12

Intensive and Extensive Production Techniques to Provide Copepod Nauplii for Feeding Larval Red Snapper *Lutjanus campechanus*

Ronald P. Phelps, Gede S. Sumiarsa, Emily E. Lipman, Hsiang-Pin Lan, Komarey Kao Moss, and Allen D. Davis

ABSTRACT

A series of studies were conducted at the Claude Peteet Mariculture Center, Gulf Shores, Alabama, to develop protocols for obtaining large quantities of copepod nauplii from organically fertilized ponds or from laboratory-based systems. Use of alfalfa meal, fishmeal, rice bran, and wheat bran as organic fertilizers to stimulate copepod production were compared. The use of aeration to improve copepod production in wheat bran–fertilized ponds was also studied. Fatty acid profiles of pond-produced nauplii were described, and the effects of acclimation and enrichment were determined.

Maximum densities of > 5,000 nauplii/L were common in most pond management protocols and averaged > 1,000/L. Aerated wheat bran–fertilized ponds averaged 5,079 nauplii/L. Zooplankton production was cyclic with rotifer abundance peaking at 6–14 days after filling and nauplii abundance at 10–14 days after filling. An average of 4.9 million nauplii were collected per trap set. When nauplii were acclimated from 10–15 ppt to 32 ppt over 6 hours, there were reductions of 44.2% in total lipids, 44.2 and 55.5% in polar and nonpolar docosahexaenoic acid (DHA), respectively, and 23.9 and 64.9% in polar and nonpolar eicosapentaenoic acid (EPA), respectively. A 3-hour enrichment in A-1 Super Selco returned total fatty acid, DHA, EPA, and arachidonic acid (ARA) levels of nauplii to values similar to that of preacclimation.

The intensive production of the cyclopoid copepod *Apocyclops panamensis* was studied. Adult stocking rates of 320, 640, 1,280, 2,560, and 5,120/L were evaluated in a series of trials. Wild-caught adult copepods were stocked into 40-L clear plastic bags filled with 30 ppt artificial seawater and *Isochrysis galbana*, at a density of 500,000 cells/mL. A mean yield of 16,942 ± 794 nauplii/L was obtained when adult *A. panamensis* were stocked 5,120/L. Production per adult female ranged from 8.1 to 8.5 nauplii per female at adult stocking densities of 320–2,560/L and 4.7 nauplii per female at 5,120 adults/L.

A closed batch system was evaluated, tracking two populations over time. A total of 10 trials, ranging from 4 to 9 days each, were conducted over a period of 77 days, restocking adults into new bags at each harvest. Production of nauplii and copepodids in a closed system varied depending on the adult density stocked. Mean pro-

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Corresponding author: Ronald P. Phelps, Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL 36849,USA; Phone: (334) 844-9317; Fax: (334) 844-9208; E-mail: RPPhelps@acesag.auburn.edu.

duction in population A was 10,939 ± 3,200/L, but ranged from 144 to 31,532/L. Mean production in population B was 14,366 ± 3,095/L and ranged from 250 to 36,800/L. Females in the closed system averaged 14.13 nauplii and copepodids per female.

INTRODUCTION

The small mouth gape of red snapper *Lutjanus campechanus* larvae requires that a small food organism be available at first feeding. Copepod eggs and nauplii are an important food for many marine fish larvae in their natural habitat (Hunter and Lasker 1981; Houde 1978; Toledo et al. 1999). In hatcheries copepods have been used as the first feed for larvae of halibut *Hippoglossus hippoglossus*, turbot *Scophthalmus maximus*, and cod *Gadus morhua* L. (Støttrup et al. 1998), mangrove red snapper, *Lutjanus argentimaculatus* (Singhagraiwan and Doi 1993), sea bream *Archosargus rhomboidalis*, bay anchovy *Anchoa mitchilli*, and lined sole *Achirus lineatus* (Houde 1975, 1978). Larval mangrove snapper raised on rotifers failed to survive after 5 days after hatching, but when the larvae were fed copepod nauplii, survival was 40% after 21 days after hatching (Doi and Singhagraiwan 1993). Daily specific growth rates of cod have been shown to increase from 2.8 to 21% with an increase in availability of copepod nauplii (van der Meeren and Naess 1993). Survival of turbot larvae was 73% when fed *Artemia* nauplii and 93% when fed copepod nauplii (Kuhlmann et al. 1979).

Hatchery techniques are not well established for the mass production of copepod nauplii. Producing copepods under a controlled environment requires complex facilities and sophisticated skill (Støttrup and Norsker 1997). In addition, culturing live food organisms in a controlled environment may not be economical for certain hatchery applications (Graves and Morrow 1988).

An alternative way to obtain live food organisms is to collect zooplankton from fertilized ponds (Graves and Morrow 1988; Misra and Phelps 1992). Species abundance, however, may vary from day to day and the act of trapping may damage organisms and affect their nutritional quality. Salinity at which copepods are produced and at which they are being given as food may differ and also reduce copepod survival and nutrient quality. Changes in salinity stimulate crustaceans to adjust their osmoregulation. It has been observed in the adult calanoid copepod *Eurytemora affinis* and zoea 1 of several decapod crustacean larvae that extracellular osmoregulation is associated with energy expenditure as part of active ion transport, involving degradation of energy-rich compounds such as lipid and protein (Gonzales and Bradley 1994; Kimmel and Bradley 2001; Torres et al. 2002). When copepod nauplii are to be used as feed for larval fish, changes in salinity may affect their nutrient quality.

Nutrient quality of several types of organisms can be improved through supplemental enrichment. Various enrichment regimes have been evaluated to improve the nutrient quality of rotifers (Støttrup and Attramadal 1992; Fernandez-Reiriz et al. 1993) and *Artemia* (van Ballaer et al. 1985; Mourente et al. 1993; Craig et al. 1994; Rainuzzo et al. 1994). Additional enrichment of copepods is also possible to ensure adequate nutrition for fish larvae (Craig et al. 1994; Salhi et al. 1997; Planas and Cunha 1999; Toledo et al. 1999).

Extensive production of copepods in ponds can vary seasonally, and not all marine hatcheries have access to ponds that can be dedicated to copepod production. Intensive production of copepods in an indoor environment offers better control over species composition and nutritional quality. The feasibility of culturing copepods in intensive indoor systems has been investigated, and maximum densities of 26,000 harpacticoid copepod *Tigriopus brevicornis* nauplii/L (Vilela 1992) and 33,000 cyclopoid copepod *Apocyclops royi* nauplii/L (Cheng et al. 2001) have been reported. Intensive copepod production systems, however, have been difficult to maintain over extended periods of time (Davis 1983; Støttrup and Norsker 1997).

The following reports on a series of trials conducted at the Claude Peteet Mariculture Center, Gulf Shores, AL, USA, to provide copepod nauplii to feed larvae of red snapper *Lutjanus campechanus*. Topics addressed include: pond production techniques, trapping, acclimation and enrichment considerations, as well as intensive production trials with *Apocyclops panamensis*.

MATERIALS AND METHODS

EXTENSIVE PRODUCTION TECHNIQUES

Pond Production

Three pond fertilization studies were conducted to optimize the production of copepod nauplii using outdoor 1,100-m^2 ponds having an average depth of 1.0 m. Details of each study are given in Table 12.1. In each study organic fertilizers were given at rate of 250 kg/ha, followed by weekly applications of 125 kg/ha using three replicate ponds per treatment. Liquid inorganic fertilizer (38-8-0) was added to all ponds initially at 20 L/ha and at 10 L/ha at each week thereafter. In Study III, one of the treatments with wheat bran was provided with aeration for 8 hours (1000–0600) per day using a 1-hp Aero-2 aspirator pump aerator.

