

**VARIABILITY IN THE LARVAL DEVELOPMENT OF
METASESARMA RUBRIPES (DECAPODA, GRAPSIDAE)
REARED IN THE LABORATORY**

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ABSTRACT

Larvae of the brackish water crab **Metasesarma rubripes** (Rathbun) originating from a mangrove area in Paraná (southern Brazil) were reared in the laboratory from hatching to metamorphosis. Larval morphology was compared with a previous description of material from Venezuela (Díaz & Ewald, 1968). Morphological differences were observed and described in all larval instars, suggesting the existence of different geographical races within this widely distributed species. Moreover, **M. rubripes** from Brazil (unlike that from Venezuela) reveals a variability in its developmental pathways, with 4 or 5 zoeal stages preceding the megalopa. The relative frequency of these pathways within a hatch was found to differ between hatches originating from different females, suggesting that the number of developmental instars may depend on genetic or other maternal factors. Only those larvae that had passed the first three zoeal stages relatively fast (compared to sibling larvae from the same hatch) were able to develop directly from the zoea IV to the megalopa, whereas relatively delayed larvae tended to pass through a zoea V stage. Morphological comparison between zoea IV larvae developing directly to a megalopa with those passing through an additional zoeal instar shows that the former are not only

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faster in development but also morphologically more advanced than the latter. The zoea V of **M. rubripes** is described for the first time; it reveals morphological characters that are intermediate between the less advanced form of zoea IV and the megalopa. Variability in developmental pathways is interpreted as a possible adaptation to extremely variable environments, where developmental flexibility may increase the chance to encounter a suitable habitat for settlement and metamorphosis.

Key Words: Crustacea, **Metasesarma rubripes**, developmental pathways.

RESUMO

Variabilidade no Desenvolvimento Larval de Metasesarma rubripes (Decapoda, Grapsidae) Criada em Laboratório. Foram criadas em laboratório larvas do caranguejo de água salobra **Metasesarma rubripes** (Ratbun) procedentes de uma área de manguezal no Paraná, desde a eclosão até a metamorfose. A morfologia das larvas foi comparada com uma descrição anterior de material da Venezuela (Diaz & Ewald, 1968). Foram observadas diferenças morfológicas e descritas em todos os estádios larvais, sugerindo a existência de diferentes raças geográficas desta espécie que está amplamente distribuída. **Metasesarma rubripes** do Brasil, ao contrário da espécie da Venezuela, revela uma variabilidade nas vias de desenvolvimento, com 4 a 5 estádios de zoea precedendo 1 de megalopa. A frequência relativa destas duas vias em larvas de uma mesma mãe e em outras originadas de diferentes fêmeas varia muito sugerindo que o número de estádios de desenvolvimento pode depender de fatores genéticos ou outros fatores maternos. Só aquelas larvas que tinham passado os três primeiros estádios larvais relativamente rápido (comparando com larvas da mesma mãe) desenvolveram diretamente de Zoea IV a megalopa; entretanto, as larvas que desenvolveram-se mais lentamente tenderam a passar por um estádio de Zoea V. Comparações morfológicas entre larvas de Zoea IV que desenvolveram diretamente a megalopa com aquelas que passaram por um estádio adicional, mostram que as primeiras não são só mais rápidas no desenvolvimento como também morfológicamente mais avançadas que

as primeiras. É descrita a Zoea V pela primeira vez, revela caracteres morfológicos intermediários entre as formas menos avançadas de Zoea IV e a megalopa. A variabilidade nas vias de desenvolvimento é interpretada como uma possível adaptação a ambientes extremamente variáveis, onde a flexibilidade no desenvolvimento pode aumentar a possibilidade de se encontrar um habitat apropriado para instalação e metamorfose.

Palavras-chave: Crustacea, **Metasesarma rubripes**, vias de desenvolvimento.

