POPULATION DYNAMICS OF THE CALANOID COPEPOD, *BESTIOLINA SIMILIS*, IN SMALL SCALE CULTURES

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By:
Kyle R. VanderLugt

Thesis Committee:
Petra H. Lenz, Chairperson
Spencer R. Malecha
Clyde S. Tamaru
Michael J. Cooney
We certify that we have read this thesis and that, in our opinion, it is satisfactory in scope and quality as a thesis for the degree of Master of Science in Animal Science.

THEESIS COMMITTEE

[Signatures]

Chairperson
Population dynamics of calanoid copepods in small-scale cultures:

I. Why do populations crash?

Kyle VanderLugt 1, Michael J. Cooney 2, Petra H. Lenz 1

1 Pacific Biosciences Research Center, 1993 East-West Rd., Univ. Hawaii Manoa, Honolulu, HI 96822

2 Hawaii Natural Energy Institute, Univ. Hawaii Manoa, Honolulu, HI 96822

Corresponding Author: Petra H. Lenz

Pacific Biosciences Research Center, U. Hawaii Manoa

1993 East-West Rd., Honolulu, HI 96822

Tel: (808) 956-8003 Fax: (808) 956-6984

e-mail: petra@pbrc.hawaii.edu

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Abstract:

Population dynamics of the calanoid copepod, *Bestiolina similis*, were assessed in 3.5 L cultures with maintained food concentrations. Populations were divided into four stage groups (N1-N2, N3-N6, C1-C4 and C5-C6) and densities of stage groups (animals L\(^{-1}\)), female egg production rates (eggs per female per day or EF\(^{-1}\)D\(^{-1}\)), recruitment rates (eggs per liter per day or EL\(^{-1}\)D\(^{-1}\)), and population mortality rates (animals L\(^{-1}\)D\(^{-1}\)), were measured over time in cultures initially stocked with wild caught adult and sub-adult animals (stages C5-C6). In all cultures we observed a consistent population cycle comprised of five distinct growth phases: lag, growth, peak plateau, decline, and post decline. The complete temporal population cycle occurred over 19 days at temperatures ranging from 24-27 °C. Despite significant temporal variation in population densities within cultures (ANOVA, p < 0.05), changes in animal abundances were predictable as a result of consistent temporal changes in female egg production rates (EF\(^{-1}\)D\(^{-1}\)), recruitment rates (EL\(^{-1}\)D\(^{-1}\)), and population mortality rates (animals L\(^{-1}\)D\(^{-1}\)). This consistency allowed for the ability to forecast population abundances including densities of nauplii. Results will be applied in future studies to improve management intervention techniques leading to sustained production of nauplii over time, and to coordinate the first feeding of marine finfish larvae with a suitable food supply.
1. Introduction

Successful propagation of difficult to rear marine food fish species (e.g. grouper, snappers, turbot, etc.), in addition to marine ornamentals (e.g. angelfishes, butterfly fishes, tangs, etc.), has been impeded by two key bottlenecks: 1) achieving consistent and reliable production of fertilized eggs from broodstock, and 2) successfully rearing larvae through the first feeding stages. In recent years, advances in broodstock technology have led to improved captive maturation and control over spawning for several marine food finfish species including, but not limited to, the red drum (*Sciaenops ocellatus*; Arnold, 1988), winter flounder (*Pleuronectes americanus*; Harmin et al., 1995), stripped mullet (*Mugil cephalus*; Lee et al., 1996), mahimahi (*Coryphaena hippurus*; Ostrowski, 2000), and several ornamental species including clown fish and angelfishes. However, largely due to a lack of a suitable first feed, difficulty in rearing larvae through metamorphosis remains a major constraint in marine finfish aquaculture.

Typically used live feeds, such as *Artemia* and ss-type rotifers, are too large and nutritionally inadequate to support the growth of small marine larvae through the first feeding stages. Alternatively, copepod nauplii and especially those of paracalanid species have been proposed as a suitable food source (McKinnon et al., 2003). Copepod nauplii are sufficiently small to fit into the small mouth gapes of marine finfish larvae and possess high levels of highly unsaturated fatty acids (HUFAs) including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Kraul, 1992; Kraul et al., 1992; Shields et al., 1999; Bell et al., 2003). These fatty acids are particularly critical to the healthy development of juvenile fishes (Bell et al., 2003; Kraul et al., 1992). Despite
these advantages large-scale production of copepod nauplii has been limited and consequently relatively few species of finfish have been successfully reared in captivity.

Production and use of copepod nauplii in finfish hatcheries has been impeded by several factors. Firstly, daily production levels are often highly variable. Although Payne and Rippingale (2001) were able to successfully rear the calanoid copepod, Gladioferens impairers for 121 days in 500 L automated harvesting systems, nauplius production levels were highly variable. Though not addressed, this variation may be explained by naturally occurring population cycles as was observed for the calanoid copepod, Parvocalanus sp. (Shields et al., 2005) in 400 L cultures harvested at 3 day intervals. A second constraint in utilizing copepods in finfish hatcheries is that the production of nauplii must be coordinated with the first feeding event of the target finfish larvae population. This event typically occurs 3 days post-hatch as the finfish larvae transition from internal lipid reserves to exogenous feeding. Consequently, coordinating the first feeding event with a suitable quantity of food requires the ability to forecast densities of nauplii.

The benefits of using copepod nauplii in finfish hatcheries cannot be realized without a better understanding of the underlying mechanisms that regulate population abundances over time. In the present study, we assess population dynamics of the calanoid copepod, Bestiolina similis in small scale batch cultures with maintained food concentrations. Populations are divided into 4 stage groups (N1-N2, N3-N6, C1-C4, and C5-C6), and fluctuating animal densities (animals L$^{-1}$) are evaluated in terms of female egg production rates (eggs produced per female per day or EF$^{-1}$D$^{-1}$), recruitment rates (eggs produced per liter per day or EL$^{-1}$D$^{-1}$), and population mortality rates (animals L$^{-1}$
Understanding how these parameters regulate population abundances over time is necessary for the ability to predict when and why population abundances will change, and thus the ability to forecast nauplius densities. These results will be used in future studies to coordinate the first feeding of marine finfish larvae with a suitable food supply.

2. Materials and methods

2.1. Copepod Collection and Maintenance

Copepods were collected from central Kaneohe Bay, Oahu, Hawaii by towing a plankton net (net: 25 cm diameter, 150 μm mesh) horizontally behind a slow moving boat for 1 to 2 minutes. Following net retrieval, collections were transported to the laboratory in 20 L containers (5 gallon white buckets, City Mill®) filled with seawater. In the lab *B. similis* were sorted from other calanoid copepods (e.g. *Acartia fossae* and *Parvocalanus crassirostrus*) by collecting individuals in 50 ml sub-samples concentrated at the edge of the container with a fiber optic light (Dolan Jenner, Industries Inc. Model 170-D) and then viewing the animals under a dissecting microscope (Wild M5) at 3x magnification. The total time from wild collection to isolation of *B. similis* into clear plastic culture jars with 3.5 L of seawater took less than 6 hours.

Adults and sub-adults (stages C5 and C6) were transferred into seawater obtained from Waikiki Aquarium. This water is naturally filtered in underground wells and required no further filtration. Cultures were maintained indoors with temperature ca. 24-
27°C and illuminated with plant and aquarium fluorescent lights (40 watt, Philips®) placed directly behind the cultures (< 30 cm). A 12L:12D photoperiod was provided for all cultures. Aeration was provided using a Dynamaster 2 piston-type air pump (E.G. Danner MFG. Inc.) pumped through 7.9 mm (OD) Tygon® plastic tubing connected to ceramic-type small pore air diffusers (Sweetwater®, Aquatic Ecosystems. Inc). Aeration was kept low using an air control valve (Airtech™ gang valve 5.5) to minimize water turbulence and to reduce stress on the animals.

2.2. Copepod Feeding and Algal Culture Maintenance

Copepods were provided with Isochrysis galbana (Prymnesiophyceae, diameter 5 - 6 μm) at a density of ~1 \( \times 10^5 \) cells ml\(^{-1}\). Food densities were kept above 0.85 \( \times 10^5 \) cells ml\(^{-1}\) by adding I. galbana from stock phytoplankton cultures three times per week. Algae concentrations in both copepod and stock algal cultures were determined by counting cells using a hemacytometer under an inverted compound microscope (Inverted IM Olympus, Japan) at a magnification of 20x. I. galbana were grown indoors with temperature ranging from 24-27°C in 1 L flasks using F/2 Pro Culture™ formula (Kent Marine, Inc.®). Light conditions were the same as for the copepod cultures. Algal cultures were renewed every 8 to 10 days by discarding 65% of the culture volume and inoculating new cultures with the remaining volume.

2.3. Time-lag Experiment
Egg production was monitored over the first 12 hours after animals were captured and transferred to the lab. Specifically, once the animals were in the lab, adults were isolated and sorted into control and treatment groups, each possessing 20 adults placed into one of four Petri dishes (100 mm x 15 mm; 5 adults per Petri) containing 50 ml of seawater. The seawater in the control groups was that taken from Kaneohe Bay with no addition of *I. galbana*. The seawater used in the treatment groups was that obtained from the Waikiki Aquarium with *I. galbana* added to a final concentration of \(1 \cdot 10^5\) cells ml\(^{-1}\) (i.e. same food regime in batch cultures). All dishes were then checked under a dissecting microscope (6x) at 2, 4, 6, 8, and 12 hours for presence of eggs. Once an egg was counted it was removed. Adults were sexed and average female egg production was determined.

2.4. Egg Hatching Experiment

The percentage and rate of eggs hatching into nauplii was determined at room temperature. *B. similis* eggs were collected from a 1.5 L non-aerated cone shaped culture with a valve at the bottom. *Chaetoceros neogracilis* was added to cultures as food source. The valve was carefully opened and eggs were collected in Petri dishes every two hours to ensure a collection of recently laid eggs. Hence, eggs were between 0 and 2 hours old prior to the start of experiment. Eggs were sorted under a dissecting microscope using a Pasteur pipette. 10 eggs were transferred into each of four Petri dishes and nauplii were then counted and removed at 0, 2, 4, 8, 10, 24, and 36 hours. Petri dishes were covered with Parafilm® to prevent water evaporation.
2.5. Population Cycle Experiment

Six 3.5 liter copepod cultures; with 2 cultures setup in January, 2 in June, and 2 in August, were initially stocked with 100 adult and sub-adults (stages C5-C6) L⁻¹. Population abundances (animals L⁻¹), stage distributions (N1-N2, N3-N6, C1-C4, C5-C6 L⁻¹), and average female egg production (eggs produced per female per day or EF¹D⁻¹) were measured in each of the 6 cultures and used to calculate average recruitment rates (eggs produced per liter per day or EL⁻¹D⁻¹), and mortality rates (animals L⁻¹D⁻¹) on days 1, 2, 4, 5, 6, 8, 9, 12, 13, 15, and 19.

