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Description of a standard bioassay with the marine crustacean *Mysidopsis bahia* (M.) for the evaluation of the nutritional effectiveness of *Artemia* nauplii and metanauplii

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Abstract

A standardized bioassay is described for the nutritional evaluation of *Artemia* nauplii and metanauplii using juvenile *Mysidopsis bahia.* This test is particularly suited to evaluate the dietary value of different strains and batches of *Artemia.* One day starved, fed or enriched *Artemia* metanauplii can also be tested.

For this test, a homogeneous population of freshly-released juveniles (≤ 24 h-old) were produced in a mysid generator-separator. The test animals were transferred into an experimental apparatus, cultured under standard conditions and offered *Artemia ad libitum.* After 12 days, data on survival, length, individual dry weight, and reproductive characteristics were recorded and treated statistically.

Introduction

Successful rearing of larval stages of aquatic animals is of primary importance for the aquaculturist, the ecologist, and the ecotoxicologist. Reproducible high survival and growth will determine the profit of the first and contribute to the credibility of the results of the latter two. The main factors affecting larval culture success include technological, environmental, and nutritional parameters. Technological and environmental parameters are relatively easy to study and to adapt while so far nutritional factors are poorly understood. Recently, several authors have stressed the importance of the nutritional quality of feeds used in marine larval rearing as an important factor affecting culture success (review in Léger *et al.*, 1986).

The nauplii of the brine shrimp *Artemia* have been widely used as a food source for the larval stages of aquatic laboratory animals and most commercial species so far cultured. However, the availability of different commercial sources of *Artemia* of varying nutritional quality can affect both hatchery production success and results of bioassays (review in Léger *et al.*, 1986).

In order to assess the nutritional quality of *Artemia* nauplii, a standardized bioassay has been developed with the marine crustacean *Mysidopsis bahia* Molenock. This mysid is particularly suited as a test organism because of its sensitivity to toxicants, its ease of culture and handling,

its short life cycle, its small size, its direct larval development, and its ecological importance in estuarine and marine food chains (Nimmo *et al.*, 1977 ; Nimmo and Hamaker, 1982). For these reasons *Mysidopsis bahia* has been accepted and is widely used in acute and chronic aquatic ecotoxicological testing (Bahner *et al.*, 1977 ; Anonymous, 1978 ; Nimmo and Hamaker, 1982). *Mysidopsis bahia* and other mysid species have also been used in energetic studies (Clutter and Theilacker, 1971 ; Reitsema, 1981) and in nutritional research (Johns *et al.*, 1981 ; Léger and Sorgeloos, 1984).

This paper describes a short-term bioassay using newly-released juveniles of *M. bahia* to assess the nutritional value of freshly-hatched and 24 h-old fed and starved *Artemia* nauplii.

Materials and methods

Mysidopsis bahia

Mysidopsis bahia (M.) is a semitropical crustacean (Mysidacea) first described by Molenock (1969). This species has been reported in West Bay, Galveston, Texas (Molenock, 1969) and South Florida (Odum and Heald, 1972). It is an estuarine organism living near the substratum, positively oriented towards the current (Nimmo *et al.*, 1977).

Mysidacea are often called 'opossum shrimp' since they carry their brood from the egg to the juvenile stage in a broodpouch (marsupium, Fig. 1b). *M. bahia* is sexually mature in 10-12 days post-hatch and three spawns may be released by a single female within 20 days of hatching (Nimmo *et al.*, 1977). The adult size varies from 4.4 mm to 9.8 mm (Molenock, 1969) while newly-released juveniles measure about 1.5 mm. After their release, the young are planktonic during the first 24 h (Nimmo *et al.*, 1977) ; thereafter they orient to the current and chase their prey. Not much is known about the feding habits of *Mysidopsis bahia,* however, Molenock (1969) describes the presence of dentated mandibulae and laciniae which allow *M. bahia* to macerate larger food particles.

In 1979 a few dozen adult *M. bahia* were transfered from the EPA Environmental Research Laboratory Narragansett (Rhode Island, USA) to the Artemia Reference Center, Gent (Belgium). Since then a reproducing mysid population has been successfully maintained in a culturing system as desribed by Léger and Sorgeloos (1982) (Fig. 2). At present, the culturing system consists of eleven 100 l and one 300 l aquaria and holds a standing stock of several thousand mysids.

For the bioassay, newly-released juveniles ($\leq$ 24 h) were used. These were harvested in a juvenile generator-separator (Fig. 3) as described in Léger and Sorgeloos (1982). To collect juveniles, gravid females from the stock cultures were transferred to a filter (1 200 µm)-basket (1) submersed in compartment A. The temperature was gradually increased by 2-4 °C, and the system was shaded from the light. Newly-released juveniles ($\leq$ 24 h old) were collected one day later, on the screen (4) in compartment B. These juveniles were then transferred to the experimental apparatus (see below).

Artemia

The bioassay described here was developed for evaluating the nutritional quality of freshly-hatched and 24 h-old *Artemia* nauplii. For comparative tests on different strains or batches of *Artemia* nauplii, all nauplii used as a food source were harvested at the instar I stage. The T_{90}

