

**Roger Williams University**  
**DOCS@RWU**

---

Feinstein College of Arts & Sciences Faculty Papers

Feinstein College of Arts and Sciences

---

2009

# Effects of temperature on reproduction and survival of the calanoid copepod *Pseudodiaptomus pelagicus*

Andrew L. Rhyne

*Roger Williams University, arhyne@rwu.edu*

Cortney L. Ohs

*University of Florida*

Erik Stenn

*AlgaGen LLC*

Follow this and additional works at: [http://docs.rwu.edu/fcas\\_fp](http://docs.rwu.edu/fcas_fp)

 Part of the [Biology Commons](https://www.biologycommons.org/)

---

## Recommended Citation

Rhyne, A.L., C. Ohs, and E. Stenn. 2009. "The Effects of Temperature on Growth, Reproduction, and Production of the Calanoid Copepod, *Pseudodiaptomus Pelagicus*, a Species with Potential of Mass Production for use in Aquaculture." *Aquaculture* 292: 53-59.

This Article is brought to you for free and open access by the Feinstein College of Arts and Sciences at DOCS@RWU. It has been accepted for inclusion in Feinstein College of Arts & Sciences Faculty Papers by an authorized administrator of DOCS@RWU. For more information, please contact [mwu@rwu.edu](mailto:mwu@rwu.edu).



## Effects of temperature on reproduction and survival of the calanoid copepod *Pseudodiaptomus pelagicus*

Andrew L. Rhyne<sup>a,\*</sup>, Cortney L. Ohs<sup>a</sup>, Erik Stenn<sup>b,1</sup>

<sup>a</sup> University of Florida, Indian River Research and Education Center, 2199 South Rock Road, Fort Pierce FL 34945, USA

<sup>b</sup> AlgaGen LLC, 2336 20th Street, Vero Beach FL 32960, USA

### ARTICLE INFO

#### Article history:

Received 6 September 2008

Received in revised form 18 March 2009

Accepted 21 March 2009

#### Keywords:

Calanoid

Copepod

*Pseudodiaptomus pelagicus*

Temperature

### ABSTRACT

Four experiments were conducted on the calanoid copepod, *Pseudodiaptomus pelagicus*, to determine the effects of temperature (24, 26, 28, 30, 32, and 34 °C) on survival, development time, reproductive output, and population growth in order to define the optimal temperature for culture. The first experiment stocked early stage nauplii into 1 L beakers and cultured them using standard procedures until five days after the first mature adults were observed; from this survival, sex ratio, time to maturation, and fecundity were measured. The second and third experiments evaluated the effects of temperature on nauplii production by stocking individual pairs and 25 pairs of adults, respectively; in both experiments nauplii production was determined daily for 10 days. The fourth experiment determined the effects of temperature on population growth and composition of the population produced by stocking 10 adult pairs and culturing them for 10 days at six temperatures. Results indicate survival from early nauplii to adult was significantly affected by temperature and those cultured from 24–30 °C had the highest mean survival. Time to first maturation and maturation of the entire population was significantly influenced by temperature and took from 6.8 to 12.8 days. Temperature significantly affected nauplii production in both individual and groups of paired adults. Temperature affected the mean daily nauplii production by decreasing the brood interval but did not affect the mean brood size. The number of nauplii produced by 25 adult pairs was significantly influenced by temperature; the optimal temperature was 27.5 °C at which 1861 nauplii were produced. The distribution of developmental stages in the population was also affected by temperature; at lower temperatures the population consisted of a greater proportion of nauplii while at 32 °C the population was comprised of more advanced staged individuals. When developing production objectives, aquaculturists must consider temperature because it has multiple effects on the culture of *P. pelagicus*. The optimal temperature range to achieve high survival and the greatest nauplii production is 26–30 °C. To maintain long-term stock cultures the best temperature may be 24 °C to slow maturation and growth while 28–32 °C may be used to maximize nauplii production by decreasing time to maturation and decreasing brood intervals.

© 2009 Elsevier B.V. All rights reserved.

### 1. Introduction

In the wild, copepods are dominant prey items for the vast majority of marine fish larvae (Hunter, 1981; Leis, 1991; Østergaard et al., 2005; Sampey et al., 2007). *Brachionus* spp. (rotifers) or *Artemia* spp. (*Artemia*) are commonly used in aquaculture because of convenience and commercial availability (Hoff and Snell, 1999). Yet, *Artemia* nauplii and rotifers are deficient in essential highly unsaturated fatty acids for many larval and juvenile marine fishes without

the addition of enrichments. Feeding copepods exclusively or in combination with other live organisms has repeatedly demonstrated superior results in terms of growth, survival, and the overall health of larval fish (Kraul et al., 1992; 1993; Shields et al., 1999; Gardner, 2000; Payne et al., 2001; Støttrup, 2000; Støttrup, 2003; Wilcox et al., 2006). Feeding copepods to larval fish species with small mouth gapes has allowed these species to be successfully cultured through the larval phase (Shields et al., 2003; Shields et al., 2005; Baensch, 2009). Currently, our ability to commercially produce marine fish is generally limited to those species that can be reared on enriched rotifers and *Artemia*.

Despite the aforementioned advantages of feeding copepods over other live feeds currently in use, the use of copepods in commercial settings is rare. This is primarily due to an inability to produce a reliable, continuous supply of copepods on a large-scale (Gapasin and Duray, 2000; Payne and Rippingale, 2001a; Støttrup, 2003). Typical culture densities for calanoid species are

\* Corresponding author. Department of Biology and Marine Biology, Roger Williams University, One Old Ferry Road, Bristol, RI 02809, USA. Tel.: +1 401 254 5750; fax: +1 401 254 3310.

E-mail addresses: [arhyne001@hotmail.com](mailto:arhyne001@hotmail.com), [arhyne@rwu.edu](mailto:arhyne@rwu.edu) (A.L. Rhyne), [chohs@ufl.edu](mailto:chohs@ufl.edu) (C.L. Ohs), [eriks@algagen.com](mailto:eriks@algagen.com) (E. Stenn).

