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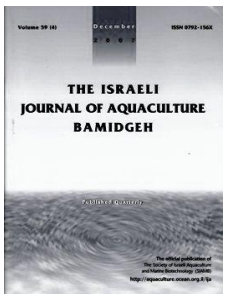

Stress Survival in Larvae of Florida Pompano (*Trachinotus carolinus*) Fed Enriched Rotifers (*Brachionus plicatilis*) and Nauplii of the Calanoid Copepod (*Pseudodiaptomus pelagicus*)

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Key words: Florida pompano larvae, copepod nauplii, stress tolerance

Abstract

The Florida pompano, *Trachinotus carolinus*, is a highly prized marine fish whose larviculture includes the feeding of live rotifers and brine shrimp nauplii. In a previous study, growth and survival of pompano larvae fed nauplii of the calanoid copepod, *Pseudodiaptomus pelagicus*, were compared to those of larvae fed enriched rotifers, *Brachionus plicatilis*. There were advantages to including the copepod in the larvae diet. The current study examines the stress tolerance of such larvae. Two trials were conducted: for seven (trial 1) and nine (trial 2) days post-hatch. Larvae were fed diets that included enriched rotifers and/or *P. pelagicus* nauplii and subjected to varying durations of air exposure ('sieve stress'). Larvae fed copepods exhibited significantly greater stress tolerance than larvae fed only enriched rotifers. In trial 1, stress tolerance increased as the number of days on which copepods were fed increased. It is possible that stress tolerance improved because of a better nutritional profile of the copepod nauplii.

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Introduction

Improved stress tolerance of fish larvae would help increase production in commercial marine fish hatcheries. Post-handling mortality can occur after moving fish from tank to tank, and during harvesting and shipping. Stress tolerance can be evaluated by challenging fish larvae with net stress (Kraul et al., 1993), salinity (Brinkmeyer and Holt, 1998), temperature, hypoxia (Kanazawa, 1997), reduced pH (Wasielesky et al., 1997), handling and transfer (Koven et al., 2001), and pathogens (Chair et al., 1994). Larvae performance in response to these stressors can be related to diet and nutrition (Kraul et al., 1993; Kanazawa, 1997; Brinkmeyer and Holt, 1998; Koven et al., 2001). In the wild, copepods are the primary prey for most marine fish larvae (Hunter, 1981; Samprey et al., 2007) and the feeding of copepods to cultured marine fish larvae is beneficial (Lee et al., 2005; Wilcox et al., 2006; Cassiano et al., 2011).

Copepods have an appropriate nutritional composition (Drillet et al., 2006), size (McKinnon et al., 2003; Lee et al., 2005), and feeding stimulant (Stottrup and Norsker, 1997) for marine fish larvae. Techniques for culturing and using copepod nauplii as a live feed have advanced (Stottrup, 2003; Lee et al., 2005). *Pseudodiaptomus pelagicus*, a marine calanoid copepod, has been in continuous culture at the University of Florida's Indian River Research and Education Center (IRREC) in Fort Pierce, FL, since 2007. The optimal temperature (Rhyne et al., 2009), salinity (Ohs et al., 2010a), and diet (Ohs et al., 2010b) for this species have been determined and its efficacy as a live feed for marine fish larvae has been examined (Cassiano et al., 2011).

The Florida pompano, *Trachinotus carolinus*, is a highly prized marine fish whose commercial larvae feeding regime for the first ~9 days post-hatch consists of enriched rotifers (Cavalin and Weirich, 2009). The benefits to growth and survival of feeding copepods to pompano larvae have been demonstrated (Cassiano et al., 2011). The present study examines the stress tolerance of pompano larvae reared on diets consisting of enriched rotifers, *P. pelagicus* nauplii, or combinations of both.

Materials and Methods

Larviculture. Pompano eggs were acquired from the Center for Reproduction and Larviculture of the Agricultural Research Service, US Department of Agriculture, located on the campus of the Harbor Branch Oceanographic Institute at Florida Atlantic University in Fort Pierce, FL. Eggs were transported to the IRREC (~30 min) and incubated in static, 200-l conical-bottom fiberglass tanks. Hatching occurred 30-37 h post-fertilization. The larvae were cultured in natural sea water (salinity 32-35 g/l), sterilized with sodium hypochlorite (150 mg/l) for 24 h, then heavily aerated until no chlorine was detected (24-48 h). The sterile sea water was pumped into the hatchery through 50-µm and 5-µm mesh bag filters and stored in a 3500-l polyethylene tank in which the water continuously circulated through an 80 Watt UV sterilizer (Emperor Aquatics Inc., PA) at 170-220 l/min, providing a sterilization intensity of 30,000-90,000 microWatts/cm^2. As the water exited the holding tank, it passed through 1.0-µm and 0.5-µm cartridge filters.

Treatments. We conducted two trials in which larvae were fed different diets. The trials were conducted in a flow-through system consisting of twenty-eight 13-l tanks with an artificial photoperiod of 14 h light:10 h dark. Newly hatched larvae, 0 days post-hatch, were volumetrically stocked into each tank at a density of 50 individuals/l. Beginning on day 2, tanks were inoculated daily with algae of the Tahitian strain *Isochrysis galbana* (T-ISO) to maintain darkness in the flow-through system. T-ISO density was 120,000 cells/ml in trial 1 and 380,000 cells/ml in trial 2. Temperature, salinity, dissolved oxygen, and pH were measured daily with a YSI Incorporated Model 556 MPS; total ammonia-nitrogen (TAN) and nitrite-nitrogen (NO_2-N) were measured daily with a Hach Spectrophotometer DR/4000U. In trial 1, the temperature was 22.7-26.8°C, salinity 34.8-35.6 g/l, dissolved oxygen 5.42-6.54 mg/l, pH 7.94-8.26, TAN 0.00-0.74 mg/l, and NO_2-N 0.0001-0.0407 mg/l. In trial 2, the temperature was 26.2-28.5°C, salinity 31.6-36.0 g/l, dissolved oxygen 5.46-6.55 mg/l, pH 8.02-8.30, TAN 0.00-0.40 mg/l, and NO_2-N 0.0018-0.0990 mg/l.

Beginning on day 2, larvae were fed enriched s-strain rotifers (*Brachionus plicatilis*) with a body width of 117.5±19.8 µm (Cavalin and Weirich, 2009) and/or *P. pelagicus* nauplii with a body width of 93.2±13.7 µm. When copepod nauplii were fed, an internal standpipe with a 50-µm nylon mesh screen was used to retain the copepods in the tanks; when rotifers were fed, an internal standpipe with a 240-µm nylon mesh screen was used. When both organisms were fed, it was necessary to constantly flush the copepods from the system. If they remained in the larvae tank they would have rapidly reproduced and become too abundant, reducing water quality and causing agitation to the larvae. Thus, when co-feeding, we accounted for the effects of rotifer 'build up' and used an internal standpipe with a 240-µm nylon mesh screen to flush excess live feeds.

Trial 1. Three diets were compared for seven days in four replicates of each treatment during trial 1: (a) a standard reference diet consisting solely of 2.5 enriched rotifers/ml fed four times daily (09:00, 13:00, 17:00, 21:00) on days 2-6, (b) a 'one-day' diet consisting of 2.43 copepod nauplii/ml on day 2 and 2.5 enriched rotifers/ml four times daily (09:00, 13:00, 17:00, 21:00) on days 3-6, and (c) a 'three-day' diet consisting of 2.43, 3.10, and 3.41 copepod nauplii/ml on days 2, 3, and 4, respectively, followed by 2.5 enriched rotifers/ml four times daily (09:00, 13:00, 17:00, 21:00) on days 5 and 6.

Trial 2. Four diets were compared for nine days in five replicates of each treatment during trial 2: (a) the standard reference diet described above on days 2-8, (b) a 'three-day' diet consisting of 2.5, 3.0, and 4.5 copepod nauplii/ml on days 2, 3, and 4, respectively, and 2.5 enriched rotifers/ml four times daily (09:00, 13:00, 17:00, 21:00) on days 5-8, (c) a copepod diet consisting of 2.5, 3.0, 4.5, 6.25, 7.8, 8.0, and 7.8 nauplii/ml on days 2-8, respectively, and (d) a mixed diet consisting of 2.0 enriched rotifers/ml and 0.5 copepod nauplii/ml four times daily (09:00, 13:00, 17:00, 21:00) on days 2-8.

Rotifer culture. Brachionus plicatilis were cultured at 26°C in a salinity of 20 g/l. They were fed T-ISO at a density of approximately 50,000 cells/ml and Culture Selco (INVE Aquaculture Inc.) following the manufacturer's instructions twice daily. The rotifers were enriched with Ori-Green (Skretting), an optimal enrichment for pompano larvae (Cavalin and Weirich, 2009), in 10-l buckets for 3 h at 27-28°C, following manufacturer instructions. Following enrichment, icepacks were used to reduce the temperature to 15°C. The rotifers were stored in a refrigerator (5-10°C) and fed to the larvae within 24 h.

Copepod culture. Pseudodiaptomus pelagicus were batch cultured at 28-30°C in a salinity of 22-25 g/l in eight 200-l cylindrical flat-bottom polyethylene tanks and one 1800-l cylindrical conical-bottom tank to simulate mass culture. During trial 1, copepods were fed T-ISO daily at a density of approximately 350,000 cells/ml. In trial 2, they were fed a 1:1 mixture of T-ISO and *Thalassiosira weissflogii* diatoms daily at a density of approximately 200,000 cells/ml. Copepod nauplii were concentrated and harvested twice daily with floating airlifts, volumetrically quantified, and fed to fish larvae.

Stress evaluation. At the conclusion of each trial, the larvae from the replicates of each treatment were combined and placed into separate gently-aerated 110-l aquaria containing water from their respective larviculture systems. Larvae were randomly selected from the aquaria for the stress experiment. Stress survival was evaluated by prolonged exposure to the air via sieve handling (Kraul et al., 1993; Kanazawa, 1997). Sieves consisted of a 0.5-l container that fit tightly into a 1.0-l container. The bottom of the 0.5-l container was removed and a 150-µm nylon mesh screen was hot glued in its place. In trial 1, five 7-day larvae were randomly selected from each aquaria using the 0.5-l screen-bottomed container. The larvae were held out of the water for 30, 120, 240, 360, or 600 seconds. Each time interval and diet combination was replicated four times. In trial 2, ten 9-day larvae were randomly selected using the 0.5-l screen-bottomed container and held out of water for 180, 360, 540, or 720 seconds. Each time interval and diet combination was replicated five times. After each interval, the 0.5-l container and larvae were gently and slowly submerged into the 1.0-l container that contained water from the culture system at 26°C. Survival was recorded one h after submersion and was defined as any movement detected within one minute.

Statistical analysis. Statistical analyses were performed with SAS version 8.02 software (Cary, NC, USA). Percentage data were arc-sine square-root transformed prior to analysis. Treatment means of dependent variables were subjected to one-way analysis of variance (ANOVA) according to the General Linear Model (PROC GLM) procedure of SAS. Fisher's least significant difference test (LSD) was used to compare treatment means for larvae survival when ANOVA was significant. Differences were considered significant when $p \leq 0.05$.

Results

Trial 1. At 240 seconds, the mean survival rates of larvae fed copepods for one or three days were significantly higher than in larvae fed the standard reference diet consisting of enriched rotifers, alone (Table 1). At 600 seconds, the mean survival of larvae fed copepods for three days was significantly greater than in larvae fed copepods for only one or no days (standard reference diet).

Trial 2. At 720 seconds, the mean survival of larvae fed copepods for three days was significantly higher than in larvae fed any of the other diets (Table 2).

Table 1. Percent survival (means±SD) of 7-day Florida pompano larvae (*Trachinotus carolinus*) fed copepods for 0 (standard reference), one, or three days and exposed to net stress for 30, 120, 240, 360, or 600 seconds.

Second	*Diet (days that copepods fed)*			*P-value*
	Standard reference	*One-day*	*Three-day*	
30	90.0±11.5	70.0±25.8	60.0±28.3	= 0.3477
120	40.0±28.3	75.0±10.0	80.0±23.1	= 0.0761
240	15.0±19.1^{b}	60.0±16.3^{a}	65.0±34.2^{a}	= 0.0351
360	10.0±11.5	30.0±11.5	35.0±30.0	= 0.2391
600	5.0±10.0^{b}	0.0±0.0^{b}	30.0±10.0^{a}	= 0.0005

Different superscripts within a row indicate statistically significant differences between means ($p \leq 0.05$).

Table 2. Percent survival (means±SD) of 9-day Florida pompano larvae (*Trachinotus carolinus*) fed different diets and exposed to net stress for 180, 360, 540, or 720 seconds.

Second	*Diet*				*P-value*
	Standard reference	*Three-day*	*Copepod*	*Mixed*	
180	54.0±11.4	56.0±19.5	80.0±12.2	76.0±20.6	= 0.0740
360	42.0±13.0	38.0±14.8	46.0±15.2	52.0±19.2	= 0.5417
540	28.0±21.7	36.0±20.7	56.0±32.1	32.0±16.4	= 0.2857
720	0.0±0.0^{c}	52.0±16.4^{a}	28.0±16.4^{b}	12.0±10.9^{bc}	<0.0001

Different superscripts within a row indicate statistically significant differences between means ($p \leq 0.05$).

Discussion

Current larvae rearing protocols for many marine fish species include the use of rotifers (*Brachionus* spp.) and brine shrimp (*Artemia* sp.) and these feeds dominate live feed production in most marine fish hatcheries. However many marine fish larvae, especially ornamental species, require other live feeds, such as copepods, to survive (Hunter, 1981; Lee et al., 2005). Thus, identifying the benefits that derive from their use will determine larvae rearing protocols. A greater understanding of live feeds and their advantages, such as the promotion of stress tolerance, will allow production of an increasing number of marine fish species.

In the present study, stress survival at 7 and 9 days was greater for pompano larvae fed copepods than for those fed the standard reference diet of *B. plicatilis* alone. The results at 7 days (trial 1) indicate that increased stress survival is associated with the number of days that copepods are fed to the pompano. At 240 seconds, stress tolerance was greater for larvae fed copepods for one or three days than for larvae that received no copepods; at 600 seconds, stress tolerance was greater for larvae fed copepods for three days than for those fed copepods only one day. At 600 seconds, at least one larva from each replicate of the three-day diet was alive, while only one larva was alive from all four replicates of the one-day and standard diets.

In contrast to the trend in trial 1, in the 9-day trial, stress survival at 720 seconds was best in larvae fed copepods for only three days although pompano in the copepod and mixed treatments were fed copepods for seven days. At 720 seconds, 14 of the 15 replicates fed a diet containing copepods had at least one larva alive after one hour while none of the larvae fed the standard reference diet were alive. The difference in trend

between the two trials may indicate that factors other than diet influenced stress survival. However, it should be noted that the authors did not intend to compare the trials and too many variables differed between them to draw reliable conclusions. Nevertheless, the improved stress survival of larvae fed copepods was clear in both trials.

Stress tolerance also improved in larvae of mahimahi (*Coryphaena hippurus*) fed the harpacticoid copepod, *Euterpina acutifrons* (Kraul et al., 1993). In an experiment similar to ours, stress tolerance was greater in larvae fed this copepod than in larvae fed enriched *Artemia* nauplii (Kraul et al., 1993). Stress tolerance was even greater in larvae fed *Artemia* with increased levels of enrichment and higher levels of docosahexaenoic acid (DHA; C22:6n3), however, mortality was significantly higher (3-4 times) than in larvae fed the copepod nauplii that had a similar level of DHA (Kraul et al., 1993). It could be that DHA from the copepod nauplii, which is a phospholipid, is more effectively incorporated by mahimahi larvae than DHA from enriched *Artemia*, which is a triglyceride (Kraul et al., 1993).

Survival and growth improved when marine fish larvae were fed copepods with an appropriate nutritional composition (Kraul et al., 1993; Shields et al., 1999; Evjemo et al., 2003; Rajkumar and Kumaraguru vasaham, 2006). In the present study, the trend was similar when stress tolerance was compared to fatty acid composition, as determined by Cassiano et al. (2011). In trial 1, stress tolerance was greater in larvae fed copepod nauplii with 18.4% DHA (% of total fatty acids) than in larvae fed rotifers with 8.9% DHA. Further, stress tolerance was greater and the percentage of DHA was higher (40.1%) in larvae fed nauplii for three days than in larvae fed nauplii for only one day (36.9% DHA), indicating a possible advantage to feeding copepods for a longer period of time. In trial 2, there were no clear relationships between stress survival and % DHA. Copepod nauplii fed the diatom, *T. weissflogii*, had higher levels (16.9%) of eicosapentaenoic acid (EPA; C20:5n3) than DHA (13.1%) which lowered the DHA:EPA ratio (0.8) and likely affected stress response (Cassiano et al., 2011).

In addition to fatty acids, other micronutrients in live feeds may improve production of marine fish larvae (Hamre et al., 2008). Copepods contain appropriate levels of vitamins, minerals, and amino acids when compared to rotifers (Hamre et al., 2008), but little information is available on the effects these micronutrients on stress tolerance in marine fish larvae. In white seabream (*Diplodus sargus*), tolerance to temperature stress was greater in larvae fed enriched rotifers supplemented with tyrosine and phenylalanine than in larvae fed solely enriched rotifers and did not differ from tolerance in larvae fed enriched rotifers supplemented with only phenylalanine (Saavedra et al., 2010). Perhaps the ability of white seabream to convert phenyalanine to tyrosine aids in greater stress tolerance (Saavedra et al., 2010). Further research is needed to understand the role of copepods in providing nutritional advantages to the stress tolerance of marine fish larvae.

In conclusion, larvae are exposed to numerous stressors in the hatchery. Therefore, their ability to withstand stress is as important to production as growth and survival. Protocols can be implemented to avoid stressors within the larvae rearing environment, but they can reduce the performance of fish larvae. Developing production protocols that enhance the stress tolerance of larvae are tantamount to optimizing growth and survival. Our data show that stress tolerance improves when pompano larvae are fed copepods rather than only enriched rotifers.

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