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EPA ENVIRONMENTAL RESEARCH BRIEF

Optimization of Environmental Factors During the Life Cycle of *Mysidopsis Bahia*

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Introduction

The estuarine mysid, *Mysidopsis bahia*, has been established through numerous toxicity tests during the last decade as members of the estuarine community most sensitive to low-level toxicant exposure (for recent reviews refer to Nimmo and Hamaker, 1982; McKenney, 1986a). Because of the relatively short life cycle, this crustacean is an excellent test species for life-cycle toxicity tests permitting not only a comparative evaluation of differential toxicity among the various life stages, but also an assessment of the impact of chronic exposures on ecologically important biological responses, such as brood size and time to sexual maturity. Indeed, for the majority of toxicants examined in life-cycle toxicity tests using this species, a sublethal reduction in reproductive success has proven to be the most sensitive criterion for chronic biological effect.

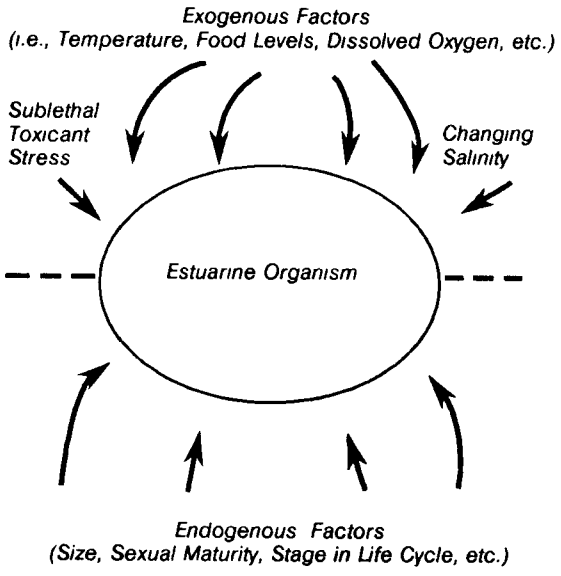
As an estuarine organism, however, *M. bahia* must be able to functionally adapt to a host of dynamically changing environmental variables, characteristic of the complex and harsh estuarine environment (Vernberg and Vernberg, 1972, 1981; Lockwood, 1976). A vast number of continually changing environmental factors (physical and chemical, abiotic and biotic) modify the physiological performance of estuarine organisms, such that, when viewed as a highly integrated system of multiple functional components, the organism is differentially influenced by these environmental variables acting in concert. Tolerance limits for these environmental parameters are controlled genetically and, within these limits, conditions exist for optimal physiological performance. The physiological capacity of these estuarine organisms, in turn, dictates the ecological performance of the population in the natural environment. Therefore, the responses of an estuarine organism to a toxicant are dictated by the simultaneous influences of a number of exogenous and endogenous variables (Figure 1) (Vernberg *et al.*, 1974; Vernberg, 1975, 1985; Lockwood, 1979).

Unfortunately, little is presently known about the basic biological requirements and physiological capacity of *M. bahia* (Mauchline, 1980), due in part to its rather recent identification as a species (Molenock, 1969). With its continued use as a test organism in marine toxicity tests, an increased understanding of this species' optimal values for the dominant environmental variables should further its successful culture while also ensuing adequate assessment of the ecological hazards of the various potential contaminants tested.

Temperature and salinity represent the dominant ecological master factors within the estuarine environment; i.e., these two factors may act either singly or in combination to modify both the physiological and ecological properties of estuarine species (Kinne, 1970, 1971; Alderdice, 1972). Furthermore, temperature and salinity stress have been shown to modify the expression of toxic responses of estuarine crustaceans and to decrease their resistance to toxicant exposure (Vernberg *et al.*, 1973, 1977; McKenney and Neff, 1979, 1981; McKenney and Costlow, 1982). Empirical determination of the optimal salinity-temperature conditions for *M. bahia* should improve laboratory culture of this organism and benefit the application of this species in toxicity assessment by eliminating extraneous environmental stress.

