wild (Sampey et al., 2007) and typically make up more than 50% of the stomach contents (Støttrup, 2000). By forming a trophodynamic link between primary and tertiary production, copepods play a key role in the cycling of nutrients and energy in marine ecosystems (Kjørboe, 1997).

It is known that early larvae of some marine fish cannot survive on the commonly used hatchery live feeds of rotifers (*Brachionus* spp.) or

Artemia spp, and this represents a major challenge to the aquaculture industry (McKinnon et al., 2003; Chesney, 2005; O'Brien and Lee, 2005), as these species include several high valued food fishes such as tropical snappers (Lutjanidae), groupers (Serranidae and Epinephelinae), as well as many marine ornamental species, for instance marine Angel fishes (Pomacanthidae) and the sea horse *Hippocampus subelongatus* (Payne and Rippington, 2001; VanderLugt and Lenz, 2008).

In contrast, marine copepods, especially calanoids, have been proven as ideal food for many cultured marine larvae (Hernandez Molejon and Alvarez-Lajonchere, 2003), showing significant benefits when compared to rotifers and *Artemia* (Chen et al., 2006). The advantages of copepods over traditional hatchery live feeds include their many naupliar and copepodite stages that provide a broad spectrum of prey sizes for cultured larvae (Chen et al., 2006). Meanwhile, the nutritional content of copepods generally matches the requirements of marine fish larvae (Støttrup, 2000; Evjemo et al.,

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2003; McKinnon et al., 2003). Additionally, the distinctive swimming behaviour of copepods is believed to stimulate stronger foraging responses in some fish larvae, resulting in improved ingestion rates (Støttrup, 2000). These advantages provide potential for the successful larval culture of species that cannot be cultured using traditional live feeds (Marcus and Murray, 2001; O'Bryen and Lee, 2005). Furthermore, inclusion of copepods in diets of already successfully cultured species could further improve their survival, development and pigmentation (Støttrup, 2000; Knuckey et al., 2005; VanderLugt and Lenz, 2008). Nonetheless, despite these promising features, the utilization of copepods in aquaculture remains sporadic (Marcus et al., 2004; Camus and Zeng, 2008). This under-utilisation is mainly attributed to their relative low productivity in intensive culture (O'Bryen and Lee, 2005), which could in turn be partially attributed to the lack of research on this field.

Food quality and quantity are probably the most important factors regulating the productivity of copepod culture. As such, the effects of different microalgal diets on egg production (Calbet and Alcaraz, 1996; Kleppel et al., 1998; Koski and Kuosa, 1999; Payne and Rippingale, 2000; Turner et al., 2001), egg hatching success (Shin et al., 2003; Milione and Zeng, 2007), mortality (Kang and Poulet, 2000; Lincoln et al., 2001) and development (Knuckey et al., 2005; Leandro et al., 2006) have been documented for several calanoid species, such as *Acartia omorii* (Shin et al., 2003), *A. sinjiensis* (McKinnon et al., 2003; Knuckey et al., 2005; Milione and Zeng, 2007), *A. tonsa* (Holste and Peck, 2006), *Calanus helgolandicus* (Lacoste et al., 2001) and *Gladioferens imparipes* (Payne and Rippingale, 2000). In contrast, few studies have investigated the response of paracalanid copepods to different food sources (McKinnon et al., 2003; VanderLugt and Lenz, 2008) and none appears to have considered the effects of various microalgal combinations.

Productivity of copepods in intensive culture is firstly linked to female egg production as it is a direct indicator of population recruitment, as well as a measure of the net production rate of adult females (Shin et al., 2003). However, subsequent egg hatching rate, naupliar and copepodite survival and development rates, all impact the productivity of cultures (Milione and Zeng, 2007). A comprehensive investigation, including assessment of the population increase after a period of culture, will therefore provide a better understanding of the effects of algal diets on copepod culture productivity.

Bestiolina similis is a small (adults < 700 µm; early nauplii < 100 µm) pelagic paracalanid copepod and is a favourite prey item for larvae of several families of tropical fish (Sampey et al., 2007). Paracalanid copepods are widely distributed in temperate and tropical waters and frequently dominate the copepod communities of surface waters (McKinnon and Duggan, 2001; Boxshall and Halsey, 2004; VanderLugt and Lenz, 2008). *B. similis*' small size, natural abundance and the fact that they are a favourite prey for a vast assemblage of marine larvae make them excellent candidates as live prey for fish larvae. McKinnon et al. (2003) suggested that among tropical copepods with high potential for aquaculture hatcheries, *B. similis* is arguably the best suited candidate for tropical larval fish rearing. More research is therefore needed to optimise their culture techniques to fully realise their potential.

Through a series of laboratory experiments, the present study examined the suitability of 10 different singular/combinations of microalgae, all of them as commonly used species in aquaculture, for the intensive cultivation of *B. similis*.

2. Materials and methods

2.1. Algal culture

All of the microalgae utilised in present experiments are common used algal species in aquaculture, therefore relatively easy to culture. They were inoculated from starter cultures supplied by the Commonwealth Scientific and Industrial Research Organization (CSIRO)

Microalgae Supply Service, Hobart, Tasmania, Australia. These algal species, including the Tahitian strain of *Isochrysis* sp. (T-Iso), *Tetraselmis chuii* (Tet), *Pavlova* 50 (Pav) and the diatom *Chaetoceros muelleri* (Chaet), were cultured in a temperature controlled room, using 20-L polycarbonate carboys filled with 1 µm filtered, autoclaved and UV irradiated seawater of salinity 30 ‰. All microalgae were cultured using f/2 medium (Guillard and Ryther, 1962), with silicates added for the cultures of the diatom *Chaetoceros muelleri*, and maintained at a temperature of 25 ± 1 °C with vigorous aeration (0.2 µm filtered air). The photoperiod was set at light:dark cycle = 12 h:12 h with a light intensity of approximately 5000 lx as measured by a MC-88 light meter. The algal cultures were in their exponential growth phase when were used for feeding copepods.