<sup>1</sup> Tel.: +1 772 978 1395.

<sup>2</sup> Edgerton Research Laboratory, New England Aquarium, One Central Wharf, Boston, MA 02110, USA.

0.5–1.0/mL (Morehead, 2004), whereas rotifers have been reported to achieve a density of 16,000/mL in intensive recirculating systems (Suantika et al., 2001).

Several species of copepods have shown commercial production potential. Payne and Rippingale (2001a,b) demonstrated successful culture methods for *Gladioferens imparipes*, and designed a system to produce 1–2 nauplii/mL on a continuous basis. *G. imparipes* is a small egg bearing estuarine calanoid copepod with a semi-benthic adult life stage (Payne and Rippingale, 2001a). Production of *Acartia tonsa* continues to be refined (Peck and Holste, 2006). Recent success with the long-term cold storage of *A. tonsa* eggs could prove to be a valuable egg banking method (Drillet et al., 2006; Holmstrup et al., 2006; Drillet et al., 2007). Several small paracalanid species have also recently shown promise for mass scale culture (McKinnon et al., 2003; Shields et al., 2005; Vanderlugt and Lenz, 2008).

In 2003, we isolated the copepod, *P. pelagicus*, from the waters of south Florida and have kept it in continuous culture for five years. *P. pelagicus* has exhibited culture characteristics very similar to *G. imparipes*, and appears well suited for mass production. *Pseudodiaptomus* spp. are semi-benthic calanoid copepods; the adults are substrate oriented and nauplii and early copepodites are pelagic (Jacobs, 1961). The genus is globally distributed from tropical to temperate waters (Walter, 1989). Predominantly an estuarine genus, they generally tolerate a wide range of environmental parameters (Chen et al., 2006). Unlike many other calanoids, *Pseudodiaptomus* spp. appear well suited to culture systems because they can tolerate heavy aeration, tolerate the presence of sediment and suspended solids, grow and reproduce well on a single readily produced microalgae species (*Isochrysis galbana*), and can achieve densities of over 5/mL (unpublished data).

Temperature is a key abiotic factor regulating the growth and reproductive potential of copepods in marine systems (Santos et al., 1999; Peterson, 2001; Isla and Perissinotto, 2004; Sullivan et al., 2007; Sun et al., 2008). Additionally, temperature is a key variable in the development of production regimes (Santos et al., 1999; Holste and Peck, 2006; Milione and Zeng, 2008). It is important to identify the impacts of key abiotic factors prior to evaluating diet and other biotic culture conditions. For example, the microalga *Rhodomonas lens*, commonly fed to copepods, is temperature sensitive and cultures decline or crash at temperatures above 26 °C. Therefore, this nutrient rich species of algae may not be feasible for feeding species of copepods which require higher culture temperatures.

A series of experiments were conducted to measure the effect of temperature on the survival, development time, reproductive output, and population growth of *P. pelagicus* with the overall objective to define the most suitable temperature or range of temperatures for commercial production and future experimentation.

## 2. Materials and methods

### 2.1. Stock cultures

*P. pelagicus* stock cultures for this experiment were obtained from AlgaGen LLC located in Vero Beach, Florida and were of the strain PP1103. Established standard culture protocols were followed which consisted of culture in 100 L static tanks at 26 °C and a salinity of 35 g/L. Gentle aeration was provided from the bottom of the tank. Water quality was maintained by exchanging 100% of the culture water every Monday and Thursday. Photoperiod was maintained at 24 h of light. All copepods were provided a daily ration of Tahitian strain *Isochrysis galbana* (T-iso) from a stock culture to obtain a feeding density between 2 and  $3 \times 10^5$  cells/mL. Unless otherwise stated, experimental culture conditions were maintained during experiments in accordance with the standard protocols developed by AlgaGen LLC described above. Adult copepods were obtained by sieving stock cultures through a 200 µm nylon screen which retained

only adults. Early stage nauplii (N1–N3) were obtained by sieving stock cultures through a 125 µm nylon screen, and collecting the nauplii on a 50 µm nylon screen.

### 2.2. Survival, sex ratio, maturation and fecundity

To determine the effects of temperature on survival, sex ratio, maturation, and fecundity, a series of experiments were conducted using early stage nauplii obtained from stock cultures at six temperatures (24, 26, 28, 30, 32, and 34 °C). All other experimental conditions were maintained per stock culture protocols. Each temperature treatment was replicated six times and cultures were maintained in a climate controlled room within constant temperature water baths. Each replicate was a covered 1 L beaker which contained 650 mL of seawater. Saline water was obtained from the Atlantic Ocean (35–35.5 g/L) and filtered before use. A total of 200 nauplii were volumetrically stocked into each replicate beaker. Volumetric stocking was conducted with a 10 mL pipette (Eppendorf Model 022472208). To determine the accuracy we conducted counts on 12 volumetric samples taken from a homogenized beaker of nauplii. In these 12 volumetric samples we were able to collect  $100 \pm 6.5\%$  of the desired number of nauplii.

Each day, replicate beakers of copepods were observed to determine the time to first maturity and time to total population maturity. When *P. pelagicus* becomes sexually mature, adults aggregate on the walls of the culture vessel and pair making it relatively easy to determine the level of maturation. Females were considered to be mature when they were carrying their first egg sac (Payne and Rippingale, 2001b). First maturity was defined as when the first female was observed to be carrying eggs. The population was determined to be totally mature when no free swimming copepodites were observed and all females were gravid. Five days after first maturity, the cultures were sieved onto a 50 µm screen and then placed in 30 mL vials and preserved in a 5% solution of neutral buffered formalin in seawater and stored in a refrigerator (4 °C) until enumeration. The entire population was counted, sexed and the number of ovigerous females was recorded. Survival was calculated by the total number of copepods harvested divided by the number of copepods volumetrically stocked (200). Copepods were sexed by observing the morphologically distinct antennae, females exhibit straight antennae and males while the right antennule bent; they also exhibit sexual size dimorphism, with the female being about 40% larger (Grice, 1969). Fecundity was determined by excising both egg sacs with fine forceps and needles from five females per replicate. Egg sac membranes were dissolved by placing each egg sac in a 5% solution of sodium hyperchlorite and gently agitating. Then the total number of eggs was quantified for each egg sac and each female with a stereomicroscope (Olympus SZ30).