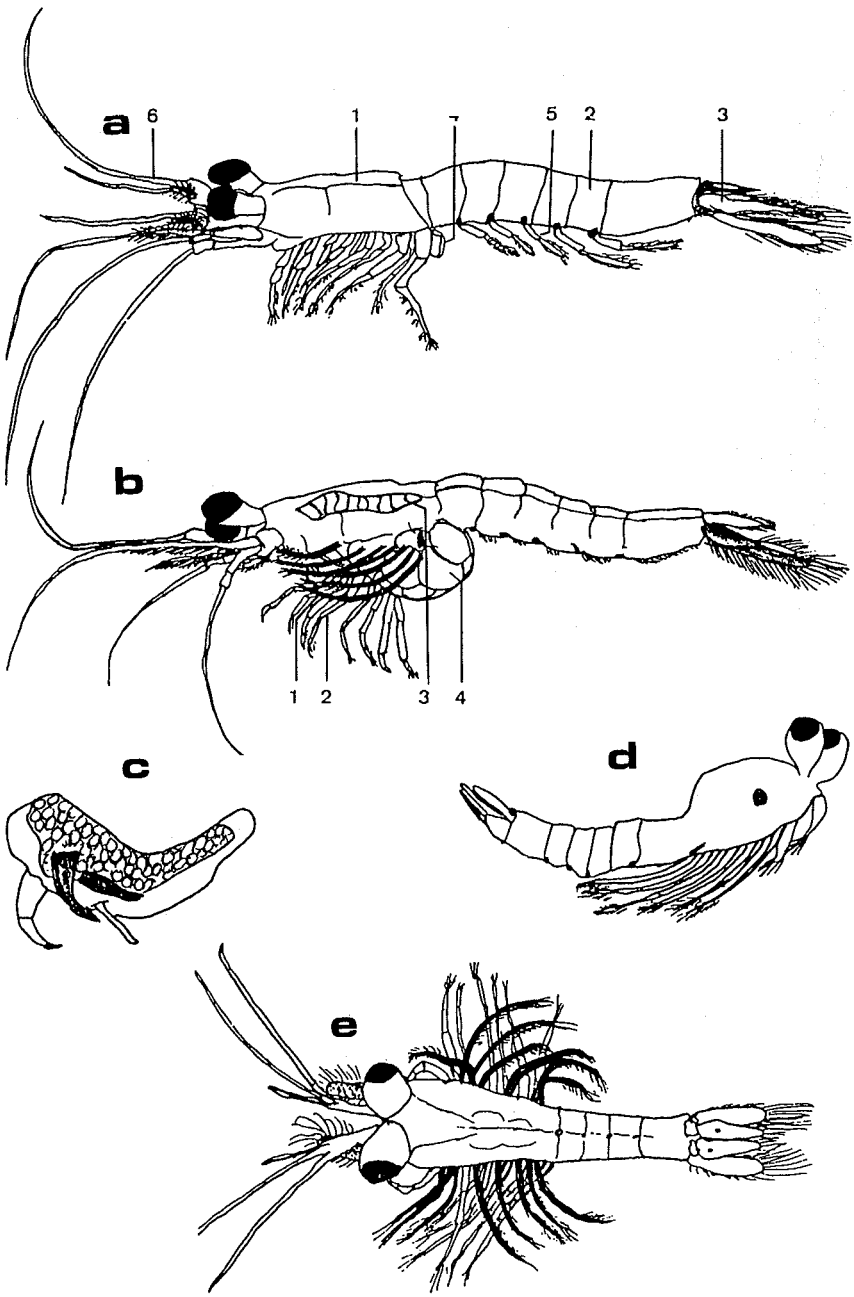

FIG. 1. *Mysidopsis bahia* Molenock. (a) adult male : (1) cephalothorax, (2) abdomen, (3) ciropodes : exo- and endopodite, (4) testes, (5) pleopods, (6) antennae ; (b) adult female : (1) thoracopods : exopodite, (2) thoracopods : endopodite, (3) ovaria, (4) marsupium filled with eggs ; (c) non-eyed juvenile removed from marsupium ; (d) eyed juvenile removed from marsupium ; (e) freshly-released juvenile.

