Early Developmental Sequences of *Aratus pisonii* (H. Milne Edwards) (Brachyura, Grapsidae) under Laboratory Conditions

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ABSTRACT


The larval development of the mangrove tree crab *Aratus pisonii* can exhibit three different sequences when reared under different salinity conditions, using larvae and water obtained from marine and estuarine environments. Such sequences consist of the suppression of one and occasionally two zoeal stages prior to metamorphosis. The occurrence of these sequences and their duration does not appear significantly affected by the origin of larvae or water used, neither by the rearing conditions (e.g., food, temperature, salinity and photoperiod). The adoption of any particular developmental sequence is interpreted as a tendency to increase fitness through developmental plasticity, consequently as an evolutionary trend of the species towards an optional reduction of its pelagic life. This allows some flexibility of response to the varying environmental conditions but it does not constitute an adaptation to any particular habitat because the occurrence of the sequences did not prove to be significantly affected by common environmental parameters.

ADDITIONAL INDEX WORDS: Larval variability, accelerated development, developmental plasticity.

INTRODUCTION

Variability of larval development in decapod crustaceans is well documented in the literature; authors have analyzed extensively its possible causes (COSTLOW, 1965; YATSUZUKA, 1962; BOYD and JOHNSON, 1963; PROVENZANO, 1967; SULKIN, 1978; among others). In most of the studies, occurrence of additional stages is frequent. However, suppression of larval stages has been observed in few cases, mainly in carideans and brachyurans; SANDIFER and SMITH (1979), GORE et al. (1981) and RABALAJES and GORE (1985) have reviewed the phenomenon. Suppression of larval stages of a given species is established when some of the larvae pass through fewer developmental stages than the majority of the larval population, reared under identical conditions before reaching the postlarval stage. The opposite, few individuals passing through larger number of instars, indicates the occurrence of additional larval stages. Among decapods, the Brachyura is the group which exhibits lower variability in the number of larval stages (COSTLOW and BOOKHOUT, 1959; PORTER, 1960; YATSUZUKA, 1962; GORE et al., 1981). This fact is attributed by COSTLOW (1966) to the strong synchronization between morphogenesis and molt frequency, both of which are independent processes. Variation in the number of larval stages could be induced by adverse environmental conditions "which prevent or delay the normal functioning of the endocrine mechanisms" (COSTLOW, 1965), thereby af-
fecting the duration of larval stages or inducing the occurrence of additional stages. Rabalais and Gore (1985) suggest skipped staging as the most common type of accelerated development.

Rearings under laboratory conditions have demonstrated that some external factors seem to be responsible, in varying degrees of importance, for variability during the larval development of several decapods. However, up to now there is not enough evidence for supporting a general conclusion concerning the underlying causes of variability. Works of several authors indicate that both temperature and salinity, combined or not, affect the larval development rate as well as the survival (Costlow and Bookhout, 1962, 1968, 1971; Costlow et al., 1966; Knowlton, 1974; Sandifer, 1973; Dawirs, 1979; Jones, 1981; Anger, 1983; among others). Some studies have implicated other factors such as diet (e.g., Broad, 1957; Regnauld, 1969; Knowlton, 1974; Sulkin and Norman, 1976; Sulkin, 1978; Anger and Dawirs, 1981), parental population (Ewald, 1969; Lucas, 1972; Sandifer, 1973; Sandifer and Smith, 1979), season (Costlow, 1967; Knowlton, 1974; Díaz and Costlow, unpublished data).

The present study was undertaken to determine if parental populations, subjected to different environmental conditions, i.e., estuarine and marine conditions, produce different sequences of larval development. The species chosen for this study is a grapsid commonly found living on the supralittoral zone, on the roots and branches of *Rizophora mangle*, the dominant species at the fringe of marine and estuarine mangrove forests. The larval development of *A. pisonii* has been described by Warner (1968), who reported 1 prezoea, 4 zoeae and 1 megalopa stages, with an approximate duration of 30 days. Variations in duration of larval stages, number of instars or its morphological characteristics were not reported.

