

Evaluation of First-Feeding Regimens for Larval Nassau Grouper *Epinephelus striatus* and Preliminary, Pilot-Scale Culture through Metamorphosis

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Abstract

Two 10-day hatchery experiments were conducted to evaluate s-type (Hawaiian strain) and ss-type (Thailand strain) rotifers *Brachionus plicatilis* and cryogenically preserved oyster *Crassostrea gigas* trochophores as first feeds for larval Nassau grouper *Epinephelus striatus*. Newly hatched grouper larvae were reared at densities of 11.2–20.8/L in 500-L tanks at 36–38 ppt salinity, 25–26 °C, and under a 11-h light : 13-h dark photoperiod. Beginning on day 2 posthatching (d2ph), prey were maintained at a density of 20 individuals/mL, while phytoplankton (*Nanochloropsis oculata*) was maintained at 500×10^3 cells/mL. In experiment 1, survival and growth were higher ($P < 0.05$) for fish fed small s-type rotifers (mean lorica length = 117 μm ; fish survival = 7.96%) selected by sieving than for fish fed non-selected rotifers (mean lorica length = 161 μm ; fish survival = 2.13%). These results demonstrated the advantage of small prey size and suggested that super-small (ss-type) rotifer strains would be beneficial. In experiment 2, three feeding regimens were compared: 1) ss-type rotifers (mean lorica length = 147 μm); 2) oyster trochophores (mean diameter = 50 μm) gradually replaced by ss-type rotifers from d5ph; and 3) a mixed-prey treatment of 50% oyster trochophores and 50% ss-type rotifers. Survival was higher ($P < 0.05$) for larvae fed mixed prey (15.6%) than for those fed rotifers (9.73%) or trochophores and rotifers in sequence (2.55%), which also showed the slowest growth. Oyster trochophores, although inadequate when used exclusively, enhanced survival when used in combination with rotifers, possibly by improving size selectivity and dietary quality. In a pilot-scale trial, larvae were cultured through metamorphosis in two 33.8-m³ outdoor tanks. Fertilized eggs were stocked at a density of 10 eggs/L and larvae were fed ss-type rotifers from d2ph–d20ph, newly hatched *Artemia* from d15ph–d18ph, 1-d-old *Artemia* nauplii from d18ph–d62ph. Survival on d62ph was 1.17%, with a total of 5,651 post-metamorphic juveniles produced.

The Nassau grouper *Epinephelus striatus* is a high-value food fish in the tropical and subtropical western Atlantic region, including the Gulf of Mexico and Caribbean. This species forms large aggregations for spawning which make it vulnerable to over-fishing, and local aggregations have disappeared in Puerto Rico, Bermuda, and the U.S. Virgin Islands (Sadovy, in press). These factors make Nassau grouper an im-

portant candidate for aquaculture and stock enhancement.

Methods for controlled breeding of Nassau grouper have been developed (Tucker et al. 1991; Watanabe et al. 1995a; Head et al. 1996), but reliable technology for rearing larvae has not been established. Because larval epinephelids have a relatively small mouth (Fukuhara 1989), adult rotifers (s- and l-types) are too large for most species of groupers (Lim 1993), including *E. tauvina* (Hussain and Higuchi 1980), *E. amblycephalus* (Tseng and Chan 1985), and *E. salmoides* (Kungvankij et al. 1986). Smaller prey types, including young rotifer

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fers, oyster and mussel larvae, sea urchin eggs, tintinnids, diatoms, and phytoplankton, have been used as first feeds (Tseng and Chan 1985; Kungvankij et al. 1986; Fukuhara 1989) but their value to larval epinephelids is still unclear.

Oyster trochophores have been difficult to obtain in sufficient quantities for use on a large scale, but availability has recently improved through bulk storage in a cryogenically preserved (cryopreserved) state. In this study, first-feeding regimens for larval *E. striatus*, including s-type rotifers, small (s-type) rotifers, super-small (ss-type) rotifers, and cryopreserved oyster trochophores, were evaluated under controlled hatchery conditions. Results of a preliminary, pilot-scale larval culture trial through metamorphosis are also reported.