Zooplankton sampling began 7 days after adding water to the pond. In Studies I and II, zooplankton was sampled every 2 days, while in Study III sampling was weekly. A 25-L composite sample, 5 L taken from the top 30 cm of water from each corner of the pond and 5 L near the drain, was taken, concentrated to 1 L over 21-μm mesh netting, and fixed in Lugol's fixative. Zooplankton was enumerated as to the abundance per liter of copepod nauplii, adult copepods, rotifers, and other organisms. Representative samples were preserved in 4% buffered formalin for later identification.

Dissolved oxygen (DO) and temperature were monitored daily between 0500 and 0600 hours using a YSI 55 DO meter. Total ammonia, pH, and salinity were monitored once a week using a LaMotte Ammonia Nitrogen Test Kit (± 0.02 ppm; LaMotte Co., Chestertown, MA, USA), a Fisher Scientific Accumet meter (Fisher Scientific International Inc., Hampton, NH, USA), and a Fisher Scientific refractometer, respectively. Pond transparency was measured weekly with a secchi disc to the nearest centimeter.

In all three studies, data collected over time by treatment were analyzed as repeated measures analysis of variance (ANOVA) using SAS Version 6.12 for Windows (SAS Institute Inc. 1996). When significant differences among treatments were detected, a least significant difference (LSD) multiple-range test was applied (Sokal and Rohlf 1981). Correlations of zooplankton abundance to other environmental parameters were analyzed and considered significant where $r^2 > 0.50$ and $P < 0.05$.

Table 12.1. Pond fertilization protocols used in Studies I, II, and III

Study	Organic fertilizer	Study period	Salinity
I	Alfalfa meal Wheat bran Fish meal	37 days	14 ppt
II	Alfalfa meal Wheat bran	30 days	12 ppt
III	Rice bran Wheat bran Wheat bran[a]	50 days	21 ppt

Note: Organic fertilizers were added at an initial rate of 250 kg/ha, followed weekly by 125 kg/ha applications. Inorganic fertilizer (38-8-0) was added at an initial rate of 20 L/ha, followed weekly by 10 L/ha applications.

[a] Aeration was provided nightly to three wheat bran–fertilized ponds.

Nutritional Quality Considerations

Fatty acid profiles were determined for unenriched copepod nauplii *Apocyclops panamensis* collected as part of fertilization trials from alfalfa meal–fertilized ponds in Study II, and from rice bran– and wheat bran–fertilized ponds in Study III. Ponds were sampled every morning (0700–0900 hours) to identify ponds in which the number of copepod nauplii was at least 90% of the total zooplankton organisms (≥ 30 μm). Copepods were trapped from such ponds using the trap shown in Fig. 12.1. The trap consisted of three cylindrical containers (140 L each) connected in series by 5.08-cm pipe. A set of 40-μm and 100-μm nylon filter bags with drawstrings around the mouth, 80-cm long and 17.5-cm diameter, were tied to each water outlet. The 100-μm mesh bag was placed inside the 40-μm mesh bag. A sump pump (1/3 HP-115 V) with a mean pumping rate of 133.81 ± 8.5 L/min was connected to a water supply pipe and suspended in the water column of the pond. Water passed through the 100-μm bag then the 40-μm bag into the surrounding container, where it then flowed back to the pond.

Nauplii were harvested from the trap and held in brackish water at 200/mL and acclimated in a plastic container with a maximum volume of 120 L. Acclimation was accomplished by dilution of the initial holding water (8~12 ppt) with 32 ppt

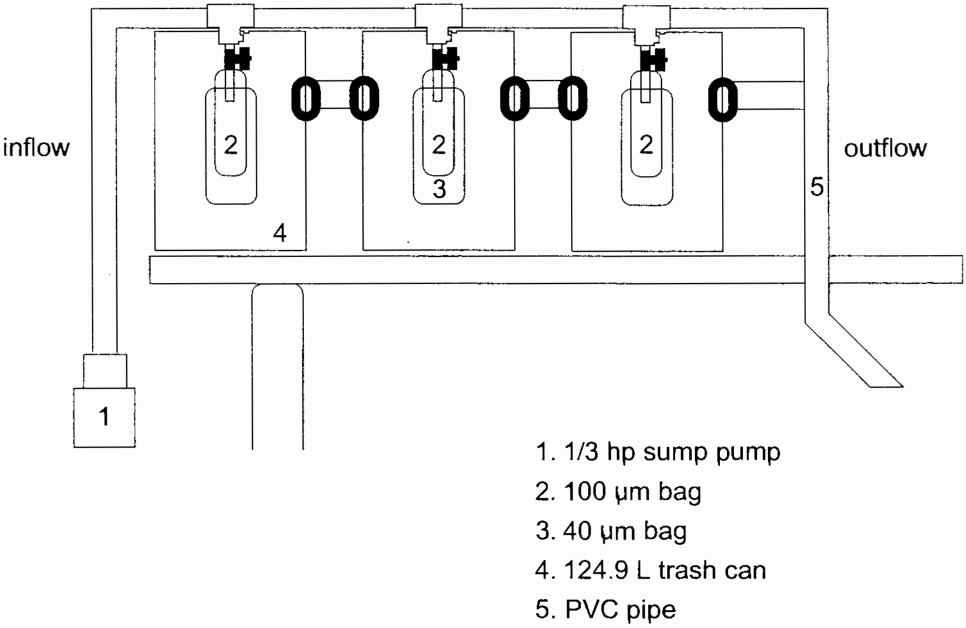

Figure 12.1. Design of trapping apparatus used to capture wild copepods from ponds, consisting of a 1/3-horsepower (hp) sump pump, 100-µm bags, polyvinyl chloride (PVC) pipe, and three 124.9-L trash cans.

seawater to achieve an end point of 32 ppt. Aeration was added at 250 mL/min to both the acclimator and the seawater supply. Copepod nauplii were not fed during the experiments. Salinity was measured using a refractometer (± 1 ppt). Water flow and salinity was monitored hourly to maintain the salinity increase evenly. Temperature and DO were measured hourly using a YSI DO meter (Model 8510; YSI Inc., Yellow Springs, OH, USA). Aeration was adjusted to maintain the DO level above 4 mg/L.

In each trial, before acclimation, at least 50 million of the collected copepod nauplii (approximately 5.0 g wet weight) were sieved through 21-µm Nitex plankton net, rinsed with approximately 1 L of distilled water, placed in screwed-cap glass jars, and stored at −20°C as described by Christie (1982). After acclimation, a minimum sample of 5.0 g (wet weight) of copepod nauplii were collected and frozen following the same procedures. All samples were held for lipid extraction and fatty acid analysis.

Enrichment of Copepod Nauplii

A series of protocols to enrich the nutrient quality of the copepod nauplii were evaluated using a commercial enrichment product, A1 Super Selco (INVE Americas Inc., Salt Lake City, UT, USA), marketed for rotifers and *Artemia*. Copepod nauplii were enriched for 3-, 6-, and 12-hour periods following the manufacturer's recommendation of 0.6 mg enrichment material pre liter of seawater. Enrichment trials were conducted indoors in 20-L plastic buckets (18 L working volume) filled with 32 ppt seawater. Density of copepod nauplii in every trial was 200 individuals/mL (3.6×10^6 copepod nauplii in each experimental unit). Two replicates were used for each treatment. Each bucket was provided with aeration of 0.4–0.6 L/min. Water quality was monitored at the beginning and end of each trial. Water temperature was measured to the nearest 0.1°C, DO to the nearest 0.01 mg/L, and total ammonia-nitrogen (TAN) to the nearest 0.01 mg/L.

Nauplii for these studies were obtained from one pond initially fertilized with 50 kg/ha of each of alfalfa meal, wheat bran, menhaden fish meal, and rice bran. In addition, two liquid fertilizers of 32-2-0 (N:P:K) and 10-34-0 were combined to give a 38-8-0, and the combination was given into each pond at an initial dose of 20 L/ha. This pond was subsequently given half the initial dose of both organic and inorganic fertilizers weekly. Nauplii were trapped and handled as described previously.