INTRODUCTION

The Brachyuran family Grapsidae shows a world-wide distribution, being very common also in South America. Some of its species live in oceanic, others in brackish, in freshwater, or in semiterrestrial conditions (Hartnoll, 1964, 1965; Burggren & McMahon, 1988). Their majority, however, lives in brackish water of estuarine areas. One species belonging to this family is **Metasesarma rubripes**. According to Hartnoll (1965), it is one of the most terrestrial crab species of mangrove ecosystems. He observed that it sometimes shares burrows with **Uca** spp. or **Cardisoma guanhumi**. Its larvae were reared in the laboratory and described for the first time by Diaz & Ewald (1968), who used material from Venezuela. These authors found consistently four zoeal stages and a megalopa. **M. rubripes** is very common also in the Paranaguá estuary near the CBM laboratory (Pontal do Sul, Brazil), and its larvae are found very frequently in plankton samples from this area (Montú, unpubl.). We reared its larvae again, in order to check them for possible morphological deviations from the caribbean material described by Díaz & Ewald (1968).

MATERIAL AND METHODS

Females of **Metasesarma rubripes** were caught in a mangrove swamp near Pontal do Sul and transferred to aquaria in the CBM laboratory, where they were maintained at 25-27°C and ca. 32‰S until larvae hatched. The larvae were mass-reared in glass

bowls with ca. 400 ml of seawater that had been filtered with Whatman GF/C glass fiber filters. Temperature was kept constant at $25 \pm 1^\circ\text{C}$ and light at a 12:12 h L:D regime. Salinities of 5, 10, 15, 25, and 32‰S were tested in a preliminary experiment. Water and food (freshly hatched San Francisco Bay Brand *Artemia* sp. nauplii) changed daily.

One experiment (at 32‰C) was carried out under individual rearing conditions, with 25 larvae in separate vials (30 ml), in order to obtain exact information on development duration in each stage.

In another experiment (also at 32‰S), we tested in larvae that had developed through the first three zoeal stages with a different speed, if the subsequent developmental pathway differed in relation to their previous rate of development: larvae from the same hatch (same age since eclosion from the egg, same mother) were separated from mass cultures on 5 subsequent days, when they moulted to stage IV, and placed in individual confinement (numbered vials, as above). Later, their development to the zoea V or megalopa, respectively, was recorded.

For morphological examination, samples of larvae and exuviae were fixed in 4% seawater-formaldehyde. Later, at least 20 individuals of each larval stage were dissected under Wild stereo microscopes. Drawings were made with the aid of a camera lucida attached to a Leitz compound microscope.

RESULTS

In our experiments, *Metasesarma rubripes* revealed two different developmental pathways. One, with four zoeal stages, was identical with that described before by Díaz & Ewald (1968); other revealed an additional developmental instar (zoea V). Both pathways occurred within the same hatch (larvae from the same female) reared under identical conditions.

All larvae reared at 5 or 10‰S died in the first zoeal stage, without developing any further. Metamorphosis to the first juvenile occurred only at salinities ≥ 15 ‰S, most frequently in full seawater (ca. 32‰S).

Development duration at 25°C and 32 ‰S was measured (in individual culture) in a second hatch:

Zoea I:	4.5 ± 0.5 d
Zoea II:	3.5 ± 0.6 d
Zoea III:	3.5 ± 0.7 d
Zoea IV:	4 d
Zoea V:	3 d
Megalopa:	12 d

Due to mass mortality (for an unknown reason; only one surviving larva) after stage III, no standard deviation can be given for later instars. Somewhat longer development time in the first as compared to the following zoeal stages suggests that the early larvae were too small to feed efficiently enough on *Artemia* nauplii. This phenomenon has been observed frequently in species with a very small first larval stage.

In 4 different hatches reared under identical conditions (32 ‰S), conspicuously different proportions of larvae developing through 4 or 5 zoeal instars, respectively, were observed. In one hatch, only 3 individuals passed through a zoea V (2 of them successfully), whereas in another hatch there was a preponderance of the longer pathway (through zoea V) and in two hatches the two different developmental pathways occurred with an approximately equal frequency.

