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The Effect of Temperature and Food Density on Survival and Growth of *Menidia peninsulae* Larvae (Pisces: Atherinidae)[1]

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ABSTRACT: Day of hatch tidewater silversides, *Menidia peninsulae,* were stocked at 5 fish per liter in 3 l of seawater at 30‰ and raised for 16 days at 20°, 25° and 30 °C. Food organisms (*Brachionus* sp. or *Artemia* nauplii) were maintained at 500, 1,000, 5,000 or 10,000 organisms per l. The influence of food density on growth of larval *M. peninsulae* was temperature dependent. At 20 °C, there was no difference in final size of fish based on food densities. But at 25° and 30 °C there was an increase in final body size as food density increased. There were no significant differences in survival among food densities in tests at 20°, 25° or 30 °C. However, for any given temperature and food density, the number of survivors in a replicate affected the final size attained. Optimal culture condition for larval *M. peninsulae,* considering both survival and growth was determined to be 5,000 food organisms per l at 25 °C.

Introduction

The tidewater silverside, *Menidia peninsulae,* is a fish common to estuarine waters of the Gulf of Mexico. Populations of this fish range from Daytona Beach, Florida, to Horn Island, Mississippi, and from Galveston Bay, Texas, to Tamiahua, northern Veracruz, Mexico (Chernoff et al. 1981). Although not commercially important, these fish represent a significant component of the ecosystems they inhabit (Lucas 1982). *Menidia peninsulae,* once considered a subspecies of *M. beryllina,* was recently separated from *M. beryllina* as a distinct species by Johnson (1975), an action confirmed by Chernoff et al. (1981). *M. beryllina,* which prefers salinities less than 19‰ (Johnson 1975), is now known as the inland silverside while *M. peninsulae,* preferring salinities greater than 15‰ (Echelle and Mosier 1982), is referred to as the tidewater silverside, a name previously given to both species (American Fisheries Society 1980).

Because of the ubiquitous nature of *M. peninsulae* in many estuarine systems along the Gulf Coast and the potential impact of agricultural, industrial and municipal wastes on these systems, the silverside would be an excellent organism for evaluating man-made environmental stress in aquatic ecosystems. However, due to the taxonomic confusion, little information exists on the life history of *M. peninsulae,* and no research has been conducted to determine conditions suitable for rearing *M. peninsullae* in the laboratory. This study was initiated in an attempt to determine optimal culture conditions of temperature and food density for larval *M. peninsulae.* Such information should improve the effectiveness of *M. peninsulae* as a test organism by enabling researchers to reduce mortality in

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controls and at the same time avoid costly overfeeding.

Materials and Methods

A population of adult *M. peninsulae,* collected from Santa Rosa Sound, Florida, was maintained at 25 ± 1 °C and 30‰ salinity in a static, recirculating system in the laboratory. Spawning was induced in this population by manipulating certain environmental parameters, including photoperiod and simulated tidal current fluctuations, and by providing a suitable spawning substrate (Middaugh and Hemmer 1984).

Spawning substrates containing eggs to be used in respective tests were removed from the spawning tanks within 12 h of fertilization and transferred to 30‰ salinity seawater at the temperature (20°, 25° or 30 °C) used in each test. On the day of hatching, larvae were transferred from the incubation container to test containers and placed in a water bath. The bath was cooled or heated to maintain the desired test temperature within ±1 °C.

The study consisted of three tests of 16 days duration. Four food densities: 500, 1,000, 5,000 and 10,000 organisms per l of seawater were utilized in each of the tests. Four replicates of each food density were maintained at 25° and 30 °C. Due to poor hatching success and asynchronous hatching of fish incubated at 20 °C, this test included only three replicates (the first two replicates from one spawn and the third replicate stocked from a spawn occurring two days later). The stocking density chosen for this study was 5 fish per l. This density has been shown to be low enough to avoid inhibition of growth or reduced survival due to crowding (Houde 1973, 1975, 1977). Replicates consisted of 15 fish in three liters of seawater in a beaker. Therefore, there were a total of 60 fish per food density at 25° and 30 °C and 45 fish per food density at 20 °C.