Survival of copepod nauplii for each enrichment protocol was determined by counting the number of live and dead nauplii per milliliter in a Sedgwick-Rafter counting cell, from three 50-mL samples taken at the beginning and the end of each enrichment period. After each enrichment trial, copepod nauplii were sieved and frozen following the same procedure as for copepod nauplii collected prior to acclimation. Wet weight of copepod nauplii in each trial was determined separately by weighing known numbers of copepod nauplii, sieved in a 21-μm Nitex plankton net (10 × 10 cm^2), in a Mettler H10 balance to the nearest 0.01 mg. Dry weight of copepod nauplii was determined following heat drying (AOAC 1970).

Lipid Extraction and Fatty Acid Analysis

Lipid extraction was conducted at the Fish Nutrition Laboratory of the Department of Fisheries and Allied Aquacultures, Auburn University, AL, USA, according to a modified method of Folch et al. (1957), where the extracted lipid was separated into polar and neutral lipid fractions using a Whatman solid-phase extraction cartridge (catalog #6804-1705). Both lipid fractions were diluted in 200 μL methylene chloride (DCM) put in a 0.3-mL insert (National Scientific Company, Atlanta, GA, USA) for fatty acid analysis. Each sample had two replicates.

The relative fatty acid methyl ester (FAME) fatty acid composition of these solutions was determined by a gas chromatograph (GC) Shimadzu GC-17A, with helium as a carrier gas. Fatty acid values were expressed as area percentage of the total fatty acids.

Statistical Analyses

Values are presented in mean ± standard error. If required, some data were transformed into square root for count data and arc sine square root for proportions before analyses. Data were analyzed using a t test and one-way ANOVA model procedure of SAS Version 6.12 for Windows (SAS Institute 1996). When significant differences among treatment means were detected, a LSD multiple-range test was applied (Sokal and Rohlf 1981). The differences were considered to be significant at a probability level of $P = 0.05$.

INTENSIVE PRODUCTION

General Procedures

Trials were conducted in an indoor laboratory using adult copepods collected from brackish water (10–27 ppt salinity) ponds using the trap design described earlier. The collected zooplankton were brought back to the lab and sieved through a series of different mesh sizes to separate copepods from rotifers, aquatic insects, and other unwanted organisms. The copepods were placed in a 19-L bucket of 1 μm filtered pond water and provided with slight aeration. Salinity of the water was increased to 30 ppt through gradual addition of 40 ppt artificial seawater. The acclimation process took approximately 30 minutes. After acclimation, the density of adult copepods in the 19-L bucket was estimated using a Sedgewick-Rafter counting cell. The number of males and females and the number of females carrying eggs were counted. Culture units were stocked based on the total number of adults required.

The culture units were 100-L, 6-mm-thick, clear, plastic bags suspended from a wooden frame. The bags were filled with 40 L of 1-μm filtered pond water and commercial sea salts to give a salinity of 30 ppt. A low-pressure blower provided aeration through weighted airstones. Light was provided by four Sylvania GTE Gro-Lux 75-W light bulbs on a 24-hour light cycle. At stocking, *Isochrysis galbana* was added to each bag at a density of 5 × 10^5 cells/mL. Water quality analyses for DO, pH, temperature, salinity, and total ammonia were measured at the beginning and end of each trial.

Experimental Trials

A series of progressively higher adult copepod stocking densities were evaluated in four trials to determine the effect of adult stocking density on nauplii production using wild-caught adults. Each culture bag was inoculated with *I. galbana* at a density of 5 × 10^5 cells/mL. Trial 1 was stocked with 320 adults/L with 10 replicates and was run for 8 days. Trial 2 was stocked at 640 and 1,280 adults/L, with four replicates per treatment. In subsequent trials the high density from the previous trial was repeated, and the second density was twice that rate. In Trial 3, adults were stocked at 1,280 adults/L and 2,560 adults/L. Trial 4 tested the stocking densities of 2,560 adults/L and 5,120 adults/L. Trials 2–4 each lasted 4 days, with four replicates per treatment.

At the end of the culture period, the bags were harvested by siphoning the water through a 150-µm sieve to capture adults and a 35-µm sieve to capture the copepodids and nauplii. The sieves were washed into 230-mL sample containers. Two samples from each sample container, four samples per bag, were counted using a Sedgewick-Rafter slide using the same method previously described, but nauplii and copepodid stages were also counted. Where rotifers and other contaminates were found, they were also enumerated.

Data were analyzed using ANOVA and multiple comparisons to find significant differences at $P < 0.05$. Multiple regressions were also run to see what factors affected production. All analyses were run on SAS, Version 6.12 (SAS Institute Inc. 1988).

Closed-System Production

An initial stock of *A. panamensis* was captured from ponds and handled as described earlier. Two 100-L, clear, 6-mm-thick plastic bags were filled with 40 L of 1-µm filtered water and commercial sea salts to give a salinity of 30 ppt. Aeration and lighting was provided as described earlier. *Isochrysis galbana* was added to each bag at a density of 5×10^5 cells/mL. Pond-caught adults were stocked into the initial bags at 320/L to establish populations A and B.

At the end of the culture period, the bags were harvested by siphoning the water through a 150-µm sieve to capture adults and a 35-µm sieve to capture the copepodids and nauplii. The sieves were washed into 230-mL sample containers to hold the retained animals. Two samples from each sample container, four samples per bag, were counted using the same method previously described, but nauplii and copepodid stages were also counted. Where rotifers and other contaminates were found, they were also enumerated.

After counting, new culture bags were prepared as described above. The adults harvested from the first trial were then stocked into bags to start a new culture, Trial 2. Population A was stocked into a new bag and population B was also stocked into a new bag, so each population was kept separate throughout the 12 trials. For each subsequent trial, the harvested adults from population A were restocked into a new bag, and the same was done for population B. Restocking density was dependent on the number of adults harvested from the previous trial. Adult stocking density ranged from 160.0 to 6,498.0 adults/L. A total of 12 trials, ranging from 4 to 9 days each, were conducted over a period of 77 days. Trials were harvested when visually the algae was cleared from the bags. This usually happened after 4 to 5 days, depending on the stocking density. When the adult stocking densities were low, < 1,000 adults/L, the cultures were run for a longer period of time (6 to 7 days) to reestablish an adult population.

Data were analyzed using ANOVA analysis and multiple comparisons to find significant differences at $P < 0.05$. Multiple regressions were also run to see what factors affected production. All analyses were run on SAS, Version 6.12 (SAS Institute Inc. 1988).

RESULTS

EXTENSIVE PRODUCTION TECHNIQUES

Pond Production of Copepod Nauplii

Abundance of copepod nauplii was highly variable, and no one organic fertilizer showed a consistent advantage (Table 12.2). In Study I the average copepod nauplii densities were 887 ± 172, 1,000 ± 361, and 1,071 ± 415/L for alfalfa-, wheat bran–, and fishmeal-fertilized ponds, respectively. In Study II the average copepod nauplii densities were 2,112 ± 594 and 2,520 ± 706/L for alfalfa- and wheat bran–fertilized ponds, respectively. The highest levels of nauplii production were achieved in Study III, where the mean densities were 3,125 ± 912, 3,391 ± 523, and 5,080 ± 1,224/L, respectively, for ponds fertilized with rice bran, wheat bran, and wheat bran with supplemental aeration.

Nauplii abundance varied over the course of each study. In Study I nauplii abundance peaked on day 10 at mean densities per fertilization regime of 4,484 ± 1,184, 3,893 ± 1,220, and 4,533 ± 1,193/L for alfalfa-, fishmeal-, and wheat bran–fertilized ponds, respectively. A second peak of 1,301 ± 203 occurred on day 32 in alfalfa-fertilized ponds and on day 34 of 3,285 ± 811 and 1,984 ± 743/L in fishmeal and wheat bran–fertilized ponds. *Acartia* sp. was the predominate copepod in the first peak. It later became infested with the parasitic protozoan *Epistylis* sp., and both adult copepod and nauplii abundance declined. Up to 79% of the adult copepods and 12.6% of the nauplii were infested. *Apocyclops panamensis,* the dominant copepod in the second wave, was not infested with *Epistylis* sp. In Study II, peak abundance of nauplii per treatment occurred on day 20 and day 26 at 3,684 ± 1,259 and 2,518 ± 571/L for alfalfa- and wheat bran–fertilized ponds. *Apocyclops panamensis* was the predominate copepod. In Study III mean peak densities per treatment were 4,566 ± 1,334, 6,070 ± 1.327, and 9,534 ± 775/L on day 35, day 49, and day 14 for the rice bran, wheat bran without aeration, and wheat bran with aeration, respectively.

