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Note

Developmental changes in the rock boring sea urchin, *Echinometra mathaei* (de Blainville) larvae exposed to macro algal extracts of *Hypnea musciformis* and *Ulva fasciata*

J. Jean Jose and A. P. Lipton*

Vizhinjam Research Centre of Central Marine Fisheries Research Institute, Vizhinjam – 695521, Kerala, India
**E-mail: liptova@yahoo.com*

Abstract

The influence of methanol extracts of *Hypnea musciformis* (Rhodophyceae) and *Ulva fasciata* (Chlorophyceae) on the development and behavioral changes in the rock boring sea urchin larvae *Echinometra mathaei* was evaluated. *H. musciformis* extract at 0.095 and 0.0095 mg/ml suppressed the development of the sea urchin larvae and retained them in the prism stage itself. In the short-term exposure, larvae up to 5th day were only affected. The extracts of *U. fasciata* did not influence the larval developmental stages at lower concentrations.

The marine macro algal metabolites are known to exhibit medicinal and pharmaceutical properties (Smit, 2004). The rapid evaluation of extracts is highly essential to detect and evaluate the biopotentials, which may influence the biochemical and behavioral responses. In this context, the sea urchin embryos offer one of the effective test models (Hose, 1985). This research note highlights the influence of methanol extracts of *Hypnea musciformis* and *Ulva fasciata* on the larval development of sea urchin, *Echinometra mathaei*.

Materials and methods

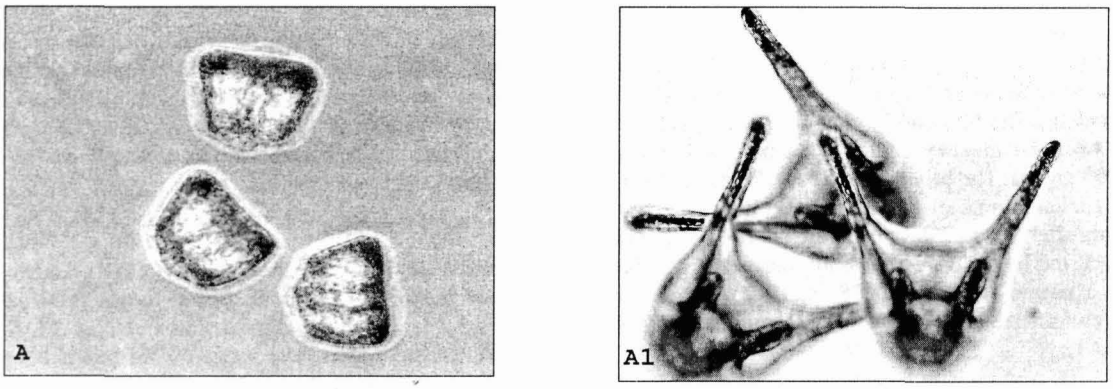
The marine macro algae, *H. musciformis* and *U. fasciata* collected from the Rameswaram (Lat 09° 25' N; Long 79° 20' E) and Thiruvananthapuram (Lat. 8° 22' N; Long. 76° 59' E) coast respectively were shade-dried and finely ground in a mechanical grinder. The secondary metabolites were extracted using methanol (Selvin and Lipton, 2004) and the respective extract was filtered and concentrated in a rotary vacuum evaporator (Buchi) at 40°C. One-day-old sea urchin larvae of *E. mathaei*, obtained by induced breeding (using 0.5 M KCl) were introduced in 2.5 ml of 0.095, 0.0095 and 0.00095 mg/ml of *H. musciformis* and 0.098, 0.0098 and 0.00098 mg/ml *U. fasciata* extract. The larvae were assessed for sensitivity and behavioral changes under 'Continuous' and by 'Short-term' exposures. For the continuous exposure trials, newly hatched prism stage larvae onwards were used and the developmental and behavioral changes were evaluated utilizing the same larvae up to 20 to 22 days or till the control larvae became competent. Each day, half of the medium was replaced and *Isochrysis galbana* was given from 48th h onwards as feed. The experiments were carried out in a humidified chamber to minimize the evaporation loss. For the 'short-term' exposure trials, different stages of larvae were collected from the stocking tank of same lot (from prism stage onwards) each day and introduced in to the specific dilutions of the extracts. The tests were performed for 3 h as triplicates. The developmental and behavioral changes were recorded and compared with the control group kept in filtered (0.2 μ) seawater.