The availability of food has been shown to influence a number of vital life processes of marine and estuarine crustaceans, including functional rates of ingestion, assimilation, growth, and reproduction (Frost, 1972; Grahame, 1983). In addition, several recent studies have demonstrated the interrelationships between alterations in the energy metabolism of assimilated food energy and

Figure 1. **Responses of an estuarine organism to a toxicant are dictated by the simultaneous influences of a variety of endogenous and exogenous variables acting in concert.**



reductions in growth and reproduction of *M. bahia* during sublethal exposure to pesticides (McKenney, 1982, 1985). Identification of the optimal food concentration for the growth and reproduction of *M. bahia* will ensure maximum expression of these physiological processes during their use in the hazard assessment of potential toxicants with this species.

The objective of the research summarized herein was to determine for *M. bahia* optimal values of several dominant environmental variables. Optimal salinity-temperature conditions will be determined for survival of the various life stages of *M. bahia* in a complete life cycle through reproductive maturation, as indicated by appearance of the female marsupial pouch. Feeding densities of *Artemia* nauplii will be determined which will result in maximum growth of *M. bahia* through its life cycle and which, ultimately, will be responsible for maximum reproductive effort. Optimization of these environmental variables during life-cycle toxicity tests with this species should enhance the ecological validity of using *M. bahia* for assessing the ecotoxicological hazards of compounds to the estuarine community.

Approach

Salinity and Temperature Study

The experimental design for the temperature-salinity study was a 5x3 factorial with salinities of 3, 10, 17, 24, and 31‰ and temperatures of 18, 25, and 32°C. Three replicates of 15 mysids each were reared at each of the 15 salinity-temperature combinations for at least 28 days or until appearance of the marsupial brood pouch in female mysids occurred.

The study was initiated by obtaining newly released juvenile *M.bahia* (<24 h old) from ovigerous females maintained in a flowing seawater culture at 24 ± 2‰ S and 25 ± 1°C. To avoid osmotic shock, juvenile mysids to be reared in either lower or higher salinities were transferred at hourly intervals in graded steps of 3-4‰ S from the original salinity of 24‰ S to the appropriate experimental salinity. Mysids to be reared in 24‰ S were transferred directly into an aquarium for the appropriate temperature condition. Water temperatures for all aquaria, originally at 25°C, were allowed to equilibrate to the test temperatures after placement in constant temperature water baths maintaining either 18, 25, and 32 ± 1°C.

Mysids were reared in groups of five in chambers constructed of a 10-cm glass petri dish to which a 15-cm-high cylinder of nylon mesh screen was attached by silicone cement. Throughout the study, all groups of mysids were fed an abundance of freshly hatched *Artemia* nauplii daily. Every third day, the 20 L of seawater in each aquarium was renewed with freshly prepared seawater of the apropriate salinity. Daily observations were made of mysid mortality and day of appearance of the female marsupium.

Feeding Study

Juvenile mysids (<24 h old) were reared in a flowing seawater system at 20 ± 2‰ S and 25 ± 1°C through an entire life cycle (29 days) under various feeding regimes using procedures described by McKenney (1986b). For the reproductive part of the study, three replicates of 15 juveniles each were reared in retention chambers receiving four different feeding regimes until maturation of the female (appearance of the marsupium), at which time the mature female was paired with a male in a smaller brood cup for observations on young production. For growth observations, other groups of mysids were reared in the same manner and subsampled at seven-day intervals for subsequent dry weight measurements.

Since past experience has shown that the feeding rates of mysids increase as they increase in size through their life cycle, feeding levels in each feeding regime progressively increased through the various life stages. Identical procedures were used for daily incubation of hydrated *Artemia* cysts to produce nauplii densities of approximately 600 per ml (mean ± standard error = 598 ± 33). For the four feeding regimes, different volumes of these nauplii were added to each replicate retention chamber in the manner described in Table 1. For the paired adults, 0.5, 1.0, 1.5, and 2.0 mls were added daily to each replicated brood cup for feeding regimes 1-4, respectively. Since the water level within the seawater system varied in the isolated replicate aquaria, water volumes in the juvenile retention chambers varied from approximately 500 to 1700 mls and in the brood cups from 100 to 300 mls.