Environmental parameters varied across studies and in some cases, among fertilization treatments within a study (Table 12.3). Mean early morning water temperatures were 28.1 ± 0.2, 29.8 ± 0.2, and 28.6 ± 0.2°C, respectively, for Studies I, II, and III. Mean salinities were 14.5 ± 0.1, 12.7 ± 0.2, and 19.0 ± 0.2 ppt, respectively, for Studies I, II, and III. Early morning DO in Study I averaged 3.8 ± 0.2, 3.2 ± 0.1, and 3.1 ± 0.2 mg/L for alfalfa-, wheat bran–, and fishmeal-fertilized ponds, respectively. In Study II early morning DO averaged 3.1 ± 0.0 and 2.8 ± 0.2 mg/L for alfalfa- and wheat bran–fertilized ponds, respectively. The use of aeration in Study III significantly increased early morning DO ($P = 0.029$). Aerated ponds averaged 4.0 ± 0.0, while rice bran- and wheat bran–fertilized ponds without aeration averaged 2.0 ± 0.0 and 2.7 ± 0.0 mg/L. In Studies I and II alfalfa-fertilized ponds were more transpar-

Table 12.2. Summary of results of zooplankton production in Studies I, II, and III

Item	Study I	Study II	Study III
Mean rotifer density (no./L)	AM: 3,193 ± 587^{a}	AM: 8,800 ± 798^{a}	RB: 3,895 ± 1,150^{a}
	WB: 4,387 ± 598^{a}	WB: 7,876 ± 1,483^{a}	WB: 2,226 ± 599^{a}
	FM: 5,671 ± 421^{a}	—	WBa: 7,881 ± 2,163^{b}
Mean adult copepod density (no./L)	AM: 219 ± 131^{a}	AM: 293 ± 116^{a}	RB: 752 ± 288^{ab}
	WB: 247 ± 66^{a}	WB: 826 ± 144^{b}	WB: 403 ± 221^{a}
	FM: 142 ± 85^{a}	—	WBa: 1,028 ± 746^{b}
Mean copepod nauplii density (no./L)	AM: 887 ± 172^{a}	AM: 2,112 ± 594^{a}	RB: 3,125 ± 912^{a}
	WB: 1,000 ± 361^{a}	WB: 2,520 ± 706^{a}	WB: 3,391 ± 523^{a}
	FM: 1,070 ± 415^{a}	—	WBa: 5,080 ± 1,224^{a}
Ectocommensal fouling disease	Yes	No	No

Note: Alfalfa meal (AM), fish meal (FM), rice bran (RB), and wheat bran with aeration (WBa) or without aeration (WB) were applied initially at 250 kg/ha.

$^{a \text{ and } b}$Values sharing the same letter in the same item column are not significantly different ($P > 0.05$).

Table 12.3. Summary of water quality parameters in Studies I, II, and III

Item	Study I	Study II	Study III
Average early morning dissolved oxygen (mg/L)	AM: 3.8 ± 0.2^{a}	AM: 3.1 ± 0.0^{a}	RB: 2.0 ± 0.0^{a}
	WB: 3.2 ± 0.1^{a}	WB: 2.8 ± 0.2^{a}	WB: 2.7 ± 0.0^{a}
	FM: 3.1 ± 0.2^{a}	—	WBa: 4.0 ± 0.0^{b}
Average secchi disk transparency (cm)	AM: 123.0 ± 3.8^{a}	AM: 83.8 ± 5.4^{a}	RB: 63.2 ± 3.9^{a}
	WB: 82.4 ± 6.6^{b}	WB: 58.1 ± 4.3^{b}	WB: 57.1 ± 2.3^{a}
	FM: 79.4 ± 5.0^{b}	—	WBa: 55.6 ± 2.2^{a}
Average salinity (ppt)	14.5 ± 0.1	12.7 ± 0.2	19.0 ± 0.2
Median pond water temperature (°C)	29.1 ± 0.2	31.7 ± 0.2	28.6 ± 0.2
Average of water temperature range (°C)	4.2 ± 0.2	3.5 ± 0.2	5.5 ± 0.3

Note: Alfalfa meal (AM), fish meal (FM), rice bran (RB), and wheat bran with (WBa) or without aeration (WB) were applied initially at 250 kg/ha.

$^{a \text{ and } b}$Values sharing the same letter in the same item column are not significantly different ($P > 0.05$).

Table 12.4. Polar and nonpolar fatty acid profiles of arachidonic acid (ARA, 20:4*n*-6), EPA (20:5*n*-3), DHA (22:6*n*-3), total omega-3 and omega 6, omega-3/omega-6, and DHA/EPA ratios, total unsaturated and saturated fatty acid (% total fatty acid) and its ratio, polar and nonpolar lipid content (% dry weight and % polar or nonpolar lipid), total lipid content (% dry weight), and individual naupliar weight (μg) of postharvest *Apocyclops panamensis* nauplii trapped from alfalfa meal (Study II) and rice bran, wheat bran, and mixed (rice and wheat bran) meals (Study III) fertilization in brackish water ponds

	Alfalfa meal (Study II)	Rice bran (Study III)	Wheat bran (Study III)	Mixed meals (Study III)
Polar fatty acids				
ARA	0.5 ± 0.0	1.5 ± 0.3	1.0 ± 0.1	1.4 ± 0.0
EPA	11.4 ± 0.7	9.8 ± 0.4^{a}	12.6 ± 0.8^{b}	13.8 ± 0.8^{b}
DHA	23.8 ± 2.7	23.0 ± 2.2	22.9 ± 0.3	22.4 ± 0.4
Σ omega-3	36.7 ± 2.0	33.4 ± 2.6	36.2 ± 1.0	38.1 ± 1.4
Σ omega-6	1.8 ± 0.1	2.5 ± 0.3	3.1 ± 0.1	3.6 ± 0.2
Omega-3/omega-6	19.7 ± 1.4	13.3 ± 0.7	11.8 ± 0.6	10.8 ± 1.0^{b}
DHA/EPA	2.1 ± 0.4	2.4 ± 0.1^{a}	1.8 ± 0.1^{b}	1.6 ± 0.1^{b}
Σ Fatty acids	89.5 ± 3.5	86.7 ± 3.5	83.0 ± 2.9	84.1 ± 1.7
Polar lipid	0.41 ± 0.07	0.51 ± 0.09	0.36 ± 0.19	0.82 ± 0.48
% Polar lipid	7.2 ± 1.1	6.5 ± 1.0	21.3 ± 1.8	11.4 ± 6.0
Nonpolar fatty acids				
ARA	1.2 ± 0.1	1.3 ± 0.0	1.3 ± 0.2	1.4 ± 0.0
EPA	16.4 ± 2.9	14.0 ± 0.1	15.6 ± 3.2	14.8 ± 0.4
DHA	26.4 ± 1.4	27.4 ± 1.6	27.0 ± 1.8	28.1 ± 2.3
Σ omega-3	43.9 ± 1.5	42.3 ± 1.6	43.7 ± 4.9	44.1 ± 1.8
Σ omega-6	2.0 ± 0.1	1.5 ± 0.1	1.9 ± 0.3	2.1 ± 0.1
Omega-3/omega-6	22.0 ± 0.4	28.2 ± 0.1^{a}	23.1 ± 0.5^{b}	21.5 ± 0.4^{b}
DHA:EPA	1.7 ± 0.4	2.0 ± 0.1	1.8 ± 0.2	1.9 ± 0.2
Σ Fatty acids	82.9 ± 1.8	82.0 ± 0.8	80.6 ± 3.6	84.5 ± 3.8
Nonpolar lipid	5.25 ± 0.07	7.26 ± 0.18^{a}	4.99 ± 0.15^{b}	6.08 ± 0.05^{a,b}
% Nonpolar lipid	92.8 ± 1.1	93.5 ± 1.0	78.7 ± 1.8	88.6 ± 6.0
Total lipid	5.66 ± 0.15	7.76 ± 0.27	6.35 ± 0.34	6.90 ± 0.52
Naupliar dry weight (μg)	0.08 ± 0.00	0.16 ± 0.01	0.13 ± 0.02	0.13 ± 0.01

Note: Values are mean ± S.E. Only parameters with significant differences ($P < 0.05$) are noted.

ent than the other fertilization regimes. In Study III, rice bran–fertilized ponds were more transparent than wheat bran ponds.