### 2.3. Nauplii production

To determine the effects of temperature on the timing and rate of nauplii production, two separate experiments were conducted to evaluate both group and individual daily nauplii production at the six treatment temperatures. The adult pairs of copepods were obtained from stock cultures at each treatment temperature.

For group production, six replicate enclosures for each treatment were stocked with 25 reproductive adult pairs (male and female attached) and cultured for 10 days. Enclosures were 1 L beakers containing a 350 mL screen enclosure (165 µm nylon mesh on the bottom of the enclosure) nested inside of the beaker. Daily, the enclosures were removed from the beakers, retaining the adults on the screen, and were immediately placed into a fresh beaker containing temperature acclimated seawater and T-iso. Daily production was determined by counting the nauplii produced during each 24 h period.

**Table 1**

Mean ( $n = 6$ ) survival, sex ratio, first maturity, population maturity, percent ovigerous, and fecundity of *Pseudodiaptomus pelagicus* cultured at the six treatment temperatures from an initial population of 200 nauplii and cultured until five days after observation of the first ovigerous female.

Temperature (°C)	Survival (%)	Sex ratio M:F	First maturity (days)	Population maturity (days)	Ovigerous (%)	Fecundity		
						Left egg sac	Right egg sac	Total
24	81.3 ± 22.7 <sup>ab</sup>	1.1 ± 0.2 <sup>a</sup>	10.7 ± 1.0 <sup>a</sup>	12.8 ± 0.4 <sup>a</sup>	50.5 ± 15.7 <sup>a</sup>	12.9 ± 1.2 <sup>a</sup>	3.2 ± 0.9 <sup>a</sup>	16.2 ± 1.5 <sup>a</sup>
26	86.3 ± 24.6 <sup>ab</sup>	1.0 ± 0.3 <sup>a</sup>	9.3 ± 1.6 <sup>ab</sup>	12.0 ± 0.0 <sup>b</sup>	11.9 ± 6.4 <sup>b</sup>	8.8 ± 1.0 <sup>b</sup>	2.0 ± 0.9 <sup>a</sup>	10.9 ± 6.4 <sup>b</sup>
28	101.8 ± 9.0 <sup>a</sup>	0.9 ± 0.2 <sup>a</sup>	7.8 ± 0.4 <sup>bc</sup>	10.0 ± 0.0 <sup>c</sup>	91.2 ± 6.3 <sup>c</sup>	20.0 ± 1.2 <sup>c</sup>	5.2 ± 0.8 <sup>b</sup>	25.2 ± 1.0 <sup>c</sup>
30	74.3 ± 9.8 <sup>ab</sup>	1.0 ± 0.2 <sup>a</sup>	6.8 ± 0.4 <sup>c</sup>	9.0 ± 0.0 <sup>d</sup>	78.8 ± 9.0 <sup>c</sup>	17.7 ± 1.9 <sup>cd</sup>	6.0 ± 0.9 <sup>b</sup>	23.7 ± 2.3 <sup>cd</sup>
32	66.7 ± 8.9 <sup>b</sup>	1.2 ± 0.1 <sup>a</sup>	6.7 ± 0.5 <sup>c</sup>	8.0 ± 0.0 <sup>e</sup>	66.7 ± 10.1 <sup>c</sup>	15.3 ± 2.2 <sup>ad</sup>	5.3 ± 1.0 <sup>b</sup>	20.6 ± 2.6 <sup>de</sup>
34	62.5 ± 10.0 <sup>b</sup>	1.2 ± 0.2 <sup>a</sup>	6.7 ± 0.5 <sup>c</sup>	8.0 ± 0.0 <sup>e</sup>	53.0 ± 13.5 <sup>a</sup>	14.0 ± 1.8 <sup>a</sup>	5.0 ± 1.1 <sup>b</sup>	19.1 ± 2.1 <sup>ae</sup>

Different superscript letters indicate statistical differences ( $p \leq 0.05$ ) among treatments.

For individual production, reproductive adult pairs were individually held in one of six 30 mL beakers per treatment, each containing 20 mL of temperature acclimated seawater and T-iso. Each day the individual pair was captured with a transfer pipette and moved to a new culture vessel containing fresh temperature adjusted seawater and T-iso. Daily nauplii production was determined by counting the number of nauplii produced during each 24 h period.

For individual production, brood interval was the number of days between broods, measured from the first day nauplii were present in two successive broods. A brood was defined as the production of greater than one nauplii in the 24 h period between water exchanges. Mean brood size was calculated from all broods during the 10 day experimental culture period.

#### 2.4. Population dynamics

To determine the effects of temperature on the development of population composition, 10 reproductive adult pairs were obtained from stock cultures and were stocked into six 1 L beakers containing 650 mL of filtered seawater and T-iso at each of the six treatment temperatures. After 10 days following standard culture protocols, the entire population was sieved onto a 50  $\mu$ m screen then placed in 30 mL vials and preserved in a 5% neutral buffered formalin and seawater solution and placed in a refrigerator (4 °C) until enumeration. The number of early nauplii, late nauplii (N4–N6), copepodites (C1–C5), and adults (C6) (Grice, 1969) was quantified for each replicate by placing the population on a zooplankton counting wheel and observing each individual with a stereo-microscope.

#### 2.5. Statistics

An analysis of variance using the general linear model (PROC GLM) of SAS (SAS, 1999) was used to determine if there were statistically significant differences between treatments for survival, time to first maturity, time to population maturity, percent ovigerous, fecundity, brood interval, brood size, and total nauplii produced. The means were separated by the Tukey's procedure of SAS (SAS, 1999). Statistical significance occurred in all analyses when the calculated  $p$ -value was  $\leq 0.05$ . All mean values are reported as mean  $\pm$  S.D.