Results

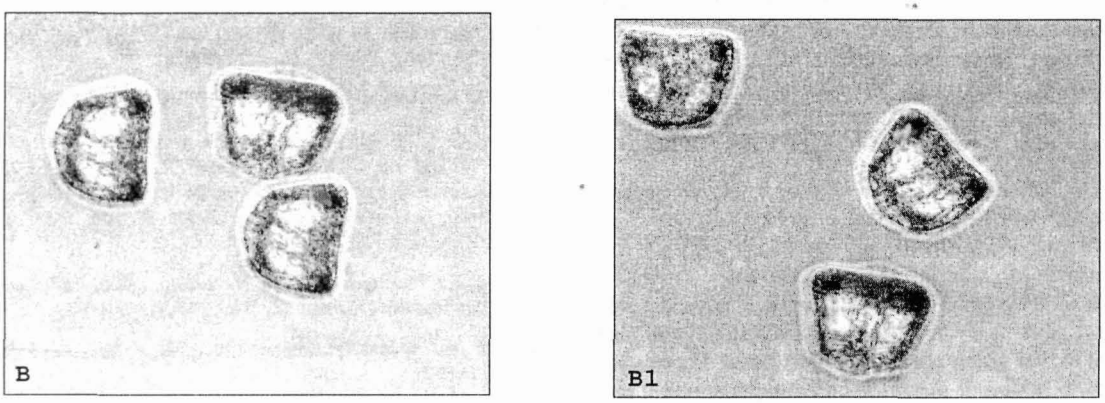
In the continuous exposure trials, the *E. mathaei* larvae remained in prism stage in 0.095 and 0.0095 mg/ml of *H. musciformis* extract up to 48 h and 96 h respectively (Fig. 1 B and B1). Mortality (66.7 %) was noted in 0.095 and 0.0095 mg/ml in two sets in 48 and 96 h respectively when they started developing, except in one set, in which development progressed further together with survival of larvae. Normal development took place in 0.00095 mg/ml (Fig. 1 C and C1) though 66.7% of mortality was noted on 8th day and the remaining developed to free-swimming stage and reached competency on the 20th day. On the contrary, the *E. mathaei* larvae exposed continuously to *U. fasciata* extract in 0.098, 0.0098 and 0.00098 mg/ml were normal in growth, behavior and development. However, 33.3% mortality was noted on 10th day in 0.098 mg/ml.

In the short-term exposure, the duration of the free swimming stage was reduced and larvae started settling at the bottom. All the larvae survived up to the observation period of 3 hours.

