USE OF PRIMARY NURSERY PONDS FOR RED SNAPPER LARVAE CULTURE

AND ASSOCIATED ZOOPLANKTON DYNAMICS

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USE OF PRIMARY NURSERY PONDS FOR RED SNAPPER LARVAE CULTURE AND ASSOCIATED ZOOPLANKTON DYNAMICS

Daniel Patrick English

A Thesis

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Degree of

Master of Science

Auburn, Alabama
May 10, 2008

USE OF PRIMARY NURSERY PONDS FOR RED SNAPPER LARVAE CULTURE

AND ASSOCIATED ZOOPLANKTON DYNAMICS

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Daniel Patrick English, son of Elizabeth Parr English and Thomas D. English, was born on January 17, 1978 in El Paso, TX. He graduated from Coronado High School in El Paso, TX in May 1996. In December 2001 he graduated with a Bachelor of Science degree in Marine Fisheries from Texas A&M University, Galveston, TX. While attending college he worked as an intern at Moody Gardens Aquarium in the fish quarantine facility and assisted the Gulf Restoration Network organization with submitting comments to the Gulf of Mexico Fishery Management Council concerning 2001 amendments to reef fish management. From December 2001 to September 2003 he worked as systems manager at Aquamarine Fish Farms, Inc. in Macungie, PA. In January 2004 he entered the Master of Science Program at Auburn University. In June 2005 he started work at El Paso Corporation in Colorado Springs, CO.

THESIS ABSTRACT

USE OF PRIMARY NURSERY PONDS FOR RED SNAPPER LARVAE CULTURE AND ASSOCIATED ZOOPLANKTON DYNAMICS

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Master of Science, May 10, 2008
(B.S. Texas A&M University, 2001)

93 Typed Pages

Directed by Ronald P. Phelps

The challenge with red snapper aquaculture has centered around meeting the food requirements for larvae at the time of exogenous feeding. Snapper larvae demand sufficient quantities of small prey organisms at the onset of first-feeding. Copepod nauplii have proved to be an appropriate prey for red snapper larvae. In 2004 experiments were conducted to formulate red snapper culture methods using marine primary nursery ponds. Twenty 0.1 ha ponds were prepared with either 250 or 500 kg/ha rice bran organic fertilizer. Two day-old red snapper larvae were stocked in ponds at either 5, 7, or 10 days post-filling (dpf). At the end of 30 d the ponds were harvested.

Zooplankton results showed high densities of copepod nauplii (averaging 774.65 ± 962.9 org/L) during the first 5 days following larval stocking in ponds receiving the 250 kg/ha fertilizer rate. These low rate ponds experienced significantly higher densities of nauplii than the 500 kg/ha ponds ($p=0.021$). A high degree of variation in nauplii abundance was observed between and within ponds during the study period. Average red snapper survival also varied greatly between treatments, ranging from nearly 0% survival in treatment 2 (250 kg/ha and stocked 7 dpf) to 1.07 % in treatment 5 (500 kg/ha, stocked 10 dpf, and continuous aeration). The treatment receiving 250 kg/ha of rice bran and stocked 10 days post-filling resulted in higher snapper survival when compared to the remaining low fertilizer treatments. Following these results recommendations for future research might suggest incorporating continuous aeration with the low rate fertilizer treatment and stocking larvae at 10 dpf. In addition to the pond study a computer model was constructed to predict the best day to stock red snapper determined by copepod nauplii density. The model used a forecasting method incorporating 29 pond parameters and resulted in an adjusted R^2 of 0.6954. The model was inconsistent at predicting nauplii abundance for 3 out-of-sample pond nauplii counts and therefore was not recommended as an appropriate management design. Alternatively, an empirical approach was evaluated to determine the number of ponds that would be needed to satisfy stocking requirements under conditions of uncertainty. This approach yielded more manageable results.

ACKNOWLEDGMENTS

Financial support for this publication was provided by USDA Cooperative Research, Education, and Extension Service Special Research Grant PO 2002-06152. I would like to thank the Auburn University staff D Maus, D. Rodriguez, and A. Ferry for their assistance and guidance in red snapper aquaculture. I would like to extend appreciation to the Auburn University undergraduate workers C. Smith, C. Merritt, T. Fleming, and L. Littleton for their help in pond management and data collection. I also appreciate the help of the Marine Resources Division staff at Claude Peteet Mariculture Center in Gulf Shores, AL for their help during harvesting and for providing materials and equipment.

Style manual or journal used:

I., II., III., IV., Transactions of the American Fisheries Society

V. Aquaculture Economics and Management

Computer software used: Microsoft Word, Microsoft Excel, SAS 9.0, SAS Enterprise Guide 3.0.0.

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I. INTRODUCTION

Red snapper, *Lutjanus campechanus* is found primarily along the continental shelf of the Gulf of Mexico and is subjected to heavy commercial and recreational fishing pressure (Hoese and Moore 1977; Allen 1985; Goodyear 1995; Coleman et al. 2000; Patterson et al. 2001; Wilson and Nieland 2001 Shipp 2003; Pruett et al. 2005; Saillant and Gold 2006). Factors such as their popularity as a food fish, high market price, and over fishing, have contributed to substantial interest in red snapper aquaculture for both commercial food fish production and fisheries stock enhancement. Efforts to replenish wild stocks through aquaculture have been plagued by low larval survival during the onset of feeding. Red snapper larvae require an abundance of small (< 100 µm), nutritious prey organisms at the time of exogenous feeding. Copepod nauplii have proved to be a suitable food for red snapper (Chigbu et al. 2002). To further complicate the issue; copepod nauplii grow faster in low salinities (10-15 ppt) but red snapper require higher salinities (35 ppt) for complete development. Because of these two very different life histories copepod nauplii and red snapper larvae are usually grown separately. To date, the preferred cultivation method for red snapper has been to rear larvae in tanks (1000 L) at full strength seawater in which copepod nauplii are added daily to maintain suitable densities. In this setting nauplii are extracted from brackish water ponds or large tanks either by towing plankton nets or by pumping into zooplankton traps (Phelps et al. 2000; Lan et al. 2001; Chigbu et al. 2002; Lemus et al.

2002; Lemus et al. 2004; Lindley 2004; Rhodes 2005). Survival rates for fingerlings (24 days post-hatch) ranged from 0.3 to 18 % (Chigbu et al. 2002) using the above described method. The acclimation of nauplii from the brackish water zooplankton ponds to full strength seawater tanks where the larvae are reared without compromising density and nutritional value poses a challenge for scientists. A new method of rearing red snapper with their prey needed to be explored.

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II. LITERATURE REVIEW

Nursery pond production techniques

Primary nursery ponds are used for a variety of freshwater fish (Geiger 1983; Buttner 1989; Barkoh 1996; Buurma et al. 1996; Ludwig 2002; Ljunggren et al. 2003) as well as a few marine species (Colura et al. 1976; Procarione et al. 1989; Jenkins and Smith 1997). The objective of a primary nursery pond is to provide a natural food base for larval fish during the first 30-45 days post-hatch (dph). Ponds are usually prepared and filled a few days to weeks in advance in order to synchronize zooplankton prey abundance with fry stocking. Organic and inorganic fertilizer is added to the pond to promote zooplankton growth for the arrival of fish larvae. Organic fertilizers provide nutrients for bacteria and algae which serve as food sources for zooplankton (Schroeder 1978; Moriarty 1997; Ludwig et al. 1998). The amount and type of fertilizer used is dependent upon the desired zooplankton needed for the incoming larvae. Agricultural by-products such as grains and livestock manures have been used extensively in pond culture as organic fertilizer sources. Finely ground grain fertilizers appear to be more popular because they decompose more rapidly than manures; however cost and accessibility are important considerations to the farm manager as well as crop yield. Geiger et al. (1985) found that rotifers were an acceptable first food for striped bass (*Morone saxatilis*) and survival at 38 dph was 63% when ponds were prepared with cottonseed meal and inorganic fertilizer. The use of alfalfa meal (17% protein) in initial concentrations of 200 kg/ha has proved successful in producing striped bass fingerlings in freshwater nursery ponds (Barkoh 1996). Buttner (1989) suggests using wheat shorts at 450 kg/ha during pond filling to encourage copepod growth for incoming walleye (*Stizostedion vitreum*). Geiger (1983) increased striped bass survival by combining chicken litter fertilizer and *Daphnia pulex* inoculations at 12,500/ha three days before stocking fry. Pig manure at daily additions of 750 kg/ha was shown to improve rotifer growth in freshwater nursery ponds (Li et al. 1996). Cottonseed meal is beneficial as an organic fertilizer for the production of rotifers; an important first-food for many saltwater species. Southern flounder (*Paralichthys lethostigma*) nursery ponds that were fertilized with 450 kg/ha of cottonseed meal resulted in 6% survival (Jenkins and Smith 1997). Porter and Maciorowski (1984) reported 9% survival of spotted sea trout (*Cynoscion nebulosus*) when applying 568 kg/ha cottonseed meal to brackish water ponds and stocking fish 26 days after filling.

Rice bran is inexpensive, accessible, and effective in stimulating copepod nauplii production (Turk et al. 1981; Ogle and Lotz 2000; Lemus et al. 2002). Lemus et al. (2004) found that adding rice bran to a brown-water mesocosm system proved beneficial in copepod nauplii production. The key to a successful primary nursery pond is to provide the proper type, size, and abundance of food organisms throughout the period of larval fish growth. Timing the peak of target food organisms with larval stocking is also crucial to survival (Anderson 1993; Valdenberg et al. 2006) and red snapper aquaculture has proved to be no exception. The onset of exogenous feeding and rapid absorption of yolk reserves have posed a serious challenge in red snapper nursery production.

Risk assessment and pond modeling in aquaculture

The main function of a primary nursery pond is to provide live food resources for developing fry. In order to achieve success the manager must monitor water quality and zooplankton biomass throughout the culture period and adjust fertilizer inputs accordingly. Ensuring that the proper zooplankton succession occurs in the pond is the primary goal for the manager. The desired zooplankton progression will differ for each fish species cultured and there are dramatic differences between freshwater and marine systems. Different culture conditions will dictate the pond management strategy utilized; it is important to note that not all ponds are created equal (Boyd 1979). To add further challenges; zooplankton composition can change seasonally and temperature, light, water chemistry, food availability, and predation can dramatically influence the community structure (Geiger 1983). For all the above mentioned reasons it is critical to synchronize larval stocking with target food abundance. The manager must accurately predict (by experience or through sampling) the appropriate time to stock fry. In recent years risk analysis and computer modeling have begun to surface as tools to help managers deal with uncertainties in pond aquaculture (Giovannini and Piedrahita 1994; Nelson et al. 2001; Knud-Hansen et al. 2003; Engle and Kouka 1998). One of the first comprehensive software packages developed was POND (Biosystems Analysis Group) targeted at pond managers and educators (Ernst et al. 2000). The program is a decision support system that allows users to predict the economic and ecological (i.e. fish biomass) impacts of different variables during pond production. Decision support systems are computer-based models designed to be interactive, flexible, and adaptable for supporting non-structured management strategies (Turban 1995). Using simulations from real applications the model provides a reliable tool for users. Drawbacks to the program include limited species selection, large parameter choice, and most importantly the simulations are abstracts of real outcomes and may not provide enough information for routine pond management. This problem of simulating a small number of specific ponds/management situations is common in most forecasting models. The appreciation for uncertainty in farm management decision making has resulted in replacing decision support system modeling with probability risk approaches.

Risk is essentially the combination of the probability of a negative event and its consequences. Elevating the goal level will inevitably increase the risk. Mitigating risk in aquaculture is of primary concern for the farm manager. Increasing the profitability of an aquaculture farm is the main objective; however net returns and economics are subject to great uncertainty (Hatch et al. 1987). In aquaculture risk is highly associated with increasing complexity of the operation (i.e. indoor recirculating tank culture vs. ponds). In pond catfish culture for example risk is reduced as a result of fewer technological inputs, but due to a low profit margin mitigating risk is still of great concern to the manager. Because of the above mentioned characteristics and its popularity as a food fish, primary consideration has been given to evaluating the economic risks in catfish production (Engle and Hatch 1988; Engle and Valderrama 2001; Pomerleau and Engle 2003). It is likely that similar risk management approaches will include other fish species.

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III. RED SNAPPER (*LUTJANUS CAMPECHANUS*) AQUACULTURE IN MARINE PRIMARY NURSERY PONDS.