A chi square analysis was conducted on the male:female sex ratio, and when comparing the number of eggs in the left and right egg sacs, to determine if the results were significantly different from the expected 1:1 ratio (SAS, 1999). The regression curves and formulas were generated with Sigma Plot Version 8.0 software (Sigma Plot, 2002).

### 3. Results

#### 3.1. Survival, sex ratio, maturation and fecundity

Survival of *P. pelagicus* from early nauplii to adult was significantly ( $p = 0.004$ ) affected by culture temperature (Table 1). Copepods

cultured between 24–30 °C had the higher mean survival than other temperatures and the highest survival of 101.8  $\pm$  9.0% was recorded at 28 °C. Survival significantly declined in the 32 °C and 34 °C treatments to 66.7  $\pm$  8.9% and 62.5  $\pm$  10.0%, respectively. Sex ratios of the final populations did not significantly ( $p > 0.05$ ) differ from the expected 1:1 male:female ratio and the six treatments were not significantly different from each other ( $p = 0.0869$ ). Time to the first reproductive female and to the total population maturity was strongly influenced by temperature. Development time from an early nauplii stage to the first observed reproductive female was highly significant ( $p < 0.0001$ ) and ranged from 10.7  $\pm$  1.0 days to 6.7  $\pm$  0.5 days and followed a decreasing trend with increasing temperature. Likewise, time to total population maturity was significantly influenced ( $p < 0.0001$ ) by temperature, taking 12.8  $\pm$  0.4 days at 24 °C and 8.0  $\pm$  0.0 days at 34 °C. Fecundity was significantly affected ( $p < 0.0001$ ) by rearing temperature, ranging from 25.2  $\pm$  1.0 eggs to 16.2  $\pm$  1.5 eggs per female, with the highest fecundity measured at 28 and 30 °C. In all treatments, females always had a greater number of eggs in their left egg sac compared to their right ( $p < 0.0001$ ). At the time of sampling the 28 and 30 °C treatments displayed a significantly higher ( $p < 0.0001$ ) percentage of ovigerous females (91.2  $\pm$  6.3% and 78.8  $\pm$  9.0%, respectively) than other treatments.

#### 3.2. Nauplii production

Temperature significantly affected nauplii production in both individual pairs ( $p = 0.0012$ ) and in groups of pairs ( $p < 0.0001$ ), however, the mean brood size was not affected by temperature ( $p = 0.8991$ ) (Table 2). Temperature affected the mean daily nauplii production by decreasing the amount of time required between broods (brood interval) as temperature increased. The brood interval decreased from 1.9  $\pm$  0.5 days at 24 °C to 1.3  $\pm$  0.1 days at 32 °C. Water temperature of 34 °C impeded reproductive function and an analysis was not possible due to the low number of broods. In groups, a significant decrease ( $p = 0.0014$ ) occurred in the total number of nauplii produced, once temperatures reached 32 °C (Fig. 1). For individual pairs, a significant decrease ( $p < 0.0001$ ) in total nauplii produced only occurred at 34 °C (Table 2).

**Table 2**

Mean ( $n = 6$ ) brood interval, size, and total nauplii production for *Pseudodiaptomus pelagicus* cultured in individual pairs at the six treatment temperatures.

Temperature (°C)	Brood interval (Days)	Brood size	Total nauplii production
24	1.9 $\pm$ 0.5 <sup>a</sup>	16.0 $\pm$ 4.0 <sup>a</sup>	87.8 $\pm$ 10.7 <sup>a</sup>
26	1.6 $\pm$ 0.2 <sup>ab</sup>	14.5 $\pm$ 4.0 <sup>a</sup>	80.8 $\pm$ 17.3 <sup>a</sup>
28	1.4 $\pm$ 0.1 <sup>ab</sup>	15.1 $\pm$ 3.9 <sup>a</sup>	98.7 $\pm$ 25.8 <sup>a</sup>
30	1.4 $\pm$ 0.2 <sup>b</sup>	16.2 $\pm$ 3.7 <sup>a</sup>	90.3 $\pm$ 36.5 <sup>a</sup>
32	1.3 $\pm$ 0.1 <sup>b</sup>	15.1 $\pm$ 3.0 <sup>a</sup>	86.7 $\pm$ 37.2 <sup>a</sup>
34	–	14.8 $\pm$ 2.9 <sup>a</sup>	21.8 $\pm$ 21.7 <sup>b</sup>

Different superscript letters indicate statistical differences ( $p \leq 0.05$ ) among treatments.

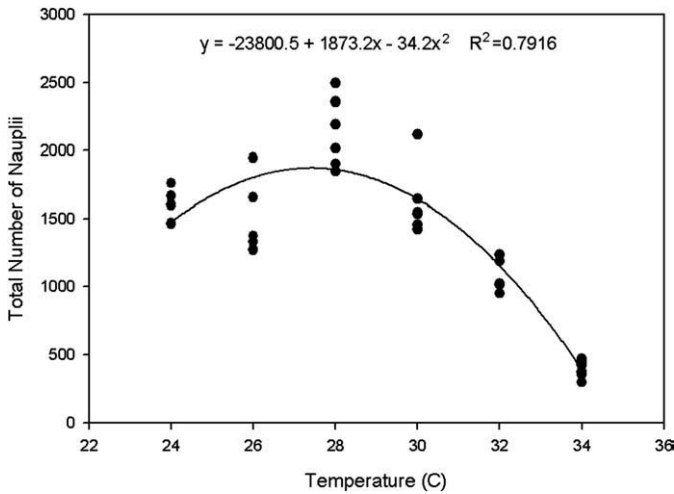


Fig. 1. Total daily nauplii (*Pseudodiaptomus pelagicus*) produced at the six treatment temperatures during 10 day group experiment ( $n = 6$ ).