**MATERIAL AND METHODS**

Ovigerous females of *A. pisonii* were collected from two geographically separated locations off the Venezuelan coast: 1) from mangrove areas under a marine regime with slight salinity fluctuations, 36.2 ‰ (Morrocoy National Park, 10° 52’ N—68° 16’ W); 2) from mangrove areas associated with an estuarine coastal lagoon, where salinity ranges from 1 to 40 ‰ (Tacarigua Lagoon National Park 10° 20’ N—68° 55’ W). The large salinity fluctuations of this area are the result of daily tidal fluctuations as well as the seasonal rain regime (Gamboa et al., 1971).

Only ovigerous females carrying eggs in late embryonic stage were collected and individually transported to the laboratory in plastic bags containing water from the site of collection. At the laboratory, gravid females were transferred to individual glass bowls and kept in culture cabinets until hatching, which commonly occurred on the night of collection. Temperature was kept at 26 ± 1° C and a 12 L:12Dh photoperiod was maintained.

In order to minimize potential parental effects (Ewald, 1969; Sandifer and Smith, 1979), larvae from simultaneous hatches, obtained from at least five females from the same locality, were pooled. Culture sets of at least 40 larvae from such a pool, showing vigorous swimming, were chosen and individually placed in small finger bowls. This procedure allowed us to follow their larval development uniquely. Offshore water with salinity adjusted to 15, 25 and 35 ‰ S, and mangrove systems water at 25 ‰ S were used in the cultures. Prior to its use in rearings, water was paper filtered, salinity was adjusted either by diluting with distilled water or by freezing in order to concentrate the salt. Not less than five culture sets were used for each treatment and for each group of marine and estuarine larvae (locality). Larvae were fed throughout their development with recently hatched *Artemia salina* nauplii from the San Francisco Bay area (USA). Larvae were transferred daily to filtered and salinity-adjusted water in clean finger bowls; cultures were examined for exuviae; and live individuals of each stage were counted. No antibiotics or fungicides were used.

**RESULTS**

Since *Aratus pisonii* larvae were reared individually, it was possible to observe that megalopa could originate from the IV or the III zoeal stage and in few cases, even from the II zoeal stage. The occurrence of this variable number of zoeal stages was analyzed in relation to culture treatment: salinity, water origin, and locality where the parental adults were collected, respectively.

Frequencies of individuals attaining the megalopa stage, following each of the observed sequences of development in each treatment, were compared by means of the G-test (Sokal and Rohlf, 1981). No significant difference was detected.

among the treatments used \( (G_{0.05}^2) = 0.517 \), for salinity effect; \( G_{0.05}^2 = 1.826 \), for origin of water effect, N.S.; \( P > 0.05 \), in both cases). As shown in Table 1, for all treatments considered, highest frequency of individuals reached the megalopa stage after the zoea IV stage, a lower frequency was observed after the zoea III, and the lowest frequency occurred after zoea II. The latter case occurred in only two of the treatments. The other two sequences were observed in all treatments used, except in the 15 \%/\text{oo} S regime. In this case, larvae from the marine environment did not complete development, and only one larva from the estuarine mangrove reached the megalopa stage. For this reason, results from rearings at 15 \%/\text{oo} S were not taken into account for the statistical analysis. There were more megalops obtained in rearings using waters from mangrove systems.

Time to metamorphosis was compared among the three developmental sequences. For this comparison, it was considered that time spent as zoea I was similar for all cases, regardless of treatment or larval origin (DIAZ and BEVILACQUA, 1986). Thus, time to metamorphosis was estimated as time spent from the zoea II to the megalopa stage through whichever sequence followed, mean values of which are shown in Table 1. For developmental sequences 1 and 2, time to metamorphosis in all treatments were compared by means of the Kruskal-Wallis test, revealing no significant differences \( (H_{17} = 4.913, \text{N.S. and} 5.117, \text{N.S., respectively,} \ P > 0.05, \text{in both cases). However, after comparisons using Kruskal-Wallis tests, time to metamorphosis of larvae under treatments showing the three sequences (A, Dm from Table 1), significant differences were detected \( (H_{121} = 153.030* \text{ and} 29.810*, \text{respectively,} \ P < 0.05, \text{in both cases). This result might be influenced by larvae following sequence 3.}

The Wilcoxon signed-rank tests for comparing mean duration (Table 1) of developmental sequences 1 and 2 for each of the treatments, shows a trend: larvae following sequence 1 (the most frequent) spent larger amounts of time as zoeae. Time spent in the stage prior to megalopa (Table 2) was compared in the same modality as described above. These comparisons indicate that for each particular sequence, treatments do not affect significantly the time spent as the last zoea. However, durations of that particular stage appears to be longer for zoea II than for zoea III, and this was even longer than zoea IV, for those treatments where three sequences were observed (Table 1). This duration of the last zoeal stage appears to be longer in the sequence 3 (the less frequent), which in turn appears to be the way to attain the megalopa stage with less instars.

