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Project Summary

Marine Fish Larvae Growth and Survival

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This report describes the research undertaken to develop and standardize culture methods for larvae of two common marine fishes, the spotted seatrout *Cynoscion nebulosus* and the lined sole *Achirus lineatus*. Culture methods are explained and the relationships of survival, growth and yield to temperatures, food concentration and egg stocking densities were determined.

Two different diets, a laboratory-cultured diet based on the rotifer *Brachionus plicatilis*, supplemented in some experiments with brine shrimp *Artemia salina* nauplii, and a net-collected zooplankton diet were compared. Feeding rates and rations also were estimated for each species as functions of age, size, food type and, in the case of seatrout, temperature.

Despite considerable variability in survival and growth rates, it is possible to produce large numbers of larvae at any stage from hatching to metamorphosis for toxicological studies including hazard assessment and water quality bioassays. Spotted seatrout larvae are easier to culture successfully than are lined sole, which might be a factor to consider in choice of a bioassay test organism.

This Project Summary was developed by EPA's Environmental Research Laboratory, Narragansett, RI, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The larval stages of marine organisms are particularly sensitive to environmental stresses. Despite more difficulty in culturing and maintaining these stages it is useful to evaluate the potential effects of environmental contaminants on larval development. The ability to culture marine fish larvae for experimental purposes has increased greatly in the past 10 years. However, successful rearing techniques have not been standardized although there is a general recognition of important factors that affect both survival and condition of larvae. The role of food and nutrition in larval culture is most important.

The purpose of this research was to develop and evaluate standard methodology to culture larvae of two common marine fishes, so that large numbers of healthy larvae or juveniles could be predictably produced for toxicological research. Two species that are found in coastal waters of the Gulf of Mexico and southeastern United States, the spotted seatrout (*Cynoscion nebulosus*) and lined sole (*Achirus lineatus*), were selected for this project to investigate how survival, growth and condition of the larvae were affected by culture methods.

Research results reported here focused on the roles of prey type and prey availability in predictably producing metamorphosed spotted seatrout and lined sole. Feeding rates of the larvae were determined as a function of age and in relation to the culture regime. In addition to aiding design of pollution and contaminant-oriented experiments, results of this study have helped to document environmental requirements of larvae of the two species.

Results and Discussion

Both spotted seatrout and lined sole can be reared on a diet of zooplankton or lab cultured food including rotifers and brine shrimp nauplii. Survival rates at metamorphosis increased as food concentrations were raised. Embryo stocking levels had a relatively small effect on survival, but lowest survival rates usually were observed at highest stocking levels.

Spotted Seatrout

Percent survival over all factor combinations ranged up to 73.4% for spotted seatrout larvae reared on zooplankton and to 80.5% for larvae reared on rotifers (Table 1). On a zooplankton diet mean survival rate increased from 15.1 to 73.4% as food level was raised from 25 to 5000 per liter. Survival was significantly higher at 28° than at 24° or 32°C.

Survival of seatrout larvae that were fed rotifers did not differ significantly from survival of zooplankton-fed larvae. Mean survival rates over all food concentrations and stock densities were 37.9% for zooplankton-fed larvae and 43.6% for rotifer-fed larvae. Survival of larvae increased significantly as food level was raised for both diets.

Growth of seatrout larvae, expressed as standard length and dry weight, increased significantly as zooplankton concentrations and temperatures were raised (Table 2). Lengths and weights of larvae decreased as stock density increased. Growth increased as food concentration was raised for larvae reared on the rotifer diet and decreased as stock density increased, but the effects were not as clear as for zooplankton-fed larvae (Table 3). At the 1000 per liter food concentration mean dry weights of seatrout larvae were 6.1 times higher on the zooplankton diet than on the rotifer diet.

A laboratory cultured diet of rotifers-*Artemia* was similar to the zooplankton diet in supporting survival of spotted seatrout, Table 2.

Lined Sole

Survival rates, presented as 90% confidence intervals, ranged up to 63.2% over all factor combinations for larvae fed zooplankton and to 55.4% for larvae fed rotifers, (Table 3). Survival increased significantly as food levels were raised for both zooplankton and rotifer foods. There was some evidence that survival was higher on rotifers at the lowest food levels and on zooplankton at the highest food level. At food levels of 1000 per liter or higher, either zooplankton or rotifers could be used to rear large numbers of lined sole to metamorphosis.