2.2. *B. similis* stock culture

B. similis were initially obtained from a plankton tow performed at the mouth of the Ross River in Townsville, Northern Queensland, Australia, on December 5th, 2008. Immediately after collection, plankton samples were transported back to a laboratory at James Cook University and *B. similis* were isolated from the rest of the zooplankton. *B. similis* cultures were gradually scaled up and eventually kept in four 20 L carboys filled with 1 µm filtered seawater and gentle aeration. Salinity was 30 ± 1‰ and the culture temperature was maintained at 26 ± 1 °C. Light intensity was about 700 lx on a light:dark cycle of 12 h:12 h. About 20% of the culture water was exchanged daily using a siphon with a 22 µm mesh attached to the end to prevent removal of copepods. *B. similis* were fed daily with a mixture of 3 microalgae: the Tahitian strain of *Isochrysis* sp. (T-Iso), *Tetraselmis chuii* (Tet) and *Pavlova* 50 (Pav) at an equal ratio of biomass (i.e. T-Iso: Tet: Pav = 1:1:1). Carboys containing the copepod stock cultures were completely drained approximately every 10 days to remove detritus while copepods were retained on a 150 µm submerged sieve. Carboys were then cleaned and sterilised with chlorine before cultures were restarted.

2.3. Experimental design and setup

Three separate experiments were carried out to assess the influence of various microalgal diets and their combinations on major parameters related to *B. similis* culture productivity, i.e. (1) egg production rate, (2) egg hatching rate and (3) population increase over a 12 day culture period.

For all 3 experiments, the same 10 microalgal diets were used, including 4 mono-algal, 5 binary algal and 1 tri-algal diet. Details of these diet treatments were as follows:

- Diet 1: The Tahitian strain of *Isochrysis* sp. (T-Iso)
- Diet 2: *Tetraselmis chuii* (Tet)
- Diet 3: *Pavlova* 50 (Pav)
- Diet 4: *Chaetoceros muelleri* (Chaet)
- Diet 5: *Tetraselmis chuii* + *Isochrysis* sp. (Tet + T-Iso) (1 :1)
- Diet 6: *Tetraselmis chuii* + *Pavlova* 50 (Tet + Pav) (1 :1)
- Diet 7: *Isochrysis* sp. + *Pavlova* 50 (T-Iso + Pav) (1:1)
- Diet 8: *Chaetoceros muelleri* + *Tetraselmis chuii* (Chaet + Tet) (1:1)
- Diet 9: *Chaetoceros muelleri* + *Pavlova* 50 (Chaet + Pav) (1:1)
- Diet 10: *Isochrysis* sp. + *Tetraselmis chuii* + *Pavlova* 50 (T-Iso + Tet + Pav) (1:1:1)

All experiments were carried out under similar conditions as described for the stock cultures, (i.e. 26 ± 1 °C; 27 ± 1‰ and photoperiod 12 h:12 h). Microalgal concentrations were determined daily using a haemocytometer under a microscope (Leica CME). For all experiments, *B. similis* were fed with the different microalgal diets on an equal biomass based on carbon concentrations which were calculated for each species according to Strathmann (1967). Algae were fed to cultures at about 1500 µgC L⁻¹, a carbon concentration known

to saturate copepod feeding (Kjørboe et al. 1985). When *B. similis* were fed with a binary or a tri-algal diet, carbon concentration was divided equally between the 2 or 3 algae offered based on their biomass.

2.3.1. Egg production experiment

B. similis adults and late copepodites were obtained from four 20 L carboy stock cultures by draining culture water through a 150 µm sieve. Animals caught on the sieve were immediately resuspended in a Petri dish with a small amount of seawater. Groups of *B. similis* were then randomly captured using a broad-tipped pipette and distributed into thirty 1 L beakers, with groups of 3 beakers assigned to each diet treatment as replicates. *B. similis* were then fed daily in excess for 3 days with the designated algal diets to acclimatize them to respective diets and to remove any potential residual effects of previous diets.

Following 3 days of acclimatization, individual *B. similis* females that were actively swimming with intact appendages were randomly selected from beakers of a given diet and carefully transferred to 30 mL Petri dishes in order to monitor their daily egg output. There were 6 replicates per tested diet and hence a total of 60 Petri dishes. After 24 h, *B. similis* females were removed from each Petri dish and eggs they produced were counted using a Sedgewick Rafter counting cell and a compound microscope.

Following the procedure described above, new females were randomly selected daily from the stock culture beakers and transferred into a new set of sixty 30 ml Petri dishes containing fresh filtered seawater and algal diets to obtain individual 24 h egg output for each of 4 consecutive days. Daily replacement of experimental females from the pre-conditioned mixed population ensured that experimental animals were fertilised and healthy prior to introduction to the Petri dishes for the egg production experiment. For each treatment, 24 h female Egg Production Rate (EPR) was subsequently calculated by averaging data from all 6 females over 4 days.

2.3.2. Egg hatching success experiment

As in the egg production experiment, groups of *B. similis* were randomly selected from the carboy cultures and transferred into 30 1-L beakers and fed with 10 different algal diets (3 replicate beakers per treatment). After 2 days, eggs produced over the period were discarded by thoroughly syphoning the bottom of each beaker with a siphon that had a 60 µm mesh attached to the end to prevent copepodites and adults being siphoned out. This procedure ensured that eggs presented in the beakers the next day were all produced within 24 h. The following day, freshly produced eggs in each beaker were carefully sieved out using a 25 µm mesh and counted before being randomly distributed into fifty 30 ml containers for incubation at room temperature (26 ± 1 °C). There were 5 replicates (containers) per diet treatment with each replicate containing 40 to 60 eggs.

Egg hatching success (%) was estimated for each microalgal diet by calculating the difference between the number of eggs introduced and the number of unhatched eggs after 48 and 96 h incubation period respectively (Camus and Zeng, 2009). Counting of *B. similis* eggs was made using a Sedgewick Rafter counting cell as above.

2.3.3. Population increase experiment

As in the previous experiments, *B. similis* were acclimatized to each diet for 3 days in 1 L beakers before being used for the experiment. Upon completion of the acclimatization period, 10 mature *B. similis* adults (3 males and 7 females) were introduced into each 500 mL beaker, for the population increase experiment. A total of 50 replicate beakers were established with 5 replicates for each treatment. For the following 12 days, *B. similis* were fed daily with the designated diets and approximately 30% of the culture water was exchanged by gently syphoning. The siphon had a 25 µm mesh

attached to the end to prevent the removal of any eggs or post-egg-stages of *B. similis* from the culture.

After 12 days, content from each beaker was drained through a 25 µm sieve and all eggs, nauplii, copepodites and adults retained were fixed with 10% formalin and stored for later counting. *B. similis* eggs, nauplii, copepodites and adults were counted in each replicate and the final population estimated as the average of five replicates. Based on Fenchel (1974), the intrinsic rate of population increase r was then calculated for each treatment using the formulation: $r = \ln(N_0/N_1)/t$, where N_0 = population number at the beginning of the experiment, N_1 = population number at the end of the experiment while t (days) is the duration of the experiment.