2.6. Effect of Stocking Density on Population Dynamics

Adults and sub-adults (stages C5 and C6) were stocked into 3.5 liter cultures at densities of 100, 500, and 1000 animals L⁻¹. Three cultures were stocked with 100 L⁻¹, three with 500 L⁻¹, but only one with 1000 L⁻¹ as repeatedly isolating 3500 animals was unrealistic. Population abundances (animals L⁻¹) and stage distributions (N1-N2, N3-N6, C1-C4, C5-C6 L⁻¹) were estimated daily from days 1 to 8 and on day 10. Average female egg production (eggs produced per female per day or EF¹D⁻¹) was also measured in each culture on days 2, 4, 6, 8, and 10 and used to calculate average recruitment rates (average eggs produced per liter per day or EL⁻¹D⁻¹).

2.7. Water Quality
Water quality was monitored in cultures with initial stocking densities of 100, 500, and 1000 C5-C6 adult and sub-adults (stages C5 and C6) L⁻¹ described in the above experiment (section 2.6). Dissolved oxygen was measured using an Orion Research 820 Probe (Thermo Electron Corp.), and ammonia was measured using the Ammonia Palintest® test kits. Standard calibration curves were made by serial dilution of known concentrations of ammonium chloride (Sigma) dissolved in Waikiki Aquarium seawater. A conditioning reagent (Palintest®) containing lithium hydroxide was used to prevent the precipitation of salts. The intensity of the color reactions was taken to be proportional to the ammonia concentration. The absorbance was measured at 640 nm using a Cary 50 Bio UV-Vis spectrophotometer (VARIAN).

2.8. Copepod Population Dynamics

Fluctuating animal densities in individual copepod cultures were characterized similarly to that described in Aksnes et al. (1997). Briefly, changes in population abundances \((N)\) over time \((t)\) are described as the recruitment rate \((R(t))\) of animals into the population minus the rate of animals lost from the population due to mortality \((M(t))\).

\[
dN / dt = R(t) - M(t) \tag{1}
\]
Recruitment rates \( R(t) \) defined as the number of individuals entering the population over time \( t \) was determined by:

\[
R(t) = b_f(t)n_f(t)
\]  

(2)

where \( R \) is the number of eggs produced per liter \( EL^{-1} \); \( t \) is time in days, \( b_f \) is the birth rate of the average adult female in units of eggs per female \( EF^{-1} \); and \( n_f \) is the number of adult females in units of female per liter \( FL^{-1} \). 

Mortality rates \( M(t) \), as the number of animals lost from the population over time \( t \), were determined from equations 1 and 2 as follows:

\[
M(t) = R(t) - \frac{dN}{dt}
\]  

(3a)

\[
M(t) = (b_f(t)n_f(t)) - \frac{dN}{dt}
\]  

(3b)

Where \( M \) is the number of animals lost from the population in units of Animals \( L^{-1} \); and \( t \) is time in days (D).

2.9. Sampling Methods

In order to estimate recruitment rates \( R(t) \) and mortality rates \( M(t) \) described above (section 2.8), protocols were developed to estimate population densities \( N \) and birth rates \( b_f (t) \) or the number of eggs produced per female per day \( EF^{-1} D^{-1} \)
Population densities \( (N) \) were estimated from 50 ml sub-samples extracted from individual cultures. Animals in the sub-samples were anaesthetized by adding 300 \( \mu \)L of 0.2 M magnesium chloride, separated into one of four stage groups (i.e., N1-N2, N3-N6, C1-C4, or C5-C6), and counted. Animal counts per 50 ml sub-sample (animals per 50 ml) were extrapolated to obtain animal densities per liter (animals L\(^{-1}\)). The densities of the 4 stage groups were then added to obtain the total population density (animals L\(^{-1}\)).

N1-N2 stages were grouped together but separately from N3-N6 animals as N1 and N2 stage animals are smaller than 100 \( \mu \)m and considered to be appropriate food size for finfish larvae (Ostrowski and Laidley, 2001). C5 and C6 animals were grouped together but separately from C1-C4 animals as C5 and C6 animals are difficult to distinguish from each other and were used to determine adult densities and thus female densities.

Female density \( (FL^{-1}) \) was determined from the density of the C5-C6 stage group (animals L\(^{-1}\)) and assuming 1:1 sex ratio. This assumption was validated in the population cycle experiment (section 2.5), in which 36 adults were sexed on each sampling day and females comprised between 44 and 56% of the animals for the duration of the experiment. Further, greater than 90% of animals in the C5-C6 stage group are adults (stage C6) as the pre-adult stage (C5) lasts only 24 hrs (McKinnon et al., 2003; Table 3) and adults live for at least 10 days after reaching maturity (Table 1).

A slightly different method was used to determine animal densities in the two cultures stocked with 100 animals L\(^{-1}\) during the setup in January. For these cultures, the first 20 animals observed in each 50 ml culture sub-sample were classified into one of the
four staged groups as described above. The remainder of animals in each sample were staged and counted as being either a nauplius (N1-N6) or copepodite (C1-C6). Grouped stage densities were determined by multiplying the proportion of animals in each stage group by the total number of nauplii or copepodites.

Birth rates \( b_f (t) \) were determined by counting eggs produced from females isolated from individual cultures. Six adults were isolated from individual cultures in the evening (18:00 to 21:00 hrs), divided into two groups of three individuals placed into 10 mL Petri dishes containing 5 mL of seawater. To maintain consistent conditions (i.e. food levels, temperature, salinity) the seawater was taken from the same cultures as the adults. After 12 hours, the eggs, nauplii, and adult females (C6) were counted under a dissecting microscope. The average egg production rates per female were calculated for each culture by dividing the total number of eggs and nauplii by the number of adult females counted over a 12 hour period. Because overnight egg production was quantified in this study, this result was multiplied by a factor of 1.7 to obtain daily female egg production (EF\(^{-1}\)D\(^{-1}\); VanderLugt, 2005).

2.9. Sampling Error

To verify that the extrapolated stage group densities \( N_{stage,i} \) and thus total densities \( N \) accurately reflect true population densities, and thus validate estimations of recruitment and mortality (section 2.7), population densities estimated from sub-samples were compared to actual population densities at 12 and 24 hours. Specifically, two clear plastic jars (cultures 1 and 2) containing 3.5 L of sea-water were stocked with C5 and C6...
stage animals to a density of 100 L\(^{-1}\) and *L. galbana* was added to a density of 1 \(\cdot 10^5\) cells ml\(^{-1}\). Three 50 ml samples were extracted from culture 1 and three samples were extracted from culture 2 after 12 hours and 24 hours, respectively. Estimated animal densities for each stage group \((N_{stage,i})\) were determined and summed to calculate an extrapolated total density \((N)\) using the sampling methods described above (section 2.9). Next, actual stage group densities \((AN_{stage,i})\) and total population density \((AN)\) were determined by filtering and collecting the entire culture population on a 55-μm mesh sieve, re-suspending the population in 50 ml of fresh seawater, and then counting animals under a dissecting microscope. Estimation error \((E)\) was determined as the difference between actual and estimated densities determined from each of the three 50 ml samples as follows.

\[
E = 100\% - \left(\frac{(N_{stage,i})}{(AN_{stage,i}) \times 100}\right)
\]

Where \(E\) is estimation error (%); \(N_{stage,i}\) is the estimated animal density for one of the 4 stage groups; and \(AN_{stage,i}\) is the actual density of one of the 4 stage groups.

Estimation of error is inversely related to sample size such that as animal densities increase, error decreases, and therefore estimation errors are expected to decrease as population densities increase. At 12 hours, C5-C6 density was 20 animals L\(^{-1}\) and estimated C5-C6 densities ranged from 10-20 animals L\(^{-1}\). Although the average sampling error was 17%, 10 animals L\(^{-1}\) estimated in one sample resulted in a sampling error of 50%. Estimated N1-N2 densities ranged from 30-40 animals L\(^{-1}\) and were within
14% of the actual density, 35 animals L\(^{-1}\). However, even at a low total population density at 12 hours (55 animals L\(^{-1}\)), the average difference between the actual and estimated total densities was 9%.

Animal density increased from 12 hours (55 animals L\(^{-1}\)) to 24 hours (318 animals L\(^{-1}\)) and as expected, estimation errors decreased. By 24 hours, estimated total population densities were within 6% of the actual densities (average 35%; Table 2). Lower group stage densities at 24 hours, compared to total animal densities, corresponded to higher estimation errors. Estimated N1-N2 densities ranged from 200-220 animals L\(^{-1}\) and were within 8% of the actual density (218 animals L\(^{-1}\)). Estimated N3-N6 densities ranged from 60-100 animals L\(^{-1}\) and were within 25% (average 17%) of the actual density (80 animals L\(^{-1}\)). The estimated densities of all stage groups, except for one estimate of C5-C6 density, were within 25% of actual densities. The estimated density of C5-C6 in one sample was 40 animals L\(^{-1}\) and the actual density was 20 animals L\(^{-1}\). Estimation errors for stage group densities, and thus total densities, are expected to further decrease as population densities increase over time.

2.10. Statistical Analysis

Statistical analysis was done using Statistical Analysis Software (SAS 9.1). Analysis of variance (ANOVA) was used to determine the variability in population densities both within and across cultures over time. Consistent events within the temporal cycle were determined by averaging the daily population densities across cultures (n = 6) and comparing the means using a Duncan’s Multiple Range Test (MRT;
The rates and consistency of population growth were compared across cultures using multiple regression analysis. Analysis of variance (ANOVA) and Duncan's Multiple Range Test (MRT; $\alpha = 0.1$) were also used to determine the variability in female density, egg production rates per female, and egg production rates per liter both within and across cultures over time.

3. Results

3.1. Time-Lag Experiment

Average egg production rates per female ($\text{EVI}^1$) were measured over 12 hours from females maintained in Kaneohe Bay seawater (control group) and for females maintained in Waikiki seawater with food added (treatment group; Fig. 1). A time-lag of 8 hours was observed as the first eggs were produced from females in the treatment group between 6 and 8 hours. From 8 to 12 hours, average egg production from females in the treatment group increased from $3 \pm 1$ to $8 \pm 1$ eggs per female; a rate of approximately 4 eggs in 4 hours. Copepod eggs were not observed by 8 hours from females in the control group, but by 12 hours females produced an average $2 \pm 1$ eggs (control group; Fig. 1)

3.2. Egg Hatching Experiment

This experiment was performed to determine the rate and cumulative percent of eggs that hatched into nauplii. After eggs were isolated into Petri dishes within 2 hours of
being produced, 50% of the eggs hatched within 2.5 hours, and over 80% by 10 hours (Fig. 2).

Diapause eggs were not observed in this investigation.