Tests were begun on the day larvae hatched. The mixohaline rotifer, *Brachionus plicatilis,* was used as a food organism for the first 72 hours of each test at 25° and 30 °C, and for 120 h posthatch at 20 °C because of the lower growth rate of larvae at this temperature. *Artemia* nauplii were provided from 72 or 120 hours after hatching until termination of each test on day 16. *B. plicatilis* were fed *Chlorella* sp. algae daily using a procedure similar to that of Theilacker and McMaster (1971). *Artemia* cysts were hatched in large aerated separatory funnels, separated from any unhatched cysts by light splitting, then maintained in an aerated 4 l beaker until used.

The desired food densities were maintained by removing triplicate 25 ml aliquots from each test container with a pipette twice daily, at 0900 and 1600 hours. The 25 ml pipette containing the aliquot was passed under a microscope and the number of food organisms in each aliquot counted. The density of food organisms in the container was calculated from this count and food organisms were then added to bring the density back to the desired level. Records of the number of food organisms fed daily were maintained in order to estimate consumption rates. Fecal matter and other detritus was siphoned from each container as necessary. Light intensity at the surface of the water in each test container was approximately 12,375 lux.

The test system was semistatic, 10% of the volume of each container being replaced with clean seawater on alternate days. Seawater for each test was passed through a 5 μm filter, activated charcoal and U.V. sterilized before use. Deionized water was added as necessary to maintain a salinity of 30‰.

Dead fish were removed from test containers and preserved in 70% ethanol. At the conclusion of each test, all surviving fish were preserved by test group. Standard length and total length were determined with a microscope and ocular micrometer. Wet weight was measured to the nearest 0.01 mg after gently blotting fish with filter paper. Dry weight was determined by placing the fish in a drying oven at 90 °C for 24 h, cooling in a desiccator, then weighing on an electrobalance to the nearest 0.01 mg. Previously, a sample of fish from each spawn had been sacrificed on the day of hatch and 6 to 20 of these fish were measured to determine any differences which may have existed between spawns at hatching.

Analysis of Variance (ANOVA) and Duncan's Multiple Range Tests were applied to data obtained from measurements of test fish in order to determine the influence of