Unenriched Copepod Nauplii from Various Fertilization Regimes

Given in Table 12.4 are the fatty acid profile (polar and nonpolar), DHA/EPA ratio, lipid content (percentage of dry weight), and individual dry weight (micrograms) of copepod nauplii trapped from ponds of the fertilizer studies and sampled before acclimation. There was no difference in mean lipid content in copepod nauplii trapped from rice bran–, wheat bran–, and rice and wheat bran mixed meals–fertilized ponds ($P = 0.18$). Mean nonpolar lipid content of copepod nauplii trapped from wheat bran–fertilized ponds in Study III was lower than those from other fertilization regimes ($P = 0.003$). There were no significant differences in mean contents and ratios of other nonpolar fatty acid profiles in copepod nauplii trapped from three fertilization regimes in Study III ($P > 0.05$) (Table 12.4). There was also no significant difference in mean individual copepod nauplii dry weight ($P = 0.29$). Except for mean content of EPA and the DHA/EPA ratio, there were no significant differences in mean abundance and ratios of other polar fatty acid profiles in copepod nauplii trapped from three fertilization regimes in Study III ($P > 0.05$).

Table 12.5. Polar and non-polar fatty acid profile of ARA, EPA, DHA, total omega-3 and omega-6, omega-3/omega-6 and DHA/EPA ratios, total unsaturated and saturated fatty acid (% total fatty acid) and its ratio, polar and nonpolar lipid content (% dry weight and % polar or nonpolar lipid), total lipid content (% dry weight), and individual naupliar weight (μg), survival rate (%), and change (%) of postharvest and 6-hour after acclimation of *Apocyclops panamensis* nauplii trapped from mixed meals fertilization in brackish-water ponds from Study III

	After harvest	6 hours after acclimation	Change (%)
Polar fatty acids			
ARA	1.4 ± 0.0^{a}	1.0 ± 0.0^{b}	−28.6
EPA	13.8 ± 0.8^{a}	10.5 ± 0.4^{b}	−23.9
DHA	22.4 ± 0.4^{a}	12.5 ± 0.7^{b}	−44.2
Σ omega-3	38.1 ± 1.4^{a}	24.2 ± 1.1^{b}	−36.5
Σ omega-6	3.6 ± 0.2^{a}	3.0 ± 0.1^{a}	−16.7
Omega-3/omega-6	10.8 ± 1.0^{a}	8.1 ± 0.4^{a}	−25
DHA/EPA	1.6 ± 0.1^{a}	1.2 ± 0.0^{b}	−25
Σ Fatty acids	84.1 ± 1.7^{a}	68.3 ± 1.4^{b}	−18.8
Polar lipid	0.82 ± 0.48^{a}	0.62 ± 0.21^{a}	−24.4
% Polar lipid	11.4 ± 6.0^{a}	15.8 ± 4.4^{a}	38.6
Nonpolar fatty acids			
ARA	1.4 ± 0.0^{a}	1.0 ± 0.1^{b}	−28.6
EPA	14.8 ± 0.4^{a}	5.2 ± 0.3^{b}	−64.9
DHA	28.1 ± 2.3^{a}	12.5 ± 1.1^{b}	−55.5
Σ omega-3	44.1 ± 1.8^{a}	18.3 ± 0.7^{b}	−58.5
Σ omega-6	2.1 ± 0.1^{a}	1.4 ± 0.0^{b}	−33.3
Omega-3/omega-6	21.5 ± 0.4^{a}	12.9 ± 0.3^{b}	−40
DHA/EPA	1.9 ± 0.2^{a}	2.4 ± 0.4^{a}	26.3
Σ Fatty acids	84.5 ± 3.8^{a}	48.6 ± 2.0^{b}	−42.5
Nonpolar lipid	6.08 ± 0.05^{a}	3.23 ± 0.06^{b}	−46.9
% Nonpolar lipid	88.6 ± 6.0^{a}	84.2 ± 4.4^{a}	−5
Total lipid	6.90 ± 0.52^{a}	3.85 ± 0.26^{b}	−44.2
Naupliar weight	0.13 ± 0.01^{a}	0.08 ± 0.00^{b}	−38.5
Survival rate		90.4 ± 3.4	

Note: Values are mean ± S.E. Only parameters with significant differences ($P < 0.05$) are noted.

Unenriched Copepod Nauplii after Acclimation

Results of fatty acid profiles of nonpolar and polar lipid classes, survival rate (percentage), individual dry weight (microgram), and change values (percentage) of after harvest and 6-h after acclimation of copepod nauplii *A. panamensis* trapped from the mixed meals–fertilized pond in Study III are shown in Table 12.5. Acclimation from an average initial salinity of 19.7 ± 0.2 ppt up to full seawater salinity of 32.0 ± 0.0 ppt was conducted over 6 hours at a copepod nauplii density of 200 individuals per liter. Mean total lipid content was significantly lower ($P = 0.029$) after acclimation, decreasing from 6.90 ± 0.52 to 3.85 ± 0.26% dry weight after 6 hours, a 44.2% decrease. Likewise, nonpolar lipid content was significantly lower after acclimation ($P < 0.001$), with a 46.9% decrease. Mean contents of ARA, EPA, DHA, total omega-3, omega-6, omega-3/omega-6, and total fatty acids in copepod nauplii showed significant decreases after acclimation ($P < 0.05$), while other components did not decrease significantly ($P > 0.05$). There were significant decreases in mean content of polar ARA, EPA, DHA, total omega-3, DHA/EPA ratio, and total fatty acids in copepod nauplii ($P < 0.05$), while other components were not significantly

Table 12.6. Polar and nonpolar fatty acid profile of ARA, EPA, DHA, total omega-3 and omega-6, omega-3/omega-6 and DHA/EPA ratios, total unsaturated and saturated fatty acid (% total fatty acid) and its ratio, nonpolar lipid content (% dry weight and % nonpolar lipid), and total lipid content (% dry weight), naupliar dry weight (DW, μg) and survival rate (SR, %) of *Apocyclops panamensis* nauplii enriched with A-1 Super Selco for 3 hours, 6 hours, and 12 hours in normal dose