The experiment in which we separated larvae according to their time of development from hatching to the zoea IV stage showed clearly that only the fastest larvae developed directly to the megalopa, whereas those moulting later to the zoea IV passed through an additional instar (Fig. 1). No difference, however, could be detected in the survival rates of these two groups, i.e. the number of larval moults was not related to viability.

Development duration was in most of these experimental groups significantly shorter in zoea IV larvae moulting to zoea V (3.1 ± 0.5 to 3.6 ± 0.5 d) than in those moulting directly from

zoea IV to the megalopa (4.1 ± 0.9 d), or in those moulting from the zoea V to the megalopa (3.8 ± 0.4 to 4.2 ± 0.8 d in different groups, respectively).

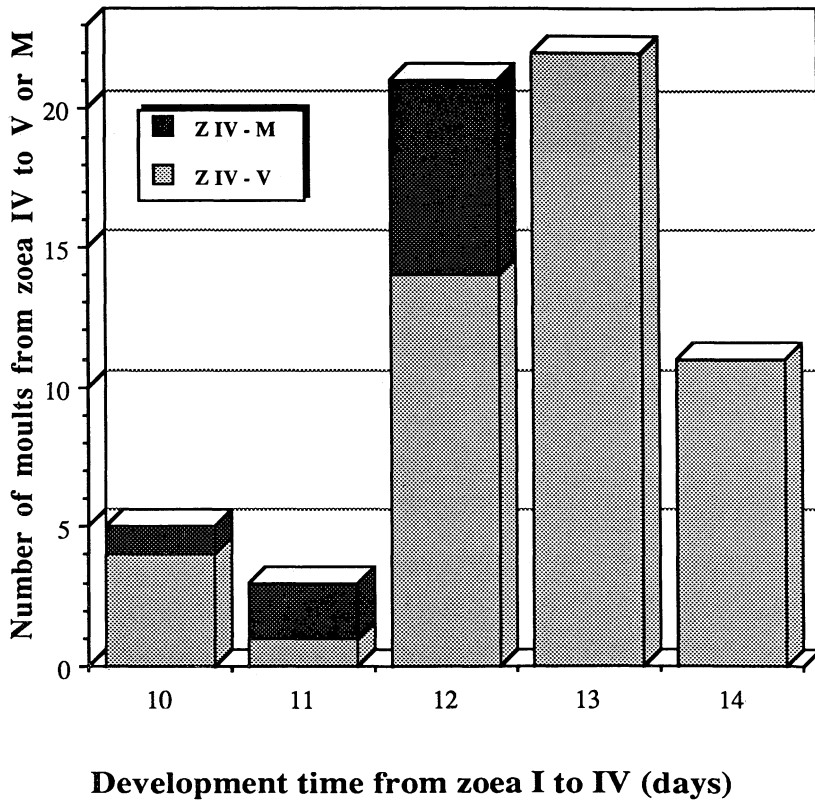


FIG. 1 — *Metasesarma rubripes*. Number of larvae moulting from the zoea IV (Z IV) or a Zoea V or directly to the megalopa (M), in relation to the time (days) required to develop from hatching (Z I) to the Z IV stage.

We found and compiled (in Table 1) a number of morphological deviations from the description given by Díaz & Ewald (1968). Moreover, morphological differences occurred between zoea IV larvae that moulted directly to the megalopa and those moulting to a zoea V stage (Table 2). In general, morphologically less advanced larvae passed through a fifth zoeal instar, whereas more advanced larvae were able to skip it. Morphological differences were observed mainly in the setation of the postero-lateral margins of the carapace, the number of aesthetascs on the antennule, the segmentation of the antenna, and the number of setae on the maxillule, the maxilla, and the branches of maxilliped 3.

The Zoea V (Fig. 2) showed characters (setation and some segmentations) that were intermediate between those of the Zoea IV and the megalopa.