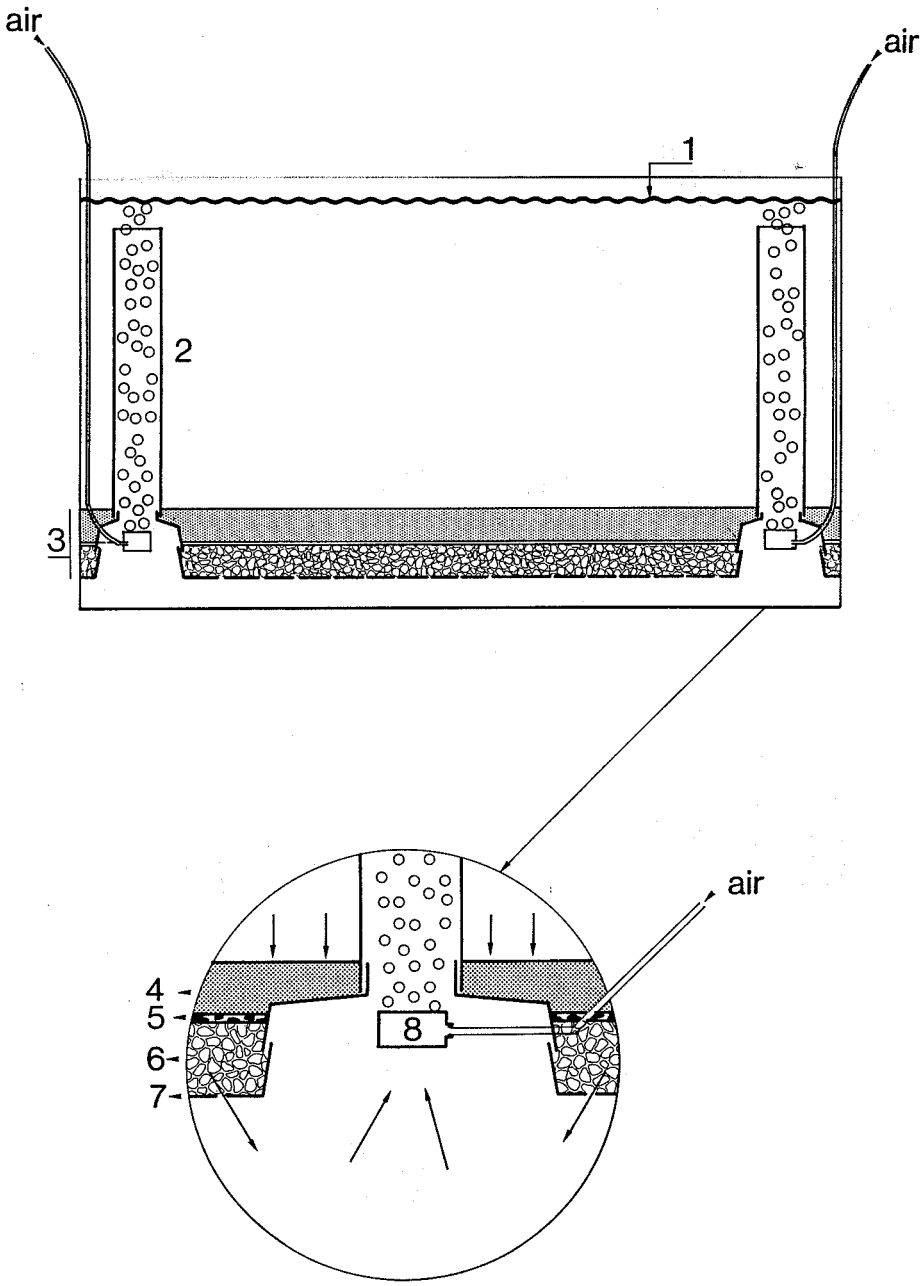

FIG. 2. Schematic diagram of stock-culture aquarium for *Mysidopsis bahia* (M). (1) automatic distribution of cold stored freshly-hatched *Artemia* nauplii ; (2) air-water lift ; (3) sub-gravel bottom filter system ; (4) fine sand ; (5) crushed sea shells ; (6) silex grains ; (7) perforated plastic bottom plate ; (8) air diffuser. (modified from Léger and Sorgeloos, 1982).

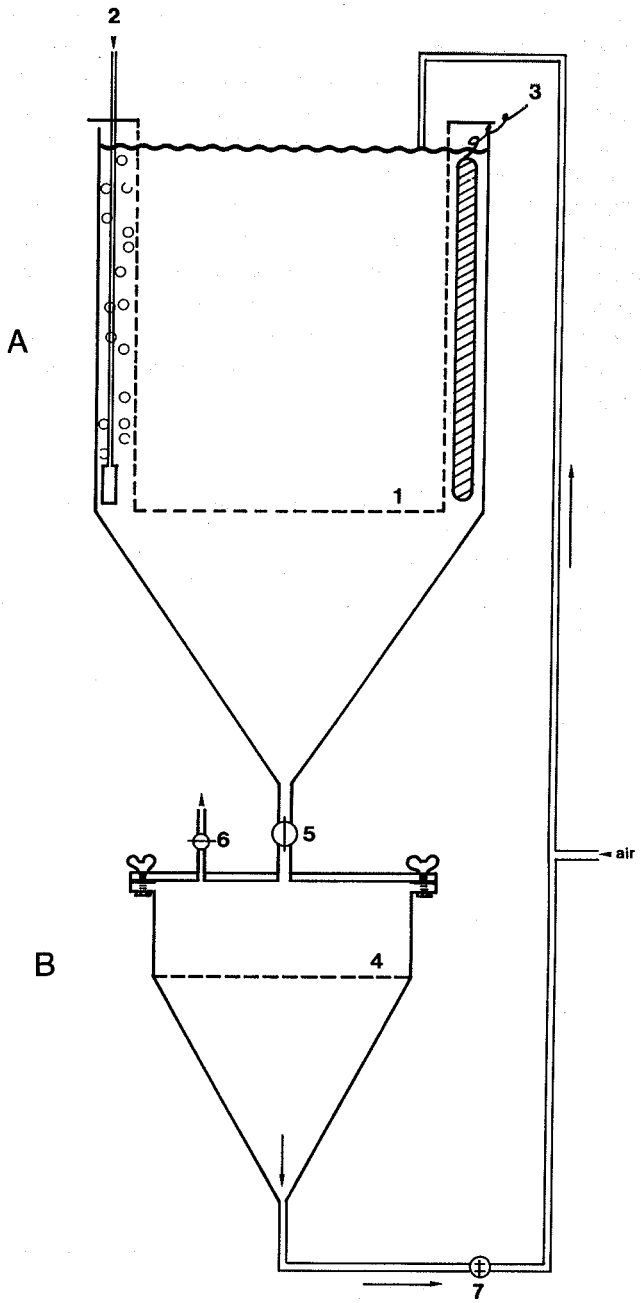

FIG. 3. Schematic diagram of the mysid juvenile generator-separator. (A) incubator for adult mysids retained in a filter basket (1), airline with diffuser (2), thermostat-heater (3) ; (B) separator box equipped with a sieve (400 μm) for retaining the juveniles (4), wide bore stopcock (5), purge valve (6), and air-water-lift with stopcock (7). (modified from Léger and Sorgeloos, 1982).

harvest time for each strain was determined in advance following the methodology of Vanhaecke and Sorgeloos (1983). Hatching of *Artemia* cysts was done in cylindro-conical glass tubes (1 l) filled with 800 ml filtered (1 µm) seawater (natural or artificial, 35 ‰, 25 °C, 2 g cysts/l) under continuous aeration and illumination (about 2 000 lux). At T_{90}, the nauplii were harvested and separated from the hatching debris using a separator box as developed by Persoone and Sorgeloos (1972). After separation, the nauplii were thoroughly rinsed and resuspended in aerated filtered (0.2 µm) artificial seawater made according to the formula of Dietrich and Kalle (1957) (Table I) in a cylindro-conical glass tube of 200 ml. From there three samples were taken with an automatic micropipet and naupliar density was calculated. The nauplii were then fed *ad libitum* to the test animals starting with an initial naupliar density of 2.5/ml. In order to maintain the remaining nauplii in the instar I stage for an eventual second feeding, the nauplii were transferred to a refrigerator and stored under continuous aeration at a temperature of about 5 °C (Léger *et al.*, 1983).

TABLE I

Composition of artificial seawater for *Mysidopsis bahia* bioassay test. (formula modified from Dietrich and Kalle, 1957). This solution was adjusted to 30.0 ‰ using deionized water (total volume approx. 400 l)

Solution A		
NaCl	8 192	g
$MgCl_2 . 6 H_2O$	3 712	g
$CaCl_2$. Anhydr.	394	g
$SrCl_2 . 6 H_2O$	1.37	g
KCl	234	g
KBr	33.9	g
Deionized water	300	l
Solution B		
$Na_2SO_4 . 10 H_2O$	3 105	g
$NaHCO_3$	143	g
NaF	0.103	g
H_3BO_3	0.925	g
Deionized water	40	l

EXPERIMENTAL SET UP

Test chambers

Circular glass bowls (diameter : 15 cm ; h : 7 cm) filled with 600 ml of artificial seawater (see below) and covered with a perforated plastic petridish were used as test chambers (Fig. 4). Each test chamber was randomly stocked with 10 newly-released juveniles (*i.e.* ± 17 cm^2 per test organism) produced as mentioned above. For this, each test chamber consecutively received two juveniles, or five times two in total. Nine replicate bowls were used per treatment (see below). All treatments were randomly assigned to the individual test chambers. The test chambers were placed in a thermostatic water bath at 25 °C ± 0.1 °C. A 14 h light : 10 h dark light regime

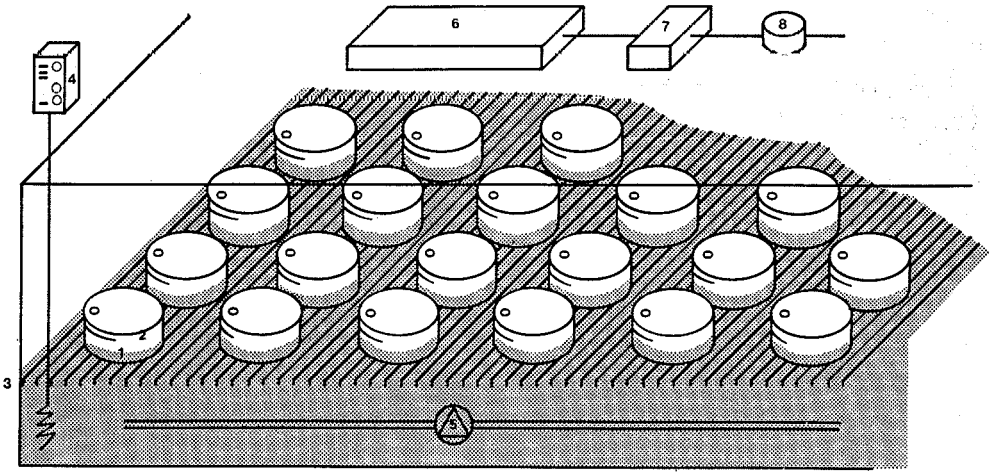

FIG. 4. Schematic diagram of the bioassay test chamber setup : circular glass bowl (1) covered with perforated plastic petridish (2), and incubated in thermostatic water bath (3) equipped with thermostat-heater (4), circulation pump (5), and photoperiod control : diffuse light source (6), dimmer (7), and timer (8).

was applied with a 30 min. twilight transition (electronic dimmer Niro Avidum 5805 equipped with timer). Indirect faint artificial light was used (about 100 lux at the water surface). Preliminary testing indicated that aeration of the test chambers was not required for successful culturing.

One control treatment was included with each bioassay. Instar I nauplii from Reference *Artemia* cysts (Sorgeloos, 1981) were used as an internal standard. The use of Reference *Artemia* cysts makes comparisons with other tests possible and with the results obtained in other laboratories.

The Reference strain has been shown to promote good survival and growth of *Mysidopsis bahia* and other experimental animals (Klein-MacPhee *et al.*, 1982 ; Seidel *et al.*, 1982 ; Léger and Sorgeloos, 1984). A starved control was also included with each bioassay. Mortality in the starved control treatment occurred between day 2 and day 3, and mortality was complete by day 5.

Seawater

Initially, natural seawater was used from the Gulf of Biscaya and diluted to 30 ‰. Preliminary testing indicated that variations in test results could be attributed to the varying quality of different batches of this seawater. To correct for this fact, artificial seawater was tested for culturing *Mysidopsis bahia.* The formula of Dietrich and Kalle (1957) gave good results when the experimental animals were acclimated over a period of two days from natural seawater to the artificial formula. All stock aquaria were switched from natural to artificial seawater. We noticed, however, that in the test chambers the pH of this artificial seawater dropped considerably in the course of a test. We therefore increased the alkalinity of the seawater by increasing the $NaHCO_3$

concentration a 20-fold (Table I). This stabilized the pH between 7.7 and 8.3 during the course of a test. Comparative culture tests with this medium guaranteed reproducible and good results.

Between 400-1 500 l artificial seawater was prepared with chemically pure salts and deionized water before testing. Two separate solutions were made up (A and B, in Table I) and poured together (B into A) under vigorous agitation. The resulting solution was then aerated for 24 h, after which it was filtered through a 1 µm and 0.2 µm cartridge filter. The required volume for one bioassay was kept at 25 °C and oxygenated using airstones.

Feeding and water exchange

In order to harvest instar I nauplii or 24 h-old metanauplii on the morning of each day of the bioassay, T_{90} values were taken into account to incubate the *Artemia* cysts at the appropriate moment. *Artemia* was fed *ad libitum* at least once per day in the morning. Preliminary tests showed that an initial density of 2.5 nauplii/ml was required to provide sufficient food for the mysids. The density for guaranteeing *ad libitum* feeding was adjusted daily and usually a final density of 10-15 nauplii/ml was reached. If needed a second feeding was administered from the chilled *Artemia* suspension (see above).