In the group experiment, daily nauplii production was much higher for day one when compared to all other days regardless of temperature (Fig. 2). This sharp decrease in production was followed by an increase in production for days three to six. Total nauplii production in the group experiment increased to 1861 nauplii at 27.5 °C, then declined gradually until 30 °C before sharply decreasing (Fig. 1).

Regardless of scale, individual pairs, or groups of pairs, the trend of temperature on mean daily nauplii production was similar. Mean daily nauplii production in the individual pairs and group experiments were both highest at 28 °C. Daily production peaked at 28 °C in the individual pair experiments, and had  $9.8 \pm 2.6$  nauplii per female per day while the group experiment also peaked at 28 °C with production of  $9.5 \pm 1.9$  nauplii per female per day.

### 3.3. Population dynamics

The effects of temperature on the population composition and the total number in the population were both highly significant

( $p < 0.0001$ ) (Figs. 3 and 4). The total population was similar from 24–30 °C and peaked at 30 °C with  $386.8 \pm 186.5$  individuals, followed by a very large decline at 34 °C to  $13.0 \pm 14.1$  individuals (Fig. 4).

The distribution of developmental stages within the population was also affected by temperature (Fig. 3). At lower temperatures, the population had a larger number of nauplii and copepodites than at higher temperatures up to 32 °C; the 34 °C treatment performed poorly. The number of adults in the population reached a maximum at 30 °C before declining sharply at 32 and 34 °C. Despite the overall decline in numbers, the 32 °C treatment was comprised of more advanced staged individuals than nauplii. The presence of gravid females increased with increasing temperatures and peaked at 32 °C. At 34 °C, the population declined greatly in number, and the distribution of life stages was no longer relevant.

## 4. Discussion

Temperature is often the most important environmental factor affecting the productivity of copepods in natural systems (Christou and Moraitou-Apostolopoulou, 1995; Siokou-Frangou, 1996). In our study of *P. pelagicus*, temperature affected survival, maturation time, the number of ovigerous females, and fecundity, but had no effect on sex ratio. Sex ratio did not deviate from the expected 1:1 in all treatments, which is consistent with the calanoid copepod, *G. imparipes* (Rippingale and Hodgkin, 1974). Survival and fecundity was highest at 28 °C, and survival was lowest at 32 and 34 °C. Fecundity was lowest at 24 and 26 °C. The percent ovigerous females followed the same trend as fecundity and was lowest at 26 °C. This may be a result of a longer interbrood duration; additionally, the time when the population was sampled may have been a time when the majority of females were between broods. Mean development time, from early nauplii to reproductive adults, decreased exponentially with increasing temperature and reached the shortest duration at 32 °C. An exponential increase in development time with a corresponding decrease in temperature is well supported in the literature. The calanoid copepod, *Pseudocalanus newmani*, was reported to experience a doubling of development time from 20.9 to 42.3 days when temperatures decreased from 15 to 6 °C (Lee et al., 2003). This trend was also observed in *A. clausi*, where development time increased from 35.4 to 74.8 days when temperatures decrease from 10 to 5 °C. This effect is also well documented in marine

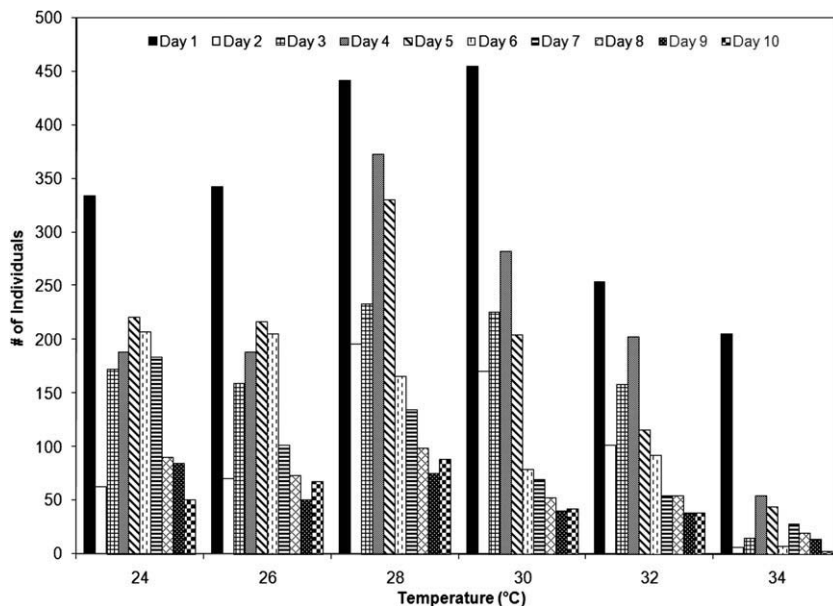


Fig. 2. Mean ( $n = 6$ ) daily production of *Pseudodiaptomus pelagicus* nauplii cultured at the six treatment temperatures in groups of pairs following 10 days of culture.

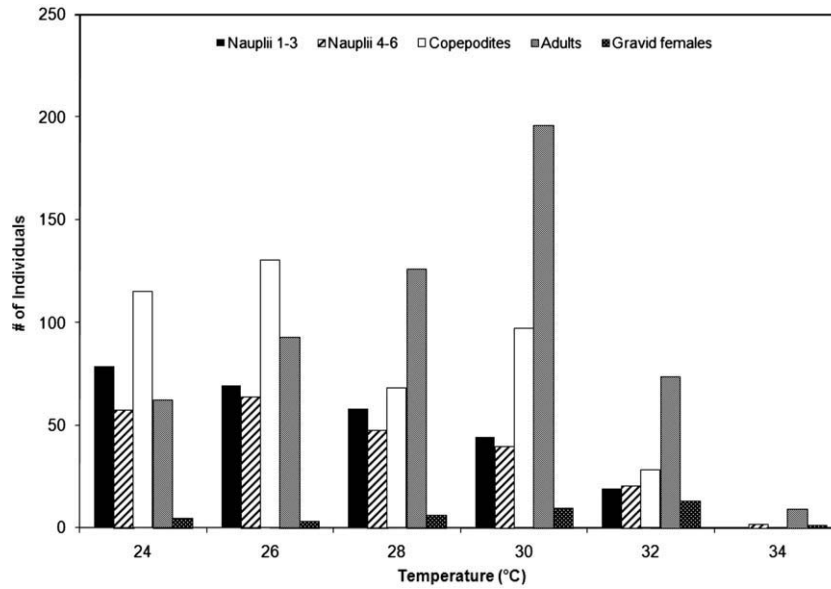


Fig. 3. Mean (n=6) number of five life stages, early nauplii (N1–N3), late nauplii (N4–N6), copepodites (C1–C5), adults (C6), and gravid females within the population of *Pseudodiaptomus pelagicus* cultured for 10 days at the six treatment temperatures from an initial population of 10 adult pairs.