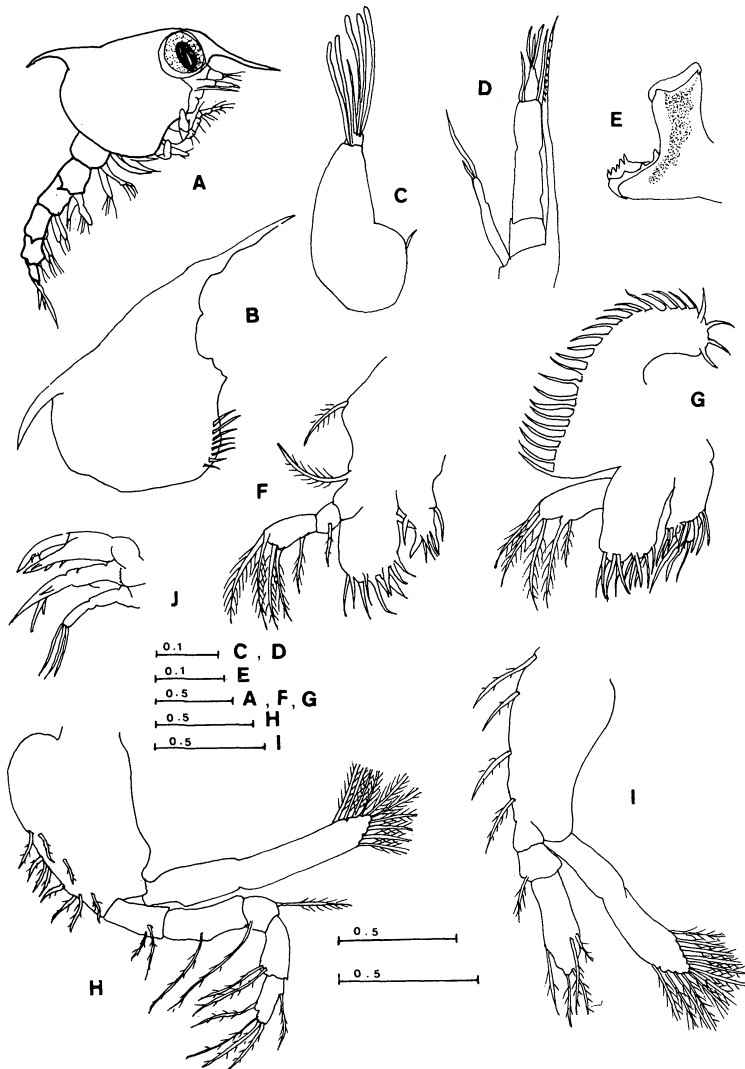


FIG. 2 — *Metasesarma rubripes*. Zoea V

A, lateral view; B, carapace; C, antennule; D, antenna; E, mandible; F, Maxillule; G, maxilla; H, maxilliped 1; I, maxilliped 2; J, pereopods.

Table 1. *Metasesarma rubripes* **Morphological differences between zoeal stages I-III described by DIAZ & EWALD (1968) and those found in the present study.** Abbreviations: a = aesthetascs, s = setae, sp = spines, pl = plumose.

	DIAZ & EWALD (1968)	Present study
Zoea I		
Antenna		
exopod:	1/3 protopod length	same length as propod
terminal s:	2 of different size	1 long + 2 smal on each side
Maxillula		
basal endite:	5 serrate sp	4 serrate sp, 1 pl s
Maxilliped		
s on endopod:	2, 2, 1, 2, 5	2, 1, 1, 2, 5 or 0. 1, 1, 2, 5 or 2, 2, 1, 2, 5
Abdomen		
lateral sp or knobs	segment 2 with 1 pair of sp, segment 3 with 1 pair of knobs	segments 2 and 3 with 1 pair of knobs each
Zoea II		
Carapace		
s on postero lateral margin	?	2
Maxilla		
coxal endite:	bilobate	bilobate
s:	8	2, 5
Maxilliped .1		
s on endopod:	2, 2, 1, 2, 5	2, 2, 1, 2, 6
Zoea III		
Carapace:	5 pl s	4 pl s
Antennule:	3 a, 1 s	2-3 a, 1 s
Maxillule		
s on basal endite:	7	5
Maxilla:		
s on scaphognathite:	3 terminal, 8 fringing	4-5 terminal, 8 fringing

Table 2. *Metasesarma rubripes*. Morphological differences between zoea IV (ZIV) described by DIAZ & EWALD (1968) and those found in the present study, depending on their later development through a zoea V (→ ZV) or directly to the megalopa. Abbreviations: as in Table 1.