2.4. Data collection and analysis

Data from all experiments were analysed using one-way ANOVA. When significant differences ($p < 0.05$) were found, Tukey's multiple comparisons test was used to determine specific differences among treatments ($p < 0.05$). All statistical analyses were conducted using Statistica version 7. Data are presented as mean \pm standard error (SE).

3. Results

3.1. Egg production

When fed 10 different microalgal diets, 24 h egg production rates (EPR; eggs female⁻¹ day⁻¹) of *B. similis* during 4 consecutive days are presented in Table 1. As no significant differences in daily egg production was detected within each diet treatment, data were pooled to calculate the overall mean 24 h EPR (Fig. 1). The results show that maternal food significantly impacted *B. similis* egg output ($p < 0.001$): the highest EPR (44.1 ± 2.8 eggs female⁻¹ day⁻¹) was produced by the tri-algal diet treatment (T-Iso + Tet + Pav), which was more than 4 times higher than that of the lowest EPR (10.0 ± 1.0 eggs female⁻¹ day⁻¹) recorded for the diatom *Chaetoceros muelleri* (Chaet). EPR in the tri-algal T-Iso + Tet + Pav treatment was significantly higher than other diet treatments ($p < 0.05$). Two binary algal treatments, T-Iso + Pav and Tet + Pav, were the next best diets, with EPR at 30.9 ± 3.1 and 30.4 ± 1.7 eggs female⁻¹ day⁻¹ respectively. In turn, these were both superior to the rest of the diet treatments ($p < 0.05$), except that of Tet + T-Iso (23.2 ± 1.6 eggs female⁻¹ day⁻¹) ($p > 0.05$) (Fig. 1). The lowest EPR produced by the Chaet treatment was significantly lower than all other diet treatments, except that of Tet and Tet + Chaet treatments (12.9 ± 1.0 and 16.6 ± 1.1 eggs female⁻¹ day⁻¹ respectively) ($p > 0.05$) (Fig. 1).

Table 1

Mean egg production rate (eggs female⁻¹ day⁻¹) of *Bestiolina similis* fed 10 different micro-algal diets over 4 consecutive days.

Microalgal Diet	Average daily Egg Production Rate			
	Day 1	Day 2	Day 3	Day 4
T-Iso + Tet + Pav	35.8 \pm 1.9	37.4 \pm 1.3	54.8 \pm 2.1	48.4 \pm 2.2
T-Iso + Pav	23.7 \pm 3.8	28.5 \pm 2.7	37.6 \pm 3.5	31.2 \pm 1.5
Tet + Pav	25.8 \pm 1.5	32.3 \pm 2.1	33.0 \pm 1.4	30.2 \pm 1.1
T-Iso + Tet	19.2 \pm 1.3	27.5 \pm 2.1	27.0 \pm 1.2	20.5 \pm 1.1
Chaet + Pav	22.2 \pm 1.0	20.5 \pm 1.5	17.8 \pm 0.9	14.8 \pm 1.3
Chaet + Tet	14.8 \pm 1.0	14.8 \pm 1.5	18.2 \pm 0.9	18.4 \pm 0.6
Pav	20.2 \pm 0.9	19.3 \pm 1.9	23.2 \pm 1.5	21.3 \pm 1.1
T-Iso	12.5 \pm 1.1	19.8 \pm 0.9	22.5 \pm 1.2	19.8 \pm 0.8
Tet	14.3 \pm 1.4	12.7 \pm 1.5	13.2 \pm 0.3	11.8 \pm 0.7
Chaet	9.6 \pm 1.1	11.3 \pm 0.6	10.6 \pm 1.1	8.6 \pm 0.6

Data are presented as mean \pm St errors.

Daily egg production rates were averaged from 6 females over 4 days.

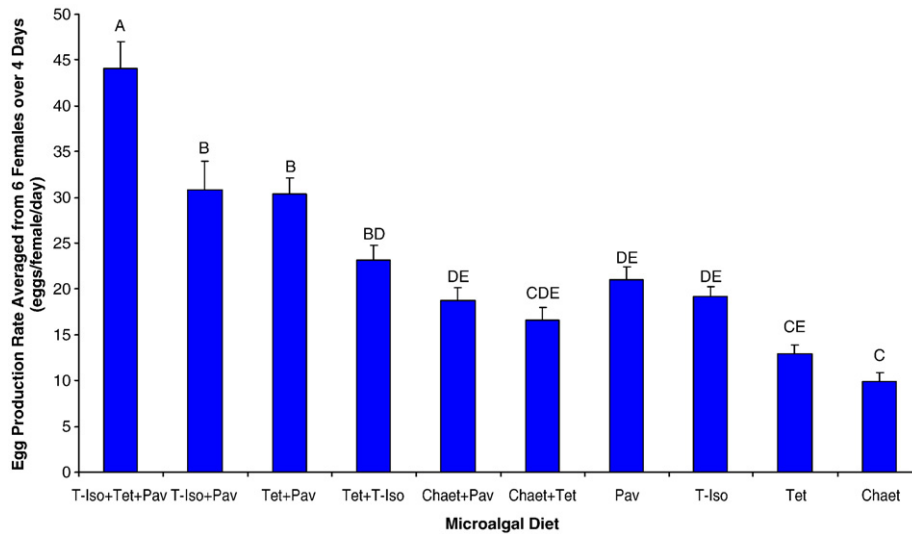


Fig. 1. Effects of 10 microalgal diets on average 24 h egg production rate of *Bestiolina similis*. For each treatment, 24 h egg production rates were averaged from 6 females over 4 days. Data are presented as mean \pm S.E. Different letters on the tops of bars indicate significant differences ($p < 0.05$).

3.2. Egg hatching rates

Microalgal diets significantly influenced both 48 and 96 h egg hatching rates (EHR) of *B. similis* ($p < 0.001$) (Fig. 2). The 48 h EHR ranged from $65.4 \pm 2.4\%$ (Chaet) to $91.0 \pm 2.0\%$ (T-Iso + Tet + Pav) for the diets tested. The highest EHR recorded for the tri-algal T-Iso + Tet + Pav was significantly higher ($p < 0.05$) than the rest of treatments except for the binary diets of T-Iso + Pav, Tet + Pav and Tet + T-Iso, as well as the monoalgal diet T-Iso ($p > 0.05$). While the diatom Chaet produced the lowest 48 h EHR, it did not differ significantly from the Chaet + Tet and the Tet treatments ($73.6 \pm 3.2\%$ and $70.1 \pm 1.5\%$ respectively) ($p > 0.05$).