Lined sole grew relatively fast on zooplankton compared to rotifers. Over all factor combinations, mean standard lengths at 16 days were 3.93 mm on the zooplankton diet and only 3.40 mm on the rotifer diet. The difference in mean weights at 16 days was even more striking; survivors averaged 209-µg on the zooplankton diet but only 123-µg on the rotifer diet. Mean standard lengths and dry weights were significantly higher at the 1000 per liter food levels compared to the 50 and 100 per liter levels for both diets.

Thirteen to 25 day old lined sole had equally good survival and slightly better growth on the lab-reared diet of rotifers-*Artemia* compared to zooplankton fed larvae, Table 4.

Length-Weight Relationships

Spotted Seatrout

The exponent in power functions that described the length-weight relationship for spotted seatrout larvae reared at three different temperatures, over three food levels (25, 100, 1000 food items

Table 1. Spotted Seatrout . *Ninety Percent Confidence Intervals about the Means for Percent Survival, Standard Length, Dry Weight Per Larva and Total Yields of Larvae at 12 Days After Hatching Reared at 28°C on Either a Zooplankton or Rotifer Diet. Confidence Limits are Placed on Means Over All Factor Combinations at the Designated Factor Level.*

	.90 Confidence Limits			
Factor and Level	*Percent Survival*	*Standard Length (mm)*	*Larval Dry Weight (µg)*	*Total Yield (mg)*
I. Zooplankton Food 28°				
Food Concentration				
25	*6.7 to 15.1*	*3.13 to 4.75*	*30.7 to 341.7*	*0.0 to 5.2*
100	*18.7 to 33.6*	*2.71 to 5.85*	*0.0 to 509.7*	*6.2 to 11.1*
1000	*49.3 to 53.1*	*4.31 to 7.51*	*0.0 to 1285.4*	*65.6 to 74.0*
5000	*65.7 to 73.4*	*5.75 to 8.81*	*205.7 to 2214.0*	*166.2 to 179.2*
Stock Density (Food Concentration - 1000/l)				
0.5	*44.5 to 51.7*	*7.23 to 10.37*	*1159.6 to 3067.4*	*5.3 to 12.5*
5.0	*37.2 to 52.0*	*5.52 to 8.48*	*697.9 to 1628.7*	*25.5 to 33.1*
25.0	*15.4 to 29.4*	*5.18 to 6.88*	*453.0 to 938.2*	*33.3 to 41.7*
II. Rotifer Food 28°				
Food Concentration				
25	*10.9 to 35.5*	*2.33 to 3.57*	*0.0 to 119.7*	*0.0 to 8.9*
100	*36.3 to 44.3*	*2.68 to 4.38*	*0.0 to 233.1*	*2.0 to 10.0*
1000	*31.8 to 53.6*	*3.42 to 4.10*	*77.2 to 122.8*	*0.0 to 13.1*
5000	*56.9 to 80.5*	*3.50 to 4.42*	*44.1 to 224.7*	*15.2 to 22.0*
Stock Density (Food Concentration - 1000/l)				
0.5	*21.3 to 49.7*	*3.88 to 5.20*	*87.3 to 372.1*	*0.0 to 6.9*
5.0	*47.9 to 53.3*	*3.78 to 4.50*	*96.1 to 197.7*	*7.0 to 12.4*
25.0	*39.9 to 50.1*	*3.26 to 3.86*	*65.1 to 115.1*	*18.2 to 24.6*

Table 2. Spotted Seatrout. *Percent Survival and Mean Length of Larvae Reared to 16 Days After Hatching at 28°C on Either a Rotifer-*Artemia *or Zooplankton Diet. Two Egg Sources, Plankton-Collected or Hormone-Induced, also Were Compared.*

Egg Source	*Food Type (1000/l)*	*Percent Survival (Stock Density 0.5/l)*	*Mean Standard Length (mm) (± 1 S.E.)*
Plankton	*Rotifer-*Artemia	*65.7*	*11.97 ± 0.19*
Plankton	*Rotifer-*Artemia	*27.1*	*8.56 ± 0.18*
Hormone	*Rotifer-*Artemia	*20.0*	*8.94 ± 0.34*
Hormone	*Rotifer-*Artemia	*62.9*	*7.72 ± 0.09*
Plankton	*Zooplankton*	*60.0*	*15.49 ± 0.22*
Plankton	*Zooplankton*	*21.4*	*14.82 ± 0.33*
Hormone	*Zooplankton*	*37.1*	*15.51 ± 0.17*
Hormone	*Zooplankton*	*64.3*	*11.69 ± 0.11*