	DIAZ & EWALD (1968)	ZIV → ZV	ZIV → Megalopa
Zoea IV			
Carapace			
s on postero-lateral margin	8	4	8 - 13
Antennule:			
1 terminal s	1 terminal s	1 s	1 s
3 terminal a	3 terminal a	4 a	3 terminal a
2 subterminal a	2 subterminal a		2 subterminal a
Antenna			
Endopod:	2-segmented	2-segmented	3-segmented
spinous process:	2/3 spinulated	2/3 spinulated	1/3 spinulated
Maxillule			
sp on basal endite:	11	9 - 10	11
Maxilla			
sp on coxal endite:	11	10 - 11	11
s on scaphognathite:	3 terminal 15 - 17 fringing	5 terminal 15 fringing	5 - 6 terminal 18 - 20 fringing
Maxilliped 3			
branches:	3, with 5, 2, 0 segments	2, unsegmented	3, with 5, 2, 0 segments

DESCRIPTION OF ZOEA V

Size: CL 1.10mm; DS-RS 1.54mm; DS 0.39mm; RS 0.46mm; TL 2.30mm

Carapace (Fig. 2 B.): with 8 setae on postero-lateral margins.

Abdomen (Fig. 2 A.): as in previous stage but with pleopods more developed.

Telson: as in previous stage.

Antennule (Fig. 2 C.): with 5 aesthetascs well developed and 1 seta.

Antenna (Fig. 2 D.): endopod 3 — segmented, spinous process 1/3 spinulated, exopod with 2 setae.

Mandible (Fig. 2 E.): as in previous stage.

Maxillule (Fig. 2 F): protopod with 2 plumose setae, basal endite with 11 plumose spines, coxal endite with 7 spines.

Maxilla (Fig. 2 G.): endopod with 5 plumose setae; basal endite bilobed, setation 5,6; coxalendite bilobed, setation 3,8; scaphognathite with 22 plumose setae and posterior lobe with 4 spines.

Maxilliped 1 (Fig. 2 H.): basis with 10 setae; endopod 5-segmented, setation 2,1, 1,2,5; exopod with 10 natatory setae.

Maxilliped 2 (Fig. 2 I.): basis with 4 setae, endopod 3-segmented with 0,1,5 setae, exopod with 12 natatory setae.

Pereiopods (Fig. 2 J.): pereiopods 1,2 and 3 more developed; cheliped with dactylus and polex developed, with 2 spines on the cutting edge.

DISCUSSION

Metasesarma rubripes is a brackish water species (Hartnoll, 1965, Capitoli *et al.*, 1977), but our experimental results show that its larvae must develop in higher saline environments than those inhabited by the adult populations: they are not able to develop successfully at salinities $<15\text{‰S}$, i.e. under conditions where their parents normally live. This differential adaptation indicates the existence of ontogenetic migrations: the larvae hatch in brackish mangrove streams, but then they must be transported by currents to the outer reaches of the estuary or to the open ocean, where they can develop. Later, when metamorphosis approaches or has been passed, there must be a re-immigration (recruitment) by megalopae or benthic juveniles into lower saline environments, where the crabs grow and eventually reach sexual maturity. Similar patterns were found in another Grapsid crab, **Sesarma angustipes** (Anger *et al.*, in prep.) and in **Uca thayeri** which lives in the same habitat (Anger *et al.*, in press).