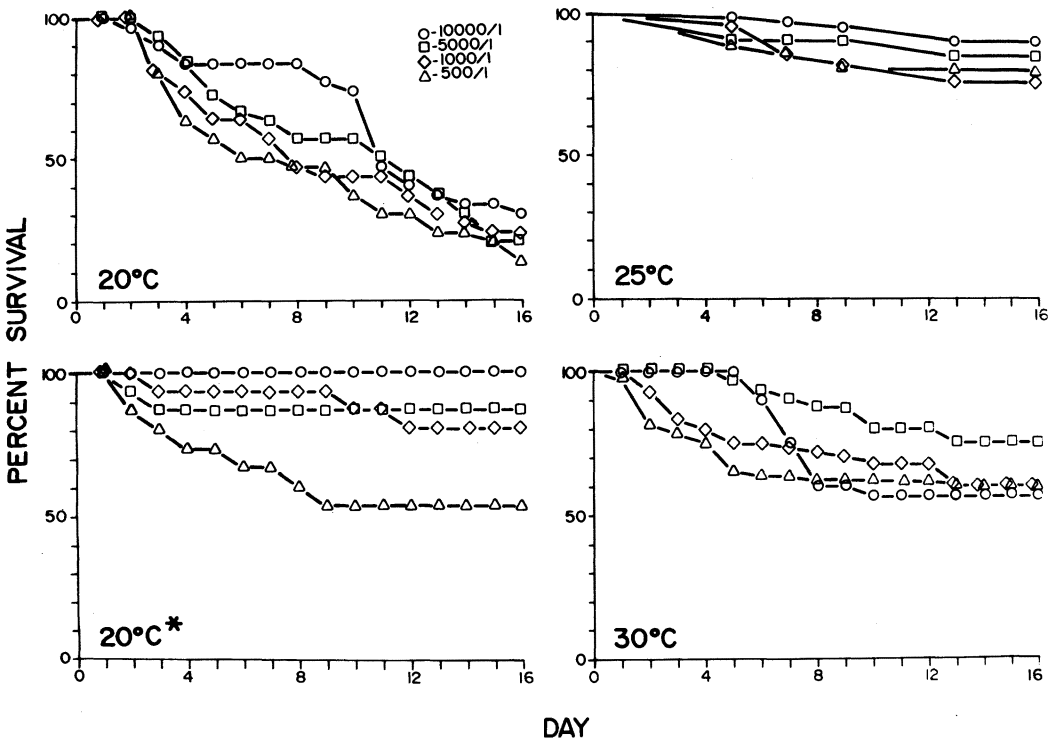

Fig. 1. Relationship between percent survival and test day for *Menidia peninsulae* cultured at four food densities and three different temperatures for 16 days. For the test at 25 °C, survival data were only available for days 5, 7, 9, 13 and 16. For all other tests, survival data were recorded daily. Asterisk denotes second spawn at 20 °C (see text).

temperature and food density on survival and growth (Steele and Torrie 1960; Sokal and Rohlf 1969). Instantaneous growth rates for *M. peninsulae* and other species, based on the exponential growth model (Ricker 1975) were calculated as follows:

$$g = \frac{\log_e W_t - \log_e W_0}{t}$$

where g is the instantaneous growth rate, W_0 is the initial weight, W_t is the final weight, and t is age in days. The weight gains were then expressed as percentage growth per day:

$$\% \text{ per day} = 100(e^g - 1).$$

A model was also developed in order to describe the influences of temperature and food density on growth and surivival of *M. peninsulae*. The model was constructed from a stepwise regression analysis, including stepwise addition (or deletion) of independent variables. Techniques included forward selection, backward elimination and maximum R^2 improvement procedures, all of which determined to what extent inclusion or deletion of a variable improve the model. This improvement is measured in terms of reduction in mean square error and increase in R^2, both of which denote how well the model describes the observed effects. A stepwise regression analysis was performed in an effort to express $\log_{10}$ standard length as a function of temperature, food density, survival and $\log_{10}$ mean day of hatch standard length. A $\log_{10}$ transformation did not appreciably reduce the error term in the ANOVA's applied to each growth and survival test but it did slightly improve the stepwise model and was therefore used. By incorporating day of hatch data into the model, it was hoped that variability due to differences inherent between spawns could be identified. Forward selection, backward elimination and maximum R^2 improvement procedures were all employed and yielded the same end-result.

TABLE 1. Summary of day of hatch data for *Menidia peninsulae* incubated at three temperatures.

Test	No. of Fish	Incubation Time (Days)	Mean Standard Length (mm) (Standard Deviation)	Mean Dry Weight (mg) (Standard Deviation)
20 °C*	15	12	3.986 (0.177)	0.034 (0.005)
20 °C	6	12	3.785 (0.251)	0.023 (0.005)
25 °C	16	7	4.572 (0.272)	0.041 (0.006)
30 °C	20	4	3.749 (0.239)	0.041 (0.004)

Results

Survival

Daily survival of larval fish for each test is given in Fig. 1. For each test temperature there were no significant differences ($\alpha = 0.05$) in survival among fish cultured at different food densities. However, at 25° and 30 °C, for a given food density, the number of fish surviving to day 16 within each replicate was significant ($\alpha = 0.05$) in explaining the variance observed in final size of fish between replicates.

Growth

Due to poor hatching success and asynchronous hatching, larvae from two spawns were used in the test at 20 °C. Fish from the first spawn were used to stock replicates 1 and 2 at each food density, while fish from a second spawn two days later were used to stock a third replicate. There were significant differences ($\alpha = 0.05$) in initial and final length and weight measurements between fish from these two spawns. Survival also differed significantly between the two spawns. For this reason, the third replicate of the 20 °C exposure was treated as a separate treatment being denoted by the term "20 °C*," while the term "20 °C" refers to data obtained from the first two replicates. Length and weight measurements, made on newly hatched fish for each test, are summarized in Table 1.