The calculated volume of the *Artemia* suspension to be fed was filtered over a 110 µm sieve. The nauplii were rinsed and transferred with filtered seawater to the test chambers. Before the first feeding in the morning the remaining *Artemia* (live and dead) were carefully siphoned off along with other debris (dead mysids, faeces, molts) ; special care was taken not to damage the experimental animals. For this a wide-bore glass tube or pipette equipped with a 400 µm screen on one side and a flexible tube on the other side was used. A complete water exchange was carried out on the morning of the 4th and 8th day of the bioassay. Preliminary testing had indicated that more frequent water exchanges (daily or every other day) did not result in a better culture success. To accomplish the water exchange, all surviving mysids from one test chamber were transferred to another test chamber filled with oxygenated, fresh artificial seawater at 25 °C ± 0.1 °C. Mysid transfer was accomplished using a wide-bore pipet or glass tube with an attached rubber bulb or syringe. Transferring the experimental animals was done as carefully and as quickly as possible in order to avoid unnecessary stress.

Duration of the bioassay

In preliminary tests, the results of mysid survival, growth, and reproductive characteristics were compared after 10, 12, and 14 days culture. Differences in survival were not significant. However, growth in terms of final length and dry weight was significantly higher after 12 days than after 10 days. The growth rate attenuated between day 12 and day 14 indicating a decreasing probability for detecting significant differences between treatments. Sexual differentiation of the mysids was about 90 %, 96 %, and 98 % after 10 d, 12 d, and 14 d, respectively. The percentage of females carrying offspring (eggs, non-eyed, and eyed juveniles) (see below) in the broodpouch was respectively 4.5 %, 36 %, and 61.5 %. Average broodsize was about eight (eggs or juveniles) in the three groups. Based on these data a 12-d test period was selected for testing.

Criteria

Survival, growth, and reproductive characteristics of the experimental animals were used to evaluate the culture success.

Survival was followed by recording daily mortality (one check in the morning and one in the afternoon) and survival at the end of the experiment. These data were represented in a graph showing the change in survivorship over time. The final survival value (%) was used for statistical analysis (after arcsine transformation, see below).

Growth was determined by measuring the individual length and dry weight at the end of the experiment. The individual length was determined by transferring the animals from each test chamber to a 7.71 ‰ ammonium formate solution, which is isotonic to 30 ‰ seawater. Through this treatment salts and impurities were washed out from the anaesthetized animals which is essential for later dry weight analysis (ammonium formate is volatile). Since the animals show a sigmoid body curvature, length measurements were made using a dissecting microscope equipped with a drawing mirror. Lines were drawn which follow an imaginary lateral line going from the base of the eyestalk to the tip of the exopodite of the uropodes, excluding the setae (Mauchline, 1973) (Fig. 5). For each replicate all lines were drawn on one page, including a standard (*e.g.* 2 mm micrometer slide). The lines were measured using a curve-meter or digitizer and the individual length was calculated for each treatment.

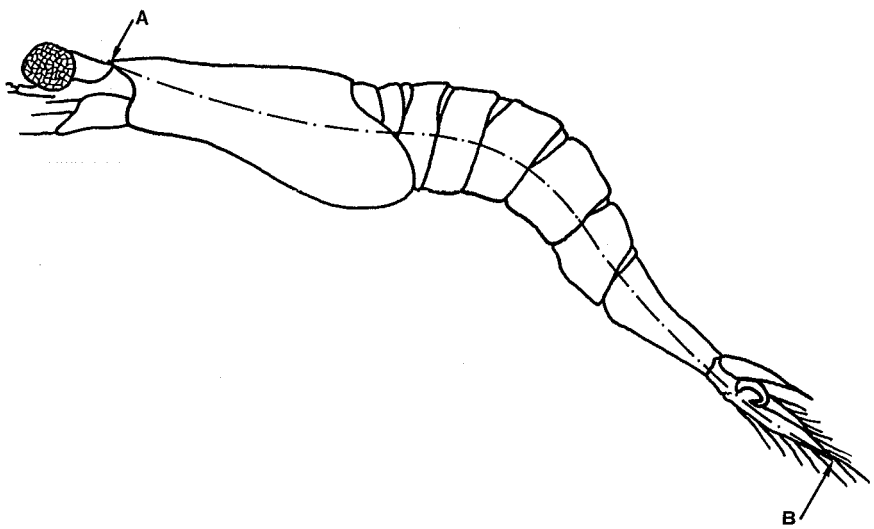

FIG. 5. For length measurements of *M. bahia* a lateral line A-B (dotted) is drawn, following the body curvature from the base of the eyestalk to the tip of the exopodite of the uropodes, excluding the setae.

Once the length measurements were completed, the individual dry weight was determined by transferring the experimental animals from one replicate into a small pre-dried and pre-weighed aluminum cup. Drying was done in a thermostatic oven at 60 °C over a period of 24 h. After cooling in a desiccator, weighing (to the nearest 1 μg) was done on an analytical balance and the individual dry weight was calculated.

The following reproductive characteristics were determined by microscopic observation at the end of the experiment when preparing the animals for length meassurement : sexual differentiation, maturity, and developmental stages of the brood carried by the females.

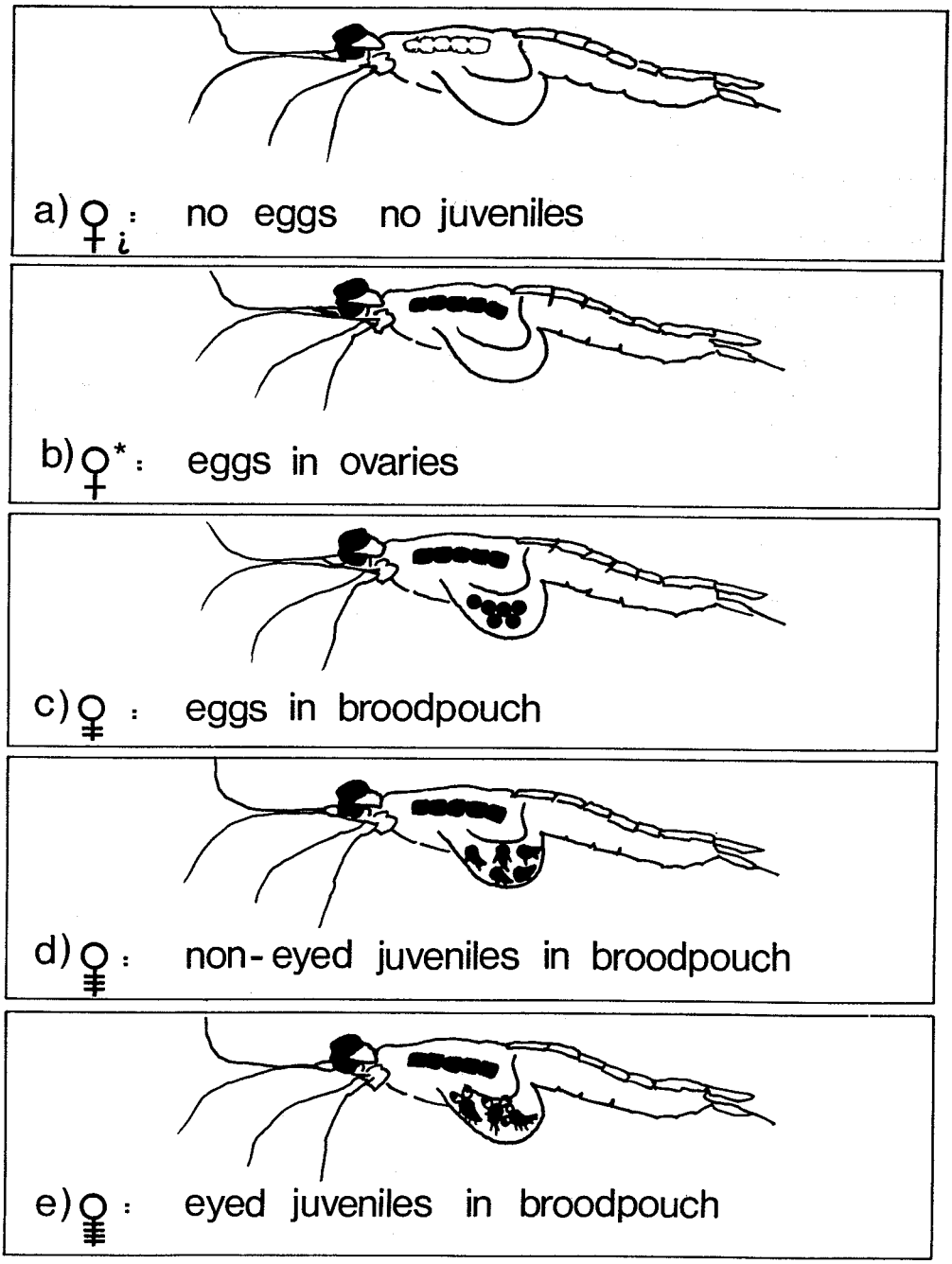

FIG. 6. Reproductive stages in *Mysidopsis bahia* (M.) females : (a) females carrying neither eggs nor juveniles ; (b) females carrying eggs in ovaries ; (c) females carrying eggs in marsupium ; (d) females carrying non-eyed juveniles in marsupium ; (e) females carrying eyed juveniles in marsupium.

Sexual differentiation as a first characteristic divides the surviving animals in two groups (expressed in %) : animals that have developed sex organs (testes, penis, ovaries, broodpouch) and those that have not. The sexually differentiated males and females are then further classified as being mature or immature. Mature males (Fig. 1a) were distinguished by the presence of: 1) two clearly developed testes near the last pair of thoracopods (transition thorax-abdomen) ; 2) presence of penis (difficult to distinguish from pleopods) ; 3) strongly-developed pleopods ; and 4) long antennal flagellae. Mature females (Fig. 1b) were distinguished by the presence of: 1) two functional ovaries and oviducts which appear as tubular crenated organs situated under the thorax above the gastrointestinal organs ; 2) a fully-formed marsupium appearing as a rounded pouch situated at the transition thorax – abdomen ; 3) poorly-developed pleopods ; and 4) antennal flagellae not as long as in mature males. The number of mature and immature animals was expressed as a percentage of the total number of surviving sexually-differentiated animals of the same sex.

Since mysids are transparent, the development of the brood from egg to juvenile can be followed in the female without dissection. The several developmental stages can be distinguished into the following categories (Fig. 6) : a) ovaries and marsupium empty ; b) ovaries filled with eggs and marsupium empty ; c) eggs present in marsupium (see also Fig. 1b) ; d) non-eyed juveniles present in marsupium (see also Fig. 1c) ; e) eyed juveniles present in marsupium (see also Fig. 1d) ; f) juveniles released by female (see also Fig. 1e).

The number of females with brood in the different developmental stages was expressed as a percentage of the total number of surviving sexually-differentiated females. The different classes of females may be represented in a histogram (see example in Fig. 7). Since the number of experimental animals was too small for statistical analysis, the reproductive characteristics were used as a supplementary evaluation criterion.

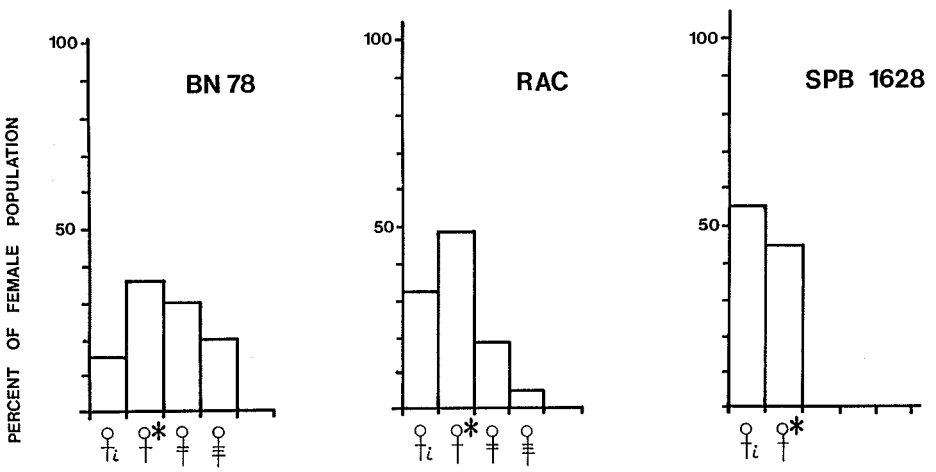

FIG. 7. Histographic presentation of results for reproductive characteristics in female *Mysidopsis bahia* fed three different strains of *Artemia* : Barotac Nuevo, Philippines (BN 78), Reference *Artemia* cysts (RAC), and San Pablo Bay, USA (SPB 1628). Legend to symbols : see Fig. 6.

Data analysis

Data for survival, individual length, and dry weight were analyzed by one-way analysis of variance. Percent survival data were normalized through arcsine transformation (arcsin $\sqrt{p}$, Snedecor and Cochran, 1967). Prior to statistical analysis, the Duncan Multiple Range Test was applied to detect significant differences among means (Goodnight, 1979).

From preliminary tests it was determined that six replicates per treatment enabled us to detect significant differences between means in a multiple range test (*e.g.* Duncan's test in Goodnight, 1979) at a level of $\alpha = 0.05$. For most tests, however, we used nine replicates and were able to detect differences at the $\alpha = 0.01$ level.

Description of testing sequence

day (- 3) : artificial seawater is prepared and aerated overnight ;

day (- 2) : artificial seawater is filtered (0.2 µm), oxygenated (airstones), and kept at a temperature of 25 °C ;

day (- 1) :
a) gravid females from stock cultures are transferred to the juvenile generator-separator. To be sure of obtaining enough juveniles to conduct a test, one gravid female should be incubated for each juvenile to be produced within the following 24 h (see also Léger and Sorgeloos, 1982).
b) daily, from day (- 1) till day (11), enough cysts are incubated for the following day's food requirements. When evaluating 24 h post-hatch (fed or unfed) *Artemia* cysts should be incubated daily, starting day (- 2) ;
c) the experimental set is prepared :
- test chambers, should be thoroughly cleaned (*e.g.* dichromate – sulfuric acid and repeatedly rinsed with deionized water) ;
- thermostatic bath is set at 25 °C ± 0.1 °C ;

day (0) :
a) the newly-released juveniles are harvested from the juvenile generator-separator and distributed at random into the required number of test chambers (nine replicates per treatment) filled with 600 ml seawater ;
b) *Artemia* nauplii are harvested and 1 500 nauplii are fed per test chamber (2.5 nauplii/ml)
The remaining *Artemia* are chilled (*e.g.* kept in refrigerator at 5 °C, see Léger *et al.*, 1983) and eventually used for a second feeding. This is done every day till day (12) ;

days (1-3) :
a) each test chamber is checked individually on a viewer (*e.g.* ground glass with light source below) :
- dead mysids are removed ;
- the surviving mysids are counted and their condition (activity, behaviour) is checked ;
- the remaining *Artemia* density is checked and one decides if a higher density is desirable in order to assure *ad libitum* feeding ;

b) the remaining *Artemia* (live and dead) and other debris are siphoned off and the mysids fed with fresh *Artemia*. The water level in the test chambers should be maintained at 600 ml. These manipulations should be done very cautiously in order not to disturb the experimental animals ;

c) a second check of the *Artemia* density is done in the late afternoon and a second feeding is given when needed ;

day (4) : a) same as day (1-3), a,

b) all surviving mysids are individually transferred to cleaned test chambers filled with fresh seawater and counted. Depending on the individual needs for food (*Artemia* density check, see above) test chambers are fed with *Artemia* nauplii.

c) same as day (1-3), c ;

days (5-7) : same as day (1-3), abc ;

day (8) : same as day (4) ;

days (9-11) : same as day (1-3), abc ;

day (12) : a) mysids in all test chambers are fed with the required number of *Artemia* nauplii ;

b) the bioassay is terminated by performing the following on each test chamber taken out of the thermostatic bath :

- dead mysids are removed and counted ;
- the water level is lowered and ammonium formate (7.71 g/l) is added in order to anaesthetize the surviving experimental animals in the shortest time ;
- mysids in each test chamber are then individually examined for survival, growth, and reproductive characteristics. To accomplish this, (surviving) mysids from one test chamber are transferred to a small Petri dish in an ammonium formate solution (7.71 g/l) and counted. The animals are individually checked under a binocular dissecting microscope. Reproductive characteristics are recorded and the individual lengths are determined (see above).

  All animals from one test chamber are then transferred with a fine forceps into a small predried and tared aluminum cup and placed for 24 h in a drying oven at 60 °C.
- the experimental set up is cleaned and prepared for a new bioassay ;

day (13) : a) individual dry weights are calculated after weighing the dried cups containing mysids ;

b) individual lengths are determined by measuring the curves drawn at day (12) ;

c) all data are treated statistically and a report is prepared.

Example of nutritional bioassay results

The following two *Artemia* strains were evaluated for their nutritional effectiveness for *Mysidopsis bahia* (M.) : a Philippine strain (BN7B) obtained after inoculating San Francisco Bay cysts in local salt ponds (Vos *et al.*, 1984) and a batch (SPB 1628) originating from the San Pablo Bay area (California, USA). Reference *Artemia* cysts (RAC) (Sorgeloos, 1981) were used as a control treatment while another series was starved.

From these results presented in Table II, Fig. 7 and 8, it is clear that the SPB 1628 *Artemia*, as compared to the RAC-*Artemia*, is a significantly inferior food source for *Mysidopsis bahia*. The

Philippine strain, on the other hand, provides excellent survival and results in the best growth (significant at $\alpha = 0.01$ level for individual dry weight). The reproductive characteristics of the test animals fed the three *Artemia* sources confirm the results obtained from determining survival and growth.

TABLE II

Results of a bioassay comparing the nutritional effectiveness of three *Artemia* strains as food for *Mysidopsis bahia*

	RAC	BN78	SPB1628
survival (%)	98.3	96.6	57.1
arcsin $\sqrt{p} \pm \sigma$	$*86.9 \pm 7.5^{aA}$	85.6 ± 10.8^{aA}	49.3 ± 10.2^{bB}
Individual length ($\mu m \pm \sigma$)	$4\,804 \pm 243^{aA}$	$4\,885 \pm 213^{aA}$	$4\,032 \pm 81^{bB}$
Individual dry weight ($\mu g \pm \sigma$)	343.2 ± 76.0^{bB}	438.3 ± 82.9^{aA}	281.4 ± 31.8^{cB}
Reproductive characteristics** (%)			
– Sexual differentiation	98.3	100	57.1
– ♂$_i$	0	0	3.5
– ♀$_i$	31.1	15.4	55.6
– ♀*	47.8	34.6	44.4
– ♀	17.4	30.8	0
– ♀	4.4	19.2	0
– ♀	0	0	0
– Average brood size	5.2	7.1	0

* Means with different superscript are significantly different at the $\alpha = 0.05$ (small letters) or $\alpha = 0.01$ (capitals) level.

** (♂$_i$, ♀$_i$) immature males respectively females ;

(♀*) females carrying eggs in ovaries ;

(♀) females carrying eggs in broodpouch ;

(♀) females carrying non-eyed juveniles in broodpouch ;

(♀) females carrying eyed juveniles in broodpouch.

Conclusions

The bioassay with *Mysidopsis bahia* (M.) is a short, simple, and inexpensive test which can be run on a year-round basis at any location in the world (*i.e.* use of artificial seawater and thermostatic bath at 25 °C). Besides a few aquaria for maintaining stock cultures, only some glassware, a dissecting microscope equipped with a drawing mirror, an analytical balance, a drying oven and some auxiliaries are required to conduct the tests. Both stock culture maintenance and bioassays can be done by one technician. This bioassay has been designed to detect differences in nutritional value among different batches and different strains of *Artemia* nauplii. Following the same procedure, 24 h old fed or unfed metanauplii can be evaluated.

Comparative culture tests with *Penaeus stylirostris* and P. *vannamei* have further shown that the results obtained in the mysid bioassay for *Artemia* nauplii and 24 h enriched metanauplii from San Francisco Bay, San Pablo Bay, and Great Salt Lake may be extrapolated to these commercially important species (Léger *et al.*, 1985, 1987).

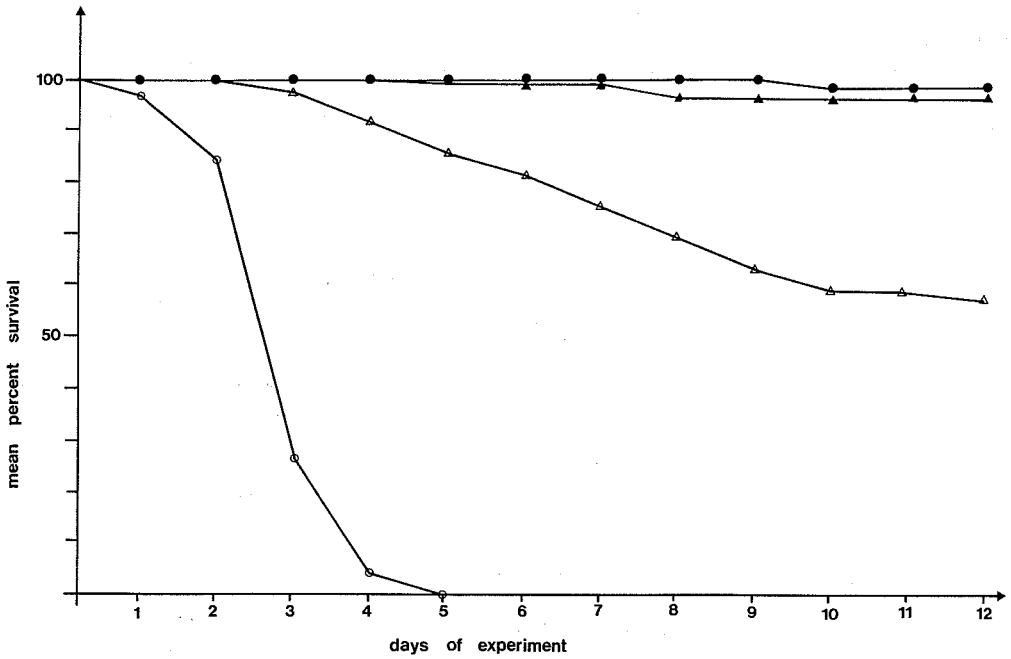

FIG. 8. Evolution of survivorship of *Mysidopsis bahia* (M.) juveniles when starved (○) or fed three different strains of *Artemia*: Reference *Artemia* (●), Barotac Nuevo, Philippines (▲), San Pablo Bay 1628 (CA, USA) (△).

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