harpacticoid copepods (Williams and Jones, 1999). As temperatures increase to the upper thermal limit of the species, the effect of temperature on development time decreases in magnitude (see Peterson, 2001 for a review).

In our study of *P. pelagicus* development, the regression curve became asymptotic at approximately 30 °C. Temperatures greater than 30 °C were detrimental to survival, percent ovigerous females, and fecundity. Culture temperatures below 28 °C had high survival and experienced lower fecundity and extended maturation time from early nauplii to adult. The optimal temperature for aquaculture purposes appears to be 30 °C, which results in the shortest duration time to adult and relatively high survivorship and fecundity.

Nauplii production from both individual pairs and groups of pairs followed similar trends with peak production at approximately 28 °C. Individual pair data revealed that increased production was not due to an increase in brood size but rather a decrease in brood interval. Brood interval followed the same trend as mean development time, and exponentially increased with decreasing temperature. Brood interval at 28 °C was 1.4 days and at 34 °C thermal stress likely impeded reproduction. At 34 °C, production was lower than all other temperatures and a brood interval could not be determined, although mean brood size was similar to other temperatures. At 34 °C, thermal stress resulted in more erratic production of nauplii between replicates with a range of 0–55 nauplii produced in a 10 day period. Also, a greatly reduced lifespan was observed at 34 °C and the maximum number of observed broods was three in one replicate but production ceased after the fourth day. This may indicate thermal stress caused energy to be allocated toward survival processes and away from reproduction. This trend was reported in *Tisbe battagliai*, where at 25 °C nauplii production ceased after 20 days while lower temperature treatments continued to produce nauplii for 36 days (Williams and Jones, 1999). Group data confirmed this with a similar pattern of production. Daily mean nauplii production was similar in the grouped and individual pairs, and the trend remained the same. The elevated production in the group experiment on the first day suggests a possible container effect and/or possible stress of the copepods having to acclimate to experimental conditions. Despite the apparent confounding effect of the enclosure, the overall pattern remains constant in all experiments, increasing temperature increases production up to 30 °C, after which as temperature increases production declines. The overall trend in the data corroborates that of the individual pairs where optimal production occurred at 26–30 °C.

Population growth and composition has recently been used to examine the effects of temperature and salinity on the aquaculture production of *A. singiensis* (Milione and Zeng, 2008). Milione and Zeng (2008) observed the highest production between 25–30 °C with a peak at 30 °C followed by a sharp decline at 34 °C. Furthermore, they related this result to the optimal temperature where mean development time was shortest and survival and egg production was highest. This is evident in our results in which the optimal observed range for production of *P. pelagicus* was 26–30 °C, with the highest at the 28 °C treatment and the optimal temperature of 27.5 °C predicted by the quadratic function. Temperatures above 30 °C elicit the sharp decline observed in Fig. 4. In addition to an increased total production, the composition of the population is directly affected by temperature. A larger proportion of the population reached maturity at 28 °C which resulted in peak performance. Above 30 °C, culture performance declines and stage composition reflects the thermal stress effects.

Population growth and composition is a good indicator of potential aquaculture performance because it shows the effects of temperature on reproduction, growth, and survival. However, this method does not provide necessary data to determine the effects of temperature on

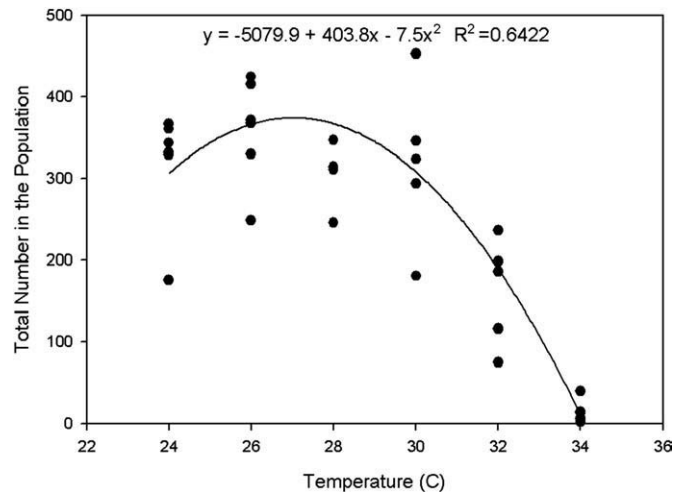


Fig. 4. Total population of *Pseudodiaptomus pelagicus* produced at the six treatment temperatures during the population dynamics experiment (n=6).

mean brood size or brood interval. Individual pair data showed the mean brood size was constant and the decrease in production at lower temperatures was due to the longer brood interval while at higher temperatures it was from decreases in survival and number of broods.

Experimental scale is an issue in copepod production studies. Few studies have examined production at a large commercial scale. Payne and Rippingale (2001a) examined three different production systems ranging from 60 to 1000 L with the peak nauplii production of 1117/L/day in 60 L batch systems compared to 520/L/day in 1000 L semi-continuous systems. It is possible that nauplii production may change when scale is increased. However, conducting replicated experiments at commercial scale can be impractical given the cost and time involved. Small scale studies, such as the present, provide valuable data concerning abiotic parameters which remain constant regardless of scale.