3.3. Population Cycle Experiment

To characterize fluctuating animal densities, animal densities (animals L\(^{-1}\); Fig. 3a) and rates of change in abundances (dN/dt; Figs. 3b), stage distributions (Figs. 4a-d), female egg production rates (EF\(^{-1}\)D\(^{-1}\); Fig. 5a) recruitment rates (EL\(^{-1}\)D\(^{-1}\); Figs. 5b), and mortality rates (animals L\(^{-1}\)D\(^{-1}\); Fig. 6) were averaged across six cultures on each sampling day. We observed a consistent temporal cycle comprised of five distinct phases: described as lag, growth, peak plateau, decline, and post decline, which occurred on days 0-1, 1-4, 4-8, 8-12, and 12-19 respectively. Naupliar development (i.e. development from N1 to N6) was completed in 3 days and copepodite development (development from C1 to C6) was completed in 5 days. Thus, development from an egg to a reproductively capable adult (C6) occurred in 8 days (Table 1).

3.3.1. Lag Phase

In the time-lag experiment, the first eggs were produced between 6 and 8 hours from the start of the experiment (treatment group, Fig. 1). Furthermore, in the egg quality experiment, eggs began to hatch within 0 to 4 hours of being produced (Fig. 2).
Assuming the rates of egg production and hatching are comparable to those in batch cultures, these results suggest that

the first nauplii were recruited into the population approximately 6 to 12 hours after stocking culture with adults and sub-adults. This assumption is validated as nauplii production within 12 hours of stocking cultures was lower than production from 12 to 24 hours (Table 1). Recruitment of the first nauplii between 6 and 12 hours also suggests that the duration of the N1-N2 stage group was less than 14 hours (Table 1), as N3 stage animals were observed in each culture by 24 hours. Although McKinnon et al. (2003), found the duration N1-N2 stage group duration to be approximately 1 day for an Australian strain of *B. similis*, differences can be expected in development times between the Australian strain and Hawaiian strain described in the present study.

From days 0 to 1 average population densities increased from 100 C5-C6 L\(^{-1}\) to 267 ± 53 animals L\(^{-1}\) (Fig. 3a); an average rate of 167 animals per liter per day (animals L\(^{-1}\)D\(^{-1}\); Fig. 3b). However, approximately 60% of the initially stocked animals died within 24 hours, as mean C5-C6 densities declined to 40 ± 13 L\(^{-1}\) by day 1 (Fig. 4d).

Mortality likely occurred within 12 hours of stocking cultures as was previously observed in the experiments used to estimate the sampling errors 12 hours after the start of a culture (Table 2). Abundances of grouped stages N1-N2 and N3-N6 on day 1 were 107 ± 10 L\(^{-1}\) and 112 ± 46 L\(^{-1}\) respectively (Figs. 4a, b). As expected from the development time, no copepodites (stages C1-C4) were detected (Table 1; Fig. 4c).

The first females were isolated in the first evening of the experiment and eggs were counted 12 hours after isolation. These females produced an average of 16 ± 3 eggs. The calculated average daily egg production rate of 30 ± 5 EF\(^{-1}\)D\(^{-1}\) (Fig. 5a) represents
the production of eggs between days 0.5 and 1.5 and thus does not take into account the initial 8 hour time-lag. Determination of recruitment rates (i.e. egg production per liter per day or EL⁻¹D⁻¹) within the lag-phase of the temporal cycle, below, requires an understanding of female egg production over the first 12 hours after transferring females from wild to culture conditions.

As females produced an average of 8 eggs per female (EF⁻¹) within the first 12 hours of being transferred to the laboratory and isolated in culture conditions (Fig. 1, treatment), and female density on day 1 was 22 FL⁻¹, recruitment from 0 to 12 hours was 176 EL⁻¹ (8 EF⁻¹ x 22 FL⁻¹). As females that were isolated 12 hours from the start of the experiment produced an average of 16 ± 3 eggs within 12 hours, recruitment from 12 to 24 hours was 352 EL⁻¹ (16 EF⁻¹ x 22 FL⁻¹). Recruitment from day 0 to 1 was thus 528 EL⁻¹D⁻¹ (sum of recruitment from 0-12 and 12-24 hours). The low growth rate during the lag-phase of the temporal cycle (ca. 167 animals L⁻¹D⁻¹) compared to that observed in the growth phase below, is explained in part by a time-lag in egg production of 8 hours upon transition from wild to culture conditions.

3.3.2. Growth Phase

The lag phase was followed by a period of rapid linear increase in population densities between days 1 and 5 (y = 669x - 560, R² = 0.97; Figs. 3a, b). From days 0 to 1, animal densities increased at rates ranging between 580 and 679 animals L⁻¹ (Fig. 3), and rates were not significantly different (multiple regression analysis, p = 0.90). The population was dominated by the naupliar stages (N3-N6), which comprised from 74 -
77% of the population between days 2 and 5. N1-N2 densities peaked on day 4 at 367 ± 155 animals L⁻¹, and densities of N3-N6 peaked on day 5 at 2100 ± 117 animals L⁻¹. As expected from the rate of animal maturation (Table 1), there was no recruitment into the C5-C6 stage group. Adult densities were 44 ± 12 (mean ± standard deviation) and did not significantly change from day 1 to 5 (ANOVA, p = 0.38; Fig. 4d). The first copepodites (C1-C4) were observed on day 4, corresponding to the expected development time to reach the C1 stage (4 days, Table 1). Population densities increased most rapidly during the growth phase of the temporal cycle, and presumably resulted from sustained female egg production described below.

Egg production rates per female remained constant at ca. 30 EF⁻¹D⁻¹ (ANOVA, p = 0.57; Fig. 5a) and female densities varied from 17 ± 6 to 25 ± 18 FL⁻¹ though without statistically significant variation among sampling dates (Duncan’s Multiple Range Test, MRT, p > 0.1). The average female density during this period was 22 ± 11 FL⁻¹ (Fig. 5a), and the average recruitment rates (i.e. egg production per liter per day or EL⁻¹D⁻¹) of 656 ± 87 to 612 ± 102 EL⁻¹D⁻¹ were found to be in good agreement with the rate of increase in population densities during the growth phase (i.e. 670 individuals L⁻¹D⁻¹; Fig. 3b).

Mortality rates from days 1 to 5 ranged from 166 ± 335 animals L⁻¹D⁻¹ on day 2 (22% of the population) to 231 ± 155 on day 5 (9% of the population; Fig. 6). Negative mortality rates on days 3 and 4 resulted from having a higher population growth rate (ca. 670 animals D⁻¹) than calculated rate of recruitment. This may have resulted from an underestimation of female densities as a result of sampling error. Sampling error on day 1 for C5-C6 density was 33% (Table 2). Error was expected to be the same from days 1
to 6 as densities of C5-C6 did not significantly change (Duncan’s Multiple Range Test, p > 0.1).

3.3.3. Peak Plateau Phase

Following the growth phase, population densities began to stabilize as the rate of increasing population densities began to decay. During this peak plateau phase, from days 5 to 8 the average density was 2424 ± 418 animals L⁻¹ (Fig. 3a) and peak densities in 4 of the 6 cultures were > 3000 animals L⁻¹. Rates of changing population densities slowed to approximately zero between days 5 and 8 (Fig. 3b), suggesting that recruitment into the population matched mortality. Although net changes in population densities was near 0, N3-N6 densities declined from 2100 ± 117 to 665 ± 103 animals L⁻¹. As densities of the stage group N3-N6 increased from days 2 to 5 (Fig. 3b), and maturation from the N3 to C1 stage occurred in 3 days (Table 1), we expected C1-C4 densities to increase during days 5 to 8. The increase in C1-C4 densities observed from days 5 (310 ± 190) to 8 (676 ± 354; Fig. 2c), however, was not statistically significant (MRT, p > 0.1).

A significant (Student’s one-tailed t-test, p < 0.1) increase in C5-C6 densities from day 7 to 8 corresponded to the maturation of eggs produced at the beginning of the experiment (day 0) to C6 on day 8 (Table 1). The increase in C5-C6 density correlated with a slight increase in N1-N2 density on day 8 (Figs. 4a, d). However, the N1-N2 densities on this day were not significantly different from the densities on days 5 and 6 (MRT, p > 0.1).
Female egg production rates significantly declined (MRT, p < 0.1) between days 5 (28 ± 5 EF⁻¹D⁻¹) and day 8 (8 ± 2 EF⁻¹D⁻¹; Fig. 5a) and this decline was inversely correlated to copepodite (C1-C4) densities (Pearson correlation, r = -0.92, df = 28, p < 0.01; Fig 7a). No significant relationship was found between female egg production rates and densities of nauplii (Pearson correlation, r = -0.16, df = 28, p = 0.84; Fig 7b).

Recruitment rates (EL⁻¹D⁻¹) declined from day 5 (612 ± 102) to day 7 (242 ± 28 EL⁻¹D⁻¹; Fig. 5b). A significant increase in recruitment on day 8 (MRT, p < 0.1; Fig. 5b), however, coincided with an increase in abundances of C5-C6 (Fig. 4d). Despite an increase in egg production rates per liter, no significant increase in N1-N2 was observed from days 6 to 8 (MRT, p > 0.1; Fig. 4a).

Mortality rates increased from day 5 (231 ± 155) to day 8 (952 ± 326 animals L⁻¹D⁻¹; Fig. 6). Densities of the stage group N3-N6 were approximately 2x higher than densities of the stage group C1-C4 (Figs. 4b, c), and high mortality may have occurred either during metamorphosis from N6 to C1, or during maturation of animals from C1 to C4 stages.

3.3.4. Decline Phase

After the peak plateau phase, average animal densities declined rapidly from day 8 (1701 ± 675) to day 9 (850 ± 417 animals L⁻¹); a rate of approximately 851 animals per liter per day (Fig. 3b). Thereafter, population densities continued to decline through day 12 (303 ± 125 animals L⁻¹; Fig. 3a). The rate of decline did not significantly differ between cultures (multiple regression analysis, p = 0.95) although the population in one
of the cultures, which was not included in the multiple regression analysis, crashed at a rate of -1180 animals L\(^{-1}\)D\(^{-1}\). Declines in population densities occurred in all developmental stages during the decline phase from days 8 to 12 (Figs. 4a-d).

Female egg production rates (eggs per female per day or EF\(^{-1}\)D\(^{-1}\)) decreased, though not significantly (MRT, p > 0.1) from day 8 (8 ± 2) to day 12 (4 ± 2 EF\(^{-1}\)D\(^{-1}\); Fig. 5a). In combination with this decline, decreasing female densities resulted in a significant decline in recruitment rates from day 8 (629 ± 238) to day 12 (112 ± 54 EL\(^{-1}\)D\(^{-1}\)). In addition to the decline in recruitment rates, mortality rates rapidly increased between days 8 to 10 (Fig. 6), resulting in a decline in all stage groups (Figs. 4a-d). The largest decline occurred in the N3-N6 stage group (from 661 ± 127 to 74 ± 53). Mortality rates declined, however, from days 10 to 12.