Unlike Díaz & Ewald (1968), we found more than one developmental pathway in **Metasesarma rubripes**. The number of zoeal stages varied in our material between 4 and 5, with marked differences in the relative occurrence of these two developmental pathways, when separate hatches from different females were compared. Variability in morphology and developmental pathways of decapod crustaceans has been observed quite frequently in caridean shrimps (e.g. Broad & Hubschman, 1962, 1963, Fincham, 1977, 1979, Haynes, 1979; Haynes, 1979; Criales, 1985; Criales & Anger, 1986), less often in anomurans (see Christiansen & Anger, in press, for references), but rather seldom in brachyurans (Costlow, 1965; Yang 1971; Gore & Scotto, 1982; Díaz & Bevilacqua, 1987 a, b). Two of the latter cases occurred within the Grapsidae; moreover, it has been observed by us also in the Chinese Mitten crab (**Eriocheir sinensis**) which belongs to the same family.

Since we reared the larvae under controlled conditions, and no influence of water salinity on the developmental pathway could be detected, the variability in larval developmental

of this species may have a genetic base. This is supported by the experiment, in which sibling larvae were grouped daily upon moulting to the Zoea IV instar, according to their development duration from hatching to this moult. The developmental pathway after the zoea IV reveals a direct relationship to the previous rate (time) and degree (morphological advancement) of development in earlier zoeal stages: only relatively fast developing and morphologically advanced larvae will proceed directly from the zoea IV to the megalopa, whereas those with a slower and morphologically stunted development will pass through a zoea V. A similar pattern of variation had been observed in **Galathea** spp. (Gore, 1979, Christiansen & Anger, in press). Sandifer & Smith (1979) proposed the hypothesis that the number of stages in an individual larva might be inheritable, whereas external factors act as selective forces favouring different developmental pathways under different conditions. However, besides genetic also other maternal factors may exert significant influences on the number of instars that have to be passed during larval development :e.g. the nutritional state of the female during the period of oogenesis and hence, the amount of yolk reserves deposited in different egg masses.

Variability was observed also in the development time of the zoea IV stage, depending on the kind of stage to which it developed. This suggests that development time depends on the degree of morphogenesis that is necessary to reach the next stage: a moult cycle from one zoea to another will require less internal morphological reconstruction and hence, less time than development from a zoea (IV or V) to a megalopa stage. The same effect had been observed also in caridean shrimp larvae where variation occurred in developmental pathways (Criales & Anger, 1986).

Morphological differences between **Metasesarma rubripes** larvae from Venezuela and Brazil suggest that there may exist different geographical races in this widely distributed species. According to Melo (1984), it occurs in tropical Central America and along the South American coasts, south to the temperate waters of Argentina. The different climatic conditions occurring in this wide area of distribution should favour separation into

physiological and eventually, morphological races, i.e. speciation.

A genetic polymorphism is suggested also by our observations on variability in developmental pathways. It may be interpreted as an adaptive strategy for enhanced survival in a very variable environment, in this case the mangrove system with its wide fluctuations in salinity and temperature. The ability to delay metamorphosis gives the larvae an opportunity to be transported to areas with more suitable environmental conditions (see Sandifer & Smith, 1979, for detailed discussion). In the literature, developmental variability has sometimes been considered as an artifact caused by the artificial conditions in a laboratory culture, however, it has been observed also in the natural pelagic environment (Haynes, 1979; Criales, 1985; Wehrtmann, 1989) and hence, deserves further attention in both laboratory and field studies. Future experiments must analyse the relative importance of possible influences exerted on developmental rates and pathways by genetic and other maternal factors, and by environmental variables.

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REFERENCES

- BROAD, A.C. & HUBSCHMAN, J.H. 1962. A comparison of larvae and larval development of species of Eastern U.S. *Palaemonetes* with special reference to the development of *Palaemonetes intermedius* Holthuis. — Amer. Zool., 2:394-395.
- . 1963. The larval development of *Palaemonetes kadiakensis* M.J. Rathbun in the laboratory. — Trans. Amer. microsc. Soc.: 82:185-197.

- BURGGREN, W.W. & MCMAHON, B.R. (EDS.) 1988. Biology of the land crabs. — Cambridge University Press, New York: 479 pp.
- CHRISTIANSEN, M.E. & ANGER, K. (in press). The complete larval development of *Galathea intermedia* Lilljeborg reared in laboratory culture (Crustacea, