

## Nutritional Evaluation of *Artemia* Nauplii for the Culture of Marine Crustacean *Mysidopsis bahia* (M.)

J P ROYAN\*, A ZEMMOURI†, P SORGELOOS & Ph LÉGER

Artemia Reference Centre, State University of Ghent, Rozier 44, B-9000, Ghent, Belgium

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A standard bioassay was carried out for the nutritional evaluation of *Artemia* nauplii of Indian and Sri Lankan strains using newly released juveniles of *M. bahia* (M.). After 12 d, results on survival (91-97%), length (4911-5430  $\mu\text{m}$ ), individual dry weight (373-428  $\mu\text{g}$ ) and reproductive characteristics were recorded and treated statistically. Both Indian and Sri Lankan strains showed high 20:5  $\omega$  3 fatty acid levels and were found nutritionally suitable for feeding larval forms.

Adult *Artemia* and their nauplii are extensively used as a source of live feed for the larvae and juveniles of many species of fish and invertebrates. As commercial fish and shrimp farming activities have increased, there is a considerable and continuing demand for large quantities of dry *Artemia* cysts. This growing demand has led to exploration and exploitation of several new sources of *Artemia*, including successful artificial inoculations<sup>1</sup>. As a result, there are nearly 300 sources spread over the 5 continents from where *Artemia* can be collected.

It has been observed that feeding the brine shrimp nauplii obtained from certain sources to larvae of fishes and crustaceans leads to lethargy and even heavy mortality<sup>2-4</sup>. These instances have been attributed to contamination with DDT, chlorinated hydrocarbons (CHC) and/or lack of essential fatty acids in the nauplii<sup>2,5</sup> used as feed.

In order to assess the nutritional quality of *Artemia* nauplii from various geographical origins, a standard bioassay has been developed using the marine crustacean *Mysidopsis bahia* (Molenock)<sup>6</sup> and nearly 50 strains of *Artemia* have been screened for nutritional evaluation. In this paper, results of a short term bioassay for nutritional evaluation of nauplii from 2 strains of *Artemia parthenogenetica*—one from Hambantota, Sri Lanka and the other from Jamnagar, India—along with a reference *Artemia* as control, using marine crustacean *M. bahia*<sup>7</sup> are presented.

\*Present address: National Institute of Oceanography, Dona Paula, Goa 403004, India

†Office National de Développement et de production Aquacole, BP 32 Rue Colonel Chabou, Bou-Ismaïl Wilaya de Tipaza, Algeria

### Materials and Methods

**Experimental animals**—*M. bahia* is a semitropical crustacean occurring in West Bay, Galveston, Texas and South Florida. The adult females carry their brood from egg to juvenile stage in a brood pouch and sexually mature in 10 to 12 d. The adults range in size from 4.4 to 9.8 mm and the freshly released juveniles measure about 1.5 mm. From juvenile to adult, *Mysidopsis* can be fed freshly hatched nauplii.

For the bioassay, freshly released juveniles of *M. bahia* approximately the same age were used. For this, a juvenile generator-separator<sup>8</sup> was used.

*Artemia* nauplii were hatched from the cysts from India (from saltpans of Tata Chemicals, Mithapur, Jamnagar) and Sri Lanka (from saltpans at Hambantota, Sri Lanka) and reference *Artemia* strain following standard procedures and only instar I nauplii were used as food. After hatching, the nauplii were separated, washed and suspended in aerated and filtered (0.2  $\mu\text{m}$ ) artificial seawater. The total density of nauplii per ml of each strain was determined prior to feeding.

**Experimental set up**—Circular glass bowls (15 cm diam.; 7 cm high) were filled with 600 ml of artificial seawater and covered with perforated plastic petridishes. Each bowl was stocked with 10 freshly released juveniles of *M. bahia*. Nine replicates were used for each strain. The bowls were placed in a thermostatic water bath at  $27 \pm 0.1^\circ\text{C}$ . A 14 h light:10 h dark photoperiod was maintained with a 30 min twilight transition (electronic dimmer Niro Avidum 5805 equipped with timer). From earlier studies, it was learnt that aeration was not necessary.