Abstract

Twenty 0.1 ha ponds were prepared as marine primary nursery ponds. Over a 3 week period ponds were filled with 35 ppt seawater and fertilized initially with either 250 kg/ha or 500 kg/ha of rice bran, and 20 L/ha inorganic (38-8-0) fertilizer. Ponds were fertilized at half the above rates each week thereafter. Four ponds receiving the 500 kg/ha treatment were also supplied with continuous aeration. Two day-old red snapper were stocked in ponds at either 5, 7, or 10 days post-filling. Zooplankton samples were collected and counted daily. Red snapper were harvested after 30 d. Survival varied greatly between treatments, ranging from 1 fish in treatment 2 to 295 fish in treatment 5. The treatment receiving 250 kg/ha of rice bran and stocked 10 days post-filling resulted in higher snapper survival when compared to the remaining low fertilizer treatments.

Introduction

Red snapper, *Lutjanus campechanus* have undergone heavy exploitation by both commercial and recreational fishing in the Gulf of Mexico (Wilson and Nieland, 2001). Restocking efforts aimed at replenishing populations are hindered due to poor larval survival (Papanikos et al. 2003). The difficulty of successful stock enhancement is due to the problem of meeting the dietary requirements of developing larvae. Characteristics such as small mouth size and limited yolk reserve at the time of first-feeding make successful red snapper aquaculture a challenge (Williams et al. 2004). Red snapper require an abundance of small (< 100 µm) prey items rich in fatty acids at the onset of exogenous feeding. Copepod nauplii have proved to be a suitable food for red snapper (Chigbu et al. 2002). Copepod nauplii are usually collected from outdoor brackish water ponds and then added to larvae in tank systems. Nauplii densities in larval rearing tanks are maintained through daily zooplankton addition. Using this method snapper survival can be compromised by the reduced nutritional value of nauplii during their transfer from the low salinity culture ponds to the full strength seawater in the rearing tanks (Lan, H. P. 2001; R. Phelps, Auburn University, personal communication). A new method of rearing red snapper with their prey needed to be explored.

Primary nursery ponds are used for a variety of freshwater fish (Geiger 1983; Buttner 1989; Barkoh 1996; Buurma et al. 1996; Ludwig 2002; Ljunggren et al. 2003) as well as a few marine species (Colura et al. 1976; Procarione et al. 1989). The objective of a primary nursery pond is to provide a natural food base for larval fish during the first 30-45 days post-hatch (dph). Organic and inorganic fertilizer is added to the pond to promote zooplankton growth for the arrival of fish larvae. The amount and type of fertilizer used is dependent upon the desired zooplankton needed for the incoming larvae. Rice bran is inexpensive, accessible, and effective in stimulating copepod nauplii development (Turk et al. 1981; Ogle and Lotz 2000; Lemus et al. 2002). Lemus et al. (2004) found that adding rice bran to a brown-water mesocosm system proved beneficial in copepod nauplii production. The key to a successful primary nursery pond is to provide the proper type, size, and abundance of food organisms throughout the period of larval fish growth. Timing the peak of target food organisms with larval stocking is also crucial to survival (Valdenberg et al. 2006). The goal of this study was to evaluate the use of saltwater primary nursery ponds for rearing larval red snapper.

Materials and methods

Pond preparation

Beginning May 4, 2004 twenty 0.1 ha ponds located at the Claude Peteet Mariculture Center in Gulf Shores, AL were prepared as primary nursery ponds. Prior to filling, ponds were tilled and sterilized with 1,000 kg/ha hydrated lime ($Ca(OH)^2$). Nylon filter socks (1,000 µm mesh) were attached to the inlet pipe during filling to prevent aquatic predators from entering the pond. Sets of ponds were filled each week over a three week period with 35 ppt seawater obtained from the Gulf of Mexico. Each pond received either 250 (low rate) or 500 kg/ha (high rate) of rice bran (Burris/Cargill Animal Nutrition, Franklinton, LA, USA) as an initial application of organic fertilizer and half those rates every week thereafter. Liquid inorganic fertilizer (38-8-0) was applied at 20 L/ha as an initial application and half this rate every week. Four ponds receiving the 500 kg/ha rate were also supplied with continuous aeration from a low-head air-lift system via a 1 hp. regenerative blower (Sweetwater, AES, Inc., Apopka, FL, USA). In total, three pond preparation protocols were evaluated: 1) initial applications of 250 kg/ha organic fertilizer (12 replicates), 2) 500 kg/ha (4 replicates), and 3) 500 kg/ha with aeration (4 replicates). Table 1 shows in detail the pond filling protocol.

Zooplankton sampling

Starting 3 days post filling zooplankton was collected daily by pumping 10 L of pond water through a 35 μm Nitex sieve. In order to obtain a random sample the pump continuously moved through the water column. Samples were preserved in 5% formalin and filtered seawater. One milliliter Sedgwick-Rafter counting chambers were used to

Table 1.—Pond management protocol according to each start block, treatment, pond filling date, and pond stocking date. Parenthetical numbers represent stocking day post-filling.

Block 1		**Block 2**		**Block 3**	
Pond filled on 5/4		**Pond filled on 5/11**		**Pond filled on 5/21**	
Pond stocked on 5/14		**Pond stocked on 5/21**		**Pond stocked on 5/31**	
Pond	Treatment (DPF)	Pond	Treatment (DPF)	Pond	Treatment (DPF)
B-8	250 kg/ha (10)	A-5	250 kg/ha (10)	B-6	250 kg/ha (10)
A-1	500 kg/ha (10)	B-3	500 kg/ha (10)	A-3	500 kg/ha (10)
B-2	500 kg/ha (10)	D-6	500 kg/ha (10) Air	A-8	250 kg/ha (10)
D-5	500 kg/ha (10) Air	D-7	500 kg/ha (10) Air	D-4	500 kg/ha (10) Air
Pond filled on 5/7		**Pond filled on 5/14**		**Pond filled on 5/24**	
Pond stocked on 5/14		**Pond stocked on 5/21**		**Pond stocked on 5/31**	
Pond	Treatment (DPF)	Pond	Treatment (DPF)	Pond	Treatment (DPF)
B-1	250 kg/ha (7)	A-2	250 kg/ha (7)	A-4	250 kg/ha (7)
B-4	250 kg/ha (7)				
Pond filled on 5/9		**Pond filled on 5/16**		**Pond filled on 5/26**	
Pond stocked on 5/14		**Pond stocked on 5/21**		**Pond stocked on 5/31**	
Pond	Treatment (DPF)	Pond	Treatment (DPF)	Pond	Treatment (DPF)
A-6	250 kg/ha (5)	B-5	250 kg/ha (5)	B-7	250 kg/ha (5)
		A-7	250 kg/ha (5)		

identify and enumerate zooplankton found in pond samples. Organisms were enumerated by counting every row of the Sedgwick-Rafter chamber.

Water quality measurements

Temperature and dissolved oxygen were measured twice daily while salinity and pH were recorded once daily. Dissolved oxygen, temperature, salinity, and pH were measured using an YSI 556 MPS multi-probe meter (Yellow Springs, Ohio, USA). A 20 cm diameter secchi disk (Aquatic Eco-Systems, Inc., Apopka, FL, USA.) was used to monitor the relative visibility of the pond water. Total ammonia-nitrogen (TAN) was measured four times during the 30 d study. Pond TAN concentrations were measured using the Nessler method and analyzed on a Spectronic 20 Genesys spectrophotometer (Spectronic Instruments, Inc., Rochester, NY, USA.).

Larval rearing

Brood stock were captured from the Gulf of Mexico using hook and line and transported to the hatchery at Claude Peteet Mariculture Center in Gulf Shores, AL where they were induced to spawn using methods described by Minton et. al (1983). Fertilized eggs were placed in 100 L incubators with continuous aeration provided at the base of the 200 µm screened stand pipe. Temperature and salinity were maintained at 27 C and 33 ppt, respectively.

Ponds were stocked at either 5, 7, or 10 days post-filling (dpf) with 33-42 h post-hatch (hph) larvae at a density of 275,000/ha or 27,500 per pond. Beginning at dawn larvae were placed in plastic bags, introduced to nursery ponds, and allowed to acclimate before release. Table 1 describes the stocking and harvesting dates for each pond. Artificial feed (Aquamax, Purina Mills, Inc., St. Louis, MO, USA.) replaced fertilizer when fish were 22 days old and continued until harvest. Weight and length measurements were taken for each fish during harvest.

Results

Snapper survival

Total survival from all twenty ponds was 577 fish with ponds D6 and A5 contributing 295 and 123 snapper respectively. Table 2 summarizes red snapper age, survival (number of fish harvested per pond), average length, and average weight at harvest. Fertilizer treatment and pond stocking day had a significant effect ($p<0.0001$) on survival. Treatments 3 and 5, which were prepared 10 d prior to stocking, had the greatest number of snapper at harvest with means 50.3 ± 50.4 and 74.3 ± 128.6 respectively.

The set of ponds filled and stocked during the same time period, or start block, had a significant effect on survival ($p<0.0001$). It is evident from table 2 that the second start block had the highest survival (68.4 ± 102.4 fish/pond). Also, ponds stocked with fish that were 42 hph had significantly higher survival ($p<0.0001$) averaging 68.4 ± 102.4. These correlations could be indicating a brood stock influence on snapper survival since each group of ponds stocked came from different spawns. There was no apparent correlation between copepod nauplii density and snapper survival ($p=0.7743$).

Snapper growth

Average weight varied between treatments ($p=0.0232$) from a single fish weighing 0.6 g in treatment 4 to 1.72 g average weight in treatment 1. There was no significant difference in average snapper total length among the treatments. The largest fish were found in treatments 1 and 4 averaging 4.79 and 4.13 cm (TL) respectively. There appears to be no significant correlation between snapper growth (average length and average weight) and survival.

Water quality data was not useful in explaining variation in snapper survival despite the fact that two ponds (B2 and D7) experienced dissolved oxygen levels below 2.0 ppm and resulted in zero survival.

In addition to red snapper, silver perch (*Bairdiella chrysoura*), spotted seatrout (*Cynoscion nebulosus*), sand seatrout (*Cynoscion arenarius*), and feather blennies (*Hypsoblennius hentz*) were also found during harvest (table 3). Treatment 3 had the fewest predatory fish species totaling 8. Treatments 1, 2, and 5 had similar amounts of predatory fish. The greatest numbers of foreign fish were found in treatment 4 with 43 silver perch, 7 spotted seatrout, 3 sand seatrout, and 5 blennies (for an average of 14 fish/pond). The presence and abundance of predatory fish had no significant impact on snapper survival (p=0.1580).

Table 2.—Red snapper stocking, survival, average length, average weight, and age at harvest.

Pond	Start block	Treatment	Stocked (dpf)	Stock age (hph)	Age at harvest	No. Harvested[a]	Av. TL Length	Av. Weight (g)
A1	1	500 kg/ha (no air)	10	34	27	0	0	0
A6	1	250 kg/ha	5	34	34	16	3.96	1.12
B1	1	250 kg/ha	7	34	36	0	0	0
B2	1	500 kg/ha (no air)	10	34	36	0	0	0
B4	1	250 kg/ha	7	34	35	0	0	0
B8	1	250 kg/ha	10	34	35	71	4.36	1.59
D5	1	500 kg/ha (air)	10	34	34	0	0	0
A2	2	250 kg/ha	7	42	34	0	0	0
A5	2	250 kg/ha	10	42	35	123	5.13	1.87
A7	2	250 kg/ha	5	42	35	6	4.5	1.32
B3	2	500 kg/ha (no air)	10	42	33	0	0	0
B5	2	250 kg/ha	5	42	34	53	4.21	1.24
D6	2	500 kg/ha (air)	10	42	33	295	3.86	0.83
D7	2	500 kg/ha (air)	10	42	20	0	0	0
A3	3	500 kg/ha (no air)	10	33	35	0	0	0
A4	3	250 kg/ha	7	33	35	1	4	0.6
A8	3	250 kg/ha	10	33	33	6	3.33	0.42
B6	3	250 kg/ha	10	33	34	1	3.1	0.3
B7	3	250 kg/ha	5	33	34	5	3.44	0.43
D4	3	500 kg/ha (air)	10	33	33	0	0	0

[a] Represents the number harvested out of 27,500 fish per pond.

Discussion

This study showed that red snapper fingerlings can be raised in marine primary nursery ponds. Using rice bran as an organic fertilizer at high and low concentrations and allowing 5, 7, and 10 days before stocking ponds we were able to successfully produce red snapper fingerlings. However, survival was as low and variable as found in tank culture where nauplii are added daily. Ponds fertilized with 250 kg/ha of rice bran and waiting 10 dpf to stock larvae (treatment 3) yielded the most consistent snapper survival among the five treatments (50 ± 58 survival and 1.04 ± .80 g average weight). Although treatment 5 had the highest total survival in a single pond, the other three ponds had zero; from a risk perspective this treatment would not be favored as a primary nursery pond production method.