Statistical Treatment of the Data

Differences in biological responses under the various salinity, temperature, and feeding regimes were analyzed by analysis of variance, using Duncan's procedure for multiple comparisons of treatment means (Zar, 1974). Data from the salinity-temperature study was regressed on the quadratic function of salinity and temperature:

$$Y = b_0x_0 + b_1SAL + b_{11}SAL^2 + b_2TEMP + b_{22}TEMP^2 + b_{12}(SAL \times TEMP)$$

Table 1. **Feeding regimes (in mls) employed in the growth and reproduction study with *Mysidopsis bahia*. Each ml contained about 600 *Artemia* nauplii.**

Feeding Regime	Days in Life Cycle				
	1-3	4-6	7-9	10-12	>12
1	1.00	1.25	1.50	1.75	2.00
2	2.00	2.50	3.00	3.50	4.00
3	3.00	3.75	4.50	5.25	6.00
4	4.00	5.00	6.00	7.00	8.00

where Y = the estimated biological response (either percentage survival or days to sexual maturity in this case); x_0 = the intercept; SAL and TEMP = the linear effect of salinity and temperature; SAL^2 and $TEMP^2$ = the quadratic effect of salinity and temperature; SAL x TEMP = the linear by linear interaction between salinity and temperature; and b_0, b_1, etc. = the regression coefficients (Alderdice, 1972). Response surface curves were generated at a range of levels of these two factors by the General Linear Model (GLM) and PLOT procedures available in the SAS statistical package (Barr *et al.*, 1976).

Results and Discussion

Salinity-Temperature Study

The combined effects of salinity and temperature differentially modified survival of the estuarine mysid, *M. bahia*, dependent on stage in the life cycle. As depicted by the isopleths of percentage survival on the various response surfaces (Figure 2), generated from empirical observations at discrete salinity-temperature conditions, survival of *M. bahia* was influenced by a salinity-temperature-life stage interaction. Juvenile mysids during their first week of life were least resistant to lower salinities and temperatures, being particularly sensitive to salinities below 10‰ S and temperatures below 20°C (Figure 2A). Optimal salinity-temperature conditions for survival of these young mysids was at 20‰ S and 28°C (center of the 95% isopleth), while 95 % survival occurred at the approximate range of 15-28‰ S and 23-32°C. During their second week of existence, juvenile mysids were more resistant to the combined effects of a broad range of salinity and temperature conditions (Figure 2B) and optimal levels for both of these factors was lowered to 17‰ S and 23°C. As shown by the survival isopleths, salinity was the dominant factor affecting survival of juveniles during their second week. After mysids matured during the end of this second week, adults were more resistant to salinity and survival was principally affected by temperature (Figure 2C). These dramatic changes in the resistance patterns of *M. bahia* to salinity during the third week of its life cycle suggest the development of osmoregulatory mechanisms in this estuarine organism concurrent with its maturation period. Older adult mysids, during the fourth week of their life cycle, appeared to develop a susceptibility to higher salinities at temperatures above 20°C (Figure 2D).

The cumulative effects of salinity and temperature on survival of *M. bahia* through a complete life cycle are shown on Figure 3. Through a 28-day period, optimal salinity-temperature conditions for survival of this estuarine species occurred at a salinity of 20‰ S and a temperature of 23°C. The finding that the optimal salinity-temperature values for survival of this estuarine mysid are located at intermediate values within the broad range of these environmental factors seen in a temperate estuary, are consistent with those for other estuarine crustaceans (McKenney and Neff, 1979; McKenney and Costlow, 1982; and references therein).

The time required for reproductive maturation of *M. bahia* was influenced by the salinity-temperature conditions in which this estuarine species was reared (Figure 4). Marsupial brood pouches developed in females in 10.5 days at 18‰ S and 29°C. Both lower and higher salinities delayed the development of these pouches in females by as much as 2 to 3 days. Lower temperatures more than doubled the time necessary for the appearance of the marsupium in female mysids.