Compared to 48 h EHR, 96 h hatching rates improved across all diet treatments with EHR higher than 85% recorded in 8 of the 10 microalgal diet treatments (Fig. 2). The highest 96 h EHR was still the tri-algal diet T-Iso + Tet + Pav ($96.3\% \pm 0.4$), although now it was only significantly higher than the Tet and Chaet treatments ($p < 0.05$). Indeed, no significant differences in 96 h EHR was found among 8 of the 10 diets tested, i.e. T-Iso + Tet + Pav, T-Iso + Pav, Tet + Pav, Tet + T-Iso, Chaet + Pav, Chaet + Tet, Pav and T-Iso ($p > 0.05$). The monoalgal Chaet produced the lowest and significantly inferior 96 h EHR ($73.4\% \pm 3.6$), which was only not significantly lower than that of the Tet treatment ($83.3\% \pm 2.7$) ($p > 0.05$) (Fig. 2).

3.3. Population increase

After 12 days of culture on different algal diets, the average final population numbers of *B. similis* are presented in three categories, i.e. 'All Stages Included' (i.e. including eggs); 'All Post-Egg-Stages' (i.e. excluding eggs); and 'Adult Only' (Table 2). When 'All Stages Included', the final number of *B. similis* population was highest for the Pav + Tet + T-Iso treatment (886.8 ± 139.4), significantly higher than all other diet treatments ($p < 0.05$). The second most productive diet was Tet + T-Iso (541 ± 53.6) although it was not statistically different from T-Iso + Pav (479.0 ± 54.1), Chaet + Pav (285.4 ± 42.2) and the monoalgal diet T-Iso (334.4 ± 43.9) ($p > 0.05$). The Tet and Tet + Chaet treatments produced the lowest population number at 26.0 ± 2.3 and 42.8 ± 8.4 respectively, significantly lower than all other treatments ($p < 0.05$), except that of the Chaet, Pav, Chaet + Pav and Tet + Pav treatments. Excluding eggs from the final population counts resulted in sustainably reduced final population number (i.e. All Post-Egg-Stages) for all treatments as eggs represented the highest counts among all life stages in all treatments. However, similar trend and statistics of dietary effects on the final population number remained (Table 2).

It is worth noting that while algal combinations generally provided better population increase over 12 days, this was not always the case for

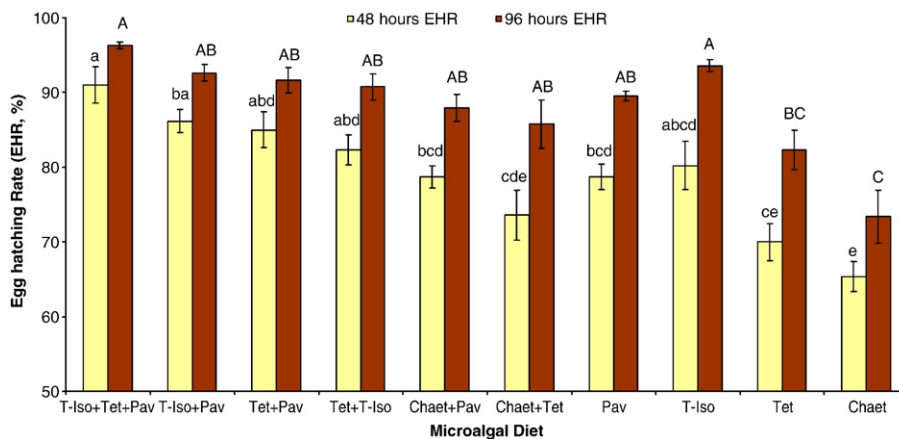


Fig. 2. The 48 h and 96 h egg hatching rates (%) of eggs produced by *Bestiolina similis* fed with 10 different microalgal diets. Data are presented as mean \pm SE. Different letters on the tops of bars indicate significant difference ($p < 0.05$).

Table 2

Final population and intrinsic rate (*r*) of population increase of *B. similis* cultured over a 12 days period fed on different microalgal diets.

Diet	Final Population Number when All Stages Included	<i>r</i> (All Stage Included)	Final Population number of All Post-Egg-Stages	<i>r</i> (All Post-Egg-Stages)	Final Number of Adults	<i>r</i> (Adults Only)
T-Iso + Tet + Pav	886.8 ± 139.4 ^a	0.37 ± 0.03 ^a	478.2 ± 63.4 ^a	0.32 ± 0.02 ^a	79.6 ± 16.2 ^a	0.17 ± 0.04 ^a
T-Iso + Pav	479.0 ± 54.1 ^{bc}	0.32 ± 0.02 ^{bc}	227.0 ± 41.7 ^{bc}	0.26 ± 0.03 ^{bc}	35.0 ± 5.2 ^b	0.10 ± 0.03 ^b
Tet + Pav	265.0 ± 34.9 ^{cde}	0.27 ± 0.03 ^{cde}	89.8 ± 9.2 ^{cde}	0.18 ± 0.02 ^{cde}	18.0 ± 1.2 ^{bc}	0.05 ± 0.02 ^{bc}
Tet + T-Iso	541.0 ± 53.6 ^b	0.33 ± 0.02 ^b	260.2 ± 36.1 ^b	0.27 ± 0.03 ^b	33.6 ± 5.5 ^{bc}	0.10 ± 0.05 ^{bc}
Chaet + Pav	285.4 ± 42.3 ^{bcde}	0.28 ± 0.03 ^{bcde}	125.4 ± 22.4 ^{bcde}	0.21 ± 0.03 ^{bcde}	18.2 ± 4.8 ^{bc}	0.04 ± 0.05 ^{bc}
Chaet + Tet	42.8 ± 8.4 ^e	0.11 ± 0.04 ^e	26.6 ± 5.6 ^e	0.07 ± 0.04 ^e	2.6 ± 0.5 ^{bc}	0.12 ± 0.04 ^{bc}
Pav	213.4 ± 37.5 ^{cde}	0.25 ± 0.03 ^{cde}	132.8 ± 29.7 ^{bcde}	0.21 ± 0.03 ^{bcde}	16.6 ± 4.5 ^{bc}	0.03 ± 0.04 ^{bc}
T-Iso	334.4 ± 43.9 ^{bcd}	0.29 ± 0.03 ^{bcd}	175.4 ± 26.4 ^{bcd}	0.23 ± 0.03 ^{bcd}	32.6 ± 5.6 ^{bc}	0.10 ± 0.04 ^{bc}
Tet	26.0 ± 2.3 ^e	0.08 ± 0.02 ^e	10.8 ± 1.0 ^e	0.01 ± 0.02 ^e	1.2 ± 0.3 ^c	0.13 ± 0.08 ^c
Chaet	110.2 ± 25.1 ^{de}	0.19 ± 0.04 ^{de}	55.6 ± 16.0 ^{de}	0.13 ± 0.05 ^{de}	6.8 ± 1.8 ^{bc}	0.05 ± 0.06 ^{bc}