Standard length and dry weight measurements recorded at the conclusion of each test are summarized in Table 2. At 20 °C, there was no increase in growth with increased food density; however, both length and weight increased as food density increased in tests at 25° and 30 °C. At 25 °C, length and weight of fish maintained at 5,000 and 10,000 food organisms per l were significantly higher than those of fish held at 1,000 per l, which, in turn, were significantly greater than those maintained at 500 per l for 16 days. In tests at 30 °C, fish maintained on 5,000 or 10,000 food organisms per l were significantly larger than those maintained at either 500 or 1,000 per l (Table 2).

Data from the two tests at 20 °C indicate

TABLE 2. Summary of survival and final size data for *Menidia peninsulae* cultured at four food densities and three temperatures for 16 days post-hatch. Means with the same grouping letter are not significantly different (Duncan's Multiple Range Test, $\alpha = 0.05$). Grouping letters are used to compare means within a test and should not be used for making comparisons between tests.

Test	Food Density (Organisms per l)	No. Alive on Day 16/ No. Stocked on Day 0	Mean Standard Length (mm)/ Standard Deviation—Grouping	Mean Dry Weight (mg)/ Standard Deviation—Grouping
20 °C*	500	8/15	7.599/0.604 A	0.206/0.065 A
	1,000	12/15	7.779/0.576 A	0.222/0.066 A
	5,000	13/15	6.574/0.883 B	0.180/0.091 A
	10,000	15/15	8.144/0.640 A	0.234/0.089 A
20 °C	500	4/30	6.569/0.857 B	0.118/0.061 B
	1,000	7/30	5.912/1.473 B	0.124/0.055 B
	5,000	6/30	5.539/0.415 B	0.083/0.033 B
	10,000	9/30	6.235/1.056 B	0.116/0.053 B
25 °C	500	48/60	9.282/0.685 D	0.708/0.236 D
	1,000	46/60	9.782/0.916 E	1.042/0.423 E
	5,000	51/60	10.459/0.819 F	1.474/0.453 F
	10,000	54/60	10.617/0.649 F	1.536/0.338 F
30 °C	500	36/60	9.714/0.941 G	1.287/0.474 G
	1,000	36/60	10.044/1.221 G	1.616/0.773 G
	5,000	45/60	10.848/1.007 H	2.205/0.730 H
	10,000	34/60	11.066/1.327 H	2.243/0.919 H