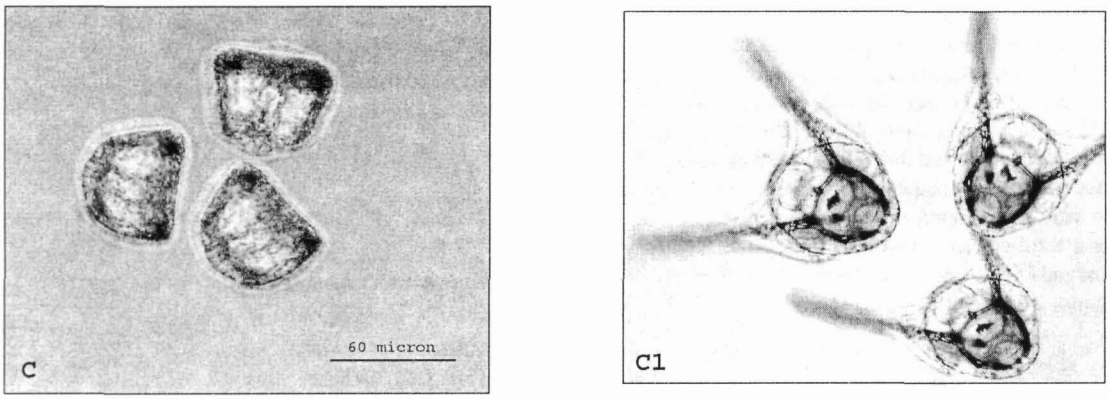
Control groups



Experiment I (*H. musciformis* extract at 0.095 and 0. 0095 mg/ml)



Experiment II (*H. musciformis* extract at 0.00095 mg/ml)



(A 1st day larvae, B 1st day larvae, C 1st day larvae, A_1 24 h larvae (Normal development), B_1 After 24 h larvae (No development), C_1 After 24 h larvae (Development of arm)

Fig.1 Development of *Echinometra mathaei* exposed to *Hypnea musciformis* and *Ulva fasciata* extract

The 1day and 2 days old larvae exposed to 0.095, 0.0095 and 0.00095 mg/ml of the *H. musciformis* extract were active as that of the control. But the activity ceased among the 3 day old larvae and they sank to the bottom at 80 minutes in 0.095 mg/ml, while the similar stage larvae were active and alive in the other two lower concentrations. The 5, 6 and 7th day old larvae sank to the bottom in 30 minutes after introduction in 0.095 and 0.0095 mg/ml. The behavior of 8th to 17th day old larvae was similar to that of the control and they sank to the bottom after 17 to 22 days of fertilization. In *U. fasciata* extract, the larvae were apparently normal in the short-term exposure in 0.098, 0.0098 and 0.00098 mg/ml and the results were comparable to the control set (Fig. I A and A1).

Discussion

The results revealed that *H. musciformis* extract influenced more adversely the development and behavior of *E. mathaei* than the *U. fasciata* extract. The persistence of macro algae in its environmental niche is possible by production of deterrents. The tropical seaweeds produce more deterrents than their temperate counterparts (Cetrulo and Hay, 2000; Nomura *et al.*, 2000; Smit, 2004).

The larvae of *E. mathaei* up to 5th day were only adversely affected during the short exposure to *H. musciformis* suggesting lesser resistance in early stages to the metabolites. Resistance appeared from 6th day onwards as they exhibited normal behavior and developmental patterns during the 8th day to 17th days as compared to that of the control set. This could be correlated to the induction of metamorphosis and substratum preference, which triggers the radical transformation of the morphology, physiology, ecology and behavior in the juvenile stage towards the coralline and regular mixed algae including *Hypnea* and *Ulva* sp. (Rahman and Ueharai, 2001). In the continuous exposure of larvae in 0.095 and 0.0095 mg/ml of *H. musciformis* extract, the larvae remained in prism stage up to 48 h and 96 h. Rahman and Ueharai (2001) inferred that a lectin-like protein diabolin isolated from *Laminaria diabolica* prevented the cleavage of the sea urchin *Hemicentrotus pulcherrimus* by developing a fertilization envelope around unfertilized eggs. Lectins and ketosteroids were isolated from *H. musciformis* (Nagano *et al.*, 2002; Ponce *et al.*, 2002). The caulerpenyne, a sesqueterpene from *Caulerpa taxifolia* affected the larval development and metamorphosis of the sea urchin, *Paracentrotus lividus* (Pesando *et al.*, 1996 and 1998). It also interfered with the microtubule-dependent events during the first mitotic cycle of sea urchin eggs as reported by Pedrotti and Lemee (1996). The exact fraction or fractions of *H. musciformis* extract bringing about the observed developmental changes in *E. mathaei* larvae remains to be evaluated.

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