Data are presented as Mean ± S.E.; different superscript letters in a same column indicate significant differences (*p* < 0.05).

some binary diet treatments. For example, the mono-algal diet T-Iso was ranked 4th of all 10 diets tested, producing better population increase than the binary algal diets Tet + Pav, Chaet + Tet and Chaet + Pav. Although the differences were not statistically significant for the Tet + Pav and Chaet + Tet treatments (*p* > 0.05), it was significantly higher than the Chaet + Pav treatment (*p* < 0.05) (Table 2). Furthermore, no significant difference in population increase was found between the monoalgal diets Pav, Chaet and Tet when compared to the binary algal diets of Tet + Pav, Chaet + Tet and Chaet + Pav (*p* > 0.05) (Table 2).

The intrinsic rates of population increase (*r*) of *B. similis* was calculated for all treatments (Table 2). When All-Stage-Included, it ranged from 0.08 ± 0.02 for Tet to 0.37 ± 0.03 for T-Iso + Tet + Pav. If eggs were excluded from final population counts, it ranged from 0.01 ± 0.02 (Tet) to 0.32 ± 0.02 (T-Iso + Tet + Pav) (Table 2).

A breakdown of *B. similis* final population composition based on 4 groups of life stages (i.e. eggs, nauplii, copepodites and adults) is shown in Fig. 3. The results show that the Pav + Tet + T-Iso treatment produced the highest numbers across all life stages and the differences among treatments were often statistically significant (*p* < 0.05) (Fig. 3).

4. Discussion

Selecting appropriate microalgal diet is crucial to the productivity of cultures of the tropical paracalanid copepod *B. similis*. In all current experiments, *B. similis* were acclimatized to experimental diets for 3 days prior to the start of any experiments. Such an acclimation

period was chosen based on Sedlacek and Marcus (2005) as well as a pilot starvation trial which showed that few *B. similis* could resist starvation for more than 3 days. As most past literature investigating effects of diets on copepod egg production and hatching success adopted pre-conditioning period of less than 48 h (e.g. Kang and Poulet, 2000; McKinnon et al., 2003; Shin et al., 2003), a 3 days acclimatisation period was judged sufficient. Among the 10 algal diets tested, the tri-algal diet T-Iso + Tet + Pav consistently performed better than others, with significantly higher mean 24 h egg production rate and population increase over 12 days as well as the highest 48 and 96 h egg hatching rates. The combination of 3 algae is likely to provide a more comprehensive and better balanced diet and is therefore recommended for the intensive production of *B. similis*. Among the binary diets, the Tet + T-Iso and Pav + T-Iso performed relatively well. As for the mono-algal diets, T-Iso stood out, while Pav also achieve reasonably good results. This suggests that both species may be used as monoalgal diet for keeping *B. similis* stock cultures at relatively low densities during non-production seasons in hatcheries to save costs and facilities required for producing multiple algal species.

Previous research on calanoid copepods have demonstrated clear effects of food quality on culture productivity (e.g. Kleppel et al., 1998; Morehead et al., 2005; Lee et al., 2006; Milione and Zeng, 2007). Egg production is one of the principal factors determining copepod culture productivity and has been linked to the maternal nutrition (Castro-Longoria, 2003), especially n-3 polyunsaturated fatty acids (PUFAs),

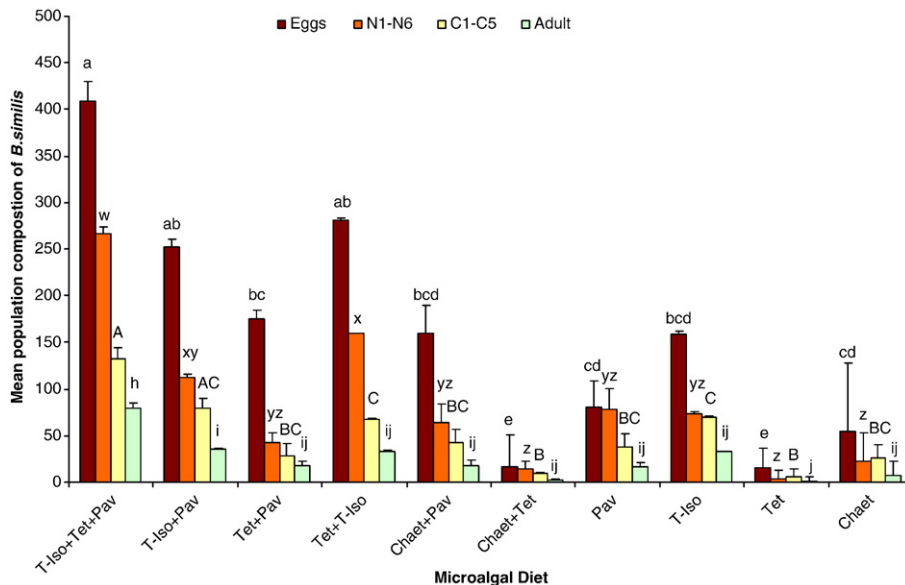


Fig. 3. Effects of microalgal diets on average population composition of *Bestiolina similis* over a 12 day culture period. The experiment started with 10 adult (7 females and 3 males) of *B. similis*. Data are presented as mean ± SE. Different letters on the tops of bars indicate significant difference (*p* < 0.05). N1–N6: naupliar stage 1 to 6; C1–C5: copepodite stage 1 to 5.

such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) (Milione and Zeng, 2007). PUFAs are known to affect hatching success and embryonic development in crustaceans and molluscs, as well as playing important roles in cell membrane functions (Lacoste et al., 2001; Anderson and De Silva, 2003). However, their exact functions for copepods is still unclear, as dietary functions of PUFAs were largely established for decapod crustaceans and were only assumed to be applicable to copepods (Lacoste et al., 2001). Some researchers indicated that no clear correlations existed between copepod fertility and their dietary EPA and DHA concentrations (Koski et al., 1998; Lee et al., 1999). Furthermore, traces of DHA and EPA were found in *Calanus* eggs when parental stock were fed diets lacking of these essential fatty acids, indicating that at least some copepod species are capable of synthesising DHA and EPA from dietary precursors when they were lacking in their diet (Lacoste et al., 2001). Table 3 summarizes the fatty acid contents of the algal species used in present study, and it can be seen that although the algae *Pavlova* 50 (Pav) possesses a relatively balanced EPA to DHA profile, this did not necessarily lead to significantly higher EPR or hatching rate when compared to T-Iso based on present results.