Means

Percent Survival	
Plankton eggs	*43.6%*
Hormone eggs	*46.1%*
*Rotifer-*Artemia *diet*	*43.9%*
Zooplankton diet	*45.7%*
Standard Length	
Plankton eggs	*12.71 mm*
Hormone eggs	*10.97 mm*
*Rotifer-*Artemia *diet*	*9.30 mm*
Zooplankton diet	*14.38 mm*

per liter), differed significantly. The exponent at 28°C, for either rotifer or zooplankton diets, was significantly higher (Analysis of Covariance) than those at 24° or 32°C. The equations are:

- zooplankton food 24°—$W = 1.071L^{3.41}$
- zooplankton food 28°—$W = 0.51L^{3.91}$
- rotifer food 28°—$W = 0.50L^{3.93}$
- zooplankton food 32°—$W = 0.83L^{3.62}$

There was no evidence that food concentrations had a significant effect on the length-weight relationships. The combined length-weight relationship for both of the food types, all temperature and food concentrations was: $W = 0.75L^{3.66}$.

Length-weight relationships, in theory, can be used as an index of larval conditions. High values of the exponent in the power function relationships indicate that larvae are relatively "fat" and presumably better conditioned. We did not find effects of food concentration on length-weight relationships of spotted seatrout. This result indicates that the length-weight relationship may not be useful to judge effects of diet on larval condition for this species. Temperature did seem to have an easily detected effect of length-weight relationships for spotted seatrout. The

Table 3. Lined Sole. *Ninety Percent Confidence Intervals About the Means for Percent Survival, Standard Length, Dry Weight Per Larva and Total Yields of Larvae at 12 Days After Hatching Reared at 28°C on Either a Zooplankton or Rotifer Diet. Confidence Limits are Placed on Means Over All Factor Combinations at the Designated Factor Level.*

	.90 Confidence Limits			
	Percent Survival	*Standard Length (mm)*	*Larval Dry Weight (μg)*	*Total Yield (mg)*
I. Zooplankton Food 28°				
Food Concentration (Stock Density 0.5/l)				
50	*0.0 to 2.0*	*3.31 to 3.33*	*56.6 to 97.4*	*0.0 to <0.1*
100	*2.2 to 12.5*	*3.32 to 3.78*	*87.8 to 161.0*	*<0.1 to 1.8*
1000	*30.8 to 63.2*	*3.97 to 4.49*	*196.3 to 313.1*	*5.3 to 26.2*
Stock Density (Food Concentration - 1000/l)				
0.5	*2.6 to 60.8*	*3.50 to 4.62*	*99.1 to 372.6*	*<0.1 to 4.6*
2.0	*0.0 to 30.0*	*3.12 to 4.52*	*34.5 to 249.3*	*<0.1 to 3.1*
8.0	*0.8 to 38.8*	*3.31 to 4.19*	*93.1 to 229.5*	*<0.1 to 35.9*
16.0	*0.0 to 21.7*	*3.51 to 3.81*	*90.2 to 249.5*	*<0.1 to 10.6*
II. Rotifer Food 28°				
Food Concentration (Stock Density - 0.5/l)				
50	*0.8 to 10.7*	*3.04 to 3.32*	*37.9 to 90.9*	*0.1 to 0.6*
100	*10.1 to 23.1*	*3.16 to 3.46*	*66.4 to 114.6*	*0.6 to 4.0*
1000	*22.5 to 47.3*	*3.40 to 3.78*	*131.7 to 169.8*	*2.9 to 10.5*
Stock Density (Food Concentration - 1000/l)				
0.5	*13.9 to 55.4*	*3.28 to 3.70*	*67.0 to 149.7*	*0.2 to 1.4*
2.0	*3.6 to 32.0*	*3.17 to 3.75*	*40.0 to 167.5*	*<0.1 to 6.9*
8.0	*4.5 to 21.9*	*3.13 to 3.59*	*54.0 to 135.7*	*0.6 to 7.9*
16.0	*1.4 to 23.3*	*2.92 to 3.44*	*67.1 to 134.0*	*0.2 to 20.3*

Table 4. Lined Sole. *Percent Survival and Mean Length of Larvae Reared to 25 Days After Hatching at 28°C on Either a Rotifer-*Artemia *or Zooplankton Diet.*

Food Type (500/l)	*Percent Survival (Stock Density, 0.5/l)*	*Mean Standard Length (mm) (± 1 S.E.)*
Rotifer-Artemia	54.3	7.72 ± 0.11
Rotifer-Artemia	75.7	7.01 ± 0.07
Rotifer-Artemia	40.0	6.84 ± 0.15
Zooplankton	51.4	6.77 ± 0.14
Zooplankton	48.6	6.29 ± 0.11
Zooplankton	70.0	6.38 ± 0.08

Means

Percent Survival	
Rotifer-Artemia *diet*	56.7%
Zooplankton diet	56.7%
Standard Length	
Rotifer-Artemia *diet*	7.19 mm
Zooplankton diet	6.48 mm

"fattest" larvae, regardless of diet type, were reared at 28°C. Larvae at lower and higher temperatures apparently were less well conditioned.