Although snapper survival was low (overall 0.10 %) it is not abnormal compared with other marine species cultured. Chigbu et al. (2002) reported red snapper (*Lutjanus campechanus*) survival between 0 to 7.6 % when cultivated in 200 L black tubs with copepod nauplii added. Colura et al. (1976) reported spotted seatrout survival between 0 and 18.6 % when raised in primary nursery ponds for 29-32 days. This study suggested that the variability in fingerling survival was a function of larval age at stocking. The researchers found that stocking 2 day old spotted seatrout resulted in lower survival when compared to the 7 day old stocked larvae.

Table 3.—Exotic fish species harvested from ponds by treatment.

Block 1	Treatment	Silver perch	Spotted seatrout	Sand seatrout	Feather blennie
A-1	4	17	1	0	1
A-6	1	2	0	0	2
B-1	2	0	0	0	0
B-2	4	23	2	3	2
B-4	2	10	0	1	2
B-8	3	1	0	0	2
D-5	5	10	0	1	1

Block 2	Treatment	Silver perch	Spotted seatrout	Sand seatrout	Feather blennie
A-2	2	0	1	0	2
A-5	3	0	0	0	0
A-7	1	0	0	0	2
B-3	4	0	0	0	1
B-5	1	1	0	0	2
D-6	5	0	0	0	1
D-7	5	0	0	0	2

Block 3	Treatment	Silver perch	Spotted seatrout	Sand seatrout	Feather blennie
A-3	4	3	2	0	1
A-4	2	2	0	0	1
A-8	3	0	0	0	2
B-6	3	1	0	1	1
B-7	1	7	0	0	2
D-4	5	1	2	0	2

Conclusion

It is difficult to identify the chief reason for low survival observed in the nursery ponds. Although there appears to be no statistical evidence supporting predation by extraneous fish, the inability of the pond filter socks to entrain invasive species still could have had a negative impact on snapper survival by either predation or direct competition for food. Also red snapper cannibalism cannot be ruled out as a cause of larval mortality. Similarly, poor water quality could have caused added stress leading to mortality, which is contrary to the statistical results in this study. Copepod nauplii density appeared to have no appreciable bearing on snapper survival. Even when comparisons were made between individual ponds with similar fish survival (during the few days following stocking), copepod nauplii abundance was very different. This is peculiar because similar studies have revealed a significant positive correlation between prey availability (density) and larval survival (Watanabe et al. 1998; Chigbu et al. 2002).

In this study we attempted to regulate major sources influencing red snapper survival and growth however, in a mesocosm pond system there are many variables that cannot be measured. It is likely that one or many of these unaccounted for variables resulted in low survival.

Future research on primary nursery pond culture of red snapper should include increased control of predaceous fish by employing smaller mesh filter socks during filling. The low rate ponds stocked at 10 dpf experienced higher survivals than the other 250 kg/ha treatments and should be replicated to reaffirm the results of this study. The addition of continuous aeration may have had a positive effect on the high rate ponds. Future experiments adding air to low rate ponds would prove valuable.

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IV. ZOOPLANKTON DEVELOPMENT IN MARINE PRIMARY NURSERY PONDS

Abstract

Twenty 0.1 ha ponds were prepared as primary nursery ponds. Ponds were filled over a 3 week period with 35 ppt seawater and fertilized initially with either 250 kg/ha or 500 kg/ha of rice bran, and 20 L/ha inorganic (38-8-0) fertilizer. Ponds were fertilized at half the above rates each week thereafter. Four ponds receiving the 500 kg/ha treatment were also supplied with continuous aeration. Zooplankton development was examined for 30 days after a pond was filled. Ponds receiving low amounts of fertilizer had higher copepod nauplii densities than the high rate ponds. During the second week the low rate pond treatment showed greater abundance than the other two treatments with an average density of 884.66 ± 1091.81 nauplii /L. Aeration had no significant effect on nauplii density. Variation in nauplii density was high between and within ponds. Adult copepod abundance was found to be greater in low rate ponds during the first 14 days after pond filling. Rotifers were late to appear in all of the ponds. Rotifer densities exceeded 20,000/L in some ponds during the last week of the study.

Introduction

Primary nursery ponds are commonly used for a variety of freshwater fish (Geiger 1983a, Buttner 1989, Barkoh and Rabeni 1990, Ludwig and Tackett 1991, Barkoh 1996, Buurma et al. 1996, Ludwig 2002, Ljunggren et al. 2003) with the objective of providing a natural food base for larval fish growth during the first 30-45 days post-hatch (dph). Ponds are prepared in anticipation of stocking young larvae by the addition of organic and inorganic fertilizers, then filling the pond and allowing phytoplankton and zooplankton to become established. The types of zooplankton and their abundance depend on the types and quantities of fertilizers used, water temperature, and other environmental factors. Geiger (1983b) increased striped bass survival by combining chicken litter fertilizer and *Daphnia pulex* inoculations at 12,500/ha three days before stocking fry. Geiger et al. (1985) found that rotifers were an acceptable first food for striped bass (*Morone saxatilis*) and survival at 38 dph was 63% when ponds were prepared with cottonseed meal and inorganic fertilizer. A goal in a primary nursery pond production system is to have the proper size food organism available at an appropriate density throughout the period of larval fish growth.

The use of primary nursery ponds for the production of marine fish is less common. However red drum (*Sciaenops ocellatus*), spotted seatrout (*Cynoscion nebulosus*), and southern flounder (*Paralichthys lethostigma*) have been produced in primary nursery ponds. The issue is the same as in freshwater ponds- trying to have the proper type and abundance of zooplankton- but it is complicated by the greater diversity of zooplankton found in a marine system. Johansen (1986) concluded that due to the rapid succession (only 4 days) of dominant zooplankters in red drum primary nursery ponds it is critical to time the filling and stocking of ponds carefully. Colura et al. (1992) was able to produce high densities of polychaete larvae for spotted seatrout (*Cynoscion* *nebulosus*) fingerlings by preparing saltwater ponds with 141-284 kg/ha cotton seed meal and waiting 28 days before stocking.

Copepod nauplii have proven to be an appropriate food for larval red snapper when cultivated in a mesocosm system (Chigbu et al. 2002, Ogle and Lotz 2000). Sturmer (1987) found copepod nauplii are abundant in red drum primary nursery ponds. For successful red snapper aquaculture it is particularly critical that adequate numbers of nauplii of the proper size be available when the snapper larvae first begin to feed. Williams et al. (2004) found that the transition period between using endogenous and exogenous nutrient sources is very brief. They determined that at first feeding only 1% of yolk and 2% oil globule remained. Therefore, a nutritionally adequate food of a proper size must be abundant as snapper larvae transition to exogenous feeding.

The purpose of this paper is to describe the zooplankton development in marine primary nursery ponds subjected to different organic fertilizer concentrations.

Materials and methods

Pond preparation

Beginning May 4, 2004 twenty 0.1 ha ponds located at the Claude Peteet Mariculture Center in Gulf Shores, AL were prepared as primary nursery ponds. Prior to filling, ponds were tilled and sterilized with 1000 kg/ha hydrated lime ($Ca(OH)^2$). Nylon filter socks (1000 µm mesh) were attached to the inlet pipe during filling to prevent aquatic predators from entering the pond. Sets of ponds were filled each week over a three week period with 35 ppt seawater obtained from the Gulf of Mexico. Each pond received either 250 (low rate) or 500 kg/ha (high rate) of rice bran (Burris/Cargill Animal Nutrition, Franklinton, LA, USA) as an initial application of organic fertilizer and half those rates every week there after. Liquid inorganic fertilizer (38-8-0) was applied at 20 L/ha as an initial application and half this rate every week. Four ponds receiving the 500 kg/ha rate were also supplied with continuous aeration from a low-head air-lift system via a 1 hp. regenerative blower (Sweetwater, AES, Inc., Apopka, FL, USA). In total, three pond preparation protocols were evaluated: 1) initial applications of 250 kg/ha organic fertilizer (12 replicates), 2) 500 kg/ha (4 replicates), and 3) 500 kg/ha with aeration (4 replicates). Table 1 shows in detail the pond filling protocol. Red snapper larvae were stocked at a density of 275,000/ha in the ponds at two days post-hatching after the ponds had been filled for either 5, 7, or 10 days. After 30 d ponds were harvested.

Zooplankton sampling

Starting 3 d post-filling zooplankton samples were collected daily by pumping 10 L of pond water through a 35 µm Nitex sieve. In order to obtain a random sample the pump continuously moved through the water column. Samples were preserved in 5% formalin and filtered seawater. One milliliter Sedgwick-Rafter counting chambers were used to identify and enumerate zooplankton found in pond samples. Organisms were enumerated by counting every row of the Sedgwick-Rafter chamber.

Table 1.—Pond management protocol according to each start block, treatment, pond filling date, and pond stocking date. Parenthetical numbers represent stocking day post-filling.

Block 1		**Block 2**		**Block 3**	
Pond filled on 5/4		**Pond filled on 5/11**		**Pond filled on 5/21**	
Pond stocked on 5/14		**Pond stocked on 5/21**		**Pond stocked on 5/31**	
Pond	Treatment (DPF)	Pond	Treatment (DPF)	Pond	Treatment (DPF)
B-8	250 kg/ha (10)	A-5	250 kg/ha (10)	B-6	250 kg/ha (10)
A-1	500 kg/ha (10)	B-3	500 kg/ha (10)	A-3	500 kg/ha (10)
B-2	500 kg/ha (10)	D-6	500 kg/ha (10) Air	A-8	250 kg/ha (10)
D-5	500 kg/ha (10) Air	D-7	500 kg/ha (10) Air	D-4	500 kg/ha (10) Air
Pond filled on 5/7		**Pond filled on 5/14**		**Pond filled on 5/24**	
Pond stocked on 5/14		**Pond stocked on 5/21**		**Pond stocked on 5/31**	
Pond	Treatment (DPF)	Pond	Treatment (DPF)	Pond	Treatment (DPF)
B-1	250 kg/ha (7)	A-2	250 kg/ha (7)	A-4	250 kg/ha (7)
B-4	250 kg/ha (7)				
Pond filled on 5/9		**Pond filled on 5/16**		**Pond filled on 5/26**	
Pond stocked on 5/14		**Pond stocked on 5/21**		**Pond stocked on 5/31**	
Pond	Treatment (DPF)	Pond	Treatment (DPF)	Pond	Treatment (DPF)
A-6	250 kg/ha (5)	B-5	250 kg/ha (5)	B-7	250 kg/ha (5)
		A-7	250 kg/ha (5)		

Water quality measurements

Temperature and dissolved oxygen were measured twice daily while salinity and pH were recorded once daily. Dissolved oxygen, temperature, salinity, and pH were measured using an YSI 556 MPS multi-probe meter (Yellow Springs, Ohio, USA). A 20 cm diameter secchi disk (Aquatic Eco-Systems, Inc., Apopka, FL, USA) was used to monitor the relative visibility of the pond water. Total ammonia-nitrogen (TAN) was measured four times during the 30 d study. Pond TAN concentrations were measured using the Nessler method and analyzed on a Spectronic 20 Genesys spectrophotometer (Spectronic Instruments, Inc., Rochester, NY, USA).

Results

Species composition

A variety of zooplankton developed in the nursery ponds but the species composition was not related to the pond management protocol. There were, however, differences in abundance of specific organisms and population dynamics related to pond preparation. The tintinnid *Parafavella* was the most evident protozoan and *Brachionus rotunda* was the most common rotifer.

In addition to the dominant organisms mentioned previously, samples contained larval forms of Cirripedia, Polychaeta, and Opisthobranchia as well as Spirotricha ciliates including *Euplotes* and other loricate ciliates. In all three treatments loricate ciliates appeared only for the first 12 days. The 500 kg/ha with air treatment experienced the highest densities of loricate ciliates approaching 300 org/L. Barnacle cypris larvae began to appear in pond samples around day 10 post-filling. The highest cypris counts were found in the high rate ponds with air (130/L). In most of the ponds barnacle densities peaked just prior to harvest. Polychaete larvae (trochophores and metatrochophores) were observed primarily during the end of the study (20-30 dpf). Maximum densities ranged from 110 to 220/L and were highest in high rate fertilized ponds supplied with air. Beginning 20 dpf a mollusk veliger was found, and later identified during pond harvest as the sea slug *Bursatella leachii*. Relative veliger abundance was highest in the 500 kg/ha ponds.