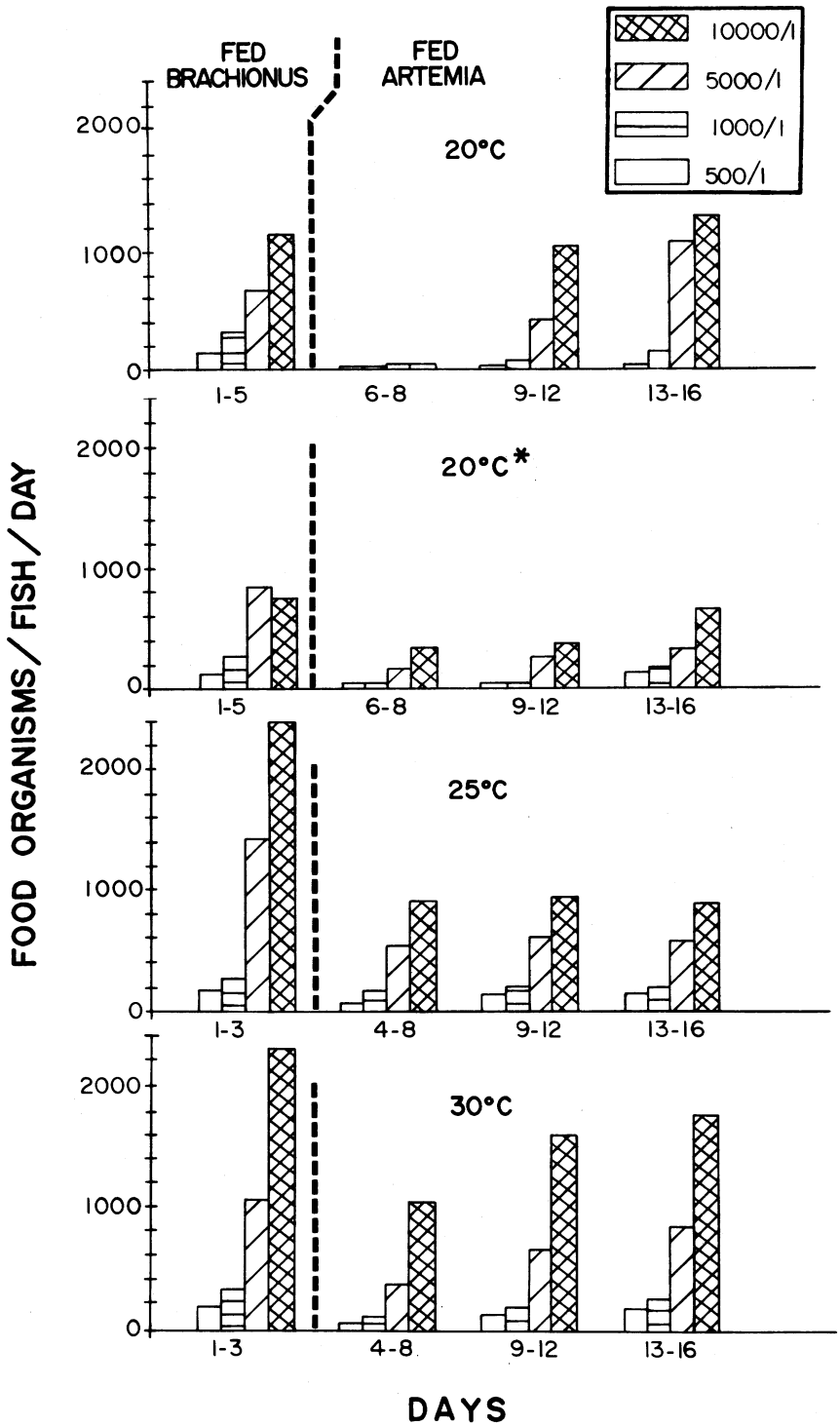

Fig. 2. Number of food organisms consumed per fish per day, over time for each test. The first group of columns in each row represents the period of time during which *Brachionus* sp. were provided as prey. The remaining three groups of columns represent consumption of *Artemia* nauplii. Asterisk denotes second spawn at 20 °C (see text).

TABLE 3. Stepwise regression analysis for the dependent variable $\log_{10}$ standard length for *Menidia peninsulae*. Main effects model $R^2 = 0.6669$.

	df	Sum of Squares	Mean Square	F	Prob >F
Regression	4	1.81466220	0.45366555	20422	0.0001
Error	408	0.90633300	0.00222140		
Total	412	2.72099520			

	B Value	Std Error	Type II SS	F	Prob >F	Contribution to R^2 Total
Intercept	−0.09642380					
T	0.019310438	0.00076156	1.42735289	642.55	0.0001	0.3867
M	0.81252770	0.07510818	0.25997374	117.03	0.0001	0.2096
F	0.00000410	0.00000063	0.09537011	42.93	0.0001	0.0506
N	0.00486006	0.00098075	0.054555001	24.56	0.0001	0.0200

T = exposure temperature.
M = $\log_{10}$ mean day of hatch standard length.
F = food density.
N = number of fish surviving to day 16 for each test beaker.

inherent differences between spawns which limit the validity of comparisons of growth and survival data among test temperatures. However, when final size data for each of the four tests were combined and an analysis of variance (ANOVA) test applied ($\alpha = 0.05$) both temperature and food density within a temperature were significant in explaining the variance associated with the length and weight data. That is, while there was a significant difference in size between fish in the 20 °C and 20 °C* tests, fish from both tests were significantly smaller than those at 25 °C which, in turn, were smaller than those maintained for 16 days at 30 °C.

Consumption

Numbers of food organisms added to test containers daily were recorded for each food density, and divided by the total number of fish alive for the time period to generate estimates of consumption rates per fish (Fig. 2). Examination of Fig. 2 indicates a higher consumption rate of rotifers at 25° and 30 °C than at 20 °C, at least at the higher food densities. Also, the conversion from rotifers to *Artemia* nauplii seems to have occurred more smoothly at the higher temperatures, with consumption rates higher at 25 °C and 30 °C than at 20 °C for the first few days on *Artemia*. Because of their smaller size, fish at 20 °C were allowed to feed on rotifers an additional 48 hours, but conversion to *Artemia* nauplii on day 5 may still have been premature, with many fish unable to prey on *Artemia*. Unsuccessful utilization of *Artemia* nauplii as a prey organism may have been responsible for some of the mortality and reduced final size in the 20 °C and 20 °C* tests. Further studies are needed to determine if a longer culture time on rotifers would alter the observed responses. Consumption rates towards the end of the 16-day test period were more uniform between temperatures.

Predictive Model

A model was developed in order to describe the influences of temperature and food density on growth and survival of *M. peninsulae*. The model chosen included all variables and is given in Table 3. This model is also expressed as the following equation:

$$L = -0.09642380 + (0.01930438)(T) + (0.81252770)(M) + (0.00000410)(F) + (0.00486006)(N)$$

where L = $\log_{10}$ standard length, T = culture temperature, M = $\log_{10}$ mean day-of-hatch standard length for each spawn, F = food density and N = number of fish surviving to day 16 for each test beaker.

Discussion

Several studies have been conducted to assess the effects of temperature, stocking density, salinity and food density on growth and survival of larvae of marine and estuarine fishes. Middaugh and Lempesis (1976) determined the effect of salinity on the percentage emergence of larval *Menidia menidia* at 22 °C and found the highest emer-

TABLE 4. Comparison of length and weight data for this study and those of Houde (1977, 1978). Grouping variables for this study were obtained from Duncan's Multiple Range Tests. Houde's variables were obtained from Student-Neuman-Keuls' Tests. Treatments with the same grouping variable are not significantly different ($\alpha = 0.05$). Grouping variables for comparisons within tests only. N/A = information not available.

Species	Temp.	Food Density (Organisms per l)	Day of Hatch Dry Weight (mg)	Final Mean Standard Length (mm)—Grouping	Final Mean Dry Weight (mg)—Grouping	Wgt. Specific Growth Rate (% per day)
M. peninsulae	30 °C	500	0.041	9.714 A	1.287 A	24.0
(This study)		1,000		10.044 A	1.616 A	25.8
		5,000		10.848 B	2.205 B	28.3
		10,000		11.066 B	2.243 B	28.4
	25 °C	500	0.041	9.282 C	0.708 C	19.5
		1,000		9.782 D	1.042 D	22.9
		5,000		10.459 E	1.474 E	25.1
		10,000		10.617 E	1.536 E	25.4
	20 °C*	500	0.034	7.599 F	0.206 F	12.0
		1,000		7.779 F	0.222 F	12.4
		5,000		6.574 G	0.180 F	11.0
		10,000		8.144 F	0.234 F	12.8
	20 °C	500	0.023	6.569 H	0.118 H	10.7
		1,000		5.912 H	0.124 H	11.1
		5,000		5.539 H	0.083 H	8.4
		10,000		6.235 H	0.116 H	10.6
Anchoa mitchilli	26 °C	50	0.018	8.24 A	0.182 A	15.6
(Houde 1977)		100		7.92 A	0.174 A	15.2
		1,000		10.64 B	0.508 B	23.2
		5,000		11.74 B	0.850 C	27.2
Archirus lineatus	28 °C	50	0.022	3.31 A	0.077 A	8.1
(Houde 1977)		100		3.55 A	0.125 A	11.4
		1,000		4.23 B	0.255 B	16.5
Archosargus	26 °C	10	0.028	4.35 N/A	0.181 N/A	12.4
rhomboidalis		25		4.57 N/A	0.178 N/A	12.3
(Houde 1978)		50		5.39 N/A	0.384 N/A	17.8
		100		6.13 N/A	0.627 N/A	21.4
		500		7.76 N/A	1.521 N/A	28.4

gence rate of larvae was 61% when eggs were maintained at a salinity of 30‰. Emergence was 56% at 20‰ and 47% at 10‰. The effect of delayed feeding on survival and growth of larvae was also determined at 20 and 30‰ at 25 °C. Survival and growth were best for larvae fed *Artemia* nauplii immediately after emergence at 30‰. Bengston (1981) determined growth of post larval *M. menidia* at four temperature regimes and found significant increases in growth as fish were exposed to higher temperatures.