## 5. Conclusion

Temperature significantly affected the growth, survival, and reproductive output of the semi-benthic calanoid copepod *P. pelagicus* as reported for other species of calanoid (Peterson, 2001) and harpacticoid copepods (Williams and Jones, 1999). The optimal range for production was observed to be 28–30 °C; this range provides for high survival, short brood interval, and decreased mean development time. Temperatures below the optimal range resulted in slower growth and longer brood intervals, which contributed to overall lower production. Additionally, temperatures above the optimal range resulted in a marked decline in production as a result of reduced survival and a decreased number of broods. Mean sex ratio and brood size were not affected by temperature.

Based upon production goals, these results provide options for commercial production. Culture water temperatures of 28–30 °C will maximize population growth and nauplii production. Temperatures from 20–24 °C will slow development and nauplii production but can be used to maintain cultures long-term with high survival while decreasing algae requirements. Manipulating temperature within a production scenario with multiple culture systems facilitates the timing of maturation and nauplii production by controlling developmental time and provides the ability to coincide or stagger the timing of nauplii production.

## Acknowledgements

We thank the Florida Department of Agriculture and Consumer Services Division of Aquaculture and the University of Florida Institute of Food and Agricultural Sciences (IFAS) Research Innovation Fund Grants Program for funding this research. We thank the three anonymous reviewers for valuable comments. We also thank Scott Grabe, Matthew DiMaggio, and Shawn DeSantis for assisting with various aspects of these experiments.