	A-1 Super Selco		
	3 hours	6 hours	12 hours
Polar fatty acid			
ARA	1.0 ± 0.2	1.3 ± 0.0	1.6 ± 0.3
EPA	10.5 ± 0.7	11.3 ± 1.2	11.9 ± 0.8
DHA	16.3 ± 0.2	20.8 ± 1.0	21.2 ± 0.6
Σ omega-3	28.2 ± 0.4	34.0 ± 2.1	36.6 ± 2.6
Σ omega-6	3.0 ± 0.3	3.3 ± 0.1	3.8 ± 0.3
Omega-3/omega-6	9.6 ± 0.7	10.3 ± 0.3	9.7 ± 0.1
DHA/EPA	1.6 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
Σ Fatty acids	73.9 ± 0.7^{a}	82.9 ± 1.6^{b}	86.4 ± 5.5^{b}
Polar lipid	1.36 ± 0.01^{a}	1.94 ± 0.12^{a,b}	2.30 ± 0.21^{b}
% Polar lipid	18.7 ± 0.2	20.7 ± 0.8	21.4 ± 1.2
Nonpolar fatty acid			
ARA	1.5 ± 0.0^{a}	1.6 ± 0.0^{a}	1.9 ± 0.1^{b}
EPA	12.5 ± 1.3	15.8 ± 0.3	16.2 ± 0.5
DHA	22.3 ± 1.8	23.1 ± 1.4	24.5 ± 0.4
Σ omega-3	36.5 ± 2.9	41.1 ± 1.1	43.1 ± 1.1
Σ omega-6	2.5 ± 0.0^{a}	2.7 ± 0.0^{a}	3.1 ± 0.1^{b}
Omega-3/omega-6	14.9 ± 1.5	15.2 ± 0.4	13.9 ± 0.8
DHA/EPA	1.8 ± 0.0	1.5 ± 0.1	1.5 ± 0.0
Σ Fatty acids	71.8 ± 3.6^{a}	80.1 ± 2.3^{b}	83.8 ± 1.2^{b}
Nonpolar lipid	5.89 ± 0.14^{a}	7.42 ± 0.09^{b}	8.44 ± 0.19^{b}
% Nonpolar lipid	81.3 ± 0.2	79.3 ± 0.8	78.6 ± 1.2
Total lipid	7.25 ± 0.15^{a}	9.36 ± 0.20^{ab}	10.74 ± 0.40^{b}
Survival rate	89.0 ± 3.5^{a}	58.6 ± 2.2^{ab}	28.1 ± 4.1^{b}
Dry weight	0.10 ± 0.01^{a}	0.27 ± 0.03^{b}	0.30 ± 0.06^{b}

Note: Values are mean ± S.E. Only parameters with significant differences ($P < 0.05$) are noted.

lower after acclimation ($P > 0.05$). Mean survival rate after acclimation was 90.4 ± 3.4%.

Enrichment of Copepod Nauplii

Nonpolar and polar fatty acid profiles of ARA, EPA, DHA, total omega-3 and omega-6, omega-3/omega-6, and DHA/EPA ratios, total fatty acids, total lipid content, copepod naupliar dry weight, and survival rate of *A. panamensis* nauplii enriched with A-1 Super Selco for 3 hours, 6 hours, and 12 hours are shown in Table 12.6. Mean concentration of nonpolar fatty acid profiles of nauplii enriched with A-1 Super Selco for 3 hours, 6 hours, and 12 hours were not significantly different ($P > 0.05$; Table 12.6), but mean content of ARA, omega-6, total fatty acids, total lipid, and neutral lipid content were significantly different ($P < 0.05$). Except for mean total fatty acids and polar lipid content, there were no significant differences in all other fatty acid profiles of polar lipid class ($P > 0.05$; Table 12.6). Means of survival rates of copepod nauplii were significantly lower after the 12-hour enrichment period ($P = 0.006$) and averaged 28.1 ± 4.1% (Table 12.6).

DO was progressively lower the longer the enrichment period, dropping from 6.32 mg/L to 3.6 mg/L in a 12-hour period even though the holding container was aerated. Ammonia was below detectable levels at the end of the enrichment period.

Table 12.7. Evaluation of *Apocyclops panamensis* when wild-caught adults are stocked at varying densities in bags containing *I. galbana* at 5 × 10^5 cells/mL and harvested after 4 days

Adults/L	Production/L	Change in adult abundance (%)	Production/female
320	1,630 ± 357.0^{d}	−117 ± 57.2^{a}	2.6 ± 0.7^{b}
640	3,044 ± 117.2^{d}	2.0 ± 10.9^{b}	8.1 ± 1.1^{a}
1,280	6,198 ± 466.4^{c}	12 ± 2.3^{b}	8.6 ± 0.9^{a}
2,560	3,616 ± 682.3^{b}	27 ± 9.1^{b}	8.2 ± 1.2^{a}
5,120	17,873 ± 654.0^{a}	39 ± 5.5^{b}	4.7 ± 0.6^{b}
P value	< 0.01	< 0.01	< 0.01

Note: Mean ± standard errors of the production of nauplii and copepodids per liter, the change in density of adults in the culture, and nauplii and copepodid production per female at harvest. Means in columns with the same letter are not significantly different.

Table 12.8. Evaluation of *Apocyclops panamensis* culture when wild-caught adults are stocked at varying densities in bags containing *I. galbana* at 5 × 10^5 cells/mL and harvested after 4 days

Adults/L	Males	Females	Females with eggs	Males (%)	Females (%)	Females with eggs (%)
320	86.0 ±20.2	143.0 ± 73.6	64.0 ± 12.0	44.3 ± 5.8^{a}	56.7 ± 5.8^{a}	72.2 ± 17.3^{a}
640	288.3 ± 40.2	394.1 ± 50.2	216.4 ± 26.8	42.2 ± 1.0^{a}	58.8 ± 1.0^{a}	55.0 ± 2.0^{a}
1,280	712.6 ± 54.6	744.4 ± 45.4	456.6 ± 34.4	48.7 ± 3.1^{a}	51.3 ± 3.1^{a}	61.3 ± 2.5^{a}
2,560	1,968.6 ± 229.8	1,999.4 ± 352.0	1,105.3 ± 160.2	51.4 ± 2.8^{a}	48.6 ± 2.8^{a}	58.8 ± 3.8^{a}
5,120	4,633.6 ± 455.9	4,010.6 ± 543.2	2,031.8 ± 237.2	53.9 ± 2.4^{a}	46.1 ± 2.4^{a}	51.1 ± 1.0^{a}
P value	—	—	—	0.23	0.23	0.43

[a] Mean ± S.E. for the number of males, females, and egg-bearing females per liter in the culture at harvest. Mean ± S.E. for the percentage of males, females in the population, and the percentage of females with eggs in the female population. Means in columns with the same letter are not significantly different.

INTENSIVE PRODUCTION

Evaluation of Adult Stocking Density

Mean abundance of nauplii and copepodids per liter reached a peak of 17,873 ± 654 at an adult density of 5,120/L. Production was significantly ($P < 0.001$) different for each stocking density, except for the two lower stocking densities, 320 and 640 adults/L, which were similarly low (Table 12.7). Production of nauplii and copepodids per female ranged from 2.6 ± 0.7 to 8.6 ± 0.9. The highest stocking density, 5,120 adults/L, and the lowest, 320 adults/L, had a significantly ($P < 0.01$) lower mean production of nauplii and copepodids per female in comparison with the other three stocking densities of 640, 1,280, and 2,560 adults/L (Table 12.8). The change in the size of the adult population at harvest, in terms of the stocking density, was significantly ($P < 0.001$) higher for the lowest stocking density, 320 adults/L, compared with the other adult stocking densities (Table 12.8). At harvest, there was no significant difference in the percentage of males ($P = 0.23$) or females ($P = 0.23$) or the percentage of the female population with eggs ($P = 0.43$) in the culture population for the different treatments (Table 12.8). Overall, DO was positively correlated with production of nauplii and copepodids per female ($R^2 = 0.47$, $P = 0.02$).