Lined Sole

The only length-weight relationships that were obtained for lined sole were those at 28°C for both the zooplankton and rotifer diet, when food concentrations were 1000 per liter. There were no significant differences in the relationships:

- zooplankton food—$W = 0.60L^{4.03}$
- rotifer food—$W = 0.63L^{4.05}$

Thus, there appeared to be no differences in condition of lined sole when reared at high food concentration for either type of food. The length-weight relationship from pooled zooplankton and rotifer-fed lined sole larvae was $W = 0.60L^{4.06}$.

Conclusions

Both spotted seatrout and lined sole can be routinely reared from hatching through metamorphosis on either a zooplankton or rotifer diet. Survival rates exceeding 50% from hatching to metamorphosis can be predictably obtained. Survival did not differ between the diets, but growth and yield were significantly higher on the zooplankton diet when equal food concentrations were compared. Survival, growth and yield can be predicted with reasonable confidence as a function of food concentration, but with less confidence as a function of stock density. At moderately high food concentrations, the degree of variability in survival and growth responses was similar on either the zooplankton or rotifer diet.

Spotted seatrout larvae survived better at 28° than at 24 or 32°C, but individual growth was best at 32°C. Growth and survival was increased as food levels were raised. Despite poorer survival and growth of individuals at high stock densities, total yields of both seatrout and lined sole increased as stocking density was raised.

A laboratory-cultured diet of rotifers and *Artemia salina* nauplii was adequate to grow spotted seatrout and lined sole well into the juvenile stage with equal survival rates compared to fish fed zooplankton. Growth of spotted seatrout was relatively poor on rotifers-*Artemia* compared to zooplankton, but lined sole growth did not differ between diets. The *Artemia* component of the lab-cultured diet was responsible for the good growth of lined sole on that diet.

Eggs of spotted seatrout from plankton collections or from hormone-induced spawning gave equal survival and growth results in larval rearing experiments.

Growth of spotted seatrout larvae was faster than that of line sole larvae at equal food concentrations. Specific growth rates of spotted seatrout fed zooplankton at 28°C ranged from 44.9 to 69.4% per day over the range of food concentrations that was fed, while lined sole specific growth rates ranged from 20.3 to 38.5% per day. Seatrout larvae growth rates were among the fastest reported for fish larvae, the maximum measured rate being 76.5% per day at 32°C and 1000 zooplankters per liter.

Feeding rates and rations were determined as functions of age and size of larvae as well as of food concentrations, and in the case of spotted seatrout the effect of temperature also was documented. Under the same conditions seatrout larvae consumed more food per unit time than did lined sole. Consumption of rotifers by 2-6 day-old seatrout larvae was higher than zooplankton consumption, but subsequently zooplankton was consumed at higher rates, as larval predatory ability increased. Both seatrout and lined sole larvae could consume much more than 100% of their body weight per day when food concentrations were high.

Length-weight relationships were determined as functions of food concentration, food type (zooplankton or rotifers) and temperature for spotted seatrout larvae. Only temperature had a significant effect on the relationship; the fattest larvae were reared at 28°C. No significant difference was found in the length-weight relationship for lined sole reared at 28° on zooplankton or rotifers at a 1000 per liter food concentration.

A routine culture procedure to provide spotted seatrout or lined sole larvae for aquatic toxicology research could be based upon 28°C rearing temperatures, 1000 per liter food concentrations (either zooplankton or rotifers) and a 5.0 per liter egg stocking density. Growth rates and survival rates of both species are higher at food levels exceeding 1000 per liter, but such levels are unlike any found in nature. If the goal of the toxicological study were to provide a large amount of assay material (i.e., yield), then egg stocking densities higher than 5.0 per liter could be used.

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The complete report, entitled "Marine Fish Larvae Growth and Survival," (Order No. PB 82-101 395; Cost: $9.50, subject to change) will be available only from:

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