## References

- Baensch, F., Tamaru, C., 2009. Spawning and development of larvae and juveniles of the rare blue Mauritius angelfish, *Centropyge debelius* (1988), in the hatchery. *Journal of the World Aquaculture Society* 40 (4).
- Chen, Q., Sheng, J., Lin, Q., Gao, Y., Lv, J., 2006. Effect of salinity on reproduction and survival of the copepod *Pseudodiaptomus annandalei* Sewell, 1919. *Aquaculture* 258, 575–582.
- Christou, E.D., Moraitou-Apostolopoulou, M., 1995. Metabolism and feeding of mesozooplankton of the eastern Mediterranean coast (Hellenic waters). *Marine Ecology Progress Series* 126, 39–48.
- Drillet, G., Iversen, M.H., Sørensen, T.F., Ramløv, H., Lund, T., Hansen, B.W., 2006. Effect of cold storage upon eggs of a calanoid copepod *Acartia tonsa* Dana and their offspring. *Aquaculture* 254, 714–729.
- Drillet, G., Lindley, L.C., Michels, A., Wilcox, J., Marcus, N.H., 2007. Improving cold storage of subitaneous eggs of the copepod *Acartia tonsa* Dana from the Gulf of Mexico (Florida – USA). *Aquaculture Research* 38, 457–466.
- Gapasin, R.S.J., Duray, M.N., 2000. Effects of DHA enriched live food on growth and survival and incidence of opercular deformities in milkfish (*Chanos chanos*). *Aquaculture* 193, 49–63.
- Gardner, T., 2000. Spawning and rearing the yellow dottyback, *Pseudochromis fuscus*. *Freshwater and Marine Aquarium* 23 (4), 126–132.
- Grice, G.D., 1969. The developmental stages of *Pseudodiaptomus coronatus* Williams (Copepoda, Calanoida). *Crustaceana* 17, 291–301.
- Hoff, F.H., Snell, T.W., 1999. *Plankton Culture Manual*, fifth edition. Florida Aqua Farms Inc., Dade City, FL, 160 pp.
- 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. *Marine Biology* 148, 1061–1070.
- Holmstrup, M., Overgaard, J., Sørensen, T.F., Drillet, G., Hansen, B.W., Ramløv, H., Engell-Sørensen, K., 2006. Influence of storage conditions on viability of quiescent copepod eggs (*Acartia tonsa* Dana): effects of temperature, salinity and anoxia. *Aquaculture Research* 37, 625–631.
- Hunter, J., 1981. Feeding ecology and predation of marine fish larvae. In: Lasker, R. (Ed.), *Marine Fish Larvae: Morphology, Ecology, and Relation to Fisheries*. University of Washington Press, Seattle, WA, pp. 33–77.
- Isla, J.A., Perissinotto, R., 2004. Effects of temperature, salinity and sex on the basal metabolic rate of the estuarine copepod *Pseudodiaptomus hessei*. *Journal of Plankton Research* 26, 579–583.
- Jacobs, J., 1961. Laboratory cultivation of the marine copepod *Pseudodiaptomus coronatus* Williams. *Limnology and Oceanography* 6, 443–446.
- Kraul, S., Brittain, K., Cantrell, R., Nagao, T., Ako, H., Ogasawara, A., Kitagawa, H., 1993. Nutritional factors affecting stress resistance in the larval mahimahi, *Coryphaena hippurus*. *Journal of the World Aquaculture Society* 24, 186–193.
- Kraul, S., Nelson, A., Brittain, K., Ako, H., Ogasawara, A., 1992. Evaluation of live feeds for larval and postlarval mahimahi *Coryphaena hippurus*. *Journal of the World Aquaculture Society* 23, 299–307.
- Lee, H., Ban, S., Ikeda, T., Matsuishi, T., 2003. Effect of temperature on development, growth and reproduction in the marine copepod *Pseudocalanus newmani* at satiating food condition. *Journal of Plankton Research* 25, 261–271.
- Leis, J.M., 1991. The pelagic stage of reef fishes. In: Sale, P.F. (Ed.), *The Ecology of Fishes on Coral Reefs*. Academic Press, San Diego, CA, pp. 183–230.
- Morehead, D., 2004. Copepod culture workshop in Hawaii. *Austasia Aquaculture* 18, 46–49.
- McKinnon, A.D., Duggan, S., Nichols, P.D., Rimmer, M.A., Semmens, G., Robino, B., 2003. The potential of tropical paracalanid copepods as live feeds in aquaculture. *Aquaculture* 223, 89–106.
- Milione, M., Zeng, C., 2008. The effects of temperature and salinity on population growth and egg hatching success of the tropical calanoid copepod, *Acartia sinjiensis*. *Aquaculture* 275, 116–123.
- Østergaard, P., Munk, P., Janekam, V., 2005. Contrasting feeding patterns among species of fish larvae from the tropical Andaman Sea. *Marine Biology* 146, 595–606.
- Payne, M.F., Rippingale, R.J., 2001a. Intensive cultivation of the calanoid copepod *Gladioferens imparipes*. *Aquaculture* 201, 329–342.
- Payne, M.F., Rippingale, R.J., 2001b. Effects of salinity, cold storage and enrichment on the calanoid copepod *Gladioferens imparipes*. *Aquaculture* 201, 251–262.
- Payne, M.F., Rippingale, R.J., Cleary, J.J., 2001. Cultured copepods as food for west Australian dhufish (*Glaucosoma hepbraicum*) and pink snapper (*Pagrus auratus*) larvae. *Aquaculture* 194, 137–150.
- Peck, M.A., Holste, L., 2006. Effects of salinity, photoperiod and adult stocking density on egg production and egg hatching success in *Acartia tonsa* (Calanoida: Copepoda): optimizing intensive cultures. *Aquaculture* 255, 341–350.
- Peterson, W.T., 2001. Patterns in stage duration and development among marine and freshwater calanoid and cyclopoid copepods: a review of rules, physiological constraints and evolutionary significance. *Hydrobiologia* 453/454, 91–105.
- Rippingale, R.J., Hodgkin, E.P., 1974. Population growth of a copepod *Gladioferens imparipes* Thomson. *Australian Journal of Marine and Freshwater Research* 25, 351–360.
- Sampey, A., McKinnon, A.D., Meekan, M.G., McCormick, M.I., 2007. Glimpse into guts: overview of the feeding of larvae of tropical shorefishes. *Marine Ecology Progress Series* 339, 243–257.
- Santos, P.J.P., Castel, J., Souza-Santos, L.P., 1999. Development time of harpacticoid copepods: some empirical models and implications. *Journal of the Marine Biological Association of the U.K.* 79, 1123–1124.
- SAS, 1999. *SAS Procedures Guide*, Version 8. SAS Publishing, 1624 pp.
- Shields, R.J., Bell, J.G., Luizi, F.S., Gara, B., Bromage, N.R., Sargent, J.R., 1999. Natural copepods are superior to enriched *Artemia* nauplii for halibut larvae (*Hippoglossus hippoglossus*) in terms of survival, pigmentation and retinal morphology: relation to dietary essential fatty acids. *Journal of Nutrition* 129, 1186–1194.
- Shields, R.J., Kotani, T., Augustin, M., Marion, K., Kobashigawa, J., 2003. Intensive culture of a calanoid copepod, *Parvocalanus* sp., and application for rearing small subtropical marine fish larvae. *World Aquaculture Annual Conference*, Salvador Brazil. *Book of Abstracts*, vol. 2. 720 pp.
- Shields, R.J., Kotani, T., Molnar, A., Marion, K., Kobashigawa, J., Tang, L., 2005. Intensive cultivation of a subtropical paracalanid copepod, *Parvocalanus* sp., as a prey item for small marine fish larvae. In: Lee, C.S., O'Bryan, P.J., Marcus, N. (Eds.), *Copepods in Aquaculture*. Blackwell Publishing, pp. 209–223.
- Sigma Plot, 2002. *Sigma Plot Users Guide*, Version 8.0. SPSS Inc. 526 pp.
- Siokou-Frangou, I., 1996. Zooplankton annual cycle in a Mediterranean coastal area. *Journal of Plankton Research* 18, 203–223.
- Støttrup, J.G., 2000. The elusive copepods: their production and suitability in marine aquaculture. *Aquaculture Research* 31, 703–711.
- Støttrup, J.G., 2003. Production and nutritional value of copepods. In: Støttrup, J.G., McEvoy, L.A. (Eds.), *Live Feeds in Marine Aquaculture*. Blackwell Science, Oxford, UK. 318 pp.
- Suantika, G., Dhert, P., Rombaut, G., Vanderberghe, J., DeWolf, T., Sorgeloos, P., 2001. The use of ozone in a high density recirculation system for rotifers. *Aquaculture* 201, 35–49.
- Sullivan, B.K., Costello, J.H., Van Keuren, D., 2007. Seasonality of the copepods *Acartia hudsonica* and *Acartia tonsa* in Narragansett Bay, RI, USA during a period of climate change. *Estuarine, Coastal and Shelf Science* 73, 259–267.

- Sun, X.H., Sun, S., Li, C.L., Zhang, G.T., 2008. The seasonal and spatial variation in abundance and egg production of *Paracalanus parvus* (Copepoda: Calanoida) in/out Jiaozhou Bay, China. *Estuarine, Coastal and Shelf Science* 79, 637–643.
- Vanderlugt, K., Lenz, P.H., 2008. Management of nauplius production in the paracalanid, *Bestiolina similes* (Crustacea: Copepoda): effects of stocking densities and culture dilution. *Aquaculture* 276, 69–77.
- Walter, T.C., 1989. Review of the New World species of *Pseudodiaptomus* (Copepoda: Calanoida), with a key to the species. *Bulletin of Marine Science* 45, 590–628.
- Wilcox, J.A., Tracy, P.L., Marcus, N.M., 2006. Improving live feeds: effect of a mixed diet of copepod nauplii (*Acartia tonsa*) and rotifers on the survival and growth of first-feeding larvae of the southern flounder, *Paralichthys lethostigma*. *Journal of the World Aquaculture Society* 37, 113–120.
- Williams, T.D., Jones, M.B., 1999. Effects of temperature and food quality of the reproduction of *Tisbe battagliai* (Copepoda: Harpacticoida). *Journal of Experimental Marine Biology and Ecology* 236, 273–290.