For the bioassay one starved control was also

run. In this treatment, mortality of the mysid larvae was noticed between 2 d and 3 d and by 5 d total mortality was observed. Instar I nauplii from reference *Artemia* cysts were used as an experimental standard control for the purpose of comparison with other tested strains. On the first experimental day, the test individuals received a ration of approximately 1500 instar I nauplii each. This ration was gradually increased as the mysids grew. On 11 and 12 d, they received 5000 nauplii and at the end of the 12 d, the experiment was terminated. On each day of the experiment, prior to feeding, the dead *Artemia* nauplii, the faeces, moults and dead mysids were removed. Survival of the mysids was recorded daily. On the 4th and 8th d of the experiment, the medium in each bowl was changed with fresh medium. At the end of the experiment, final survival, individual length, dry weight and the reproductive potential of the mysids were analysed. Data obtained were treated in a one way analysis of variance. Significant differences among means were determined using Duncan's multiple range test. Prior to analysis, survival data were normalized through an  $\text{Arcsin } \sqrt{\%}$  transformation<sup>9</sup>.

**Fatty acid analysis**—It was made for the freshly hatched instar I nauplii by capillary-gas chromatography. Nauplii were homogenized with an ultrasonic homogenizer (Sonifer, B 12). Lipid extraction, saponification and esterification were done according to the procedure described by Schauer and Simpson<sup>10</sup>. Fatty acid methyl esters were injected on a capillary column (25 m fused silica, I.D: 0.32 mm, liquid phase: SILAR 10 C, film thickness: 0.3  $\mu\text{m}$ ) installed in a Carlo Erba Fractovap 2330 gas chromatograph. Operating conditions were: solid injector, carrier gas-hydrogen; flow rate—1.9  $\text{ml}\cdot\text{min}^{-1}$ . Peak identification and quantification were done with a calibrated plotter-integrator (H.P. 3390 A) and reference standards.

### Results and Discussion

Survival of *M. bahia* was very high when fed all 3 strains (Table 1). The growth of the experimental animals in terms of individual dry weight was best when fed Indian and Sri Lankan strains as compared to the reference strain. Growth in terms of individual length however was significantly inferior when fed Sri Lankan strain. The reproductive potential (Fig. 1) was high (i.e. highest percentage of female population carrying developed brood) in *Mysidopsis* fed either Sri Lankan or Indian strains as compared to the reference strain. Further, essential fatty acid (EFA)

20:5  $\omega$  3 was higher (Table 2) in Sri Lankan (13.2%; 9.7  $\text{mg}\cdot\text{g}^{-1}$ ) and Indian (8.7%; 9.7  $\text{mg}\cdot\text{g}^{-1}$ ) strains compared to reference *Artemia* strain (4.1% and 5  $\text{mg}\cdot\text{g}^{-1}$ ).

Different geographical strains and batches of *Artemia* nauplii are known to affect the growth, survival and metamorphosis of marine organisms due to differences in energy level, fatty acid composition and residual chlorinated hydrocarbons (CHC)<sup>11</sup>. Watanabe *et al.*<sup>12</sup> have shown that the presence of essential fatty acids especially 20:5  $\omega$  3 (eicosapentaenoic acid) in the *Artemia* nauplii is important for their food value for marine predators especially crustaceans and fish. When a Canadian strain (5.2% 20:5  $\omega$  3) was fed to red seabream *Pagrus major*, 68% of the fish survived but when a San Francisco Bay strain (1.6% 20:5  $\omega$  3) was fed, only 43% survived<sup>13</sup>. These findings disproved the earlier findings of many authors who attributed lethargy, deformity and even mortality

Table 1—Survival and Growth Data for Juveniles of *M. bahia* after 12 d of Feeding Freshly-Hatched *Artemia* Nauplii from Different Origins

| Parameter                           | [Values mean $\pm$ SD] |                   |                  |
|-------------------------------------|------------------------|-------------------|------------------|
|                                     | Reference strain       | Sri Lankan strain | Indian strain    |
| % Survival                          | 92.2 $\pm$ 6.7         | 96.7 $\pm$ 5.0    | 91.1 $\pm$ 9.28  |
| Individual length ( $\mu\text{m}$ ) | 5516 $\pm$ 476         | *4911 $\pm$ 213   | 5430 $\pm$ 225   |
| Individual dry wt ( $\mu\text{g}$ ) | *372.6 $\pm$ 27.2      | 427.5 $\pm$ 35.95 | 413.4 $\pm$ 39.9 |

\*Different at 0.05 level.

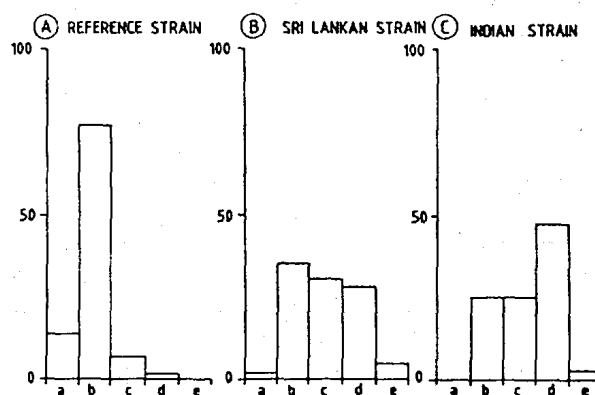


Fig. 1—Reproductive potential of *M. bahia* fed different strains of *Artemia* (Percentage values of different developmental classes within the female population are given: a—immature females; b—females with eggs in ovaria; c—females with eggs in brood pouch; d—females with non-eyed juveniles in brood pouch; and e—females with eyed juveniles in brood pouch).

Table 2 – Fatty Acid Profile of 3 *Artemia* Strains Tested

| Fatty Acid Methyl Ester | Reference strain |      | Sri Lankan strain |      | Indian strain |      |
|-------------------------|------------------|------|-------------------|------|---------------|------|
|                         | A                | B    | A                 | B    | A             | B    |
| 14:0                    | 1.3              | 1.6  | 1.1               | 1.2  | 0.5           | 0.6  |
| 14:1                    | 1.5              | 1.8  | 2.4               | 2.7  | 0.5           | 0.5  |
| 15:1                    | 0.9              | 1.1  | 0.7               | 0.8  | 0.5           | 0.5  |
| 16:0                    | 12.3             | 15.1 | 19.5              | 16.3 | 11.5          | 12.7 |
| 16:1 ω7                 | 10.5             | 12.9 | 12.5              | 14.0 | 12.0          | 13.3 |
| 16:3                    | 1.9              | 2.3  | 2.6               | 2.9  | 2.6           | 2.9  |
| 18:0                    | 2.4              | 2.9  | 4.5               | 5.0  | 4.6           | 5.1  |
| 18:1 ω9                 | 27.1             | 33.2 | 31.4              | 35.1 | 31.0          | 34.4 |
| 18:1 ω7                 |                  |      |                   |      |               |      |
| 18:2                    | 5.2              | 6.3  | 8.7               | 9.7  | 9.7           | 10.8 |
| 18:3 ω3                 | 22.3             | 27.3 | 2.5               | 2.8  | 14.7          | 16.3 |
| 20:1                    | 3.9              | 4.8  | Tr                | Tr   | 1.3           | 1.5  |
| 18:4                    |                  |      |                   |      |               |      |
| 22:1                    | —                | —    | 5.3               | 5.9  | 1.5           | 1.7  |
| 20:5 ω3                 | 4.1              | 5.0  | 13.2              | 14.8 | 8.7           | 9.7  |
| 22:6 ω3                 | —                | —    | 0.6               | 0.6  | 0.4           | 0.4  |

A = area %; B = mg.g<sup>-1</sup> dry wt.; Tr = traces.

in the predators to the levels of contamination with CHC in various strains of *Artemia* nauplii given as feed<sup>2,14-19</sup>. Similarly, an Italian strain and a Chinese strain containing very high DDT levels (422 mg.g<sup>-1</sup> and 172 mg.g<sup>-1</sup> respectively) yielded excellent survival in *Rhithropanopeus harrisi* larvae<sup>20-22</sup>. Léger *et al.*<sup>23</sup> studied the relative impact of CHC and essentially fatty acid (EFA) in *Artemia* nauplii on their food value for marine larvae and concluded that EFAs, especially 20:5 ω 3 determined their nutritional value.

A good quality *Artemia* can be considered to have fatty acid profile with a 20:5 ω 3 content higher than 4% of the total fatty acid methyl esters. Strains of *Artemia* with a 20:5 ω 3 content between 3 and 4% may or may not be good depending on other unknown factors. *Artemia* nauplii with <3% 20:5 ω 3 consistently has yielded poor growth and survival in marine organisms<sup>24</sup>.

The Indian and Sri Lankan *Artemia* strains studied are a suitable food source for *M. bahia* ensuring high survival, high growth rate and increased optimal reproductive potential. Since results in the mysid test may be extrapolated to most commercial marine shrimps and fish species studied (i.e. penaeid shrimps, bass, bream, turbot, etc.) it is suggested that the Indian and Sri Lankan strains may be confidently used by aquaculturists.

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