Materials and Methods

This study was conducted at the Caribbean Marine Research Center (CMRC) on Lee Stocking Island, Exuma Cays, Bahamas, during the months of January–May 1993 and 1994. Fertilized eggs of *E. striatus* were obtained by hormone-induced spawning of resident broodstock (Watanabe et al. 1995a; Head et al. 1996).

The experimental units consisted of 15, black, 500-L cylindroconical tanks situated in an indoor hatchery. Tanks were supplied with seawater (36–38 ppt salinity) at an ambient temperature range of 25–26 C. Gentle aeration was supplied to each rearing tank from a diffusion ring around the base of the central standpipe. Natural light through hatchery windows, supplemented with overhead fluorescent lighting, provided a photoperiod of approximately 11-h light: 13-h dark. Light intensity at the water surface of each tank (measured at 0900 and 1500 daily) averaged 2,056 lux (range = 1,316–2,795 lux).

Two 10-d experiments were conducted to evaluate various prey types and prey regimens for first-feeding larval *E. striatus*. In both experiments, prey were introduced on

TABLE 1. Survival and notochord lengths for larval *E. striatus* fed small and non-selected s-type rotifers (Experiment 1). Larvae were reared in 500-L tanks stocked with 34 eggs/L. Rotifers were fed at a concentration of 20 individuals/mL.

| Variable | Small | Non-selected |
|----------------------------|--------------------------|---------------------------|
| Hatching rate (%) | 32.1 ± 9.8a ¹ | 33.6 ± 6.5a |
| Initial density (larvae/l) | 10.9 ± 2.9a | 11.4 ± 1.9a |
| Survival (%) | | |
| Age (d posthatch) | | |
| 5 | 24.1 ± 10.4a | 23.5 ± 10.5a |
| 10 | 7.96 ± 2.0a | 2.13 ± 1.9b |
| Notochord length (mm) | | |
| Age (d posthatch) | | |
| 1 | 2.36 ± 0.10a | 2.39 ± 0.11a |
| 3 | 2.73 ± 0.04a | 2.75 ± 0.06a |
| 5 | 2.93 ± 0.11a | 2.65 ± 0.09b |
| 7 | 3.52 ± 0.12a | 3.25 ± 0.12b |
| 9 | 4.04 ± 0.40a | 3.75 ± 0.07a ² |

¹ Values are expressed as means ± SD (n = 4). Means within a row followed by a different letter were significantly different (P < 0.05).

² N = 3.

day 2 posthatching (d2ph) at a concentration of 20 individuals/mL, which was monitored and adjusted twice daily using volumetric methods. The microalga *Nanochloropsis oculata* was added daily to maintain a concentration of 500 × 10³ cells/mL.

Experiment 1: Comparison of Small and Non-Selected s-Type Rotifers

In Experiment 1, fertilized eggs were stocked (34 eggs/L) into nine tanks. Hatching (day 0) occurred from 27–32 h post-fertilization at an average rate of 32.9% (Table 1). Initial larval density averaged 11.2 larvae/L, with no significant differences observed among treatment groups (Table 1). Growth and survival to d10ph were compared for larvae fed s-type (Hawaiian strain) rotifers *Brachionus plicatilis* (mean lorica length = 161 μm; range = 74–264 μm) and small s-type rotifers (mean lorica length = 117 μm; range = 50–200 μm), selected by sieving. Four replicate tanks were maintained per treatment

TABLE 2. Survival, notochord length, and mouth widths for larval *E. striatus* reared for 10 d post-hatching under three feeding regimens: 1) rotifers (ss-type) alone; 2) oyster trochophores, with weaning to rotifers¹; and 3) a 1:1 mixture of oyster trochophores + rotifers (Experiment 2). Fish were reared in 500-L tanks stocked with 24 eggs/L. All prey were fed at a total concentration of 20 individuals/mL.

| Variable | Feeding regimen: | | |
|----------------------------|--------------------------|--------------------------------|---------------------------------|
| | Rotifers | Trochophores, wean to rotifers | Mixed (trochophores + rotifers) |
| Hatching rate (%) | 86.3 ± 4.4a ² | 88.4 ± 11.2a | 87.0 ± 7.4a |
| Initial density (larvae/l) | 20.6 ± 1.0 | 21.1 ± 2.7 | 20.8 ± 1.8 |
| Survival (%) | | | |
| Age (d posthatch) | | | |
| 3 | 67.0 ± 30.3a | 56.5 ± 16.4a | 71.1 ± 14.7a |
| 5 | 32.4 ± 4.1a | 38.8 ± 9.4a | 52.9 ± 19.9a |
| 7 | 11.7 ± 6.4b | 13.4 ± 3.5b | 25.3 ± 8.4a |
| 10 | 9.73 ± 3.45a | 2.55 ± 1.79b | 15.6 ± 7.78a |
| Notochord lt. (mm) | | | |
| Age (d posthatch) | | | |
| 0 | 1.81 ± 0.08a | 1.88 ± 0.05a | 1.86 ± 0.17a |
| 3 | 2.93 ± 0.02a | 2.88 ± 0.08a | 2.95 ± 0.10a |
| 5 | 3.47 ± 0.58a | 2.94 ± 0.18a | 3.38 ± 0.35a |
| 7 | 3.65 ± 0.20a | 2.84 ± 0.07c | 3.23 ± 0.29b |
| 10 | 4.08 ± 0.30a | 3.14 ± 0.39b | 3.69 ± 0.17a |
| Mouth width (mm) | | | |
| Age (d posthatch) | | | |
| 7 | 416 ± 25a | 333 ± 38b | 350 ± 29b |
| 10 | 467 ± 54a | 344 ± 73b | 417 ± 23ab |

¹ Beginning on d 5 p.h., trochophores were replaced with rotifers at a rate of 25% of total prey each day.

² Values are expressed as means ± SD for three to five replicate tanks. In treatment 1, two replications were omitted due to low rotifer densities. Means within a row followed by a different letter were significantly ($P < 0.05$) different

and one tank was maintained unfed as a control.

Rotifers were grown at 22 ppt salinity in a batch culture system and fed *N. oculata* and Baker's yeast *Saccharomyces cerevisiae* at an approximate 1:1 ratio. Small rotifers were selected as those which passed through a 75- μ m mesh screen and were retained on 18- μ m mesh, while non-selected rotifers were those retained on 18- μ m mesh.

Survival was measured on d1, d5, and d10ph using volumetric methods. Samples were collected at night when larvae were observed to be uniformly distributed throughout the tank. Growth was determined on d1, d3, d5, d7, and d10ph by measuring notochord lengths of 20 larvae per tank using a dissecting microscope equipped with an ocular micrometer.

Static conditions were maintained until

d4ph when water was exchanged at a rate of 20% per d, increasing to 30% per d by d7ph. Water temperature, dissolved oxygen, and pH were measured daily, while total ammonia nitrogen was measured on alternate days.

Experiment 2: Evaluation of ss-Type Rotifers and Oyster Trochophores

Fertilized eggs were stocked (24 eggs/L) into 15 tanks. Hatching rate averaged 87.2% and initial larval density averaged 20.8 larvae/L, with no significant differences observed among treatment groups (Table 2). Growth and survival to d10ph were compared for larvae fed three prey regimens: 1) super-small (ss-type, Thai strain) rotifers (mean lorica length = 147 μ m, range = 67–237 μ m); 2) oyster trochophores (*Crassostrea gigas*) (mean diameter = 49.9 μ m, range = 35–60 μ m) with

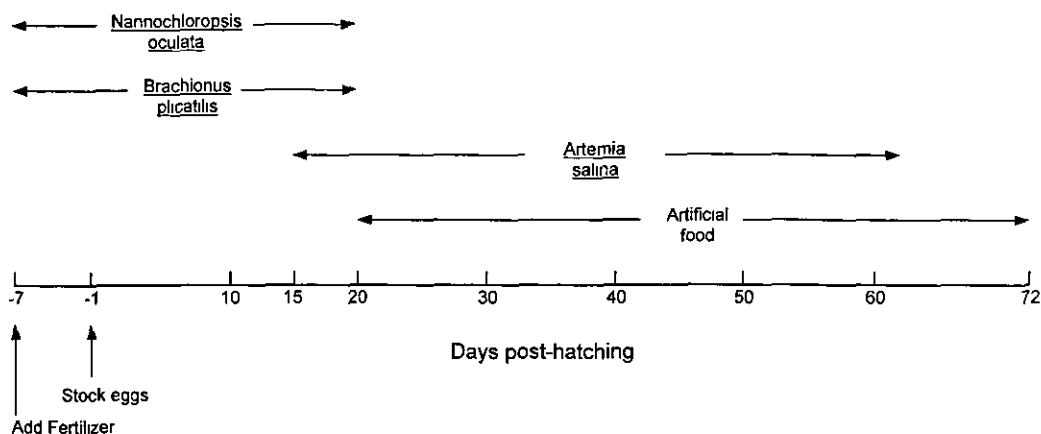


FIGURE 1. Summary of feeding regimen used during pilot-scale culture of Nassau grouper from egg to metamorphic stages in 33.8-m³ tanks (see text for explanation).

weaning to ss-type rotifers; and 3) a 1:1 mixture of ss-type rotifers and oyster trochophores. In treatment 2, larvae were weaned to ss-type rotifers starting on d5ph by replacing trochophores with rotifers at a rate of 25% of total prey each day until 100% rotifers were fed by d8ph. Five replicate tanks were maintained per treatment.

Cryopreserved oyster trochophores were obtained from a commercial source (Innovative Aquaculture Products, Skerry Bay, British Columbia, Canada) and were maintained in liquid nitrogen at -196 C. Trochophores were thawed in 30 C sea water immediately before use.

Survival and growth of larvae in each tank were monitored on d0, d3, d5, d7, and d10ph. Mouth width was determined (10 larvae per tank) on d7 and d10ph as the widest part of the upper jaw measured from a dorsal view.

Water was exchanged and water quality monitored as described above.

Statistical Analyses

Survival, notochord lengths, and mouth widths were compared among treatments by analysis of variance (ANOVA). Arcsine (survival data) or natural log (length data) transformations were performed prior to analysis when variances were not homogeneous. If a significant ($P < 0.05$) ANOVA

was indicated, the Ryan-Einot Gabriel-Welch multiple-range test was used to compare treatment means (Day and Quinn 1989).

Pilot-Scale Culture to Metamorphosis

Between 17 January and 30 March 1994 survival and growth of larval *E. striatus* from egg through metamorphic stages were assessed under pilot-scale hatchery conditions.

Two above-ground, rectangular tanks (7.5 × 4.5 × 1 m) constructed of framing lumber and lined with black high-density polyethylene (volume = 33.8 m³), were filled with unfiltered seawater. Tanks were enclosed by a polyethylene greenhousing and the roof was covered by a 70% light-occluding shade cloth. Light intensity at the water surface of each tank, measured at 0815 and 1520 daily, ranged from 6,688 to 10,857 lux. Moderate aeration was provided using three aeration hoses (A. John Hinde Co., Lake Bluff, Illinois, USA) placed equidistantly across the width of each tank.

Fig. 1 summarizes the feeding regimen used during the pilot-scale trial. One week prior to the start of the trial, tanks were fertilized with ammonium phosphate (50 g), monopotassium phosphate (15 g), urea (2.5 g), Fe-EDTA (7.5 g), and trace metal mix (0.5 g) (Oceanic Institute, Honolulu, Ha-

TABLE 3. Survival and growth of larval *E. striatus* from hatching through metamorphic stages during pilot-scale culture in two, 33.8-m³ tanks. Fertilized eggs were stocked in each tank at a density of 10 eggs/L.

| Age (d post-hatching) | Survival ¹ | | Notochord length (mm) |
|-----------------------|-------------------------|---------------------|-----------------------|
| | (larvae/L) | (%) ² | |
| 0 | 7.03 (6.48–7.57) | 100 | nd ³ |
| 5 | 4.19 (2.53–5.84) | 58.1 (39.0–77.1) | 3.59 (3.21–3.96) |
| 10 | 2.97 (2.24–3.69) | 41.7 (34.6–48.7) | 4.50 (4.43–4.57) |
| 15 | 2.20 (2.09–2.30) | 31.3 (30.4–32.3) | 5.96 (5.76–6.15) |
| 20 | 1.08 (1.00–1.16) | 15.4 (15.3–15.4) | 7.00 (6.41–7.59) |
| 25 | 0.18 (0.13–0.23) | 2.50 (2.0–3.0) | 8.21 (7.16–9.26) |
| 35 | nd | nd | 10.7 (10.4–10.9) |
| 62 | 0.084 (0.059–0.1081) | 1.17 (0.92–1.43) | nd |
| 72 | nd | nd | 29.8 (29.1–30.4) |

¹ Values are expressed as averages for two tanks, with range in parentheses.

² Percentage of initial (day 0) larval density.

³ No data available.

waii, USA), then inoculated with *N. oculata* (150×10^3 cells/mL) and ss-type rotifers (1/mL).

Seven days after inoculation, each tank was stocked with fertilized eggs of *E. striatus* at a density of 10 eggs/L. Aeration levels were reduced prior to hatching. Hatching rate averaged 70.3% and initial larval density averaged 7.03 larvae/L (Table 3).

Rotifer and algae concentrations were monitored daily and replenished as available. Rotifers concentrations (individuals/mL) averaged 12 (range = 6–18) from d2ph–5ph, 17.6 (range = 8.0–27.2) from d6ph–15ph, then declined to 4 (range = 1–7) from d16ph–20ph. Algae was maintained at approximately 25×10^3 cells/rotifer per mL. Algae and rotifers were not added after d20ph.

Newly hatched *Artemia* nauplii (San Francisco Bay strain) were introduced on

d15ph and were fed for 3 d. One-day-old *Artemia* were added from d18ph to d62ph. *Artemia* concentrations (individuals/L) averaged 374 (range = 279–468). Nauplii were enriched with either high-DHA Super Selco (*Artemia* Systems, NV-SA, Gent, Belgium) or Frippak Booster (France Aquaculture, Plouzane, France) for 15–22 h after harvest.

Commercially-prepared feed (Nippai, Nippon Formula Feed Manufacturing Co. Ltd., Yokohama, Japan) was introduced on d20ph and fed at least three times daily until d62ph. Feed particle size was increased from 250–440 μ m on d62ph to 880–1,025 μ m by d62ph.

Water was exchanged initially at a rate of 10% per d and gradually increased to a maximum of 100% per d by d30ph. Aeration was gradually increased during the rearing period. To prevent loss of larvae, standpipes were screened (250- μ m mesh) and provided with an air diffuser ring. Mesh size was gradually increased as fish grew and water exchange was increased.

A styrofoam skimmer was used to remove oily surface film (Lim 1993) and was cleaned twice daily. Tank bottoms were siphoned on d40ph–44ph and on d51ph–52ph.

Survival was monitored using volumetric methods on d5, d10, d15, d20, and d25ph. Survival was not determined after d25ph since larval distribution was patchy. On d62ph, total number of fish remaining in each tank was determined gravimetrically.

Growth was monitored on d5, d10, d15, d20, d25, and d35ph by measuring notochord lengths of 20 fish from each tank. Growth sampling was discontinued after d35ph since larger fish were able to evade capture. On d72ph, a sample of 100 individuals from each tank were individually weighed and measured.

Temperature, dissolved oxygen, and pH were monitored twice daily. Total ammonia nitrogen was monitored weekly.

Results

Experiment 1: Comparison of Small and Non-Selected s-Type Rotifers

Survival fell to 0% in the unfed control tank by d5ph, while averaging 23.8% for larvae fed small or non-selected rotifers, with no significant differences observed among treatments (Table 1). By d10ph, survival of larvae fed small rotifers (7.96%) was markedly higher ($P < 0.05$) than those fed non-selected rotifers (2.13%) (Table 1).

Notochord lengths of larvae averaged 2.38 mm at d1ph, with no significant differences observed between treatment groups (Table 1). Notochord lengths diverged from d5ph and were higher among larvae fed small rotifers than those fed non-selected rotifers, with significant ($P < 0.05$) differences observed from d5ph (Table 1).

Average daily water temperature (26.6 ± 0.02 C), dissolved oxygen (mean \pm SD = 5.93 ± 0.05 mg/L), pH (8.19, range = 8.17–8.33), and total ammonia nitrogen (0.13 ± 0.08 mg/L) did not differ significantly between treatments ($P > 0.05$).

Experiment 2: Evaluation of ss-Type Rotifers and Oyster Trochophores

Significant ($P < 0.05$) treatment differences in survival were evident from d7ph (Table 2). On d10ph, survival was higher ($P < 0.05$) among larvae fed mixed prey (15.6%) and those fed rotifers (9.73%) than among those fed trochophores and rotifers in sequence (2.55%) (Table 2).

Growth (notochord length) of larvae in all treatments was similar through d5ph (Table 2). Significant ($P < 0.05$) treatment differences were observed from d7ph, when larvae fed rotifers alone generally exhibited higher growth than those fed mixed prey, while larvae fed trochophores and rotifers in sequence showed slowest growth.

On d7 and d10ph, average mouth width showed differences among treatments that were similar to those in growth. Mouth width was larger ($P < 0.05$) in larvae fed

rotifers than in those fed mixed prey or trochophores and rotifers in sequence.

Average daily water temperature (25.4 ± 0.04 C), dissolved oxygen (6.02 ± 0.01 mg/L), and pH (7.98; range = 7.97–7.98) did not differ significantly among treatments ($P > 0.05$). Total ammonia nitrogen (0.07 ± 0.05 mg/L) decreased ($P < 0.01$) from 0.13 mg/L in larvae fed rotifers to 0.04 mg/L in larvae fed trochophores and rotifers in sequence.

Pilot-Scale Culture to Metamorphosis

Survival fell abruptly in both tanks to an average of 58.1% by d5ph, then declined steadily to 2.50% by d25ph (Table 3). After this, survival declined gradually to 1.17% on d62 ph (Table 3) when a total of 5,651 post-metamorphic juveniles were produced. Transition from a planktonic to a benthic mode of existence, the primary criterion used to estimate timing of metamorphosis, varied among individuals from approximately d45ph to d62ph.

Average notochord length increased from 3.59 mm at d5ph to 29.8 mm on d72ph (Table 3). On d72ph, post-metamorphic fish averaged 0.82 g (range = 0.22–3.93 g), with 43.6% ranging from 0.51 to 0.80 g (Fig. 2).

Average daily water quality parameters during the culture period were as follows: temperature (25.0 ± 1.4 C), dissolved oxygen (6.91 ± 0.86 mg/L), pH (8.25, range = 7.8–8.7), and total ammonia nitrogen (0.04 ± 0.08 mg/L).

Discussion

The importance of prey size to successful first-feeding (Hunter 1980) was demonstrated in this study (Experiment 1) by higher survival and growth of larval *E. striatus* fed small, selected s-type rotifers than in those fed non-selected rotifers, presumably identical in nutritional quality. Since selection of smaller rotifers by sieving is wasteful of the larger individuals, feeding regimens utilizing a super-small (ss-type) rotifer strain

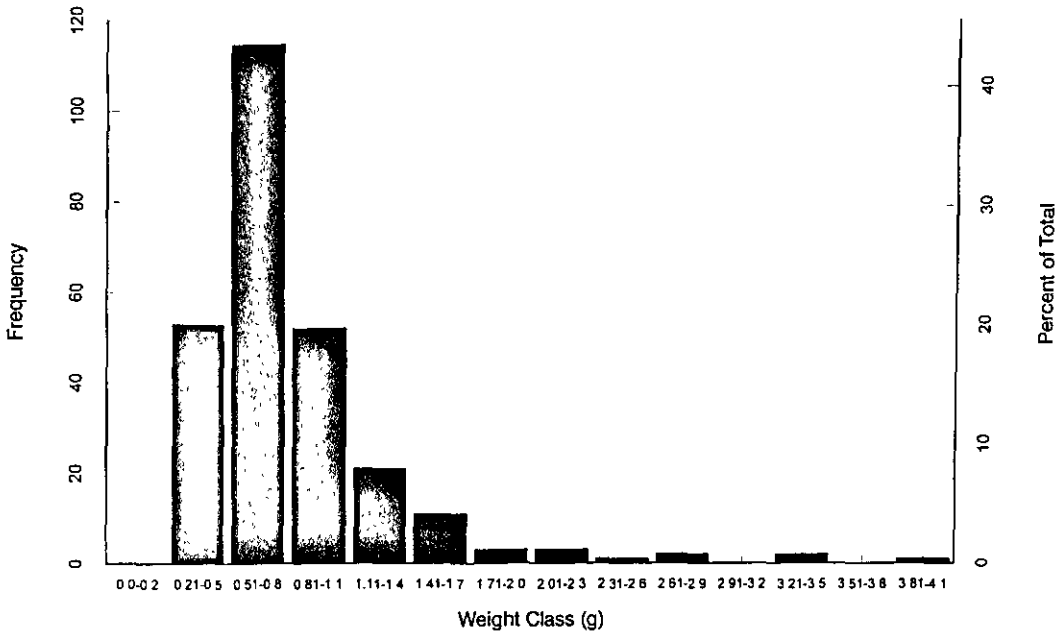


FIGURE 2. Frequency distribution of body weights for post-metamorphic *E. striatus* (72 d posthatching cultured from egg stages in 33.8-m³ tanks).

and/or oyster trochophore larvae were evaluated in Experiment 2.

In Experiment 2, a mixed-prey regimen consisting of equal concentrations of ss-type rotifers and oyster trochophores produced higher survival than regimens using rotifers alone or trochophores and rotifers in sequence. The benefits of the mixed-prey regimen were probably related to the small size and low motility of oyster trochophores, which enabled a greater percentage of larvae to ingest prey at time of mouth opening (Yufera et al. 1993). Higher survival, but smaller average size of larvae fed the mixed prey regimen is consistent with this idea.

Whereas transition from a slow-moving oyster trochophore to a much larger, more mobile rotifer may be difficult for smaller larvae when these prey are introduced sequentially, availability of both prey types at first feeding may allow larvae to adapt to these dissimilar prey. Thus, a mixed-prey regimen allows larvae to feed selectively and accommodates individual differences in development (Tseng and Chan 1985). Eda

et al. (1990) concluded that, in grey mullet *Mugil cephalus*, continued use of rotifers with artemia from day 15 produced higher survival than weaning from rotifers to artemia between days 12 and 15, presumably by increasing feeding of smaller larvae.

In addition to facilitating prey capture by smaller, weaker larvae, a mixed-prey regimen may improve nutritional quality. The highly unsaturated fatty acids, 20:5n3 and 22:6n3, essential for marine fish larvae (Watanabe et al. 1983), are each present at 15% of total fatty acids in cryopreserved oyster trochophores (Innovative Aquaculture Products, Skerry Bay, British Columbia, Canada).

Despite the apparent benefits of oyster trochophores as a first prey, their exclusive use during the first three days (d2ph–d4ph) of exogenous feeding produced the lowest survival and growth to d10ph, even though rotifers were introduced from d5ph. This was probably due to a lack of prey of adequate size for growing larvae. Optimal prey size in first-feeding marine fish larvae in-

creases rapidly from 25% to 50% of the mouth width within a few days (Hunter 1984). At 26 C, average mouth width in *E. striatus* increases from 240 μm (range = 180–290 μm) at first feeding (d3ph) to 293 μm (range = 240–390 μm) by d5ph (W. O. Watanabe et al., unpublished data), suggesting prey size optima of 60 μm (range = 45–73 μm) and 147 μm (range = 120–195 μm), respectively. Thus, by d5ph, oyster trochophores are much smaller than ideal.

Average mouth width of *E. striatus* increases to 366 μm by d7ph and 409 μm by d10ph (Table 2), indicating prey size optima of 183 μm and 205 μm , respectively. This suggests that s- or l-type rotifer strains (mean lorica lengths = 160–216 μm) may be optimal at these stages while small *Artemia* nauplii (mean length = 449 μm) (Pryor and Epifanio 1993) may be too large.

Under a mixed-prey (trochophores + rotifers) regimen, survival of *E. striatus* to d10ph, averaged 15.6%, a value considerably higher than reported for the greasy grouper *E. tauvina* (5.5%) fed ss-type and s-type rotifers (Lim 1993). In contrast, a survival of 24.4% (to d12ph) was obtained with the brown-marbled grouper *E. fusco-guttatus*, a species which is able to feed directly on an s-type rotifer (Lim 1993).

Maximum survival of *E. striatus* to d10ph in 500-L tanks (15.6%) was much lower than that (41.7%) achieved in large, pilot-scale tanks. Although larvae in large tanks were only fed ss-type rotifers, survival was possibly improved by the availability of alternative planktonic prey (e.g., copepods and protozoans) which were likely introduced with seawater and proliferated during the period of inoculation. Heterogeneous feeding regimens may be advantageous, but are difficult to manage and replicate in large systems. The application of mixed-prey feeding regimens, including ss-type rotifers and oyster trochophores, therefore warrants testing on a large scale.

High mortality as well as slow growth (or atrophy) was generally observed from d3ph to d7ph in 500-L tanks. Greatest mortalities

also occurred by d5ph during culture in large, pilot-scale tanks. High early mortality may be attributable to factors unrelated to availability of prey (e.g., biochemical quality of eggs and environmental conditions) (Watanabe et al. 1995b), as well as to difficulties in transitioning from endogenous to exogenous nutrition (May 1974). High mortality during the first-feeding stages in cultured groupers has previously been reported in the brown-spotted grouper *E. tauvina* (Chen et al. 1977; Hussain and Higuchi 1980) and the white-spotted green grouper *E. amblycephalus* (Tseng and Chan 1985).

Due to their small size and fragility, larval epinephelids are considered among the most difficult marine finfish to rear. Under pilot-scale conditions, *E. striatus* larvae were reared to metamorphosis (d45ph–62ph) at an average survival rate of 1.17%, in the range of maximum survival rates (<1 to 10%) to metamorphosis (d35ph–60ph) reported for various species (Hussain and Higuchi 1980; Fukuhara 1989; Lim 1993).

Post-metamorphic juveniles produced in the pilot-scale trials were characterized by considerable size variation (Fig. 2), postulated to be a major cause (and effect) of cannibalism (Hecht and Pienaar 1993). Cannibalism may therefore have been an important cause of mortality during the late (pre- and post-metamorphic) stages of the pilot-scale trial. This is supported by a high incidence of cannibalism observed among hatchery-reared juvenile *E. striatus* (S. C. Ellis et al., unpublished data). Studies to minimize size variation, including optimal feeds and feeding regimens, stocking densities, and grading practices (Hecht and Pienaar 1993) will be important toward economic culture of this species.

Results of this study emphasize the importance of prey size to early survival of larval *E. striatus* and the benefits of a mixed prey dietary regimen, including ss-type rotifers and oyster trochophores. In order to reduce the costs of using cryopreserved oyster trochophores on a large, com-

mercial scale, optimum densities and durations of their use in combination with rotifers, should be determined.

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