3.3.5. Post-Decline Phase

From days 12 to 19 population densities averaged 202 ± 155 animals L\(^{-1}\) and in 3 of 6 cultures densities were below 100 animals L\(^{-1}\) (Fig. 3a). There was no significant increase in population density in any culture following the decline phase and rates of change in animal densities were near zero (Figs. 3a, b). By day 13 nauplii (N1-N6) and copepodites (C1-C6) comprised 45 ± 20% and 65 ± 18% of the total population density, respectively. Population densities were < 400 animals L\(^{-1}\) but remained stable until the termination of cultures on day 19 (Fig. 3b). Stable population densities suggest that the population had stabilized at low densities with recruitment matching mortality. Egg production remained low at approximately 3 ± 1 EF\(^{-1}\)D\(^{-1}\) and female densities (10 ± 5 FL\(^{-1}\)
were stable from days 13 to 19 (Fig. 5a). As expected from stable population
densities, recruitment rates of ca. 30 EL⁻¹D⁻¹ matched mortality rates (30 animals L⁻¹D⁻¹).

3.4. Effect of Stocking Density on Population Dynamics

To determine the effects of initial stocking density on population dynamics,
population abundances (animals L⁻¹) and egg production rates (EL⁻¹D⁻¹) were measured
over time in cultures initially stocked with 100, 500, and 1000 C5-C6 L⁻¹ (hereafter
referred to as 100, 500, 1000 L⁻¹). On day 2, female egg production rates (EF⁻¹D⁻¹) in
cultures stocked with 100, 500, and 1000 L⁻¹, were 28 ± 2, 16 ± 1, and 11 ± 2 EF⁻¹D⁻¹
respectively and were significantly different (ANOVA, p < 0.005; Fig. 8a). Female egg
production rates declined over time in each treatment. As expected, egg production in
cultures stocked with 100 L⁻¹ were sustained from days 2 (28 ± 2) to 4 (28 ± 2) and
subsequently declined by day 8 (4 ± 1 EF⁻¹D⁻¹). Egg production rates (EF⁻¹D⁻¹) in
cultures with 500 L⁻¹ also did not significantly change from days 2 (16 ± 1) to 4 (14 ± 1;
Student’s t-test; p = 0.73) but subsequently declined by day 8 (5 ± 3). In contrast, egg
production in cultures with 1000 L⁻¹, declined from day 2 (11 ± 2 EF⁻¹D⁻¹) to day 4 (6 EF⁻¹
D⁻¹). Cultures with 1000 L⁻¹ were not replicated as stocking multiple cultures with 3500
animals was not realistic. No significant difference in egg production rates was found
between the three treatments on day 8 (ANOVA, p > 0.1).

On day 2, recruitment rates (EL⁻¹D⁻¹) in cultures with 500 and 1000 L⁻¹, were
2865 ± 21 and 3080 EL⁻¹D⁻¹ respectively (Fig. 8c). As only one culture contained an
initial stocking density of 1000 C5-C6 L⁻¹, significant differences in recruitment rates
between cultures with 500 and 1000 could not be determined. In cultures with 100 L\(^{-1}\), recruitment was 1030 \(\pm\) 80. Despite increasing the stocking density by 10x, from 100 to 1000, initial recruitment only increased by a factor of 3x, from ca. 1000 to 3000 EL\(^{-1}\)D\(^{-1}\). Increasing the stocking density by 5x, from 100 to 500, also resulted in an initial 3x increase in recruitment.

In addition to the rapid decline in female egg production rates (EF\(^{-1}\)D\(^{-1}\)) in cultures with 1000 L\(^{-1}\), female density also decreased from day 2 (500 FL\(^{-1}\)) to 4 (190 FL\(^{-1}\); Fig. 8b). Due to the combination of a decline in female abundance and egg production rates per female, recruitment rapidly declined from day 2 (3080 EL\(^{-1}\)D\(^{-1}\)) to day 4 (1140 EL\(^{-1}\)D\(^{-1}\)). Recruitment in cultures with 500 L\(^{-1}\), though less rapid, also decreased from days 2 (2865 \(\pm\) 21) to 4 (1880 \(\pm\) 283 EL\(^{-1}\)D\(^{-1}\)). Recruitment in cultures with 100 L\(^{-1}\) did not significantly change from day 2 (1020 \(\pm\) 87) to 4 (920 \(\pm\) 87 EL\(^{-1}\)D\(^{-1}\); Student’s one-tailed t-test, p > 0.1), however subsequently declined to day 8 (340 \(\pm\) 105). No significant difference was found between the recruitment rates between the three treatments on day 8 (ANOVA, p > 0.1).

Similar rates of increase in animal abundances (animals L\(^{-1}\)D\(^{-1}\)) were observed in cultures with 1000 and 500 L\(^{-1}\) from days 0 to 2 (y = 1220x + 960; and 1050x + 360, respectively). However, rates of increase in cultures stocked with 100 L\(^{-1}\) (y = 394x + 227; Fig. 9) occurred from days 1 to 5. Integrating these growth rates shows that nearly 2000 EL\(^{-1}\) were achieved in each treatment. Thus, despite higher stocking densities, net growth was the same in each treatment.

3.5. Dissolved Oxygen and Ammonia
Figs. 11a and 11b show ammonia and dissolved oxygen concentrations over time in cultures initially stocked with 100, 500, and 1000 C5-C6 L⁻¹. Ammonia concentrations were consistently low but exhibited some variability. The highest ammonia concentration (189 μg L⁻¹) was observed on day 8 in a culture initially stocked with 500 C5-C6 L⁻¹ (Fig. 11a). Concentrations of dissolved oxygen reached peak levels (5.1 mg L⁻¹ or 91% saturation) by day 2 and did not significantly change over time (ANOVA, p > 0.05; Fig. 11b).

4. Discussion

4.1. Population Cycle in Batch Cultures

Utilizing copepod nauplii for the first feeding of marine finfish larvae is challenging as copepod population densities in cultures significantly fluctuate over time. To better understand when and why population abundances change over time, we have assessed population dynamics of *B. similis* in small-scale batch cultures with maintained food levels. To the best knowledge of these authors, temporal assessment of female egg production rates (EF⁻¹D⁻¹), recruitment rates (eggs produced per liter per day, EL⁻¹D⁻¹), and mortality rates (%), had not been used to assess fluctuating animal densities in copepod cultures prior to this study.

Copepod populations were characterized by a consistent temporal cycle comprised of 5 distinct phases: lag, growth, peak plateau, decline, and post-decline. This
population cycle, occurring within 12 days, was largely driven by a series of cascading events where on day 5 female egg production rates (EFID-I) began to rapidly decline, on day 6 mortality rates began to increase, and on day 8 population abundances began to severely and rapidly decline. The population crash was thus due to the combination of a rapid and severe decline in egg production rates and an increase in mortality.

The decline in female egg production rates preceded the population crash. Though the onset of this decline occurred on day 5, at high animal densities, female egg production rates did not increase following a subsequent decline in population abundances. Thus, temporal variation in female egg production rates occurred independently of animal densities. The causes and physiological processes underlying the decline in female egg production rates could not be ascertained in this investigation.

In other studies, a decline in fecundity has been explained by low food availability (Checkley 1980a, b; Cahoon, 1981; Strøetrup and Jensen, 1990; White and Roman, 1992; Sedlacek and Marcus, 2004), temperature (Miller et al., 1977; Calbet and Agusti, 1999), hypoxia (Marcus et al., 2004; Sedlacek and Marcus, 2005), acute and chronic ammonia toxicity (Buttino, 1994), and density-dependent cannibalism from females and sub-adults (Ohman and Hirche, 2001). In the present study, however, food and dissolved oxygen levels remained high, ammonia concentrations were below acute and chronic toxicities reported for *Acartia clausi* (Buttino, 1994), *B. similis* has not been reported to be cannibalistic (Kimmerer, 1984; McKinnon et al. 2003), and animals were maintained within their ambient temperature range.

Alternatively, the decline in fecundity may have resulted from negative effects associated with animal crowding. In our experiments, in which the population consisted
of a single calanoid species, maximum densities of greater than 3000 animals L\(^{-1}\) were frequently achieved. These abundances are far greater than calanoid densities found in natural populations. Dominant copepod species such as the *Acartia tranteri* in Western Port Bay Australia reach up to 12 animals L\(^{-1}\) (Kimmerer and McKinnon, 1987). In Kaneohe Bay, where the animals in this study were collected, copepod abundances can reach 200 animals L\(^{-1}\) but are typically less than 40 animals L\(^{-1}\) (Peterson, 1975).

Production of chemicals from animals maintained at high densities in an enclosed environment may have conditioned the culture seawater, resulting in the observed decline in female egg production, as was found to occur for the freshwater rotifer *Synchaeta pectinata* (Kirk, 1998), saltwater rotifer *Brachionus plicatilis* (Yoshinaga et al., 1999), and in *Daphnia* (Seitz, 1984; Goser and Rattle, 1994; Burns, 1995). Even as animal densities began to decline during the decline phase of the temporal cycle, fecundity would not be expected to increase from females maintained in conditioned seawater.

### 4.2. Improving Egg Production by Optimizing Culture Conditions

Although female egg production rates consistently declined prior to the population crash, rates were consistently around 30 eggs per female per day (EF\(^{-1}\)D\(^{-1}\)) from days 1 through 5. These production levels may further be increased by improving the feeding regime. McKinnon et al. (2003) achieved egg production rates of 48 EF\(^{-1}\)D\(^{-1}\) when feeding *Bestiolina similis* with *Heterocapsa niei*—a dinoflagellate (20 x 14 \(\mu\)m; length x width) larger than the *Isochrysis galbana* strain used in this study (6 x 6 \(\mu\)m; length x width). When feeding *B. similis* with *Chaetoceros neogracilis* (apical axis 10 –
15 μm), densities of nauplii were low while adult densities and survival were high (Clauberg, 2004). A mixed nutritious diet, composed of a large strain suitable for adults and a smaller strain necessary for nauplii, may be optimal for achieving high reproductive performance and high nauplius survivorship.

Female egg production rates may also be increased by manipulating the initial stocking density of adults and sub-adults (stages C5 and C6). In this study, female egg production rates were highest at the lowest stocking densities (100 L⁻¹). As such, fecundity may be increased by stocking cultures with less than 100 C5-C6 L⁻¹. Studies by both Medina and Barata (2004), and Peck and Holste (2006), on the calanoid copepod, *Acartia tonsa*, also show that female egg production rates are higher in cultures with lower population densities. Peck and Holste, for example, found that females produced ~20 and 40 EJ⁻¹D⁻¹ at densities of 425 and 65 adults L⁻¹, respectively. However, recruitment rates (EL⁻¹D⁻¹) in the present study were higher in cultures with an initial stocking density of 500 L⁻¹, although the duration of increasing population densities was shorter compared to cultures with lower recruitment rates. Thus, net production in cultures with stocking densities of 100 and 500 L⁻¹ were approximately the same.