Table 1. Aratus pisonii developmental sequences in different treatments. Frequency of larvae reaching megalopa stage and mean duration (days) of the process, excepting duration of the first zoeal stage.

<table>
<thead>
<tr>
<th>Treatment (salinity, water origin)</th>
<th>Larval Origin</th>
<th>Initial Number of Larvae</th>
<th>Sequence 1 (z IV to megalopa)</th>
<th>Sequence 2 (z III to megalopa)</th>
<th>Sequence 3 (z II to megalopa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) 25 %/\text{oo} S m.</td>
<td>284</td>
<td>30</td>
<td>13.63</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>B) 25 %/\text{oo} S e.</td>
<td>230</td>
<td>31</td>
<td>12.03</td>
<td>14</td>
<td>11.93</td>
</tr>
<tr>
<td>C) 35 %/\text{oo} S o.</td>
<td>288</td>
<td>4</td>
<td>13.25</td>
<td>3</td>
<td>12.33</td>
</tr>
<tr>
<td>D) 25 %/\text{oo} S o.</td>
<td>288</td>
<td>6</td>
<td>13.00</td>
<td>3</td>
<td>10.33</td>
</tr>
<tr>
<td>E) 15 %/\text{oo} S o.</td>
<td>292</td>
<td>--</td>
<td>--</td>
<td>--</td>
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</tr>
<tr>
<td></td>
<td>238</td>
<td>--</td>
<td>--</td>
<td>--</td>
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</tr>
</tbody>
</table>

m.: mangrove from marine regime
e.: mangrove from estuarine coastal lagoon
o.: offshore

DISCUSSION

The environmental factors indicated in the literature (referred to in the previous section) as responsible for the variability in the developmental pattern of several decapod species might be interacting with the internal (endocrine) mechanism controlling the molting of the larvae. As a result of such interactions, variable number of larval stages, variable length of instars, or both, could be observed. In the long term, variation in duration of larval stages and in number of stages, might be inherited (SANDIFER and SMITH, 1979). Larvae from a single egg batch might show variations in development (KUNISCH and ANGER, 1984); through several generation cultures, it might be tested if alternative developmental patterns and variable zoeal stage duration are heritable characters which have been selected for serving both concomitantly for diminishing death risks. Since environmental factors (i.e., temperature, salinity, availability and quality of food) may vary in temporal and geographical scales, developmental patterns might be expected to vary accordingly. For estuarine species, daily salinity fluctuation might induce delayed effects on the mortality rate or upon the development time of the megalopa as suggested by ROSENBERG and COSTLOW (1979) for Rhithropanopeus harrissii. For species inhabiting different kinds of environments, much of the variability they might exhibit would be due to developmental plasticity of a particular genotype in such environments. PIANKA (1978) proposed that in “unpredictable changing environments, reproductive successes may usually be maximized by production of offspring with a broad spectrum of phenotypes.” Thus, for species with a dispersive larval phase, as in crustaceans, to have phenotypic varieties, would result in a developmental pattern that is selectively advantageous because it allows some flexibility of response to the varying environmental conditions and, therefore, optimize the larval phase and enhances the organism’s fitness.

A dispersive larval phase allows for a wide distribution of the species; new areas could be colonized if the conditions are suitable for the species (MILEIKOVSKY, 1966, 1971; STRATHMANN, 1974). Dispersive larvae could restock existing populations contributing to genetic exchange between geographically separated populations (SCHELTEMA, 1971). Gene flow between and among populations subjected to different environmental factors would result in an increase of genotypic variations.

The internal mechanism controlling molting are expressions of the individual genetic load, which in turn is a consequence of genetic variability. The response of those mechanisms to environmental stimuli, such as amount and quality of food, the presence or absence of organic or inorganic traces dissolved in the culture media, salinity and temperature, could be different for each case or their combinations depending on the particular phenotypic component.

Among crustaceans, the brachyuran larvae show the least variability in the number of larval stages. The occurrence of variable developmental sequences have been reported: the portunids, Callinectes sapidus (COSTLOW, 1965), Neptunus pelagicus and N. japonicus (YATSUZUKA, 1962), as well as the xanthids, Menippe mercenaria, M. nodifrons (PORTER, 1960; SCOTTO, 1979), Micropanope bar-

<table>
<thead>
<tr>
<th>Treatment (salinity, water origin)</th>
<th>Larval Origin</th>
<th>1 (z IV to megalopa)</th>
<th>2 (z III to megalopa)</th>
<th>3 (z II to megalopa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) 25 /%o S</td>
<td>m.</td>
<td>4.83</td>
<td>5.38</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>e.</td>
<td>4.61</td>
<td>6.36</td>
<td>7.50</td>
</tr>
<tr>
<td>B) 25 /%o S</td>
<td>m.</td>
<td>5.11</td>
<td>4.14</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>e.</td>
<td>4.33</td>
<td>5.50</td>
<td>--</td>
</tr>
<tr>
<td>C) 35 /%o S</td>
<td>m.</td>
<td>4.50</td>
<td>6.33</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>e.</td>
<td>4.85</td>
<td>5.67</td>
<td>--</td>
</tr>
<tr>
<td>D) 25 /%o S</td>
<td>o.</td>
<td>4.33</td>
<td>5.00</td>
<td>11.50</td>
</tr>
<tr>
<td></td>
<td>e.</td>
<td>5.36</td>
<td>8.60</td>
<td>--</td>
</tr>
</tbody>
</table>

m. : mangroves from marine regime  e. : mangroves from estuarine coastal lagoon  o. : offshore

Table 2. Mean duration (days) of the last premetamorphic stage of three development sequences of Aratus pisonii.
badiensis (Gore et al., 1981), the grapsids Eriocheir japonicus and Sesarma dehaani (Yatsuzuka, 1962) and the ocypodid Uca subcylindrica (Rabaïais and Cameron, 1983) may exhibit supernumerary stages. Attaining the megalopa stage after fewer zoeal stages does not necessarily mean that developmental time would be considerably shortened consistently (Gore et al., 1981), the variability observed in the larval developmental sequence of Aratus pisonii consists in a frequent “skipping” of one or, less frequently, two zoeal stages, prior to metamorphosis. Such variations might involve shortening of their developmental time (Rabaïais and Gore, 1985). Different reproductive strategies in marine invertebrates have been widely discussed, and several models have been proposed to interpret the general cases (Vance, 1973a, b; Underwood, 1974; Strathmann, 1974, 1977; Christiansen and Fenchel, 1979). These works suggest the existence of a compromise between a larval development with a large planktonic life, allowing a wider dispersion (Mileikovsky, 1966, 1971) but also an increase in the time of exposure to predation, accidents and starvation (Strathmann, 1978); and a shorter planktonic development with a concomitant decrease in larval mortality but at the same time restricted dispersal (Palmer and Strathmann, 1981).

From our results it is difficult to indicate a clear cause of the observed variability; however, some of the factors mentioned above could be discounted. Diet used in our experiments was the same for all rearings, the amount of early Artemia nauplii exceeded the daily ingestion capacity of the larvae. The achievement of the complete larval development of A. pisonii suggests that the diet used was adequate for the species (Díaz and Bevilacqua, 1986). Since temperature and photoperiod were similar for all culture series, the occurrence of the developmental sequences appear as independent of these parameters. The estuarine condition and the presence of mangroves in a lagoon of relatively low water circulation (as in the Tacarigua Lagoon), mangroves in a marine bay with higher water flow (as in Morrocoy National Park) or, finally, marine waters (about 10 km offshore), might be assumed to represent three degrees of “mangrove influence” (in terms of organic or inorganic dissolved substances) or, at least, as three different kinds of water which did not affect significantly the adoption of any particular developmental sequence or their duration.

Phenotypic variations expressed in the larval development of A. pisonii are exhibited as a survival gradient response according to the origin of the larvae and the salinity of culture water. This survival gradient was interpreted as a selective advantage because the implicit tolerance to salinity changes (Díaz and Bevilacqua, 1986).

Since the developmental sequences and their mean duration did not exhibit significant differences with regard to larval origin, it seems that the variability observed for A. pisonii does not correspond to an adaptation to any particular habitat. It seems that having different developmental sequences with different durations for a particular rearing condition may be important in terms of species dispersion and in terms of death risk involved. Such a risk emerges from nutritional vulnerability (sensu Sulkin, 1978), the probability of becoming a suitable prey for other zooplankters, or physiological malfunctioning in the days near the molting date.

An extended time in the last zoeal stage for the case of a more direct developmental pattern (i.e., megalopa after zoea II) does not result in an extension of the entire larval development time. The death risk is presumably being reduced when planktonic life is shortened. It appears as a strategic compromise, where dispersion might have a higher selective value. Those larvae exhibiting a shorter developmental time would insure restocking a population closer to their parental population. The wide distribution of A. pisonii suggests an effective and extensive larval dispersal, which appears to be supported on this three developmental sequences system. However, Warner (1968) did not find variability in the larval development of A. pisonii from Jamaica, this argues in favor of geographical variability. Consequently, it appears as necessary to test if such changing developmental sequences would be exhibited by the species along its ample geographical distribution.

Even if the causes of the developmental sequences observed in A. pisonii are not wholly elucidated, the problem can be focused on in terms of its evolutionary implications. Sulkis (1978), in his analysis of larval development of brachyurans, suggested that long pelagic periods, with large and variable numbers of molts and large amount of eggs per brood might induce nutritional vulnerability of the larvae due to the absence of favorable prey. This, as suggested by the same author might be considered a characteristic of primitive reproduction. Thus, there would be an evolutionary tendency towards a reduction of the pelagic phase (planktotrophy) and an increase of the embryonic
period (lecithotrophy) diminishing the food dependency or nutritional vulnerability. Consequently, there would be a reduction in the number of larval stages, duration of larval life, an increase in size of the larva at hatching and, finally, a lesser number of embryos produced per brood.

Since the adoption of alternative and shorter larval history patterns by A. pisonii does not seem to depend upon environmental parameters, maintaining developmental plasticity seems to be a sound strategy to increase fitness, and consequently an evolutionary trend of the species towards an optional reduction of its pelagic phase.

CONCLUSIONS

(1) Three larval developmental sequences were observed in laboratory rearings of Aratus pisonii using larva and water obtained from marine and estuarine environments. Sequences consisted of four, three and two zoal stages prior to megalopa; the latter sequence was very infrequent.

(2) Neither the adoption of any particular developmental sequence exhibited by A. pisonii, nor its duration, was significantly affected by the origin of larvae or water, neither by the rearing conditions, diet, temperature, salinity and photoperiod.

(3) Due to the independence shown by the adoption of sequences from environmental parameters and origin of A. pisonii larvae, adaptation to any particular habitat should not be invoked for explaining these developmental sequences. The adoption of any alternative larval history pattern is interpreted as a tendency towards enhancing fitness through developmental plasticity, and consequently as an evolutionary trend of the species towards an optional reduction of its pelagic life.

ACKNOWLEDGEMENTS

We are grateful to J. Alió from Universidad Simón Bolívar, Caracas, to J.E. Conde from Universidad Francisco de Miranda, Coro, Venezuela, and to unidentified reviewers for their valuable suggestions on the statistics and on the manuscript.

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El desarrollo larval del cangrejo de mangle Aratus pisonii puede exhibir tres diferentes secuencias cuando la crianza se realiza bajo distintas condiciones de salinidad, utilizando larvas y aguas obtenidas de ambientes marino y estuarino. Dichas secuencias consisten en la supresión de una, y ocasionalmente dos fases de zoea, sucediéndose entonces la metamorfosis. La ocurrencia de estas secuencias y su duración no parece ser afectada significativamente por el origen de las larvas o del agua utilizadas, ni tampoco por las condiciones de crianza (es decir: alimentación, temperatura, salinidad y fotoperiodo). La adopción de cualquier secuencia de desarrollo en particular se interpreta como una tendencia al aumento de la adaptación a través de la plasticidad de desarrollo, como consecuencia de una tendencia evolutiva de la especie hacia una reducción opcional de su vida pelágica. Esto permite cierta flexibilidad de respuesta a condiciones ambientales cambiantes, pero no constituye una adaptación a un habitat en particular, ya que la ocurrencia de las secuencias no mostró ser significativamente afectada por los parámetros ambientales comunes.