The range of egg production rate (EPR) found in current study for *B. similis*, calculated from 6 replicate females over 4 days, are either comparable or substantially better than those observed in the wild. McKinnon and Duggan (2001) reported egg outputs ranging from 8.5 to 17.3 eggs⁻¹ female⁻¹ day⁻¹, increasing to a maximum of 37.2 eggs female⁻¹ day⁻¹ for *B. similis* in waters adjacent to Australia's North-West Cape, and a mean EPR of 25 eggs female⁻¹ day⁻¹ was reported from the waters of the Great Barrier Reef (McKinnon et al., 2003). In the present experiment, a four day average of 44.1 eggs female⁻¹ day⁻¹ and a maximum of 54.8 eggs female⁻¹ day⁻¹ on a single day was found for *B. similis* fed on the diet of T-Iso + Tet + Pav. Although a maximum EPR of 48 eggs female⁻¹ day⁻¹ has also been reported by McKinnon et al (2003) when feeding *B. similis* with *Heterocapsa niei* (Dinophyceae), this algal species is known to be difficult to culture.

McKinnon et al (2003) has further reported that a mono-algal diet of *Pavlova salina* led to low EPR for *B. similis* although this species of algae possesses a good nutritional profile. In the present study, *Pavlova* 50, a different strain of *Pavlova*, was found to support reasonably good EPR and EHS as a monoalgal diet. However, dead *B. similis* were sometimes found next to their moult during culture, indicating that Pav might be nutritionally deficient in promoting successful moulting, especially for the moult from N-6 to the first copepodite stage. This was not observed when *B. similis* were fed the monoalgal T-Iso, and may be explained by the lower DHA to EPA ratio of Pav (Table 3), which could impede moulting when offered as the sole food source.

It is worth noting that difference in hatching rates between 48 and 96 h were much more substantial for treatments with low 48 h EHR, leading to no significant differences in 96 h EHR with T-Iso + Tet + Pav in several treatments that 48 h EHR were significantly lower than T-Iso + Tet + Pav (e.g. Chaet + Tet, Chaet + Pav and Pav). This result points to the possibility that better nutrition provided by the tri-algal

diet might lead to faster embryonic development than some of the other diets.

The diatom *Chaetoceros muelleri* provided significantly lower EPR and EHR when compared to T-Iso and Pav. Interestingly, McKinnon et al. (2003) found that *C. muelleri* produced the best EPR when compared to *Pavlova*, *Isochrysis* and *Tetraselmis*. This could be explained by different feeding ration or experimental protocols. Diatoms have been recently revealed potentially toxic to some copepods (Miralto et al., 1999), and are now associated with negative effects on reproduction for several species, although it is often difficult to differentiate diatom toxicity from their low nutrition quality (Irigoien et al., 1998; Jones and Flynn, 2005). It appears that the diatom Chaet may have affected egg formation of *B. similis*, as improperly formed eggs, often in batches of 3 instead of the usual 4, with smaller diameter or more transparent appearance were regularly observed during the egg production experiment, which may have contributed to the lowest hatching rate observed for the diet among all others.

Tetraselmis chunii (Tet) has been reported as a poor diet for paracalanid egg production (McKinnon et al., 2003) and this was confirmed in current experiments. Tet provided the second lowest EPR and EHR, as well as the poorest population increase, with on average less than 2 adults and fewer than 20 post-egg-stages remaining after 12 days of culture. This suggests that a total crash might happen if Tet is offered as a sole food source for *B. similis*. Such a poor result may be attributed to the fact that Tet has relatively big cell size (Table 3), therefore may not be easily ingested by *B. similis* early naupliar stages. However, Milione and Zeng (2007) reported that Tet as a mono-algal diet did not lead to mass naupliar mortality when fed to *Acartia sinjiensis* and could produce reasonably good population increase over a 8 day culture period. Such a clear difference confirms that the suitability of micro-algal diets for various copepods is species-specific, and that feeding experiments are required for each species that is considered having culture potential.

Regular culture dilution was suggested as a way to produce high number of *B. similis* nauplii by VanderLugt and Lenz (2008), these authors reported that high adult density impaired the production of nauplii, leading to subsequent population crash within a few days. However, *Isochrysis* was the only alga offered to *B. similis* during their study and hence, nutritional deficiencies might also have contributed to crashes of populations at high culture densities. A combined algal diet may help improve the situation.

In summary, copepod reproduction and subsequent development involve multiple life stages and biological processes for which the best suited diet may vary at different life stages or for different biological functions. On this basis, a single species of microalgae may become nutritionally limiting whereas appropriate combinations of algae are likely to offer better balance of required nutrients. However, this does not mean that any combinations of microalgae will lead to better culture productivity. As illustrated in the present study, some mono-algal diets were actually equally or even more productive than some of binary algal diets used for *B. similis* culture. Hence, comprehensive

Table 3
Major features and fatty acid composition of the four microalgae used as diets for *Bestiolina similis* in the present study.

Species name	Class of algae	Acronym	Strain Code	Cell Size (µm)	Total fatty acids (pg/cell)	DHA 22:6n-3 (% total fatty acids)	EPA 20:5n-3 (% total fatty acids)	Linolenic acid 18:3n-3 (% total fatty acids)	Reference
<i>Chaetoceros muelleri</i>	Bacillariophyceae	Chaet	CS-176	7.0	2.23	0.8	12.8	0.3	Zhukova and Aizdaicher, 1995
<i>Tetraselmis chunii</i>	Prasinophyceae	Tet	CS-26	13.0±2.0	2.7–4.5	Trace	4.3–10.8	11.1–21.7	Pernet et al., 2003 Muller-Fuega et al., 2003 Renaud et al. (1999)
Tahitian strain of <i>Isochrysis</i> sp.	Prymnesiophyceae	T-Iso	CS-177	5.0±0.8	1.2–1.46	6.4–25.9	0.2–0.5	3.6–7.0	Fernandez-Reiriz et al. (1989) Pernet et al. (2003)
<i>Pavlova</i> 50	Prymnesiophyceae	Pav	CS-50	4.5±1.0	1.0–2.7	8.4–9.2	23.5–25.0	1.4–2.0	Volkman et al. (1991)

research is clearly needed to gain better understanding of the best feeding practice for any candidate copepod species that is considered having potential in aquaculture hatcheries.