Zooplankton succession

The progression of zooplankton regardless of treatment exhibited the same patterns of dominant organisms; however, succession was more dramatic in ponds receiving the 250 kg/ha of rice bran fertilizer. Table 2 shows mean densities over the 30 day period of the dominant organisms sampled by treatment.

Population dynamics of most abundant species

Protozoans

The tintinnid protozoan *Parafavella* occurred in high densities during the first 10 days after pond filling. Highest densities observed overall were in the low (2422.73 ± 2451.52/L) and high fertilizer rate ponds receiving aeration (1885.97 ± 2001.54/L) on days 4 and 6 respectively (Figure 1 and 2). Following day 15, the protozoan had virtually vanished from all ponds and averaged 8.26 ± 51.12/L.

Rotifers

Rotifers were late to appear in all the ponds; reaching their highest densities during the final week (days 22 to 30). Many ponds experienced very high densities of rotifers (>10,000/L), and some exceeded 20,000/L. Rotifer succession in the low rate treatment typifies patterns observed for all ponds (Figure 1). Mean abundance is illustrated in Figure 5 and progressively increased with additional inputs (fertilizer and aeration); however there was no difference between treatments (p=0.35). The high rate with air ponds experienced greater rotifer densities and variation (Table 1).

Ponds that were filled during the first week (start block one) experienced significantly lower rotifer densities than ponds filled during the second and third week (p<0.0001).

Table 2.—Mean abundance of dominant zooplankton found between treatments for the 30 day study period.

Zooplankton	Treatment	Number of obs	Mean ± SD
Parafavella			
	250 kg/ha	360	238 ± 1246a
	500 kg/ha (no air)	120	63 ± 467a
	500 kg/ha (air)	119	106 ± 629a
Nauplii			
	250 kg/ha	360	523 ± 730a
	500 kg/ha (no air)	120	396 ± 666a
	500 kg/ha (air)	119	379 ± 402a
Adult copepods			
	250 kg/ha	360	159 ± 261a
	500 kg/ha (no air)	120	87 ± 128b
	500 kg/ha (air)	119	88 ± 81b
Rotifers			
	250 kg/ha	360	847 ± 2277a
	500 kg/ha (no air)	120	1071 ± 2824a
	500 kg/ha (air)	119	1296 ± 4525a

Copepod nauplii

The target organism, copepod nauplii, were observed throughout the culture period, although their densities were highest in the low rate ponds (1213.23 ± 1317.76/L) during the second week after filling (Figure 1). During the first two weeks after pond filling, mean density increased with decreasing fertilizer concentration and aeration (Figure 3). Low rate ponds averaged 884.66 ± 1091.81 nauplii/L for days 7 through 14 while during the same time period the high rate ponds receiving air experienced only 398.27 ± 347.64/L. Abundance was significantly higher in low rate ponds than in the

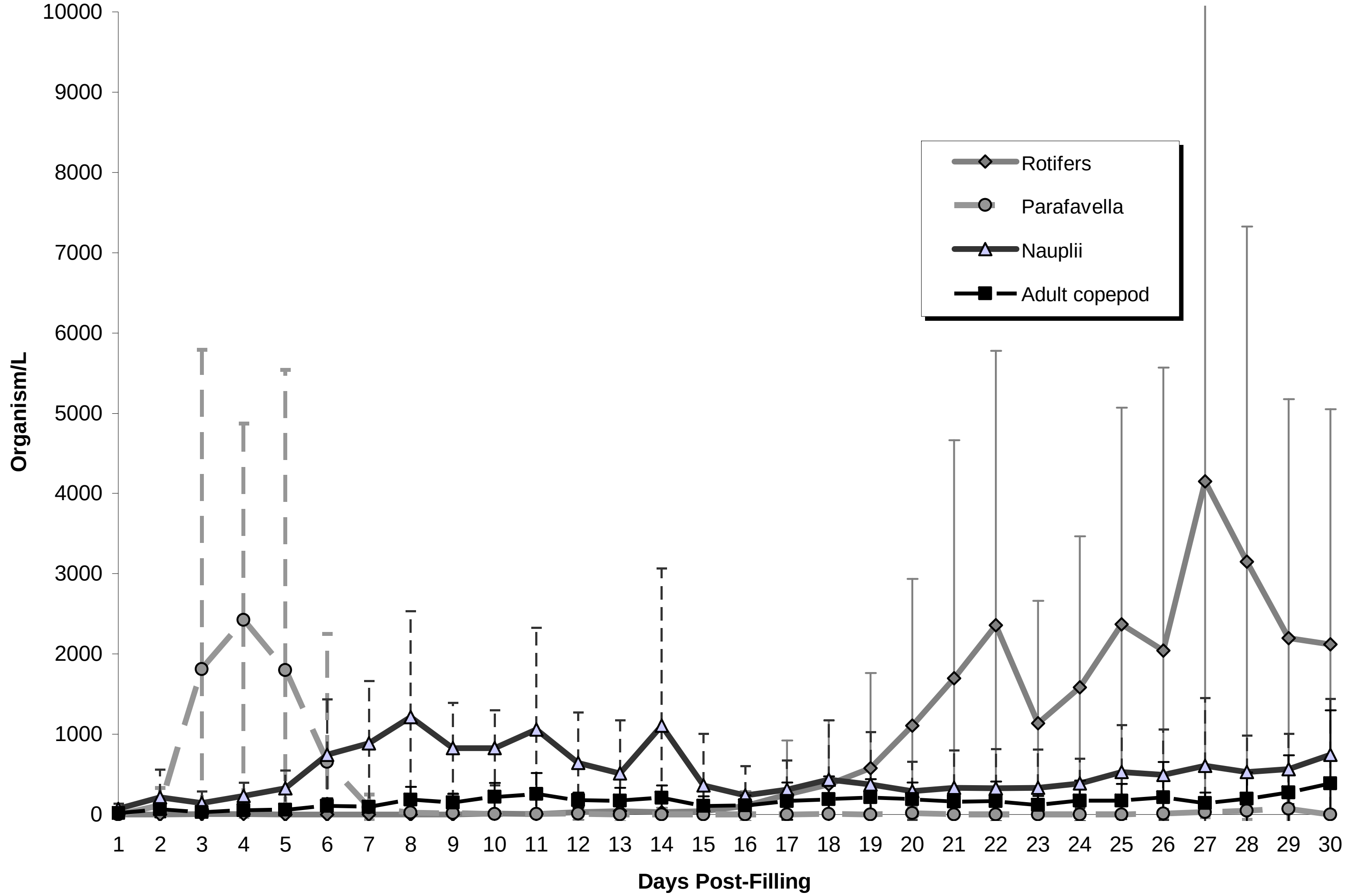

Figure 1.—Zooplankton succession in low rate (250 kg/ha) ponds.

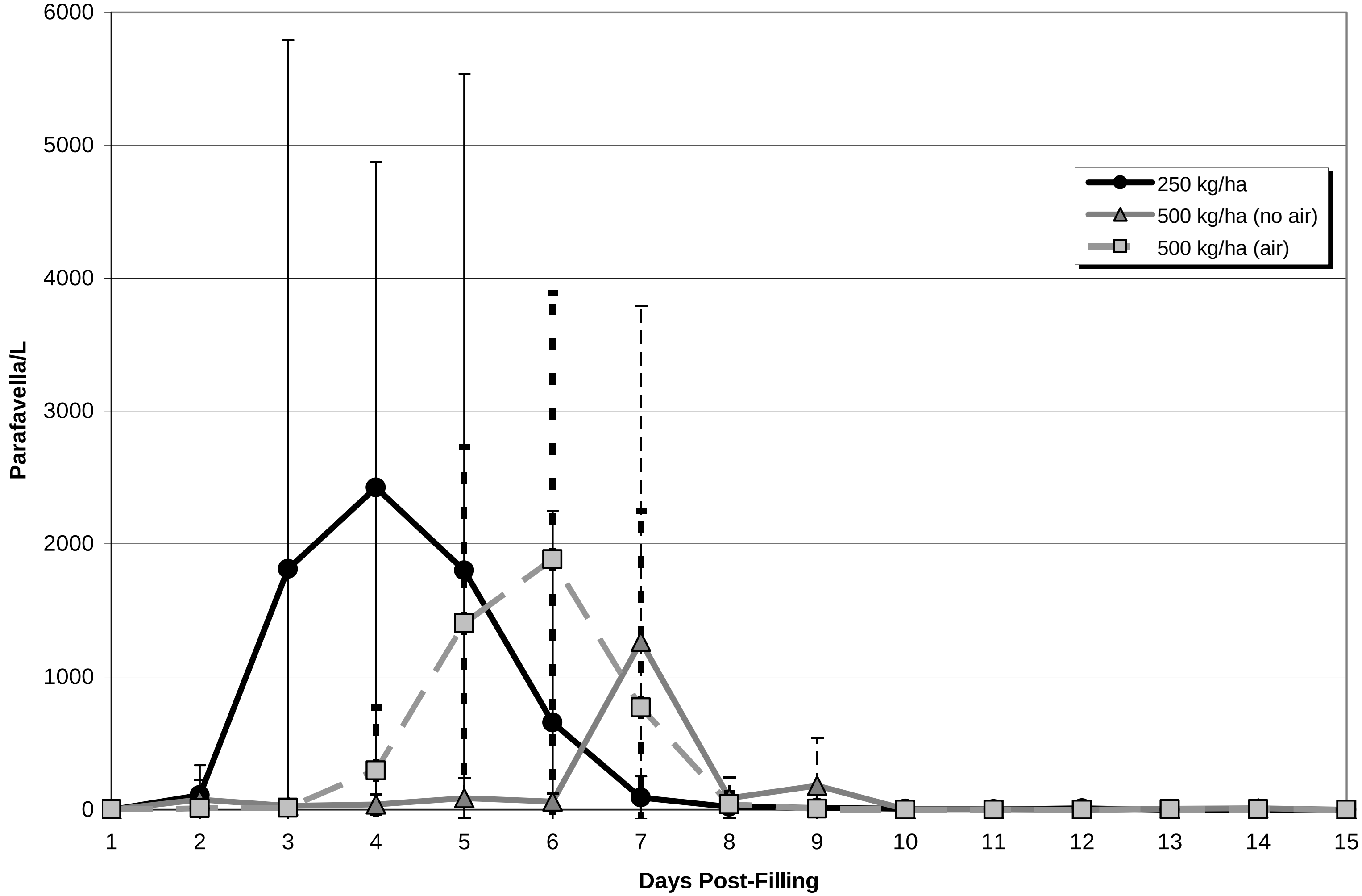

Figure 2.—Parafavella abundance in ponds receiving three different treatments of rice bran fertilizer: 250 kg/ha, 500 kg/ha (without air), and 500 kg/ha (with air).