Feeding Study

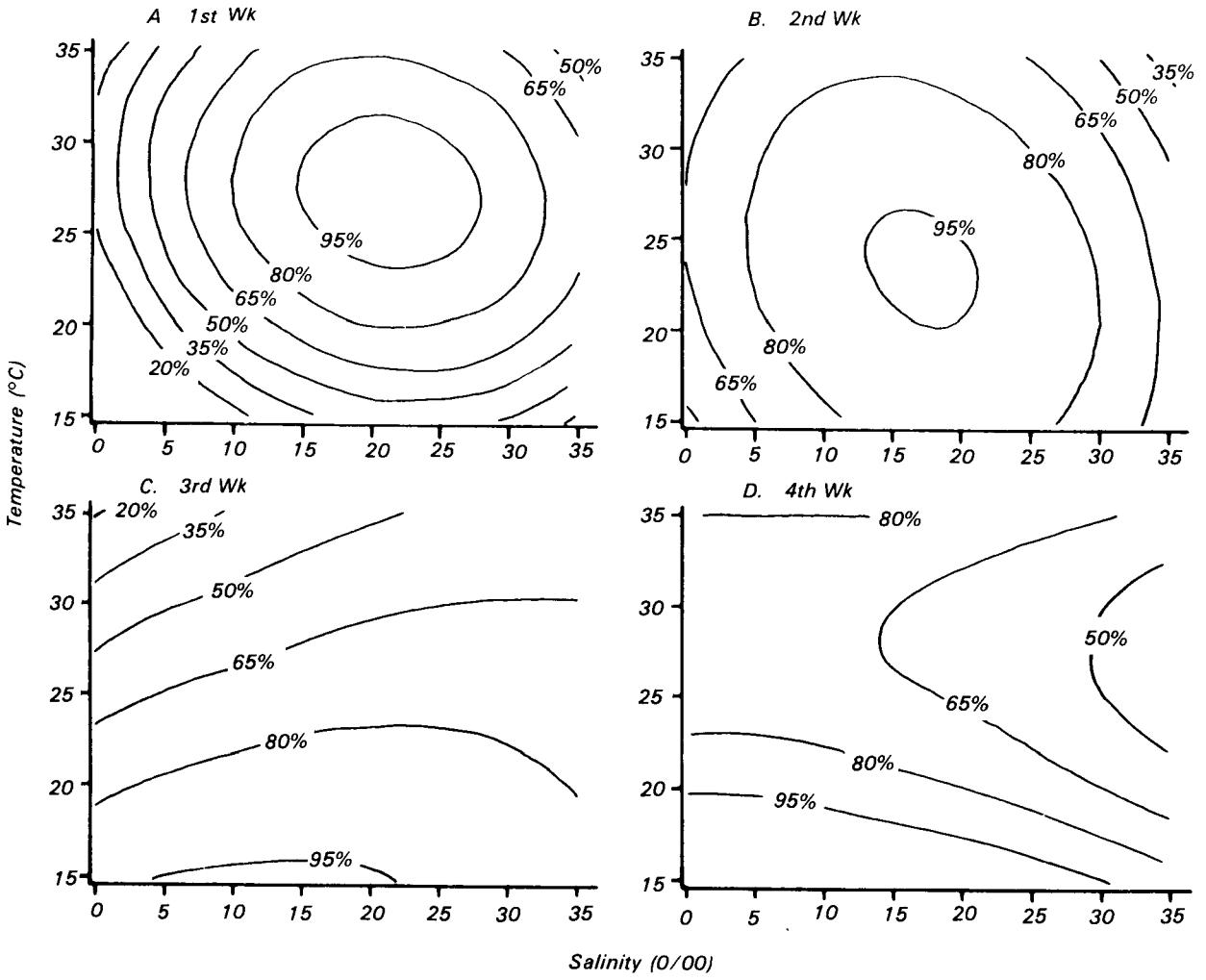
Food availability altered growth patterns of *M. bahia* through its life cycle (Table 2). The four progressively higher feeding regimes produced a linear increase in the size of juvenile mysids after one week. These weights, however, were not significantly ($P<0.05$) different between the various feeding regimes. Two weeks of exposure to the different feeding levels produced significantly smaller mysids at the next to lowest level. At the end of three weeks, again, a direct linear relationship existed between feeding level and dry weights of the mysids. Only the weights of mysids in the lowest feeding level, averaging approximately 0.5-1.0 *Artemia* nauplii per ml of seawater, were significantly lower than the three higher feeding regimes after three weeks of growth.