Higher nauplius yields may be temporarily achieved by optimizing culture conditions and/or increasing culture volume size, however, evidence from this study suggests that high egg production rates cannot be maintained over long periods of time in cultures with zero water exchange. Further, *B. similis* were not observed to produce diapause eggs in this study. Thus their eggs cannot be collected and stockpiled in long term cold storage such as may be possible for other species of calanoids which produce diapause eggs including *Acartia sp.* (Castro-Longoria and Williams, 1999; Castro-
Longoria, 1999), *Centropages hamatus* (Jo and Marcus, 2004), and *Eurytemora affinis* (Ban, 1992; Ban and Minoda, 1992). Despite these challenges, improving egg production capabilities is warranted as *Bestiolina similis* nauplii are a suitable first feed for small marine finfish larvae (McKinnon et al., 2003).

4.3. Consistency and Predictability of Temporal Cycle

Despite significant variation in population abundances over time, fluctuating densities were consistent and predictable. This consistency allowed for the forecasting of naupliar densities, which is essential for the ability to coordinate the first feeding of small marine finfish larvae with a suitable density of copepod nauplii. Hawaiian pink snapper (*Pristipomoides filamentosus*), for example, are fed copepod nauplii from days 3 to 8 days post-hatching, a total of 5 days before being weaned to ss-type rotifers (pers. communication Chris Kelley). The first food organism will typically take the larval fish through the first five to eight days following the onset of first feeding (Baensch, 2002, 2003). In this study, high egg production was sustained from days 1 to 5. As such, high copepod egg production beginning on day 1 must be coordinated with the first feeding of marine finfish larvae 3 days post egg hatch.

In addition to the timing of the first feeding, results from study can also be used to develop a suitable food supply for a target marine finfish species. Optimal predator prey ratios of striped mullet larvae (*Mugil cephalus*) to rotifers (*Brachionus rotundiformis*) for example, have been estimated to be between 10-20 fish larvae L⁻¹ to 10-20 rotifers ml⁻¹ (Tamaru et al., 1991). Likewise, marine pygmy angelfishes (genus *Centropyge*) which
produce up to 800 larvae in a single spawn, are typically stocked into tanks at densities of 10 - 20 individuals L⁻¹ (Tamaru & Baensch, 2001; Baensch 2002; 2003). This concentration allows a single brood to be reared in an 80 L tank, and assuming a live food density of approximately 10 - 20 nauplii ml⁻¹ day⁻¹ is provided, this equates to approximately 1.6 million copepod nauplii required per day to support one brood of 800 pygmy angelfish. Assuming production levels of 700 animals L⁻¹ D⁻¹ can be achieved in larger cultures under the same culture conditions, our results predict that a 2300 L (~575 gallons) container would be required to produce 1.6 million copepod nauplii per day.

4.4. Temporal Variation in Animal Densities

The variability in population abundances observed in the present study appears to agree with that observed in other studies regardless of culture volume. Animal densities in the present study, ranged from 100 at the start of the experiment to approximately 3000 animals per liter during the peak plateau phase of the temporal cycle. Schipp et al. (1999) observed similar changes in animal densities for the calanoid copepod, *Acartia spp.* in 1000 L tanks. Animal densities increased from approximately 250 animals L⁻¹ (day 0) to 2750 animals L⁻¹ (day 7), before declining to approximately 2000 animals L⁻¹ on day 8. Further, average peak naupliar abundances on day 7 were approximately 2000 nauplii L⁻¹ and thus were similar to those observed in the 3.5 L cultures in the present study (ca. 2300 nauplii L⁻¹). Shields et al. (2005) also found that populations of the calanoid copepod, *Parvocalanus sp.*, fluctuated cyclically in 400 L tanks that were harvested at 3 days intervals. Though not addressed, the decline in nauplius densities in the studies by
Schipp et al. (1999) and Shields et al. (2005) may be explained by decline in female egg production rates (EF\(^{-1}\)D\(^{-1}\)) and/or recruitment rates (EL\(^{-1}\)D\(^{-1}\)).

The present study demonstrates that although population abundances significantly fluctuate over time in copepod cultures, changes in animal densities were predictable as a result of consistent temporal changes in female egg production rates (EF\(^{-1}\)D\(^{-1}\)), recruitment rates (EL\(^{-1}\)D\(^{-1}\)) and population mortality rates (%). Furthermore, the variability in animal densities over time observed in the present study is comparable to populations of other calanoid copepod species maintained in larger culture volumes. This suggests that the information generated from this study can be utilized to characterize fluctuating densities in large scale cultures. Such information is useful for developing and coordinating a large-scale food supply with the first feeding of small marine finfish larvae.
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Table 1: Development times for *B. similis*. Initial development time indicates the age at molting into the first stage in each group. The duration of a stage group reflects the time between an animal entering and exiting a stage group.

<table>
<thead>
<tr>
<th>Stage Group</th>
<th>Initial development time (Days)</th>
<th>Duration of stage group (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>0</td>
<td>0.1 - 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>N1-N2</td>
<td>0.1</td>
<td>0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>N3-N6</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>C1-C4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>C5-C6</td>
<td>7</td>
<td>~10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> 50% of eggs hatch within 0.1 days (2 to 4 hrs) and 80% hatch within 0.5 days (10 to 12 hrs)

<sup>b</sup> The first nauplii were recruited to the population between 6 to 12 hours (0.4 days) and N3 were consistently observed by 24 hours suggesting development from N1-N2 occurred in approximately 0.6 days (15 hours)

<sup>c</sup> Value determined from maintaining animals in a glass viewing dish with *Isochrysis galbana* at 1 · 10<sup>5</sup> cells ml<sup>-1</sup> (data not shown).
Table 2: Estimated sampling errors (%) determined for each of the 4 stage groups described in this study after 12 and 24 hours of stocking cultures with 100 C5-C6 L⁻¹.

<table>
<thead>
<tr>
<th>Actual densities at 12 hrs</th>
<th>N1-N2</th>
<th>N3-N6</th>
<th>C1-C4</th>
<th>C5-C6</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample 1: Estimated densities</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>55</td>
</tr>
<tr>
<td>sample 2: Estimated densities</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>sample 3: Estimated densities</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>average: Estimation error (%)</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Actual densities at 24 hrs</th>
<th>N1-N2</th>
<th>N3-N6</th>
<th>C1-C4</th>
<th>C5-C6</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample 1: Estimated densities</td>
<td>218</td>
<td>80</td>
<td>0</td>
<td>20</td>
<td>318</td>
</tr>
<tr>
<td>sample 2: Estimated densities</td>
<td>220</td>
<td>60</td>
<td>0</td>
<td>40</td>
<td>300</td>
</tr>
<tr>
<td>sample 3: Estimated densities</td>
<td>200</td>
<td>100</td>
<td>0</td>
<td>20</td>
<td>320</td>
</tr>
<tr>
<td>average: Estimation error (%)</td>
<td>6</td>
<td>18</td>
<td>0</td>
<td>17</td>
<td>3</td>
</tr>
</tbody>
</table>
Fig. 1. Cumulative average egg production per female (EF⁻¹) over 12 hours from females (n = 8) maintained in Kaneohe Bay seawater without food addition (control group; open circles, dotted line) and females (n = 7) maintained in Waikiki Seawater with food addition (treatment group; open squares, solid line). A time-lag of 8 hours was observed as the first eggs were produced between 6 and 8 hours. Values are mean ± s.d.
Fig. 2: Cumulative average egg hatching (%) over 36 hours. Values are mean ± s.d of % hatch from 4 groups with 10 eggs in each group.
Fig. 3: (A): Animal abundances (N) in units of (animals L\(^{-1}\)) estimated over time (days) in population cycle experiment. (B): Rates of change in animal densities (dN/dt) between consecutive sampling days in units of (animals L\(^{-1}\)D\(^{-1}\)). Animal densities were estimated and averaged across 6 batch cultures on each sampling day. Error bars are ± s.d. Rates of change were determined by the difference in animal abundances (N) between consecutive sampling days; (N\(_{t}\) - N\(_{0}\)) / (t\(_{1}\) - t\(_{0}\)).
Fig. 4. Group stage densities (animals L\(^{-1}\)) over time (days) in population cycle experiment. Animal densities were estimated and average across 6 batch cultures on each sampling day. Error bars are ± s.d. Summation of grouped stages for each sampling day is total population density (see Fig. 1). Note difference in scales.
Fig. 5. (A): Birth rates per female \( b_r(t) \) in units of eggs produced per female per day \( (EF^{-1}D^{-1}) \), and female abundance \( n_f \) in units of female per liter \( (FL^{-1}) \) over time (days). (B): Recruitment rates \( R(t) \) in units of eggs produced per liter per day \( (EL^{-1}D^{-1}) \) over time (days). Birth rates per female \( (EF^{-1}D^{-1}) \) were multiplied by the abundance of females \( (FL^{-1}) \) to obtain recruitment rates \( (EL^{-1}D^{-1}) \). Values were averaged across 6 cultures on each sampling day. Error bars are ± s.d. A slight increase in female density was observed from day 1 to 6 (not shown in A), which was likely due to sampling error. Female densities were averaged from days 1 to 6 and the average was used to calculate \( EL^{-1}D^{-1} \).
Fig. 6. Population mortality rates (M(t)) in units of (animals L⁻¹D⁻¹) over time (days). Values are mean ± 95% confidence interval from six cultures.
Fig. 7: (A): Densities of the stage group C1-C4 (animals L⁻¹) compared against female egg production rates (EF⁻¹D⁻¹). (Two-tailed Pearson's correlation, r = -0.91, df = 28, p < 0.01). (B): Densities of the stage group N1-N6 (animals L⁻¹) compared against EF⁻¹D⁻¹. (Two-tailed Pearson's correlation, r = -0.16, df = 28, p = 0.83). Values obtained from sampling days 1, 2, 5, 6, 8 (n = 5) for each culture (n = 6).
Fig. 8. (A): Birth rates per female ($b_f(t)$) in units of eggs produced per female per day (EF$^{-1}$D$^{-1}$). (B): Female abundance ($n_f$) in units of female per liter (FL$^{-1}$) over time (days). (B): Recruitment rates ($R(t)$) in units of eggs produced per liter per day (EL$^{-1}$D$^{-1}$) over time (days) in cultures initially stocked at densities of 100 L$^{-1}$ ($n = 3$; open circles, solid line), 500 L$^{-1}$ ($n = 3$; open squares, dotted line), and 1000 L$^{-1}$ ($n = 1$; filled circles, solid line). Values were averaged across cultures on each sampling day. Error bars are ± s.d.
Fig. 9. Total animal densities (animals L\(^{-1}\)) over time (days) in cultures initially stocked with 100 (n = 3, open circles, solid line), 500 (n = 3; open squares, dotted line), and 1000 C5-C6 L\(^{-1}\) (n = 1; filled circles, solid line). Values are averaged across cultures on each sampling day. Error bars are ± s.d.
Fig. 10. Concentration of *Isochrysis galbana* \(10^5 \text{ cells ml}^{-1}\) measured over time (days). 6 cultures were set up over the course of a year with 2 cultures set up in Jan. 2 in June and 2 in August. Values were averaged across the 2 cultures on each sampling day. Error bars are ± s.d. Algal concentrations were maintained at \(1 \cdot 10^5 \text{ cells ml}^{-1}\) (solid line) by adding fresh algae 3x/wk by adding algae from stock cultures.
Fig. 11. (A) Concentration of ammonia (µg L⁻¹) and (B) Concentrations of dissolved oxygen (mg L⁻¹) over time (days). Values were averaged across cultures with initial stocking densities of 100 C5-C6 L⁻¹ (n = 3), 500 C5-C6 L⁻¹ (n = 3) and 1000 C5-C6 L⁻¹ (n = 1). Error bars are ± s.d. Concentrations of ammonia were 4X lower than the LC₅₀ value—the lethal concentration that kills 1% of the population—reported for *Acartia clausi* (Buttino, 1994; dotted line) but were higher than the range found in Kaneohe Bay (0 - 10 µg L⁻¹; dashed line). By day 10 ammonia concentrations fell below concentrations measured in Waikiki Aquarium sea-water and were thus not detectable.
Population dynamics of calanoid copepods in small-scale cultures:

II. Sustaining nauplius production by diluting cultures.

Kyle VanderLugt

Pacific Biosciences Research Center, 1993 East-West Rd., Univ. Hawaii Manoa, Honolulu, HI 96822

Corresponding Author: Kyle VanderLugt

Pacific Biosciences Research Center, U. Hawaii Manoa

1993 East-West Rd., Honolulu, HI 96822

Tel: (808) 956-8003 Fax: (808) 956-6984

e-mail: vanderlu@hawaii.edu

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Abstract:

Populations of the calanoid copepod, *Bestiolina similis* were periodically harvested during or before the peak plateau of the temporal cycle using three dilution techniques: re-seeding, up-scaling, and re-seeding with adult enrichment. Population dynamics were assessed in individual cultures in each of the three experiments to better understand how periodically diluting populations, within the context of population cycles, affects population abundances over time. Specifically, populations were divided into 4 stage groups (N1-N2, N3-N6, C1-C4, and C5-C6) and animal densities (animals L\(^{-1}\)) were measured over time. Female egg production rates (eggs produced per female per day, EF\(^{-1}\)D\(^{-1}\)) were also measured in the up-scale experiment and used to calculate recruitment rates (eggs produced per liter per day, EL\(^{-1}\)D\(^{-1}\)). Changes in animal densities over time in periodically diluted cultures were characterized by variation among three parameters: 1) duration of a time-lag before onset of increasing animal densities, 2) rate at which animal densities increased, and 3) duration of increasing animal densities. Optimized harvesting conditions which minimize the duration of the time-lag, promote a rapid increase in populations densities (i.e. promote high nauplius production) and which is sustained for long periods of time, are discussed from an aquaculture management perspective. Results from this study improve the understanding of how to consistently achieve high nauplius production over long periods of time.
1. Introduction

Copepod nauplii, especially those of paracalanid species, are suitable for the 1st feeding of small marine finfish larvae (McKinnon et al., 2003) and offer several advantages over traditionally used live feeds such as rotifers and Artemia (Shields et al., 1999; Støttrup, 2000). When provided as a first feed, copepod nauplii promote healthy development and improve the survival rate of difficult to rear marine finfish larvae such as grouper (Toledo et al., 1999), snappers (Singhagraiwan and Doi, 1993; Doi et al., 1994a, b; Schipp et al., 1999; Ogle and Lotz, 2000), and turbot (Witt et al., 1984). Several calanoid copepod species are now captive bred in large-scale (Toledo et al., 1997; Schipp et al., 1999; Payne and Rippingale, 2001; Shields et al., 2005). Copepod nauplii, however, remain underutilized in marine finfish hatcheries as 1) animal densities in cultures are well below those achieved for rotifers and Artemia and 2) yields of harvested nauplii are often highly variable (Payne and Rippingale, 2001; Shields et al., 2005).

To better understand the underlying mechanisms regulating population abundances over time, and to thus understand why daily harvest levels are highly variable, VanderLugt et al. (2007), assessed population dynamics of the calanoid copepod, *Bestiolina similis*, in small scale cultures. Fluctuating animal densities were characterized by a consistent temporal cycle comprised of 5 distinct phases: lag, growth, peak plateau, decline, and post-decline. Following a 5 day period of increasing animal densities, female egg production rates (eggs produced per female per day, EF⁻¹D⁻¹) and thus production of nauplii rapidly declined during the peak plateau phase.
This decline is particularly problematic for finfish hatcheries that rely on sustained production of a food supply suitable for the first feeding of finfish larvae.

Although copepod cultures have been maintained in marine finfish hatcheries over long periods of time by periodically harvesting populations (Schipp et al., 1999; Payne and Rippinagale, 2001), the effects of the harvesting techniques on population abundances over time has not been addressed. As a result, a harvesting regime which promotes sustained high nauplius production has not been developed. In the present study, populations of the calanoid copepod, Bestiolina similis, are periodically harvested within context of the population cycle described by VanderLugt et al. (2007). Specifically, populations were harvesting during or before the peak plateau phase of the temporal cycle. Population dynamics are assessed in individual copepod cultures to better understand the effects of harvesting populations on population abundances over time. Results from this study improve the understanding of how to sustain high nauplius production.

2. Materials and Methods

2.1 Copepod Collection, Culture Conditions, and Copepod Feeding

Materials and methods for the collection of copepods, copepod culture setup and conditions, and copepod feeding have been described previously (VanderLugt et al., 2007). Briefly, copepods were collected from central Kaneohe Bay, Oahu, Hawaii and transferred to the laboratory, where Bestiolina similis were sorted from other calanoid
copepods. Adult and sub-adults (stages C5-C6) were stocked into cultures at a density of 100 animals L⁻¹. The process from wild collection to the isolation of *B. similis* into their culturing jars took less than 6 hours.

Copepod cultures were maintained indoors with temperature ranging from 24-27°C and plant and aquarium fluorescent lights (40 watt, Philips®) were placed directly behind cultures (< 30 cm). A 12L:12D photoperiod was provided. Aeration was provided using a Dynamaster 2 piston-type air pump (E.G. Danner MFG. Inc.) pumped through 7.9 mm (OD) Tygon® plastic tubing connected to ceramic-type small pore air diffusers (Sweetwater®, Aquatic Ecosystems. Inc). The seawater, in which copepods were maintained, came from the Waikiki Aquarium and is naturally filtered in underground wells and required no further filtration.

Copepods were provided with *Isochrysis galbana* (Prymnesiophyceae, diameter 5 - 6 μm) at a density of ~1 · 10⁵ cells ml⁻¹. Food densities were kept above 0.85 · 10⁵ cells ml⁻¹ by adding *I. galbana* from stock phytoplankton cultures three times per week. Stock cultures of *I. galbana* were grown indoors with temperature ranging from 24-27°C in 1 L flasks with F/2 Pro Culture™ formula (Kent Marine, Inc.®). Algal cultures were renewed every 8 to 10 days by discarding 65% of the culture volume and inoculating new cultures with the remaining volume.

2.2. Assessment of Population Dynamics

Population dynamics were assessed in individual copepod cultures in three experiments (section 2.3) by measuring population abundances (N) in units of animals
per liter (animals L\(^{-1}\)) including stage distributions (N1-N2, N3-N6, C1-C4, and C5-C6 L\(^{-1}\)) at 1 to 2 day intervals. During periods of increasing animal densities, populations were occasionally measured every 3\(^{rd}\) day as rates of increase were linear and consistent for a minimum of 5 days. Birth rates (\(b_r\)) defined as the number of eggs produced per female per day (EF\(^{-1}\)D\(^{-1}\)), were also measured on each sampling day in the up-scale experiment (section 2.3) and were used to calculate the rate at which individuals were recruited to the population (\(R(t)\)), defined as the number of eggs produced per liter per day (EL\(^{-1}\)D\(^{-1}\))

Protocols for the measurement of animal abundances (\(N\)) and birth rates (\(b_r\)) have been described previously (VanderLugt et al., 2007). Briefly, population densities (\(N\)) were estimated from 50 ml culture sub-samples extracted from individual cultures. Animals in sub-samples were separated into four stage groups N1-N2, N3-N6, C1-C4, or C5-C6 and animal counts for each stage group were extrapolated to obtain stage group densities (animals L\(^{-1}\)). The extrapolated total animal density was determined by summing all four stage group densities.

Birth rates, defined as the average egg production rates per female per day (EF\(^{-1}\)D\(^{-1}\)), were determined by counting eggs produced from females that were isolated from individual cultures. Adults were isolated in the evening (18:00 to 21:00 hrs). After 12 hours the eggs, nauplii, and adult females (C6) were counted under a dissecting microscope (6x). The average number of eggs produced per female per 12 hours was calculated for each culture by dividing the total number of eggs and nauplii by the number of adult females. Recruitment rates, defined by the number of eggs produced per liter per day (EL\(^{-1}\)D\(^{-1}\)), were calculated by multiplying egg production rates per female
by female density ($FL^{-1}$). Female density was determined from the density of the C5-C6 stage group (animals L$^{-1}$) and assuming that 50% of animals were males and 50% were females (i.e. 1:1 sex ratio). This assumption was validated in the upscaling experiment (section 2.3) in which 24 adults were sexed on each sampling day and females comprised between 42 and 58% of the animals for the duration of the experiments. Further, greater than 90% of animals in the C5-C6 stage group are adults (stage C6) as the pre-adult stage (C5) lasts only 24 hrs (McKinnon et al., 2003; Table 3) and adults live for at least 10 days after reaching maturity (Table 1).

2.3. Dilution Experiments: Reseed, Upscale and Time-lag

In the first experiment, referred to as the reseeding experiment, four 1 gallon clear plastic jars with 3.5 L of seawater were diluted or reseeded by extracting 1 L culture volume and adding 2.5 L of fresh seawater. 3.5 L culture volume was maintained throughout the experiment. The four cultures were reseeded on the same days (days 6, 21, and 34), when copepod populations reached peak densities during the peak plateau phase of the temporal cycle.

In a second experiment, referred to as the up-scaling experiment, populations were diluted into consecutively larger culture volumes. Copepod populations in 1 L culture volumes were added to 2.7 L of fresh seawater on day 5, and the 3.7 L culture volume was subsequently diluted with 12.3 L of seawater on day 17. Dilutions in this experiment were also performed at peak population densities during the peak plateau phase of the temporal cycle.
In the third experiment, referred to as the time-lag experiment, the effects of adult density and the timing of the dilution on population abundances over time were assessed. Twelve one gallon culture containers with 3.5 L of seawater were set up in a randomized complete block design (RCBD), with 4 treatments each with 3 replicates. Replicates were blocked. The four treatments were 1) early dilution with adult enrichment 2) early dilution without adult enrichment 3) late dilution with adult enrichment and 4) late dilution without adult enrichment. All dilutions were performed by extracting 1 L culture volume and adding 2.5 L of fresh seawater. In early dilution treatments, culture dilutions were performed 4 days after the onset of an increase in animal densities. Late dilutions were performed 6 days after the onset of an increase in animal densities. Adults were not harvested in adult enrichment treatments. After diluting a culture (by extracting 1 L from a 3.5 L culture), the remaining 2.5 L of culture volume were gently filtered through a 253 μm mesh sieve and animals collected on the sieve were returned to diluted cultures. This sieving process was not performed in treatments without adult enrichment.