Closed Batch Production

Net production (nauplii and copepodids) varied considerably over the 12 trials (Table 12.9 and Table 12.10). Mean production in population A was 10,939 ± 3,200/L, but ranged from 144.0 to 31,532/L. Mean production in population B was 14,366 ± 3,095/L, and ranged from 250 to 36,800/L. This variability was due in part to adult stocking density. Over the 12 trials, the adults were harvested from one trial and restocked to begin the next trial. As a result, the number of

Table 12.9. Closed system production of *Apocyclops panamensis*

Trial	Culture period (days)	Initial adult density/L	Final adult density/L	Change in abundance of the adult population (%)	Yield. (No./L)	Production No./Female
1	7	320	160	−50.0	3,640	31.7
2	9	160	3,705	2,215.6	9,670	3.5
3	4	3,705	2,202	−40.6	31,531	22.6
4	4	2,202	1,908	−13.4	26,625	26.1
5	7	1,908	4,370	129.0	25,465	8.1
6	5	4,370	2,081	−52.4	24,575	19.9
7	5	2,081	1,605	−22.9	4,575	4.7
8	4	1,605	488	−69.6	5,150	17.9
9	6	488	250	−48.8	1,500	6.0
10	6	125	1,007	705.6	383	0.8
10	7	125	115	−8.0	144	5.0
11	7	1,007	690	−31.5	4,000	10.7
12	6	690	900	30.4	4,950	7.3

Note: Initial adult density, final adult density, the percentage of change in density of adults in the culture, yield of nauplii and copepodids, and nauplii and copepodid production per female at harvest for population A. There were no significant differences at $P < 0.05$.

Table 12.10. Closed system production of *Apocyclops panamensis*

Trial	Culture period (days)	Initial adult density/L	Final adult density/L	Change in adult population abundance (%)	Yield. (No./L)	Production No./Female
1	7	320	170	−46.9	3,480	38.7
2	9	170	1,290	658.8	4,255	4.0
3	4	1,290	1,115	−13.5	11,550	15.8
4	4	1,115	688	−38.3	18,350	42.4
5	7	688	6,498	844.5	15,895	3.0
6	5	6,498	2,392	−63.2	28,395	16.0
7	5	2,392	4,227	76.7	24,150	8.8
8	4	4,227	1,985	−53.0	9,320	10.2
9	6	1,985	2,250	13.4	250	0.2
10	6	750	4,492	559.1	16,033	2.0
11	7	4,710	4,974	5.6	36,800	12.0
12	6	4,974	6,025	21.1	27,900	8.9

Note: Initial adult density, final adult density, the percentage of change in density of adults in the culture, yield of nauplii and copepodids, and nauplii and copepodid production per female at harvest for population B. There were no significant differences at $P < 0.05$.

adults stocked per trial varied over time (Table 12.9 and Table 12.10). In general there was a significant relationship between adult stocking density and nauplii and copepodid production for both populations A and B (A: $R^2 = 0.65$, $P < 0.01$; B: $R^2 = 0.52$, $P = 0.01$).

Production in population A was positively correlated to water temperature over the range of 25.8 to 31.1°C ($R^2 = 0.47$, $P = 0.01$). This was not as evident in population B (25.7 to 31.0°C, $R^2 < 0.01$, $P = 0.96$). All other regressions of water quality parameters were not significant. Production per female ranged from 0.8 to 31.7 nauplii and copepodids per female in population A and 0.2 to 42.4 per female in population B (Table 12.9 and Table 12.10). The change in abundance of the adult population, from stocking to harvest, was a weak predictor (negative) of the production of nauplii and copepodids per female in population B ($R^2 = 0.31$, $P = 0.04$). When the adult population increased 844.5%, production of nauplii and copepodids per female was 3.0. When the population of adults decreased −46.9%, production of nauplii and copepodids per female was 38.7. This relationship was not evident in population A.

In population A, the percentage of males in the population averaged 37.3% over the course of the 12 trials and ranged from 0 to 74.78% ($P = 0.59$). The percentage of females averaged 62.7% with a range of 25.2 to 100% ($P = 0.60$). The percentage of the females carrying eggs ranged from 0 to 53.7% ($P = 0.87$) and with an average of 27.1%. In population B, the average population composition was 25.4% males and 74.7% females, with 41.2% of the females carrying eggs. The percentage of males over the course of the 12 trials ranged from 10.6 to 43.9% ($P = 0.03$) and from 56.1 to 89.4% ($P = 0.03$) for females. The percentage of females carrying eggs in population B ranged from 31.7 to 50.0% ($P = 0.26$). Though there was a significant difference in the composition of adult population in population B, there appeared to be no relationship between the percentage of the males and females in the population and the productivity of the culture.

DISCUSSION

COPEPOD NAUPLII PRODUCTION IN PONDS

Pond production of copepods is a successful way to produce high densities of adults and nauplii. All but one of the fertilization regimes in this study produced mean nauplii densities of *A. panamensis* greater than 1,000 nauplii/L. In natural temperate and tropical brackish water, densities of 0.6–100 calanoid, harpacticoid, and cyclopoid copepod nauplii per liter are common (Fukusho 1991; Mishra and Panigrahy 1996; Shansudin et al. 1997; Watanabe et al. 1998).

Pond production of copepods has the advantage in that large quantities of nauplii can be obtained using ponds that are often available at a hatchery, while to obtain similar quantities of nauplii from a laboratory-based system requires additional dedicated floor space, increased labor, and capital (Støttrup and Norsker 1997). Pond production of zooplankton, using organic fertilizer and inorganic fertilizers (Geiger 1983; Geiger et al. 1985; Colura et al. 1987; Doi et al. 1994; Kurten et al. 1999), has yielded average densities of 19 *Acartia* sp. nauplii per liter (Kurten et al. 1999) to 374 *Acartia* sp. and *Oithona* sp. nauplii per liter (Colura et al. 1987). Average nauplii densities produced in outdoor ponds with various fertilization regimes in this study ranged from 887.2 ± 171.8 individuals/L (both *Acartia* sp. and *A. panamensis* in alfalfa-fertilized ponds in Study I) to 5,079.7 ± 1,224.3 *A. panamensis* nauplii/L (wheat bran with aeration–fertilized ponds in Study III), with a mean peak of 9,533.7 ± 774.6 individuals/L, respectively. The average density of *A. panamensis* nauplii in wheat bran with aeration–fertilized ponds in Study III was comparable to the average density (5,150 nauplii/L) reported by Støttrup et al. (1986) for nauplii of the harpacticoid copepod *Tisbe holothuriae* in a laboratory system.

The type of organic fertilizer has been show to influence the zooplankton species composition. Geiger et al. (1985) found a significantly higher average copepod (adult and nauplii) abundance in cottonseed meal compared with chicken manure– and chicken litter–fertilized ponds. Bootes (1998) found that average copepod nauplii density in outdoor tanks was significantly higher in those with a high C/N fertilizer ratio (hog feed and cottonseed meal) compared with those with a lower C/N ratio (fishmeal). In the three studies reported here, one animal-origin (fishmeal) and three plant-origin (alfalfa meal, rice bran, and wheat bran) organic fertilizers, which varied in the quantity and quality of nutrients, were evaluated and added to the ponds, but no one fertilizer produced significantly more nauplii.

The relationship between copepod abundance, water quality, and other environmental parameters is complex. In Study III, DO in wheat bran with aeration was significantly higher than those of the other fertilization regimes ($P = 0.018$), and both rotifer and adult copepod densities for this fertilization regime were significantly higher than those of the other regimes ($P < 0.05$), and to a lesser extent, a higher density of copepod nauplii ($P = 0.072$). Geiger (1983) found that aeration and circulation of pond water seems to accelerate primary production, which can support a large zooplankton population. Ito and Satomi (1975) found that a vertical pump reduced biochemical stratification, but increased primary productivity and the zooplankton and phytoplankton population, compared with the control ponds.

There were relatively clear waves of abundance for rotifers, adult copepods, and copepod nauplii in Study I. Such waves were not as distinct in Studies II or III. Waves in zooplankton abundance were reported by Harrell and Bukowski (1990) in brackish water ponds fertilized with inorganic and organic (alfalfa cubes and soy meal) fertilizers. Allen (1976) reported that populations of copepods, with their longer life cycles, greater capacity for selective feeding, and greater ability to avoid predators, tend to reach their peak abundance following the rotifer peak.

Zooplankton production is highly variable even among similarly managed ponds. Standard errors of average densities of zooplankton in wheat bran–fertilized ponds across the studies were between 14 and 55%. High pond-to-pond variances in term of standard deviation or standard error have been commonly encountered in other fertilization studies in outdoor ponds. Geiger (1983) and Geiger et al. (1985) encountered 63–225% and 98–322% standard deviation values of average zooplankton densities, respectively. Kurten et al. (1999) had 17–162% of standard error values of average zooplankton densities in their study. Producing copepod nauplii in ponds can be highly variable, and several ponds need to be in production at one time to ensure one that has adequate numbers of nauplii for trapping.

Nutritional Characteristics

It has been suggested that variation in lipid content of copepods is greatly affected by available diets in natural waters (Fraser et al. 1989; Norrbin et al. 1990). In this study mean total lipid content of copepod nauplii was similar across fertilization regimes. Other nonpolar lipid characteristics of nauplii, however, differed among the fertilization regimes. Copepod nauplii trapped from wheat bran–fertilized ponds had a significantly lower mean content of ARA than the other regimes ($P = 0.022$). In addition, the omega-3/omega-6 ratio in the rice bran regime had significantly higher values than those of other regimes ($P = 0.02$). The DHA/EPA ratios of neutral lipid class were similar for the three fertilizer protocols in Study III, ranging from 1.6 ± 0.1 to 2.1 ± 0.4. Lokman (1994) and Shansudin et al. (1997) reported a DHA/EPA ratio of natural zooplankton (> 60% was *Oithona* sp.) between 1.0 and 2.3. Fraser et al. (1989) reported the DHA/EPA ratio in three copepod species from natural waters to be between 0.7 and 2.4 in nonpolar fatty acids. There were no significant differences in other fatty acid profiles of nonpolar lipids ($P > 0.05$), suggesting that *A. panamensis* nauplii propagated from any of the three fertilization regimes in this study were similar in nutritional value.

Among the polar lipids of copepod nauplii from the different fertilization regimes, there were only significant differences in EPA and the DHA/EPA ratio. Mean content of EPA in copepod nauplii trapped from the rice bran regime was at a significantly lower level than those of other regimes ($P = 0.004$). Ranges in the polar lipid DHA/EPA ratio from copepod nauplii were 1.6 ± 0.1 to 2.4 ± 0.1, with the ratio in the rice bran regime being the highest among the regimes ($P = 0.035$). Fraser et al. (1989) and Norrbin et al. (1990) reported DHA/EPA ratios in polar lipid fatty acids in adult copepods of 1.3–1.4 and 0.8–1.2, respectively. In general, Sargent et al. (1999) suggested that the optimal DHA/EPA ratio in lipids for marine finfish larval diets be about 2.0. The DHA/EPA ratio in both lipid classes was approximately 2.0.

Acclimation reduced the neutral lipid level of copepod nauplii (−46.9%) significantly more than that of the polar lipid (−24.4%; $P = 0.043$). It is suggested that energy reserves from triglycerides and wax ester (neutral lipids) are used in greater proportion due to unfed and crowding conditions during the acclimation. Bourdier and Amblard (1989) reported that almost entirely neutral lipid in the calanoid copepod *Acanthodiaptomus denticornis* was used up after 20 days of starvation. This study demonstrated that a salinity acclimation over 6 hours, from average salinities of 12.7 ± 0.1 to 19.7 ± 0.2 to full seawater salinity of 32–34 ppt clearly decreased nutritional quality of *A. panamensis* nauplii.

Enrichment of Copepod Nauplii

Copepod nauplii are considered to be of prime nutritional quality as live feed for marine fish larvae (Watanabe 1993), but as demonstrated above, the harvesting and acclimation of nauplii from brackish to full-strength seawater significantly reduces the nutritional quality. Enrichment of rotifers (Fernandez-Reiriz et al. 1993; Tamaru et al. 1993; Craig et al. 1994; Robin 1998) and *Artemia* (van Ballaer et al. 1985; Webster and Lovell

1990; Takeuchi et al. 1992; Han et al. 2000) with various species of phytoplankton and enrichment oil for 3–72 hours is common. Copepod nauplii also respond to enrichment. Mean content of ARA, total omega-6 fatty acids, total fatty acids, nonpolar lipid, and total lipids showed significant increase ($P < 0.05$) after prolonged enrichment with A-1 Super Selco (Table 12.6). Payne and Rippingale (2001) enriched nauplii of the calanoid copepod *Gladioferens imparipes* with *I. galbana* and *Nannochloropsis oculata* for 30 minutes up to 6 hours at 23°C, and they found a significant increase in total fatty acid content, with DHA/EPA ratio ranges of 4.9–7.0. In another study, Payne et al. (2001) compared the nutritional quality of enriched *G. imparipes* nauplii versus rotifers and *Artemia* using Super Selco and microalgae *I. galbana* and *N. oculata* for 6 hours. They found a higher mean content of DHA and a higher DHA/EPA ratio in enriched copepod nauplii.

The extensive approach for obtaining copepod nauplii is effective for producing large quantities of nauplii, but additional steps are required before they are suitable for feeding copepod nauplii. Acclimation to full-strength seawater reduces nauplii nutrient quality, but that can be restored by enrichment. Acclimation and enrichment with A-1 Super Selco, however, significantly reduced nauplii survival ($P = 0.006$). The extensive approach used at the Claude Peteet Mariculture Center is a tradeoff of high-volume production coupled with additional effort to ensure the nauplii are suitable for feeding red snapper larvae.

INTENSIVE PRODUCTION

Apocyclops panamensis appears to be suitable for intensive culture. Nauplii yields increased as stocking rates of wild-caught adults increased, with a peak of 17,872.5 ± 654.0 at an adult density of 5,120/L. In a closed system higher yields were possible. A yield of 36,800/L was obtained from a 6-day culture period when domesticated adults were stocked at 4,710/L. Maximum densities of 26,000 harpacticoid copepod *Tigriopus brevicornis* nauplii per liter (Vilela 1992) and 33,000 cyclopoid copepod *Apocyclops royi* nauplii per liter (Cheng et al. 2001) have been reported. Production trials with *Acartia* spp. have yielded up to 5,150 nauplii/L (Schipp et al. 1999). Shirgur (1989) produced *Apocyclops dengizicus* at densities averaging 16,000/L.

Total yield and production per female *A. panamensis* was related to change in the adult copepod populations. The greater the number of adults present at harvest relative to the number stocked resulted in greater total production of nauplii and copepodids per liter ($R^2 = 0.41$, $P < 0.0001$), and a decline in the production per females ($R^2 = 0.48$, $P < 0.0001$). This suggests that during a 4-day production cycle, it was possible for some of the nauplii to reach maturity and reproduce, thus increasing the total number of females (mature and immature) and, in turn, resulting in a decrease of production per female.

The optimum stocking density depends on the availability of adult copepods and other production-related resources. In these trials overall nauplii and copepodid yield per liter continued to increase as the stocking density increased ($R^2 = 0.87$, $P < 0.001$). At the wild-adult stocking densities of 640, 1,280, and 2,560, the production of nauplii and copepodids per female were similar. Production per female, however, declined when the adult stocking density was increased to 5,120/L. If space for production were available, a greater number of nauplii and copepodids could be obtained by stocking two production units at 2,560 adults/L rather than one at 5,120/L. In this study, space and adult copepod availability were limiting factors. Therefore, a stocking density of 2,560/L would give a high yield while also maintaining a high reproduction rate per female. Maintaining a closed population of *A. panamensis* giving *I. galbana* as the sole food source appears to be practical. Higher total yields of nauplii and nauplii per female were obtained using domesticated adults rather than wild-caught adults.

CONCLUSION

Copepod nauplii for feeding marine fish larvae can be provided through extensive or intensive production systems. Extensive production requires dedicated ponds prepared to optimize nauplii production. Harvesting and acclimating nauplii can impact their nutritional quality, but that can be restored through fatty acid enrichment. Species such as *A. panamensis* can be produced intensively with yields of 20,000–30,000 nauplii/L possible in a 4- to 6-day production period.

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