The availability of food during the complete life cycle of *M. bahia* influenced its reproductive capacity. The lowest feeding regime delayed the onset of reproduction in this mysid species (Figure 5). Mysids being provided with food densities averaging greater than 1 nauplii per ml (Feeding Regimes 2-4) released their first brood a minimum of five days prior to those fed lower average food densities. Young production, both of individual females and of the total mysid population (Figures 6A and 6B), was modified by feeding level. The two highest feeding regimes, receiving average food densities greater than 2 *Artemia* per ml of seawater, resulted in more young being produced. As has been observed in functional responses of other aquatic crustaceans to food concentration (Frost, 1972; Grahame, 1983), there appeared to exist a "threshold" concentration of food, above which the functional response remains maximally stable. For maximum reproductive success in *M. bahia*, this "threshold" concentration appears to be a feeding regime averaging approximately 2-3 *Artemia* nauplii per ml of seawater.

Conclusions

When considering both survival capacity of *M. bahia* through a complete life cycle and time required for juvenile mysids to become reproductively mature, salinity-temperature conditions of 20‰ S and 25°C appear optimal for this estuarine crustacean. Optimization of growth and reproduction in this species requires a feeding density of

Figure 2. **Estimated percentage survival of *Mysidopsis bahia* at weekly intervals through its life cycle based on fitted response surface to observed survival under 15 combinations of salinity and temperature. Curved areas of the isopleths cover those salinity- temperature ranges within which specified survival percentages are predicted.**



2-3 *Artemia* nauplii per ml of seawater. For *M. bahia* this food density results in maximum growth, shortest duration prior to initiation of reproduction, and maximum young production.

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Figure 3. **Estimated percentage survival of *Mysidopsis bahia* through a complete life cycle in 28 days based on fitted response surface to observed survival under 15 combinations of salinity and temperature.**

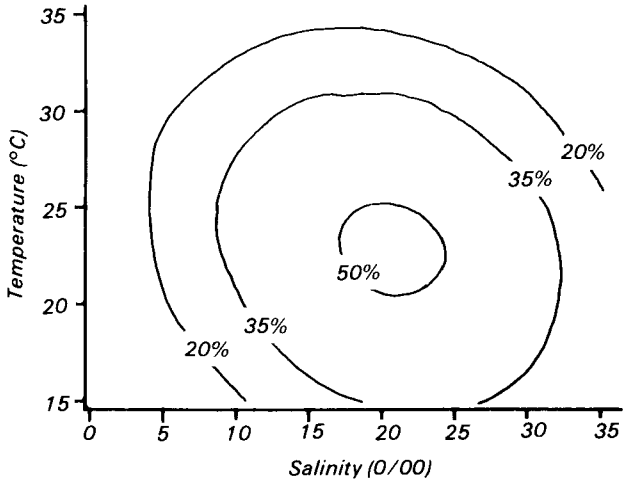

Figure 4. **Estimated number of days required for appearance of marsupium in female *Mysidopsis bahia* based on fitted response surface to observed values under 15 combinations of salinity and temperature.**

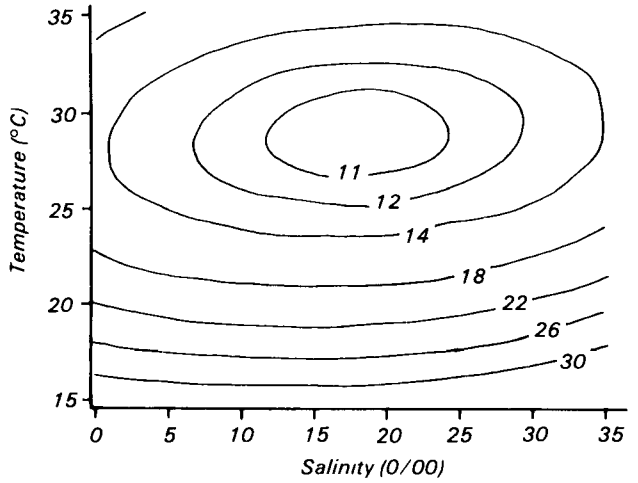

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Table 2. **Dry weights (µg) of different aged Mysidopsis bahia as influenced by different feeding regimes through an entire life cycle. Each value represents the mean ± standard error.**

Feeding Regime	Days in Life Cycle		
	7	14	21
1	148 ± 7	424 ± 16	508[a] ± 35
2	155 ± 9	359[a] ± 20	663 ± 41
3	166 ± 22	479 ± 25	690 ± 24
4	183 ± 9	470 ± 29	723 ± 33

[a] Significantly lower than other dry weights at that day in the life cycle ($P < 0.05$).