2.4. Statistical Analysis

Statistical analysis was done using statistical analysis software (SAS 9.1). Population densities both within and across cultures were assessed over time using analysis of variance (ANOVA). Population densities were averaged across cultures (n = 4 in both reseeding and up-scaling experiments; n = 3 in each treatment of time-lag experiment), on each day in each experiment. Daily averages were compared using a
Duncan's multiple range test (MRT; $\alpha = 0.1$). Rates of increasing animal densities were compared across cultures in the reseeding and up-scaling experiments using multiple regression analysis. Egg production rates per female, female density, and egg production rates per liter were also averaged across cultures on each sampling day were assessed using ANOVA and MRT.

3. Results

3.1. Reseeding experiment

Animal densities (animals L$^{-1}$; Fig. 1a) including stage distributions (N1-N2, N3-N6, C1-C4, and C5-C6 L$^{-1}$; Figs. 1b-1c) were measured at 1 to 2 day intervals in cultures that were diluted when populations reached peak densities during the peak plateau phase of the temporal cycle. Dilutions were performed on days 6, 21, and 34 and populations were maintained for 51 days. Time-lags were observed following culture dilutions, in which population abundances remained $< 1000$ animals L$^{-1}$ for periods of time ranging from 1 to 5 days prior to the onset of increasing animal densities (Table 1, Fig.1). The rates at which animal densities increased ($dN/dt$) were determined using regression analyses on periods of increasing animal densities (Table 1). Rates of increase ranged from 554 to 270 animals per liter per day (animals L$^{-1}$D$^{-1}$) and which decreased following consecutive dilutions (Table 1).

3.2. Up-scaling experiment
To determine if nauplii production could be sustained while up-scaling culture volume, total population abundances (animals L\(^{-1}\); Fig. 2a), stage distributions (N1-N2, N3-N6, C1-C4, C5-C6 L\(^{-1}\); Figs. 2c-f), female egg production rates (eggs per female per day or EF\(^{-1}\)D\(^{-1}\); Fig. 3a), and recruitment rates (eggs per liter per day or EL\(^{-1}\)D\(^{-1}\); Fig. 3b) were estimated for populations that were diluted into consecutively larger culture volumes: from 1 to 3.7 to 16 L cultures. A time-lag, ranging from 7 to 8 days, occurred after each dilution (Table 2). Following these time lags, population densities increased linearly (Table 2). No significant difference was found among all growth rates within and across the reseeding and up-scaling experiments (multiple regression analysis, \(p = 0.45\); Fig. 4).

Female egg production rates (eggs produced per female per day, EF\(^{-1}\)D\(^{-1}\)) significantly declined over time (ANOVA, \(p < 0.01\); Fig. 3a). Female egg production rates (EF\(^{-1}\)D\(^{-1}\)) measured from females isolated before and after the 1\(^{st}\) and 2\(^{nd}\) dilutions did not significantly differ (1\(^{st}\) dilution; Student's two tailed t-test, \(p = 0.44\); 2\(^{nd}\) dilution, Student's two tailed t-test, \(p = 0.87\)). Recruitment rates (i.e. eggs produced per liter per day, EL\(^{-1}\)D\(^{-1}\)) remained constant at 428 ± 191 EL\(^{-1}\)D\(^{-1}\) (average ± s.d.), from days 1 to 5 (Duncan's Multiple Range Test, \(p > 0.1\); Fig. 3b) and coincided with a linear increase in animal densities (Fig. 3b). However, an increase in animal densities was not observed from days 7 to 11, following the 1\(^{st}\) dilution, despite similar recruitment rates (463 ± 190 EL\(^{-1}\)D\(^{-1}\)). Increasing animal densities following the 1\(^{st}\) and 2\(^{nd}\) dilutions coincided with an increase in recruitment rates (Fig. 3b), which in turn coincided with an increase in female densities (Fig. 3a).
3.3. Time-Lag Experiment

Total animal densities (animals L\(^{-1}\)) were measured over time (days) in 4 dilution treatments; early dilution with adult enrichment (Fig. 5a), early dilution without adult enrichment treatment (Fig. 5b), late dilution with adult enrichment (Fig. 5c), and late dilution without adult enrichment (Fig. 5d), in order to identify the harvesting conditions which reduce the duration of the time-lag and promote sustained production of nauplii. Although densities of each of the four stage groups, (N1-N2, N-N6, C1-C4, and C5-C6 animals L\(^{-1}\)), were measured over time for each treatment, only densities of the stage groups N1-N2 and C5-C6 are shown for each treatment in Figs. 6a–d respectively.

In the early diluted treatments, animal densities increased from days 1 to 8 (Figs. 5a, b), though the increase from days 6 to 8 was not significant (MRT, p > 0.1). N1-N2 densities, in early diluted treatments (Figs. 6a, b) were constant from days 1 to 4, and increased from day 4 to day 7. In contrast, total animal densities in the late diluted treatments (Figs. 5c, d), increased from days 1 to 5 and then decreased to day 6. Densities of the stage group N1-N2 (animals L\(^{-1}\)) also increased from days 1 to 5 in the late diluted treatments (Figs. 6c, d), and thereafter decreased to day 6. Abundances of N1-N2 (animals L\(^{-1}\)) were significantly higher from days 6 to 8 in the early diluted treatments (Figs. 6a, b), than compared to the late diluted treatments (Figs. 6c, d).

In the early dilution treatment with adult enrichment (Fig. 5a), animal densities increased following each dilution. Though population abundances increased in the late dilution with adult enrichment treatment (Fig. 5c), the rate of increase, determined using
regression analysis, was approximately 100 animals L^{-1}D^{-1} (y = 97x + 385; R^2 = 0.98).

In contrast, animal densities in the early dilution without adult enrichment treatment (Fig. 5b) did not significantly increase following the second dilution. Animal densities in the late dilution without adult enrichment treatment (Fig. 5d) did not significantly increase following the 1st dilution.

Average C5-C6 abundances in the adult enrichment treatments increased to greater than 100 C5-C6 L^{-1} following each of the dilutions (Figs. 6a, c). In contrast, average C5-C6 abundances in treatments without adult enrichments remained < 100 C5-C6 L^{-1} throughout the experiment (Figs. 6b, d). In treatments with adult enrichment, densities of C5-C6 were positively correlated to N1-N2 densities in both early diluted cultures (Pearson’s correlation, r = 0.87) and late diluted cultures (Pearson’s correlation, r = 0.87; Fig. 7a). N1-N2 abundances were correlated to C5-C6 in early diluted cultures (Pearson’s correlation, r = 0.1) and late diluted cultures (r = 0.48; Fig. 7b).

4. Discussion:

4.1. Copepod populations in diluted cultures

Periodically harvesting populations of Bestiolina similis, is essential for sustaining high nauplius production over time. In batch cultures, fluctuating densities of B. similis are characterized by a consistent temporal cycle comprised of 5 distinct phases: lag, growth, peak-plateau, decline, and post-decline in which during the peak-plateau phase, nauplius production rapidly declined (VanderLugt et al., 2007). By diluting cultures
during or before the peak plateau phase, however, nauplius production was sustained. Several copepod species have now been successfully maintained for multiple generations by periodically harvesting populations, (*Acartia tonsa* Dana, Støstrup et al., 1986; *Acartia spp.*, Schipp et al., 1999; *Gladioferens imparipes*, Payne and Rippingale, 2001; *Parvocalanus sp.*, Shields et al., 2005). Though nauplius densities in these studies were highly variable over time, they were comparable to those achieved in the present study. Fluctuating densities were assessed in the present study, however, to identify the harvesting conditions which promote sustained production of nauplii over time.

Culture dilutions were followed by a time-lags ranging from 1 to 8 days before animal densities began to increase at rates ranging from 270 to 670 animals L\(^{-1}\)D\(^{-1}\). In a similar study, Schipp et al. (1999) periodically diluted populations of the calanoid copepod, *Acartia spp.*, by reseeding 5000 L tanks with 50 adults L\(^{-1}\) every 8 days. A one day time time-lag was consistently observed in these tanks before nauplius abundances increased from 50 animals L\(^{-1}\) on day 1 to 2000 animals L\(^{-1}\) on day 7; a rate of approximately 300 nauplii L\(^{-1}\)D\(^{-1}\). Although nauplius production rates were on average higher in the present study, production rates achieved by Schipp et al. (1999) were more consistent and predictable. In contrast, peak production levels achieved by Payne and Rippingale (2001) were higher than those in the present study but were highly variable and therefore less consistent. Payne and Rippingale (2001) harvested an average of 878 nauplii L\(^{-1}\)D\(^{-1}\) for the calanoid copepod, *Gladioferens imparipes* for 420 days in 500 L automated nauplius harvesting systems; though daily harvest yields ranged from 40 to 2226 nauplii L\(^{-1}\)D\(^{-1}\). Although production of *Bestiolina similis* nauplii was comparable to those achieved for other calanoid copepods in larger systems, a harvesting system which
provides consistently high nauplius production over long periods of time has yet to be developed.

4.1. Sustaining high egg and nauplius production

Sustaining high female egg production rates (eggs produced per female per day, \( \text{EF}^{-1}\text{D}^{-1} \)), is necessary to maintain high nauplius production and is achieved by regularly diluting copepod populations. Although female egg production rates (\( \text{EF}^{-1}\text{D}^{-1} \)) significantly declined over time in the up-scaling experiment, egg production rates remained greater than 10 \( \text{EF}^{-1}\text{D}^{-1} \) for 30 days. In contrast, female egg production rates in batch cultures with the same culture conditions (i.e. food levels, aeration, temperature, animal densities), rapidly and severely declined from approximately 30 \( \text{EF}^{-1}\text{D}^{-1} \) (day 5) to 5 \( \text{EF}^{-1}\text{D}^{-1} \) (day 7). Although egg production was not measured in the reseeding and time-lag experiments, animal densities consistently increased following all culture dilutions. This suggests that fecundity in the reseeding and time-lag experiments also remained higher than in batch cultures. However, the causes and physiological processes underlying the irreversible decline in female egg production rates could not be ascertained in this investigation.

The decline in fecundity is not assumed to have resulted from a decline in food quantity or quality as was found in studies by Kiørboe (1989), Jónasdóttir (1994), and Ianora et al. (1995). Fresh \( I.\text{galbana} \) was added to copepod cultures 3x/week and food levels were maintained above \( 0.85 \cdot 10^5 \) cells ml\(^{-1} \) in all cultures. The decline in female egg production rates is also not assumed to have resulted from a decline in water quality.
associated with increasing animal densities. Although water quality was not monitored in the present study, ammonia and dissolved oxygen concentrations measured in batch cultures did not explain the decline in female egg production rates (VanderLugt et al., 2007). Furthermore, peak animal densities in both the up-scaling experiment and in non-diluted cultures were approximately 4000 animals L\(^{-1}\). Thus ammonia concentrations associated with animal densities in the present study are assumed to be similar those in batch cultures. Though the causes for the decline in female egg production rate were not determined, egg production rates remained higher in diluted compared to non-diluted cultures. Sustaining higher egg and nauplius production may be achieved, however, by improving the harvesting regime.