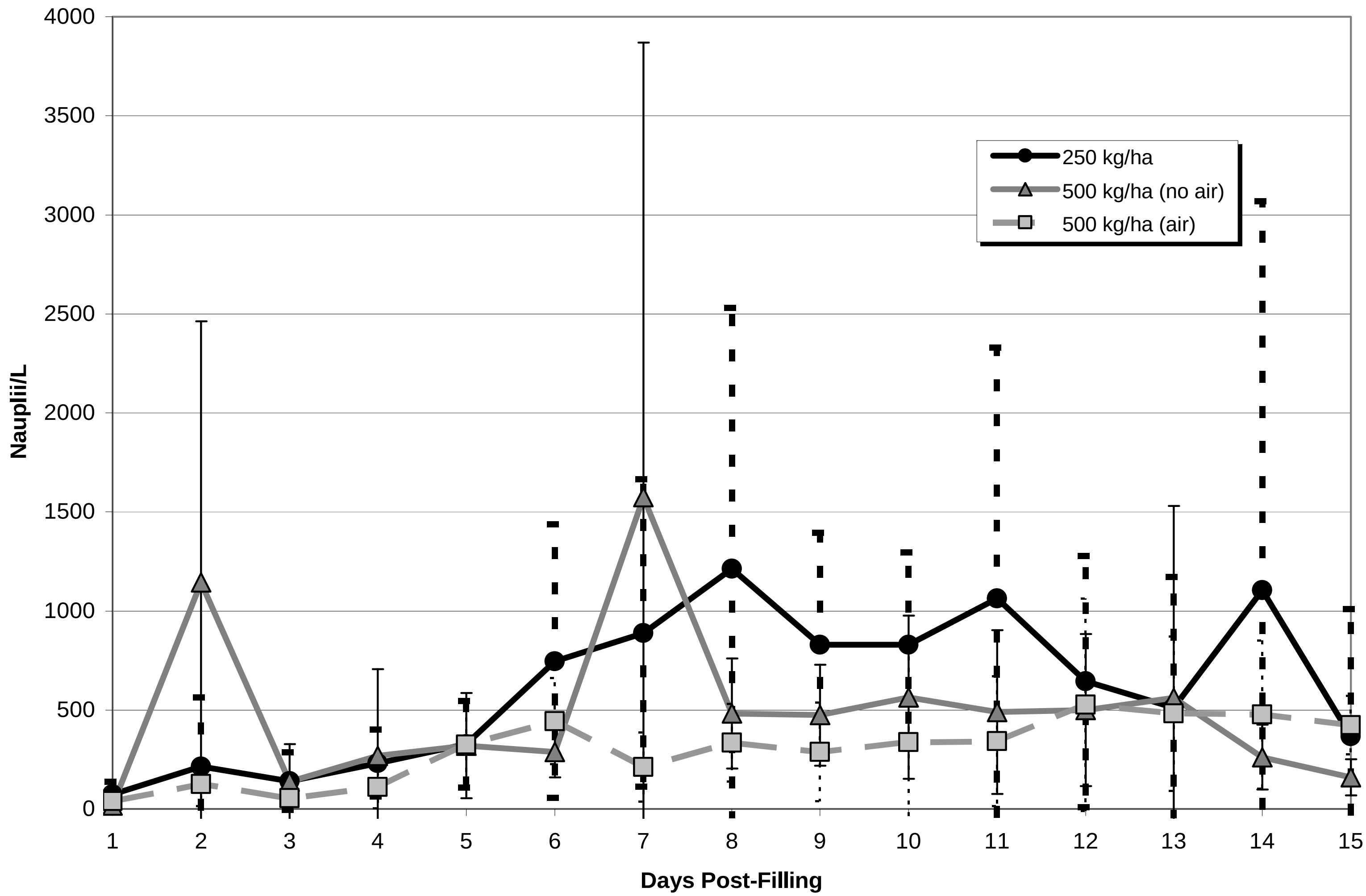

Figure 3.—Copepod nauplii abundance in ponds receiving three different treatments of rice bran fertilizer: 250 kg/ha, 500 kg/ha (without air), and 500 kg/ha (with air).

high rate with air (p=0.021) during the second week. A high degree of variation in nauplii density was observed between and within ponds during the study period.

Copepod adults

Adult copepods became more evident after the first week in the low rate ponds (Figure 1). Comparing between treatments, abundance was significantly higher in the low rate ponds than the other two treatments ($p<0.0001$) for the first 14 d. Mean adult density in low rate ponds (128.02 ± 142.38/L) was twice as high as the other treatments (64.64 ± 67.72/L) during 14 dpf. Figure 4 shows the average abundance of adult copepods for the three treatments. Three orders of copepods were identified, Calanoida, Cyclopoida, and Harpacticoida. Copepod species representing *Acartia* (calanoid), *Apocyclops* (cyclopoid), and *Tisbe* (harpacticoid) were observed. *Acartia* were the most common adult copepod during the first 14 dpf in all treatments, with their densities significantly higher ($P<0.0001$) in the low rate ponds. After the second week copepod composition in low and high rate (without air) ponds shifted to cyclopoids, which continued until the end of the study. Calanoid abundance was persistent in ponds supplied with aeration throughout the study.

Water quality

Morning and afternoon temperatures were similar among treatments averaging 28.9 ± 1.65 and 31.4 ± 1.95 degrees C respectively. Salinity ranged from 26.7 ppt in the high with air to 40.5 ppt in ponds receiving 250 kg/ha. Average salinity was near full strength at 33.6 ± 2.82. Water clarity was slightly lower in the low rate ponds, with secchi disk visibility averaging 33.4 ± 23.35 cm. Average morning dissolved oxygen was significantly higher

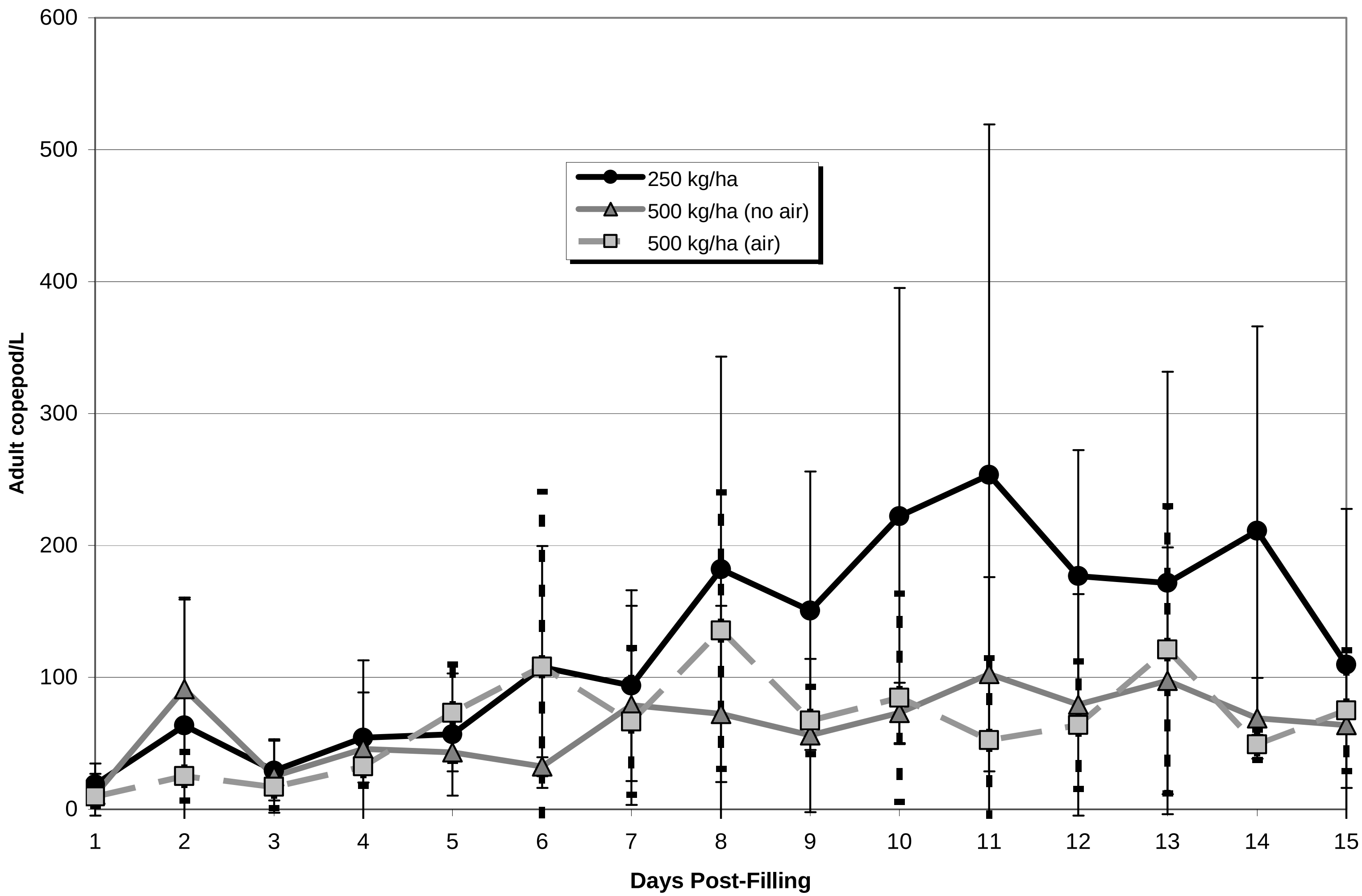

Figure 4.—Adult copepod abundance in ponds receiving three different treatments of rice bran fertilizer: 250 kg/ha, 500 kg/ha (without air), and 500 kg/ha (with air).

($p<0.0001$) in the 500 kg/ha ponds without air (5.19 ± 1.63 ppm) than those with aeration (4.36 ± 1.31 ppm). Afternoon oxygen levels were similar for the two high rate treatments ranging from 1.67 to 11.72 ppm. Pond total ammonia-nitrogen averaged 0.10, 0.09, 0.05 ppm for the low fertilizer rate, high rate with aeration, and high rate without aeration, respectively.

Discussion

Both protozoans and copepod nauplii have shown to be among the first foods of larval marine fish. Tintinnids have been found in the intestinal tract of larval surgeon fish (*Paracanthurus hepatus*) (Nagano et al. 2000). Stoecker and Govoni (1984) found larval gulf menhaden (*Brevoortia patronus*) consuming the tintinnid *Favella sp*. Parafavella abundance increased rapidly reaching densities of 14,000 org/L on day 3 post-filling in the ponds receiving 250 kg/ha fertilizer. However, the abundance of Parafavella in the experimental ponds was short lived and remained above 2,000/L for only 6 days. Protozoans have a short doubling time allowing for a rapid population expansion. Taguchi (1976) found that *Parafavella sp*. in Akkeshi Bay, Japan had a doubling time of 1.5 days. *Euplotes plicatum* and *E. vannus* were found to double their numbers in just one day (Wang et al. 2005).

Copepod nauplii have proven to be an important first food for larval red snapper when given at a density of 1/ml (Chigbu et al. 2002). Nauplii densities in ponds during the first 5 days post-filling averaged 774.65 ± 962.9 org/L with the 250 kg/ha treatment giving the most sustained abundance of nauplii averaging 700/L or more for 6 days.

Although the 500 kg/ha treatment without air experienced very high densities of nauplii on day 2 post-filling, this was influenced by an outlier pond recording an abundance of 2,360/ L.

There were significant differences in nauplii abundance between treatments (p=0.014). The 250 kg/ha ponds observed higher densities of nauplii than the 500 kg/ha ponds receiving air. The increased density of adult copepods in the 250 kg/ha treatment may also have contributed to higher nauplii abundance.

Although ponds receiving the 500 kg/ha without air treatment experienced high densities of nauplii, the variation was too great to be considered favorable for future snapper aquaculture. For example one pond had high nauplii densities (2000 org/L) on day 13 post-filling but the next day recorded 273 nauplii/L. Aeration seemed to have no appreciable impact to nauplii abundance when compared to the high rate without air treatment. In fact, the aerated ponds experienced more consecutive days with dissolved oxygen levels below 4 ppm than the other treatments making them questionable for successful snapper aquaculture. Sumiarsa (2003), in contrast, found that aeration was beneficial to nauplii production when wheat bran was given at 250 kg/ha. The low rate treatment was the most appropriate for raising snapper because it had the highest average nauplii densities and provided a stocking window between 6 and 14 dpf where counts remained above 500/L (Figure 3).

The rapid development and reduction of tintinnid ciliates in this study shows a typical pattern for a static marine system (Naas et al. 1991). Protozoa play a major role in nutrient cycling by providing a link between bacteria and very small phytoplankton on one side and larger zooplankton on the other (Porter et al. 1984). Moriarty (1997) ranked protozoans second after heterotrophic bacteria when describing primary productivity in aquaculture ponds. Naas et al. (1991) concluded that protozoan growth can be hastened with nutrient inputs (nitrate fertilizer) and turbulence. In that study additions of organic and inorganic fertilizer along with aeration were successful in stimulating protozoan growth which in turn provided forage for copepods. The detection of cypris larvae in pond samples at 10 dpf could be either a result of barnacle reproduction or larvae which passed through the filter sock during pond filling.

It is possible that higher salinities during the first 15 dpf delayed rotifer development. Rotifers appear to be more numerous in low salinity waters (Cuzon du Rest 1963). The low abundance of rotifers in the first block could be explained by observed differences in salinity between the first and remaining start blocks in which the salinity averaged 36.2 and 32.1 ppt, respectively. The highest rotifer densities seemed to coincide with minimum salinity readings for the final days of the study, although no statistical test performed could support this hypothesis (Figure 5). Colura et al. (1987) found that in low salinity ponds (10 ppt) rotifers seemed to dominate zooplankton densities, also rotifer abundance increased after 15 days post-filling in higher salinity ponds (15 and 20 ppt). The filling date (start block) had more of an effect on rotifer abundance than that of treatment type. Temperature had no appreciable impact on rotifer abundance. Competition by nauplii may also have prevented rotifer dominance. Whatever the reason, late rotifer development is preferred for red snapper larviculture.

At first-feeding red snapper are unable to consume rotifers. However, the delay in rotifer succession can provide additional forage during early growth.

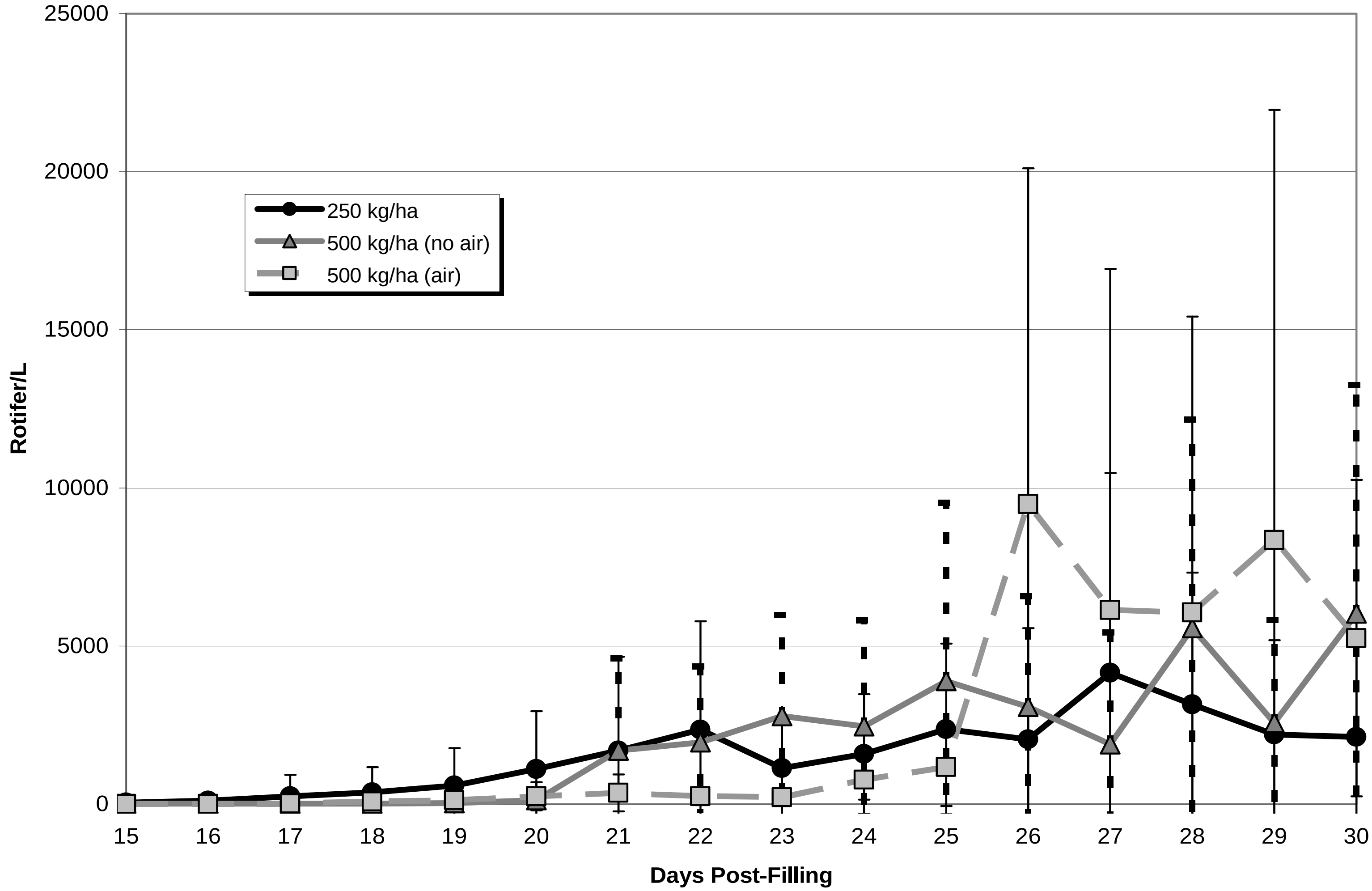

Figure 5.—Rotifer abundance in ponds receiving three different treatments of rice bran fertilizer: 250 kg/ha, 500 kg/ha (without air), and 500 kg/ha (with air). Where rotifer abundance averaged less than 26.6 ± 297.36 org/L from day 0 to 14.

Conclusion

Ponds filled with seawater and prepared as primary nursery ponds for red snapper larvae develop a variety of zooplankton, much of which are considered as acceptable food organisms. Copepod nauplii, the most desired form of zooplankton, were abundant in all management protocols tested. The initial application of 250 kg/ha rice bran with weekly additions of 125 kg/ha resulted in greater and more sustainable populations of copepod nauplii during the first two weeks after filling. Therefore, this low rate treatment is preferred for red snapper larval pond production.

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V. PREDICTABILITY OF ZOOPLANKTON POPULATIONS IN MARINE PRIMARY NURSERY PONDS

Abstract

Primary nursery ponds are prepared to provide an abundance of zooplankton as food when stocked with larval fish. Like many marine fish, lutjanids require small live prey during the onset of larval feeding. Copepod nauplii possess qualities that make them the preferred choice. Such nauplii can be produced in outdoor ponds but the timing of nauplii abundance to match the availability of fish larvae can be an issue. Twenty 0.1 ha ponds were prepared as primary nursery ponds in summer 2004 and stocked with red snapper (*Lutjanus campechanus*).

Various modeling approaches were evaluated for their accuracy in predicting the optimal stocking day after pond-filling to have an adequate availability of copepod nauplii. Stepwise regression models were used to forecast nauplii abundance by incorporating 29 parameters. However, the models did not consistently predict nauplii densities for out-of-sample ponds and so were not useful for a management scheme. Alternatively, an empirical approach was evaluated to determine the number of ponds that would be needed to satisfy stocking requirements under conditions of uncertainty. This approach yielded more manageable results. In order to ensure adequate nauplii densities it became apparent that a larger number of ponds would be needed than initially expected.

Introduction

Risk is encountered in every agricultural industry, but few sectors are more susceptible than that of aquaculture. The difficulty for aquaculture is the complexity of aquatic ecosystems; this makes it more risky than land animal production. Uncertainties in the form of environmental, economic, marketing, or production conditions, are all major concerns for the fish farmer (Jolly and Clonts 1993). Economic, marketing, and production can often be managed and mitigated by the grower but the complexity of interactions in the biological world makes outcomes to change hard to predict.

The hatchery stage is usually the riskiest production component in finfish aquaculture. Substantial investment is needed in hatchery management, including highly skilled labor and specialized equipment, in order to provide the optimum growing conditions for fish larvae. There are two approaches to the production of young fish. One option is to increase control in the laboratory over food quantity and quality, water quality and fish density. Such an approach facilitates greater inventory management but requires high skill levels and more elaborate facilities. A second option is to take a more extensive approach where less direct control over larval production is given. Outdoor ponds are prepared to nurse the larvae for 30-45 d through metamorphosis into a juvenile. Both organic and inorganic fertilizers are used to stimulate the growth of zooplankton in the culture pond. Successful pond preparation depends on providing the optimum density of an appropriate zooplankton to coincide with larval availability (Geiger 1983; Johansen 1986). However, interactions of physical and biological factors often do not give predictable zooplankton production.

Many warm water marine fish larvae have a brief endogenous period lasting only 2-3 days post-hatch (dph) and require an abundance of small, live food organisms at first-feeding (Kayano 1988; Doi et al. 1997a; Tucker 1998). Larval mortality is primarily the result of starvation, following the onset of exogenous feeding when a suitable size food is not sufficiently abundant (Kamler 1992; Kohno 1998). In intensive culture, live food is added to the larval culture unit several times a day to insure that an adequate abundance of a suitable size and quality food is available, but in extensive culture the culture pond must produce the proper type, size and abundance of food organisms.

Rotifers (*Brachionus sp.*) are commonly produced at high densities and fed to fish held in the hatchery but are too large as a first food for some species of marine fish, including red snapper. Copepod nauplii are a desirable food for many larval fish, particularly those requiring a small first food. Many species of copepods have nauplii that possess key qualities for being an acceptable first food for marine fish larvae. Copepod nauplii provide high levels of polyunsaturated fatty acids crucial for larval development (Stottrup 2003). Many newly hatched nauplii are small, measuring less than 80 μm (Buskey et al. 1993). Schipp et al. (1999) successfully raised golden snapper (*Lutjanus johnii*) using *Acartia spp*. nauplii which measured 65 μm in width. Doi et al. (1997a) found that 3 dph red grouper *Epinephelus coioides* larvae preferred small copepod nauplii over rotifers of similar size. Nauplii have proven to be effective prey items for other marine fish larvae such as red snapper *Lutjanus argentimaculatus* (Doi et al. 1997b), mahimahi *Coryphaena hippurus* (Kraul et al. 1991), pink snapper *Pagrus auratus* (Payne et al. 2001), and turbot *Scophthalmus maximus* (Stottrup and Norsker

1997). Copepod nauplii have been found to support red snapper growth and survival (Chigbu et al. 2003).

Copepod nauplii are not commonly used in intensive fish larval production due to difficulties in producing the high densities needed to support an intensive feeding protocol. Another approach is a more extensive production of larval fish where ponds are prepared to provide an abundance of zooplankton and the fish larvae are stocked into the pond (a primary nursery pond). Abundant densities of copepod nauplii can be produced in outdoor ponds (Stottrup and Norsker 1997; van der Meeren and Naas 1997) to provide valuable food for larval fish.

Timing is critical when using a primary nursery pond, particularly for red snapper larvae. A proper size and abundance of food must be available as soon as the mouth parts of a larval snapper are functional. Wide variation in zooplankton populations can occur even when ponds are managed identically (Sturmer 1990). It is therefore critically important for the manager to have a management protocol that will give a predictable abundance of nauplii in a pond at the specific time when fish larvae are ready to feed. Once brood snapper are spawned the manager is committed to having to provide food for the larvae approximately five days later. Currently no pond protocol has been able to predictably provide an adequate density of nauplii in a given pond on a specific day.

The focus of this study was to assess the potential for timing copepod nauplii density to coincide with the stocking of red snapper larvae in primary nursery ponds. The objective of this paper was to evaluate two approaches, forecasting and risk analysis, as part of management strategies to insure adequate nauplii densities appropriate for optimal snapper larvae production. In the first approach, regression models were specified using zooplankton and water quality data collected from summer 2004. The models were then validated by forecasting nauplii abundance five days ahead on two "out-of-sample" sets of ponds not included in the model specifications. In the second approach, a risk analysis model was used to examine the probability of a pond meeting or exceeding a selected nauplii target on a given day. This risk approach would then show the manager the proportion of ponds ready for stocking on a given day, and the likelihood of stocked ponds remaining above target nauplii densities through succeeding days.

Methods

Biological and water quality data were collected from pond studies conducted in summer 2003 and summer 2004 at the Claude Peteet Mariculture Center in Gulf Shores, AL.

Pond preparation and data collection

2003 Pond study for copepod nauplii production

In 2003 nine ponds were prepared to produce copepod nauplii which were later trapped and fed to snapper larvae in a laboratory setting. Lindley (2004) conducted a trial to evaluate collection of copepods from fertilized ponds. Organic fertilizer (rice bran) was applied at 500 kg/ha initially and a liquid inorganic (38-8-0) fertilizer was applied at 4 L/ha initially. Both fertilizers were applied at half those rates each week thereafter. Ponds were filled with brackish water (3-10 ppt) obtained from the Gulf Intracoastal canal. Water was filtered through 1000 μm filter socks. Zooplankton samples were collected daily by pumping 10 L water through a 30 μm mesh sieve and concentrating the sample to 100 ml with 30 µm filtered pond water. Samples were counted on 1.0 ml Sedgwick-Rafter counting chambers with one drop of Lugol's Iodine solution. Salinity and morning temperature were recorded daily. Zooplankton and water quality measurements from this trial were used as out-of-sample observations to test the second regression model's ability to predict nauplii abundance in brackish water ponds.

2004 Pond study: use of primary nursery ponds to produce red snapper larvae

Beginning May 4 2004, twenty 0.11 ha ponds were prepared over a three-week period with either 250 or 500 kg/ha rice bran, and 20 L/ha of inorganic fertilizer (38-8-0) as initial applications, and half those rates each week thereafter. Four ponds receiving the 500 kg/ha rate were also supplied with continuous aeration from a low-head airlift system. Unlike the 2003 study, the ponds were filled with full-strength (34 ppt) seawater obtained from the Gulf of Mexico. Filter socks (1000 µm mesh) were used to entrain predacious fish and invertebrates. Zooplankton samples were collected daily by pumping 10 L water through a 35 µm mesh sieve. In order to obtain a random sample the pump continuously moved through the water column. Samples were preserved in a 5% formalin/filtered seawater mixture and counted on 1.0 ml Sedgwick-Rafter counting chambers. An YSI 556 MPS probe (Yellow Springs, OH, USA) was used to measure pond temperature and dissolved oxygen twice daily, and salinity and pH once daily. Relative pond visibility was measured daily using a 20 cm diameter secchi disk (Aquatic Eco-Systems, Inc., Apopka, FL, USA). Solar radiation data was obtained from a weather station located approximately 35 km away in Fairhope, AL (AWIS Weather Services, Inc. 2005).

Wild caught snapper were induced to spawn using techniques described by Minton et al. (1983). Larvae were stocked in ponds according to the methods described previously.

Regression model construction

Two stepwise regression models were constructed to predict copepod nauplii density in saltwater primary nursery ponds. The first model used 17 ponds from the 2004 study to predict nauplii density five days ahead in the remaining three out-of-sample ponds. The variables available to the stepwise procedure consisted of 7 lags each of 16 zooplankton population indicators, 8 water quality parameters, 4 treatment effects, and 1 meteorological indicator, along with the 16 pond dummy variables that were forced into the model. Because the window of opportunity for practical larval stocking occurs during the first 15 days post-filling (dpf) it was considered infeasible to use observations in the model taken earlier than 7 days prior to a projected stocking date.

The second model used all 20 ponds from the 2004 study to predict nauplii density five days ahead in the 9 ponds from 2003. This model was very similar to the first except that fewer variables were measured in 2003, so the number of parameters available to the model was considerably less (7 vs. 29 in the first model). These variables consisted of 3 biological, 2 physical, and 2 treatment variables each lagged up to 7 days, with 19 pond dummies forced into the model. Figure 1 summarizes the data used to produce the two models, together with a description of each variable. For brevity figure 1 displays only the three out-of-sample ponds from the 2004 study, however all of the ponds from 2004 included the same parameters during model construction. This figure is helpful to explain for the two models which explanatory variables were available to them during construction as well as the individual out-of-sample ponds assigned to each model. For example, the 2003 pond study second model had only 7 explanatory variables available for use during construction (Nauplii, Totals, Rotifers, Temp_1, Salinity_1, Fert_spike, and liqspike). Also in the same example the individual 2003 Ponds (C1 through C9) were used as out-of-sample tests to examine the 2003 pond study second model.

Regression models were specified using the STEPWISE procedure in the SAS Enterprise Guide software version 3.0.0, with significance levels set at 0.15 for a variable to enter or stay (SAS Institute Inc., Cary NC, USA). The dependent variable was the five-day-ahead nauplii count (lead5naup). Starting with the variable contributing the largest F statistic, the SAS STEPWISE selection method adds variables to the model one by one as long as the F statistic after including a new variable is significant at least at the 0.15 level. After a variable is added the stepwise method evaluates all the variables already included in the model and deletes any variable that does not produce an F statistic significant at 0.15 or better. The STEPWISE process is complete when no additional variable has an F statistic that achieves the p-value and every variable in the model is significant at 0.15 or when the variable to be added to the model was the variable that was just deleted from it. Individual pond idiosyncrasies were represented by dummy variables that were forced into the model. The models were used to predict nauplii density in the out-of-sample ponds each day for days seven through twenty after pond filling. The adjusted R-squared, mean squared error, and residual plots were all used to evaluate performance and the practical significance of the models.

Variables	2004 Ponds	2003 Ponds
	A7 B4 B6	C1 C2 C3 C4 C5 C6 C7 C8 C9
Calanoid_		
Cyclopoid		
Harpacticoid		
Parafavella		
Cae		
Cye		
Hae		
BL		
DF		
LC		
PC		
Euplotes		
Veliger		
Temp_2	2004 Pond Study First Model	
DO_1		
DO_2		
pH		
Secchi_		
Feed		
Aeration		
meantemp		
Solarrad		
Nauplii		
Totals		
Rotifers		
Temp_1		2003 Pond study Second Model
Salinity_1		
Fert_spike		
liqspike		

Fig. 1 Explanatory variables available to the two regression models.
Zooplankton variables: Calanoid_, Cyclopoid, and Harpacticoid are copepod orders; Totals=sum of the copepod orders; Cae, Cye, and Hae are orders of female copepods with eggs; nauplii=copepod nauplii; BL=barnacle larva; DF=dinoflagelates; LC=loricate ciliates; PC=polychaetes; Parafavella=tintinnid protozoan *Parafavella*; Euplotes=ciliate protozoan *Euplotes*; Veliger=opisthobranch veliger larva *Bursatella leachii*; Rotifers=Rotifera. Water quality variables: Temp_1 and Temp_2 represent morning and afternoon temperatures (Cº); meantemp=average daily temperature ((Temp_1+Temp_2)/2); DO_1 and DO_2 represent morning and afternoon dissolved oxygen (mg/L); pH=pH; Salinity_1=afternoon salinity (g/L); Secchi_=secchi disk visibility (cm). Treatment variables: Fert_spike and liqspike represent applications of rice bran (kg/ha) and inorganic (L/ha) fertilizers, respectively; Aeration=continuous aeration supplied to ponds (designated as either one or zero); Feed=50% protein feed added to all ponds. The weather variable was Solarrad=surface solar radiation (Watt-hours/m^2).

Empirical risk model construction

Because it requires a five day period from collection of brooders to stocking of larvae, estimating the odds of a pond being "ready" (i.e. having an adequate zooplankton population to provide food for the larval fish) can also assist the manager in planning spawning activities.

Pond preparation and fish spawning are the most expensive portions of the hatchery phase; therefore the goal is to have the maximum number of ponds with acceptable levels of zooplankton on the day the fish are ready to feed. These management constraints provide an opportunity to incorporate risk analysis into red snapper nursery pond production. A risk/probability model was constructed using the 12 ponds for the 250 kg/ha treatment in the 2004 study, since this treatment had the highest average nauplii counts during the 15 days after filling. The model was designed to (1) determine the number of ponds that could be stocked on a given day and (2) calculate the probability of a pond exceeding a specified target nauplii density on a particular day. Three target nauplii densities were chosen: 500, 750, and 1000/L. In his review of the literature on nutrition sources used for marine fish larvae, Tucker (1998) documented recommendations for copepod nauplii densities between 500 and 10,000/L.

Because spawning requires substantial coordination of effort, the method should also suggest the most appropriate day to stock all ponds at the same time. Moreover, because red snapper larvae require adequate nauplii densities for several days after exhausting their yolk reserves (Chigbu et al. 2002), the method must also take into account the probability of nauplii exceeding a given target density for at least three consecutive days.

Results

Regression models

Tables 1a, 1b, and 2 summarize the variables used in the two models, along with their parameter estimates, means, and R^2 contributions. The first model included 47 explanatory variables (in addition to the 16 pond dummies). Only one of these variables (lag7 calanoid females with eggs) contributed more than 4% to the R^2. The idiosyncratic pond dummies accounted for almost half of the R^2 in nauplii densities (33.3% out of the 76.47%).

The second model included only 15 variables (excluding 9 pond dummies) and only two supplied more than 2% to the explanation of nauplii variation within the pond (table 2). The pond dummies accounted for over half of the total variation in the model 23.83% out of 45.26%. Analysis of variance is summarized in tables 3 and 4 for the two models. Both models had relatively modest explanatory power, generating adjusted R^2 values of only 69.54% and 40.18 % for the first and second model, respectively.

A plot of actual vs. predicted nauplii abundance illustrates the practical usefulness of the first model as it forecasted out-of-sample nauplii abundance in the three validation ponds from 2004 (figure 2). Light and dark bars connecting the actual and predicted values reveal daily forecasting errors. When forecasting nauplii for a target density, four possible outcomes arise: (1) the predicted value advises stocking of a pond when the actual value is too low to stock, (2) the predicted value advises not to stock when the actual value supports stocking, (3) the predicted value suggests not to stock and the actual value concurs, (4) and both the predicted and actual values agree on stocking.

Table 1a First stepwise regression model summary containing 47 explanatory variables (excluding pond dummies). List of explanatory variables: Lag1 through Lag7 indicate observations one day through seven days in the past; Cae, Cye, Hae are copepod adults with eggs (org/L); BL is barnacle larvae (org/L); DO_1 and DO_2 are dissolved oxygen for morning and afternoon respectively (ppm); LC are loricate ciliates (org/L); naup is copepod nauplii (org/L); Fert_spike indicates when organic fertilizer was applied; liqspike indicates when liquid fertilizer was applied; PC is polychaete larvae (org/L); solarrad is daily solar radiation measured (Watt-hours/m²); totals is the sum of adult copepods (org/L); veliger represents sea slug (*Bursatella leachii*) larvae (org/L); Temp_1 and Temp_2 are morning and afternoon temperatures respectively (°C); meantemp represents average daily temperature observed (°C); Salinity_1 and Salinity_2 represent morning and afternoon salinity respectively (ppt).

Variable	# Variables in	Partial R^2	Model R^2	Parameter estimate	T-Value	Means
Intercept	16	-	0.333	-7760.02719	-4.33	0.000
lag7Cae	17	0.042	0.375	117.49813	2.77	0.079
lag3Harpacticoid	18	0.027	0.403	8.57855	10.69	24.935
lag3naup	19	0.017	0.456	-0.1859	-4.99	557.783
LC	20	0.019	0.475	-26.58664	-9.86	0.966
lag6DO_1	21	0.020	0.495	44.11544	2.31	5.800
lag3Hae	22	0.013	0.507	-167.48905	-6.08	0.175
lag5Harpacticoid	23	0.019	0.527	-3.06466	-5.10	24.098
lag2totals	24	0.013	0.540	-2.58958	-4.51	123.860
Nauplii	25	0.014	0.553	-0.08452	-2.68	551.027
lag6meantemp	26	0.009	0.563	-197.58873	-4.37	29.283
lag4BL	27	0.008	0.570	47.45316	3.67	0.326
lag7Salinity_1	28	0.007	0.578	120.43061	5.60	33.730
lag4Harpacticoid	29	0.007	0.593	-2.51032	-4.33	23.953
lag5temp_1	30	0.008	0.600	238.37668	4.49	28.195
DO_1	31	0.010	0.616	-115.21684	-4.94	5.226
lag1temp_1	32	0.011	0.627	-110.59595	-4.17	28.713
lag4DO_1	33	0.007	0.634	118.10435	4.43	5.658
lag1naup	34	0.007	0.641	-0.13038	-3.84	572.479
lag1pH	35	0.007	0.647	404.19264	2.91	8.299
DO_2	36	0.007	0.651	40.44133	2.45	8.087
lag2LC	37	0.006	0.652	-1.41469	-1.72	4.158
lag6Calanoid_	38	0.005	0.657	-1.11008	-3.46	59.719
lag6Harpacticoid	39	0.005	0.662	1.86295	3.02	23.628
lag2Cye	40	0.007	0.669	-52.10305	-3.52	0.403
lag3Cyclopoid	41	0.008	0.678	2.30112	3.27	25.841
lag3Rotifers	42	0.005	0.682	-0.12974	-1.60	43.988
lag6Rotifers	43	0.004	0.695	0.14744	1.89	26.587
lag2Cyclopoid	44	0.003	0.701	3.60524	3.99	28.415
lag2Rotifers	45	0.004	0.705	-0.22635	-2.71	61.408
lag7Fert_spike	46	0.004	0.709	0.7512	3.07	36.871
Veliger	47	0.004	0.711	-178.59623	-3.05	0.096
lag7Cyclopoid	48	0.004	0.712	0.65761	2.53	31.051
BL	49	0.004	0.716	21.47263	2.93	0.662
lag3BL	50	0.004	0.721	29.61666	3.19	0.426
lag4pH	51	0.004	0.724	427.22431	3.13	8.273
solarrad	52	0.004	0.726	-0.03828	-1.87	5898.110

Table 1b First stepwise regression model summary containing 47 explanatory variables (excluding pond dummies).

Variable	# Variables in	Partial R²	Model R²	Parameter estimate	T-Value	Means
lag3totals	53	0.004	0.730	-0.72836	-2.18	0.000
lag2Calanoid_	54	0.004	0.733	1.82896	2.75	0.000
lag1Harpacticoid	55	0.003	0.736	1.30435	2.07	0.000
Hae	56	0.004	0.741	-49.23641	-1.99	0.000
lag5DO_2	57	0.004	0.744	-54.05502	-2.81	0.000
lag5Rotifers	58	0.003	0.747	-0.13523	-1.75	0.000
lag7Calanoid_	59	0.003	0.750	-0.94569	-2.80	0.000
lag2BL	60	0.004	0.754	13.83239	1.94	0.000
lag7naup	61	0.004	0.758	0.0892	2.11	0.000
lag3solarrad	62	0.004	0.762	-0.03941	-1.94	0.000
lag7PC	63	0.003	0.765	-0.25108	-1.67	0.000

Table 2 Second stepwise regression model summary containing 15 explanatory variables (excluding pond dummies).