Finally, it is worth noting that this study was conducted at laboratory scale over relatively short periods of time, therefore the results might not be fully reproducible in large-scale cultures. However, it clearly served the purpose of identifying the optimal microalgal diets for culturing *B. similis*, which are likely to be applicable in larger-scale cultures. Based on the findings of this study, to achieve maximum productivity of *B. similis* culture for aquaculture purposes, the species should be cultured using the tri-algal diet T-Iso + Pav + Tet.

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References

- Anderson, T., De Silva, S., 2003. Nutrition. In: Lucas, J.S., Southgate, P.C. (Eds.), *Aquaculture: Farming Aquatic Plants and Animals*. Blackwell, Oxford, pp. 146–171.
- Boxshall, G.A., Halsey, S.H., 2004. An introduction to copepod diversity. *Ray Society Series 166*. With S. H. Halsey. 2 Parts [Vols]. The Ray Society, London, U.K. 940 pp. [Part I 421 p., Part II 519 p].
- Calbet, A., Alcaraz, M., 1996. Effects of constant and fluctuating food supply on egg production rates of *Acartia grani* (Copepoda: Calanoida). *Mar. Ecol. Prog. Ser.* 140, 33–39.
- Camus, T., Zeng, C., 2008. Effects of photoperiod on egg production and hatching success, naupliar and copepodite development, adult sex ratio and life expectancy of the tropical calanoid copepod *Acartia sinjiensis*. *Aquaculture* 280, 220–226.
- Camus, T., Zeng, C., 2009. The effects of stocking density on egg production and hatching success, cannibalism rate, sex ratio and population growth of the tropical calanoid copepod *Acartia sinjiensis*. *Aquaculture* 287, 145–151.
- Castro-Longoria, E., 2003. Egg production and hatching success of four *Acartia* species under different temperature and salinity regimes. *J. Crust. Biol.* 23, 289–299.
- Chen, Q., Sheng, J., Lin, Q., Gao, Y., Lv, J., 2006. Effect of salinity on reproduction and survival of the copepod *Pseudodiaptomus annandalei* Sezall, 1919. *Aquaculture* 258, 575–582.
- Chesney, E.J., 2005. Copepods as live prey: a review of factors that influence the feeding success of marine fish larvae. In: Lee, C.-S., O'Brien, P.J., Marcus, N.H. (Eds.), *Copepods in aquaculture*. Blackwell Scientific Publication Ltd, Melbourne, pp. 133–150.
- Evjemo, Ovej, Reitan, IngeK., Olsen, Y., 2003. Copepods as live food organisms in the larval rearing of halibut larvae (*Hippoglossus hippoglossus* L.) with special emphasis on the nutritional value. *Aquaculture* 227, 191–210.
- Fenchel, T., 1974. Intrinsic rate of natural increase: the relationship with body size. *Oecologia* 14, 317–326.
- Fernandez-Reiriz, M.J., Perez-Camacho, A., Ferreira, M.J., Blanco, J., Planas, M., Campos, M.J., Labarta, U., 1989. Biomass production and variation in the biochemical profile (total protein, carbohydrates, RNA, lipids and fatty acids) of seven species of marine microalgae. *Aquaculture* 83, 17–37.
- Guillard, R.R.L., Ryther, J.H., 1962. Studies on marine planktonic diatoms: I. *Cyclotella nana* (Hustedt), and *Detonula confervacea* (Cleve) Can. *J. Microbiol.* 8, 229–239.
- Hernandez Molejon, O.G., Alvarez-Lajonchere, L., 2003. Culture experiments with *Oithona oculata* Farran, 1913 (Copepoda : Cyclopoida), and its advantages as food for marine fish larvae. *Aquaculture* 219, 471–483.
- Holste, L., Peck, M.A., 2006. The effects of temperature and salinity on egg production and hatching success of Baltic *Acartia tonsa* (Copepoda: Calanoida): a laboratory investigation. *Mar. Biol.* 148, 1061–1070.
- Irigoin, X., Verheye, H.M., Harris, R.P., Harbour, D., 1998. Effect of food composition on egg production and hatching success rate of two copepod species (*Calanoides carinatus* and *Rhincalanus nasutus*) in the Benguela upwelling system. *J. Plankton Res.* 27, 735–742.
- Jones, R.H., Flynn, K.J., 2005. Nutritional status and diet composition affect the value of diatoms as copepod prey. *Science* 307, 1457–1459.
- Kang, H., Poulet, S.A., 2000. Reproductive success in *Calanus helgolandicus* as a function of diet and egg cannibalism. *Mar. Ecol. Prog. Ser.* 201, 241–250.
- Kjørboe, T., Mohlenberg, F., Hamburger, K., 1985. Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. *Mar. Ecol. Prog. Ser.* 26, 85–97.
- Kjørboe, T., 1997. Population regulation and role of mesozooplankton in shaping marine pelagic food webs. *Hydrobiologia* 363, 13–27.
- Kleppel, G.S., Burkart, C.A., Houchin, L., 1998. Nutrition and the regulation of egg production in the calanoid copepod *Acartia tonsa*. *Limnol. Oceanogr.* 43, 1000–1007.
- Knuckey, R.M., Semmens, G.L., Mayer, R.J., Rimmer, M.A., 2005. Development of an optimal microalgal diet for the culture of the calanoid copepod *Acartia sinjiensis*: effect of algal species and feed concentration on copepod development. *Aquaculture* 249, 339–351.
- Koski, M., Klein Breteler, W., Schogt, N., 1998. Effect of food quality on rate of growth and development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda, Calanoida). *Mar. Ecol. Prog. Ser.* 170, 169–187.
- Koski, M., Kuosa, H., 1999. The effect of temperature, food concentration and female size on the egg production of the planktonic copepod *Acartia bifilosa*. *J. Plankton Res.* 21, 1779–1789.
- Lacoste, A., Poulet, S.A., Cuffe, A., Kattner, G., Ianora, A., Laabir, M., 2001. New evidence of the copepod maternal food effects on reproduction. *J. Exp. Mar. Biol. Ecol.* 259, 85–107.
- Leandro, S.M., Tiselius, P., Queiroga, H., 2006. Growth and development of nauplii and copepodites of the estuarine copepod *Acartia tonsa* from southern Europe (Ria de Aveiro, Portugal) under saturating food conditions. *Mar. Biol.* 150, 121–129.
- Lee, H.W., Ban, S., Ando, Y., Ota, T., Ikeda, T., 1999. Deleterious effect of diatom diets on egg production and hatching success in the marine copepod *Pseudocalanus newmani*. *Plankton Biol. Ecol.* 46, 104–112.
- Lee, K.W., Park, H.G., Lee, S.M., Kang, H.K., 2006. Effects of diets of the growth of the brackish water cyclopoid copepod *Paracyclops nana* Smirnov. *Aquaculture* 256, 346–353.
- Lincoln, J.A., Turner, J.T., Bates, S.S., Leger, C., Gauthier, D.A., 2001. Feeding, egg production, and egg hatching success of the copepods *Acartia tonsa* and *Temora longicornis* on diets of the toxic diatom *Pseudo-nitzschia multiseries* and the non-toxic diatom *Pseudo-nitzschia pungens*. *Hydrobiologia* 453, 107–120.
- Marcus, N.H., Murray, M., 2001. Copepod diapause eggs: a potential source of nauplii for aquaculture. *Aquaculture* 201, 107–115.
- Marcus, N.H., Richmond, C., Sedlack, C., Miller, G.A., Oppert, C., 2004. Impact of hypoxia on the survival, egg production and population dynamics of *Acartia tonsa* Dana. *J. Exp. Mar. Biol. Ecol.* 301, 111–128.
- McKinnon, A.D., Duggan, S., 2001. Summer egg production rates of paracalanid copepods in subtropical waters adjacent to Australia's North West Cape. *Hydrobiologia* 453 (454), 121–132.
- McKinnon, D., Duggan, S., Nichol, P.D., Rimmer, M.A., Semmens, G., Robin, B., 2003. The potential of tropical paracalanoid copepods as live feeds in aquaculture. *Aquaculture* 223, 89–106.
- Milione, M., Zeng, C., 2007. The effects of algal diets on population growth and egg hatching success of the tropical calanoid copepod, *Acartia sinjiensis*. *Aquaculture* 271, 656–664.
- Miralto, A., Barone, G., Romano, G., Poulet, S.A., Ianora, I., Russo, G.L., Buttino, I., Mazzarella, G., Laabir, M., Cabrini, M., Glacobbé, M.G., 1999. The insidious effect of diatoms on copepod reproduction. *Nature* 402, 173–176.
- Morehead, D.T., Battaglene, S.C., Metillo, E.B., Bransden, M.P., Dunstan, G.A., 2005. Copepods as a live feed for striped trumpeter *Latris lineata* larvae. In: Lee, C.-S., O'Brien, P.J., Marcus, N.H. (Eds.), *Copepods in Aquaculture*. Blackwell Scientific Publications Ltd, Melbourne, pp. 195–208.
- Muller-Fuega, A., Moal, J., Kaas, R., 2003. The microalgae of aquaculture. In: Stottrup, J.G., McEvoy, L.A. (Eds.), *Live Feeds in Marine Aquaculture*. Blackwell Science, Oxford, pp. 206–243.
- O'Brien, P.J., Lee, C.S., 2005. Culture of copepods and applications to marine finfish larval rearing workshop discussion summary. In: Lee, C.S., O'Brien, P.J., Marcus, N.H. (Eds.), *Copepods in Aquaculture*. Blackwell, Melbourne, pp. 245–255.
- Payne, M.F., Ripplingale, R.J., 2000. Evaluation of diets for culture of the calanoid copepod *Gladioferens imparipes*. *Aquaculture* 187, 85–96.
- Payne, M.F., Ripplingale, R.J., 2001. Intensive cultivation of the calanoid copepod *Gladioferens imparipes*. *Aquaculture* 201, 329–342.
- Pernet, F., Tremblay, R., Demers, E., Roussy, M., 2003. Variation of lipid class and fatty acid composition of *Chaetoceros muelleri* and *Isochrysis* sp. Grown in a semicontinuous system. *Aquaculture* 221, 393–406.
- Renaud, S.M., Van-Thinh, L., Parry, D.L., 1999. The gross chemical composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. *Aquaculture* 170, 147–159.
- Sampey, A., McKinnon, A.D., Meekan, M.G., McCormick, M.I., 2007. Glimpse into guts: overview of the feeding of larvae of tropical shore fishes. *Mar. Ecol. Prog. Ser.* 339, 243–257.
- Sedlacek, C., Marcus, N.H., 2005. Egg production of the copepoda *Acartia tonsa*: the influence of hypoxia and food concentration. *J. Exp. Mar. Biol. Ecol.* 318, 183–190.
- Shin, K., Jang, M., Jang, P., Ju, S., Lee, T., Chang, M., 2003. Influence of food quality on egg production and viability of the marine planktonic copepod *Acartia omorii*. *Oceanography* 57, 265–277.
- Støttrup, J., 2000. The elusive copepods: their production and suitability in marine aquaculture. *Aquacult. Res.* 3, 702–711.
- Strathmann, R.R., 1967. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. *Limnol. Oceanogr.* 12, 411–418.
- Turner, J.T., Ianora, A., Miralto, A., Laabir, M., Esposito, F., 2001. Decoupling of copepod grazing rates, fecundity and egg-hatching success on mixed and alternating diatom and dinoflagellate diets. *Mar. Ecol. Prog. Ser.* 220, 187–199.
- Vanderlugt, K., Lenz, P.H., 2008. Management of nauplius production in the paracalanoid, *Bestiolina similis* (Crustacean: Copepoda): effect of stocking densities and culture dilution. *Aquaculture* 276, 69–77.
- Verity, P.G., Smetacek, V., 1996. Organism life cycles, predation, and the structure of marine pelagic ecosystems. *Mar. Ecol. Prog. Ser.* 130, 277–293.
- Volkman, J.K., Dunstan, J.K., Jeffrey, S.W., Kearney, P.S., 1991. Fatty acids from microalgae of the genus *Pavlova*. *Phytochem.* 30, 1855–1859.
- Zhukova, N.V., Aizdaicher, N.A., 1995. Fatty acid composition of 15 species of marine microalgae. *Phytochem.* 39, 351–356.