4.3. Optimum harvesting conditions

Results from diluted and batch cultures indicate that high nauplius production may be sustained by 1) harvesting the population during or before the peak plateau phase of the temporal cycle and 2) maintaining C5-C6 abundances between 100 and 500 L\(^{-1}\). Cultures in the present study were initially stocked with 100 C5-C6 L\(^{-1}\) and population consistently increased at a rate of approximately 500 animals L\(^{-1}\)D\(^{-1}\) for a period of 5 days. Following culture dilutions, densities of C5-C6 were consistently reduced to < 100 animals L\(^{-1}\). Populations in diluted cultures were characterized by a variable time-lag lasting up to 8 days followed an increase in animal densities comparable to those achieved in cultures stocked with wild caught animals. In general, however, the duration of the time-lag decreased (Fig. 8b) and animal densities increased more rapidly (i.e.
nauplius production was higher) in cultures with higher initial abundances of C5-C6 (Figs. 8c).

Animal densities increased most rapidly (i.e. nauplius production was highest) in cultures with initial stocking densities of 500 C5-C6 L\(^{-1}\). Further increasing the initial stocking density did not result in more rapid rates of increasing densities because female egg production rates (EF\(^{-1}\)D\(^{-1}\)) decreased with increasing adult animal densities (VanderLugt et al., 2007). Studies by Medina and Barata (2004), and Peck and Holste (2006), on the calanoid copepod, *Acartia tonsa*, also show that female egg production rates are higher in cultures with lower population densities. As a result of the trade off between stocking densities and female egg production rates, recruitment rates (i.e. egg production per liter per day) reach a plateau at approximately 500 C5-C6 L\(^{-1}\) in which stocking densities of > 500 C5-C6 L\(^{-1}\) does not result in an increase in recruitment of nauplius production rates (Fig. 9).

Although animal densities increase more rapidly in cultures with higher adult and sub-adult densities (C5-C6 L\(^{-1}\)), higher rates of increase were sustained for shorter periods of time (Fig. 8d). Shields et al. (2005) achieved rapid rates of increasing densities (i.e. high nauplius production) by harvesting populations of the calanoid copepod, *Parvocalanus sp.* at three day intervals and maintained high adult densities. Similar to the results in the present study, populations were characterized be time-lags ranging from 1 to 3 days before populations increased for approximately 4 days.

Furthermore, Lemus et al. (2004) achieved the highest quantity of harvested nauplii from the calanoid copepod *Acartia spp.* when harvesting 75% of the population, compared to 0, 25, and 50% culture volume, every other day over 16 days. When stocking batch
cultures with 500 C5-C6 L⁻¹ VanderLugt et al. (2007) achieved a rate of increase rate of approximately 1100 animals L⁻¹D⁻¹. Harvesting 75% of the population daily equates to 825 nauplii L⁻¹D⁻¹, a similar harvesting yield achieved by Payne and Rippingale (2001) for the calanoid copepod, *Gliadioferens imparipes*. Assessment of fluctuating animal densities in the present study indicates that high nauplius production may be achieved by maintaining high adult densities while frequently harvesting the population during or before the peak plateau phase of the temporal cycle.
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Table 1. Summary of animal densities over time in the reseeding experiment (Fig. 1).

<table>
<thead>
<tr>
<th>Dilution (Day)</th>
<th>Time-lag (Days)</th>
<th>Period of Increasing Densities (Days)</th>
<th>dN/dt (^b) (Animals L(^{-1}) D(^{-1}))</th>
<th>Cumulative production (^b) (Animals L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start (Day 0)</td>
<td>0</td>
<td>Days 0 to 5 (5 days)</td>
<td>548</td>
<td>2740</td>
</tr>
<tr>
<td>1(^{st}) dilution (Day 6)</td>
<td>Days 6 to 11 (5 days)</td>
<td>Days 11 to 21 (10 days)</td>
<td>468</td>
<td>4680</td>
</tr>
<tr>
<td>2(^{nd}) dilution (Day 21)</td>
<td>Days 21 to 22 (1 day)</td>
<td>Days 22 to 31 (9 days)</td>
<td>292</td>
<td>2628</td>
</tr>
<tr>
<td>3(^{rd}) dilution (Day 34)</td>
<td>Days 34 to 38 (4 days)</td>
<td>Days 38 to 44 (6 days)</td>
<td>270</td>
<td>1620</td>
</tr>
</tbody>
</table>

\(a\) dN/dt is the rate of increase in animal densities over time and was determined using regression analysis on animal densities during periods of increasing densities.

\(b\) Cumulative production was determined by multiplying duration of increasing animal densities by rate of increase.
Table 2. Summary of animal densities over time in the up-scaling experiment (Fig. 2a).

<table>
<thead>
<tr>
<th>Dilution (Day)</th>
<th>Time-lag (Day)</th>
<th>Period Increasing Densities (Days)</th>
<th>$dN/dt$ $^a$ (Animals L$^{-2}$D$^{-1}$)</th>
<th>Cumulative production $^b$ (Animals L$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start (Day 0)</td>
<td>0</td>
<td>Days 0 to 5 (5 days)</td>
<td>476</td>
<td>2380</td>
</tr>
<tr>
<td>1$^{st}$ dilution (Day 5)</td>
<td>Days 5 to 12 (7 days)</td>
<td>Days 12 to 17 (5 days)</td>
<td>673</td>
<td>3365</td>
</tr>
<tr>
<td>2$^{nd}$ dilution (Day 17)</td>
<td>Days 17 to 25 (8 day)</td>
<td>Days 25 to 30 (9 days)</td>
<td>478</td>
<td>2390</td>
</tr>
</tbody>
</table>

$^a$ $dN/dt$ is the rate of increase in animal densities over time and was determined using regression analysis on animal densities during periods of increasing densities.

$^b$ Cumulative production was determined by multiplying duration of increasing animal densities by rate of increase.
Fig. 1. Population abundances (N) in units of (animals L$^{-1}$) over time (days) in reseeding experiment. (A): Total population abundances were determined from the summation of the 4 stage groups; (B): N1-N2 (C): N3-N6. (D) C1-C4 and (E): C5-C6. Animal densities were averaged across 4 cultures on each sampling day. Error bars are ± s.d. Note different scales.
Fig. 2. Population abundances (N) over time (days) in reseeding experiment. (A): Total population abundances (animals L$^{-1}$). (B): Total animal abundances ($10^3$ animals per culture) determined by multiplying animal densities by culture volume. (C): N1-N2 (D): N3-N6. (E) C1-C4 and (F): C5-C6. Animal densities were averaged across 4 cultures on each sampling day. Error bars are ± s.d. Note different scales.
Fig. 3. Egg production rates over time in up-scale experiment. (A): Birth rates ($b_f(t)$) in units of eggs produced per female per day (EF$^{-1}$D$^{-1}$), and number of females ($n_f$) in units of females per liter (FL$^{-1}$), over time (days). (B): Recruitment rates ($R(t)$) in units of eggs produced per liter per day (EL$^{-1}$D$^{-1}$) and total animal abundances ($N$) in units of animals per liter (animals L$^{-1}$) over time (days). $R(t)$ was determined by multiplying $n_f$ by $b_f$. Values were averaged across 4 cultures on each sampling day. Error bars are ± s.d.
Fig. 4. Animal abundances (N) in units of animals per liter (animals L$^{-1}$) over time (days) in reseeding and up-scaling experiments. Animal densities during time-lags (tables 1 and 2) are not shown. Animal densities were averaged across 4 cultures in each experiment on each sampling day. Error bars are ± s.d. Regression lines were used to determine rate of increasing animal densities following all dilution in each experiment (tables 1 and 2). No significant difference was found among all rates of increase (multiple regression analysis, p = 0.45).
Fig. 5. Animal abundances (N) in units of animals per liter (animals L$^{-1}$) over time (days) in 4 dilution treatments. (A): Early dilution with adult enrichment. (B): Early dilution without adult enrichment. (C): Late dilution with adult enrichment. (D): Late dilution without adult enrichment. Animal densities were averaged from 3 cultures in each treatment on each sampling day. Error bars are ± s.d.
Fig. 6. Animal abundances (N) of the stage groups N1-N2 and C5-C6 (animals L⁻¹) over time (days) in 4 dilution treatments. (A): Early dilution with adult enrichment. (B): Early dilution without adult enrichment. (C): Late dilution with adult enrichment. (D): Late dilution without adult enrichment. Animal densities were averaged from 3 cultures in each treatment on each sampling day. Error bars are ± s.d.
Fig. 7. (A): Densities of the stage group N1-N2 (animals L⁻¹) compared against densities of the stage group C5-C6 (animals L⁻¹) in adult enrichment treatments. Densities were correlated in both early diluted cultures (Pearson correlation, r = 0.87) and late diluted cultures (Pearson correlation, r = 0.87). (B): Densities of the stage group N1-N2 (animals L⁻¹) compared against densities of the stage group C5-C6 (animals L⁻¹) in treatments without adult enrichment. Densities were correlated in both early diluted cultures (Pearson correlation, r = 0.1) and late diluted cultures (Pearson correlations, r = 0.48).
Fig. 8. (A): Summary of animal densities (animals L\(^{-1}\)) over time (days) in cultures with varying initial densities of C5-C6 (animals L\(^{-1}\)). Lines of best fit are polynomial of the form \(y = ax^2 + bx + c\). Population data were taken from several experiments as follows; 500 and 1000 (VanderLugt et al. 2007); 100 averaged across reseeding and up-scale experiment (\(n = 8\)); 73 from up-scale experiment (\(n = 4\)); 13 from reseeding experiment (\(n = 4\)). Changes in animal densities were characterized by 3 parameters; duration of time-lag, rate of increase (\(dN/dt\)) in animal densities (Animals L\(^{-1}\)D\(^{-1}\)), and the duration of increasing densities. (B): Duration of time-lag (days) compared against initial stocking density of C5-C6 (animals L\(^{-1}\)). (C): Rate of increase (\(dN/dt\)) in animal densities (animals L\(^{-1}\)D\(^{-1}\)) compared against initial stocking density of C5-C6 (animals L\(^{-1}\)). (D): Duration of increase (days) compared against rate of increase (\(dN/dt\)). Filled values are for populations following capture and isolation of wild animals into cultures. Open values represent animal densities following dilutions in the three reseeding experiments in the present study.
Fig. 9. Densities of C5-C6 (animals L\(^{-1}\)) compared against egg production rates per liter (EL\(^{-1}\)D\(^{-1}\) or recruitment) and densities N1-N2 (animals L\(^{-1}\)).