Variable	# Variables in	Partial R²	Model R²	Parameter estimate	T-Value	Means
Intercept	19	-	0.238	569.58289	0.29	0.000
Fert_spike	20	0.047	0.286	8.31418	6.38	27.401
Salinity_1	21	0.045	0.331	-22.64509	-2.05	24.334
liqspike	22	0.030	0.361	-144.24086	-4.67	1.025
Temp_1	23	0.016	0.377	-132.78481	-2.80	28.935
lag3temp_1	24	0.011	0.388	134.395	2.82	28.780
Nauplii	25	0.013	0.401	0.17228	3.34	1081.780
Rotifers	26	0.010	0.411	-0.02587	-1.93	1550.090
lag1Fert_spike	27	0.007	0.418	4.75531	3.70	27.401
lag6Fert_spike	28	0.009	0.426	1.39626	2.70	55.727
lag2Fert_spike	29	0.007	0.434	1.81349	2.32	27.401
lag1liqspike	30	0.004	0.438	-66.44552	-2.16	1.025
lag4totals	31	0.004	0.442	0.13046	1.92	481.748
lag5Rotifers	32	0.004	0.445	0.01728	1.44	1711.970
lag1naup	33	0.004	0.449	0.08795	1.80	1089.530
lag2Rotifers	34	0.004	0.453	-0.02012	-1.58	1589.010

From figure 2 only pond A7 exceeded a 1,000/L target density for more than one day. In pond B4 on day nine the model predicted 4,195 nauplii/L when the actual count was just 259/L (indicated by a light bar). This situation would have prompted the manager to stock larvae when the actual nauplii density would have been significantly below the target and therefore would negatively affect fish survival. The reverse condition, which is not quite as egregious as the first, is illustrated by pond A7 on day 8; the prediction advises not stocking on each of the five days that densities were in fact above target. In general, the model is disappointingly ultra-conservative, in that it almost never advises stocking.

The second forecasting model correctly predicted that 7 ponds would be ready to stock before day 15 post-filling when using a target nauplii density of 1000/L. All ponds exceeded this target within the desired stocking time. It is important to note that these ponds were filled with low salinity water (3-10 ppt) which allows nauplii growth (Colura et al. 1987) but is not a salinity suitable for red snapper larvae.

Table 3 Analysis of variance for the first stepwise regression model.

Analysis of Variance					
Source	**Degrees of freedom**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	63	82922658	1316233	11.04	<.0001
Error	214	25517459	119240		
Corrected Total	277	108440117			
Root MSE	345.31212	**R-Square**	0.7647		
Dependent Mean	399.68022	**Adj R-Sq**	0.6954		
Coeff Var	86.3971				

Table 4 Analysis of variance for the second stepwise regression model.

Analysis of Variance					
Source	**Degrees of freedom**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	34	330651742	9725051	8.9	<.0001
Error	366	399851802	1092491		
Corrected Total	400	730503544			
Root MSE	1045.22307	**R-Square**	0.4526		
Dependent Mean	825.98778	**Adj R-Sq**	0.4018		
Coeff Var	126.5422				

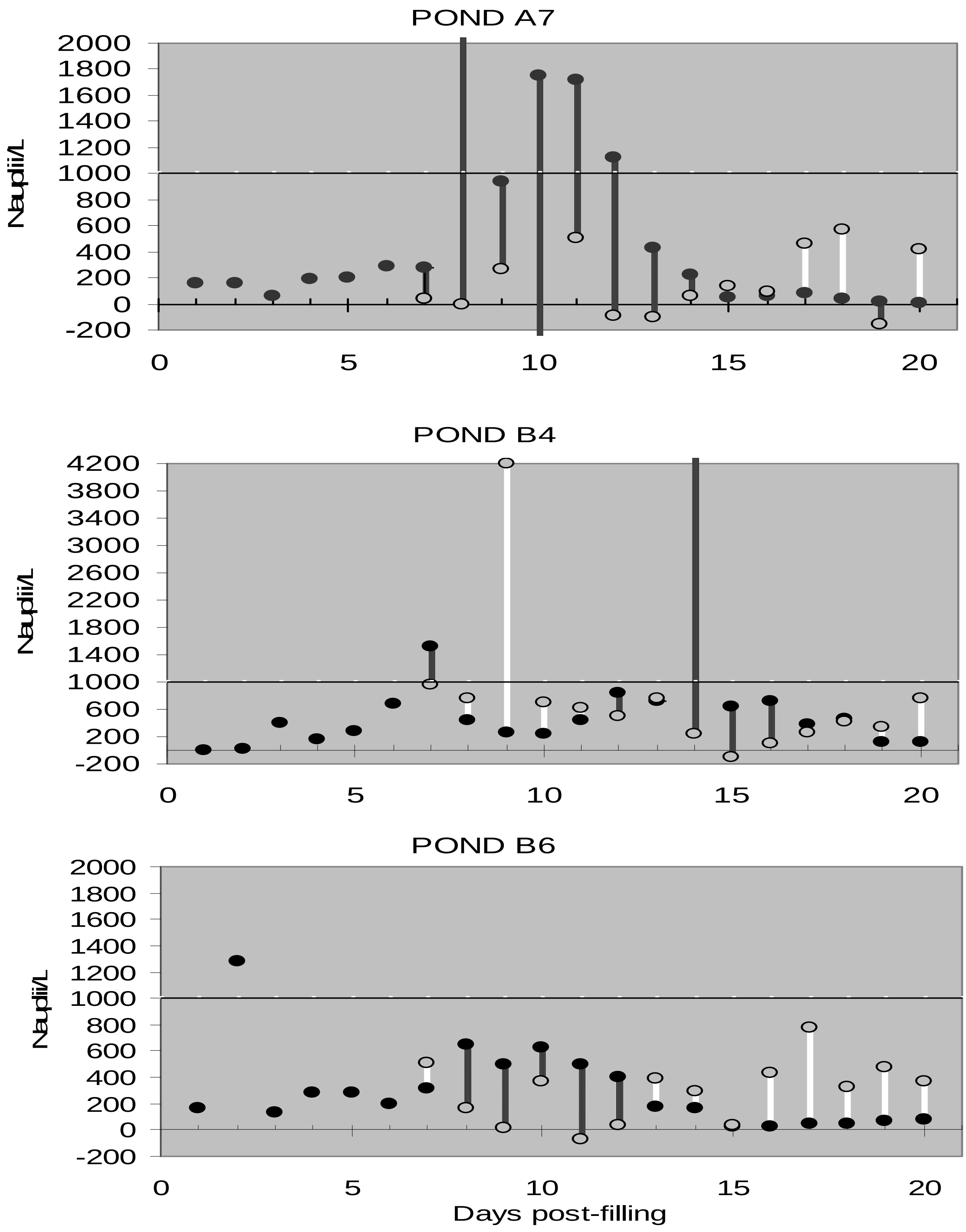

Fig. 2 Actual vs. predicted nauplii abundance for three out-of-sample ponds from 2004 pond study. Black and grey dots represent actual and predicted nauplii counts, respectively. Light and dark bars indicate daily prediction error above and below the actual value, respectively.

Empirical risk model

A number of nauplii density targets (1000, 750, and 500 nauplii/L) were selected for this model to test the probability of a pond to be on target to receive fish. The model was further extended by using the criterion that nauplii should exceed a specific target for three or more consecutive days. Table 5 demonstrates pond performance as a function of target density. The table illustrates the large variation in nauplii abundance within and between ponds even when the same management protocol was used.

A target density of 1000 nauplii/L was achieved in 9 of the 12 ponds at least once during the 15 d period but on a given day the most ponds meeting the target was 4 of 12, that occurring on day 10. When the criteria of a pond being on-target for three or more consecutive days was considered then only one of the four ponds having 1000 nauplii/L on day 10 met the criteria.

A target of 750 nauplii/L was achieved at least once in 10 of the 12 ponds, with 7 of 12 ponds having this density for 3 d or more. The largest number of ponds simultaneously meeting the target was 6 of 12 on days 9, 10 and 11. The most ponds on target for $\geq$3 d was 4 beginning day 9, 2 beginning day 10, and 2 beginning day 11.

A target of 500 nauplii/L was more achievable with all ponds meeting the target at least once in the 15 d period. On day 10, 10 out of 12 (83.3%) ponds met the target, but only 4 of them remained on target from day 10 to 12.

Discussion

The goal of a primary nursery pond is to provide an appropriate type and quantity of zooplankton as food for larval fish during the first weeks of feeding. Having the proper type and quantity of zooplankton in the first few days after larval fish are stocked into a primary nursery

Table 5 Risk analysis summary for exceeding targets of 1000 (black), 750 (dark grey), and 500 (light grey) nauplii/L in twelve ponds given as a function of probability per day and per pond.

	Nauplii counts per liter										percent	percent	percent
Day=>	6	7	8	9	10	11	12	13	14	15	>1000	>750	>500
Pond													
A2	121	1650	449	808	1136	936	919	379	278	40	20%	50%	50%
A4	549	624	492	696	630	380	162	90	96	60	0%	0%	40%
A5	468	1147	558	336	180	216	75	90	100	130	10%	10%	20%
A6	1456	626	2201	1723	808	4840	288	33	240	90	40%	50%	60%
A7	285	276	4280	937	1752	1721	1120	424	222	53	40%	50%	50%
A8	325	360	458	963	830	456	154	190	185	105	0%	20%	20%
B1	2412	2831	3270	2048	1458	285	88	138	22	36	50%	50%	50%
B4	684	1513	434	259	234	446	832	725	6490	650	20%	30%	60%
B5	756	651	905	994	1117	821	328	193	102	60	10%	50%	60%
B6	193	319	644	495	631	497	402	180	165	28	0%	0%	20%
B7	1458	440	164	306	660	1165	2196	1600	2355	2184	60%	60%	70%
B8	248	234	705	371	511	980	1164	2095	3012	954	30%	50%	70%
Ponds ready by day:											Average probability:		
Percent > 1000											23%	35%	48%
Percent > 750													
Percent > 500													

pond is critical for fish survival. However, the complexity of pond ecology can result in highly variable zooplankton populations even in ponds prepared in the same manner. Anticipating when appropriate nauplii densities will be available for stocking of larval fish has proved to be a challenge for hatchery managers. Colura et al. (1976) found large differences in copepod abundance between spotted seatrout ponds sharing the same fertilizer treatment and fill date.

This paper attempted to mitigate this risk by applying forecasting and probability assessment to determine the best day to stock ponds with red snapper larvae. The two models attempted to forecast nauplii under severe constraints imposed by the modelers including: pond idiosyncrasies not used in forecasting; small sample size (only 20 ponds) from only one year; a narrow forecasting window (7 to 20 days post-filling) which reduced the number of usable observations; and the models could not start forecasting until seven days of data had been collected and then had to forecast no less than five days ahead as the stocking window moved from day 7 to day 20. Additional challenges were placed on the second model as it used high salinity ponds (2004 study) to forecast nauplii abundance in low salinity ponds (2003 study). The large contribution of pond idiosyncrasies is not surprising considering that Knud-Hansen (1992) found that nearly 50% of the variability in net fish yield in experimental research ponds was due to between-pond differences in fertilizer applications in previous studies.

The risk approach showed that, using a target of 500 nauplii /L and stocking on day 10 post-filling, only 4 of the 12 ponds (33%) would have remained above target for the next two consecutive days (table 5, ponds A2, A7, B7 and B8). In a practical setting the unused ponds could be restarted, but there is no assurance that the same zooplankton dynamics would occur during the second round.

Conclusion

The main goal in a primary nursery pond is to produce adequate numbers of target organisms at the time when first-feeding fish larvae are stocked. Meeting these criteria is fundamental in red snapper aquaculture. Copepod nauplii need to be in abundance during the stocking of snapper larvae, however having predictable quantities can be a challenge.

This study incorporated two management strategies to predict when this stocking window would occur in marine primary nursery ponds. Using a forecasting approach rendered an overly conservative outcome; advising the manager never to stock larvae at the 1000 nauplii/L target level. This model is not recommended due to its inability to consistently predict an appropriate stocking day. The second model used a risk assessment approach which incorporated the probability of a pond being ready to stock on a day when nauplii would be in abundance to support larvae. This model proved to be more applicable for the manager by elucidating the risk of survival for a group of primary nursery ponds. The manager may choose to stock larvae in all ponds on a specific day (according to the desired nauplii target) while incurring the risk that a certain percentage of the ponds will not meet the target goals. In our case the greatest frequency of ponds meeting a targeted nauplii abundance of 500/L occurred on day 10 when 83.3 % of the ponds met the goal.

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