The influence of food density on growth of larval *M. peninsulae* was temperature dependent. Growth of larval *M. peninsulae* at 20 °C was independent of food density or competitive interactions, being solely temperature-limited for the food densities tested. Apparently, growth was inhibited by the low temperature, preventing other variables from playing a role. At 25° and 30 °C growth was a function of food density, with a marked increase in final size ocurring between fish held at 1,000 and 5,000 food organisms per l. Above 5,000 per l, the gain in size with increased food density was not significant. Therefore, using final size as the criterion, optimal culture conditions would be a food density maintained at or above 5,000 per l at 30 °C. However, survival must also be taken into account. In this study, survival was lower at 30 °C than at 25 °C. Moreover, there is evidence that several related species, including *Menidia beryllina* and *Menidia menidia,* cease to spawn at temperatures above 30 °C (Hubbs and Bailey 1977; Middaugh 1981). Gonadosomatic indices suggest that spawning also may be

inhibited in *Menidia peninsulae* when summertime water temperatures exceed 30° to 31 °C (Middaugh and Hemmer, unpubl.). In addition, the effect of survival rate on final size proved to be significant. For a specific food density at 25 °C or 30 °C, an analysis of covariance ($\alpha = 0.05$) showed that the number of fish alive in each test container at the end of the test affected the size to which those individuals grow. Growth appeared to be food-limited with a decrease in the number of fish in the water column reducing competition for prey and increasing the number of food organisms available per fish. Consumption rates may not be good indicators of growth potential. Near the end of the 16-day test period, fish in the 20 °C and 20 °C* tests seem to have accepted *Artemia* as a food source and appeared to feed at a rate not substantially less than fish at 25 °C and 30 °C. However, the size of the fish maintained at 20 °C was significantly less than those at the higher temperatures. Lower temperature may reduce the assimilation efficiency of *M. peninsulae* larvae so that they might consume as many prey organisms as fish in warmer water but be less able to assimilate food. We recommend that *M. peninsulae* be cultured at a food density of 5,000 organisms per l and a water temperature of 25 °C.

In analyzing the stepwise regression model, several observations can be made. From the "Contribution to R^2 Total" column in Table 3, it is obvious that test temperature was the primary factor affecting growth in this study. Also, the size of the fish on day of hatching was significant. However, eggs for each test were incubated at the respective test temperature and incubation (time from spawning to emergence) was affected by temperature differences. At 30 °C, incubation time was approximately 3 days, increasing at 25 °C to 6 days and at 20 °C to 11 days. The exact relationship between incubation time, incubation temperature and size at hatching is unclear and makes the treatment of temperature and day of hatch standard length as independent variables difficult.

Houde (1974, 1975, 1977, 1978, 1980) has examined effects of stocking density and food density on growth and survival of laboratory-reared bay anchovy, *Anchoa mitchilli,* lined sole, *Archirus lineatus,* and sea bream, *Archosargus rhombiodalis.* While it is difficult to compare results of this study with those of other researchers due to differences in experimental designs, some comparisons can be made between our findings and those of Houde (1977, 1978) (Table 4).

Houde's research involved growth tests at several stocking densities of fish for each food density. His results indicated no differences in growth and survival due to larval stocking density. Therefore, values given in Table 4 are the result of pooling data for all larval stocking densities at a given food density. All tests were of 16 days duration and wild zooplankton was used as food.

There are several similarities between weight specific growth rates of *M. peninsulae* cultured at 25° or 30 °C and values reported by Houde (1977, 1978) for *A. mitchilli* and *A. rhomboidalis,* Table 4. Growth rates for *M. peninsulae* held at 25 °C and a food density of 1,000 or 5,000 organisms per l were nearly identical to values reported for *A. mitchilli* grown under similar conditions. However, because of the likelihood of differences in the survival and growth potential of larval marine fishes relative to food concentration, environmental factors, water quality and the volume of test containers, such comparisons must be made with caution.

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