Figure 5. **Influence of four different feeding regimes on onset of reproduction in *Mysidopsis bahia*. Columns not sharing same letter (A or B) are significantly different ($P < 0.05$).**

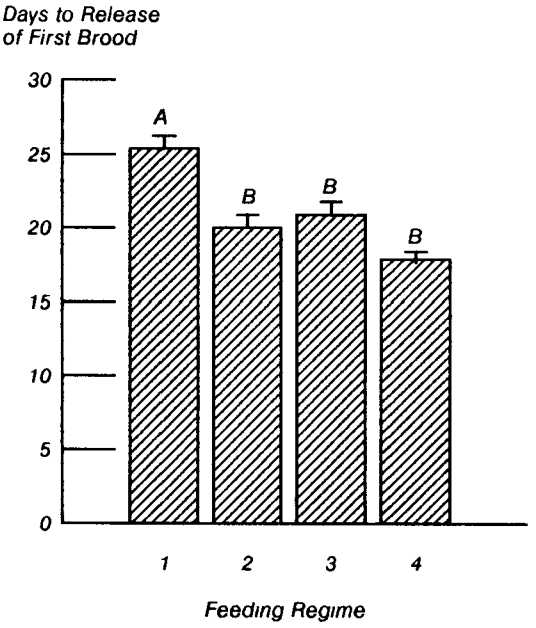

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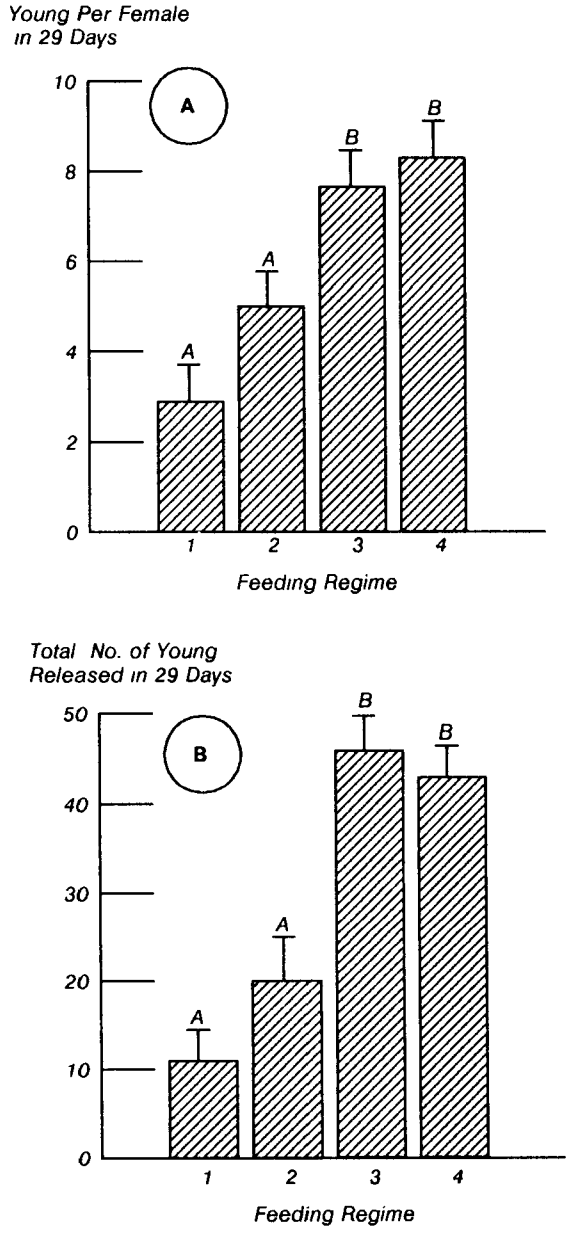
Figure 6. **Influence of four different feeding regimes on the reproductive success of *Mysidopsis bahia*. Columns not sharing same letter (A or B) are significantly different ($P < 0.05$).**



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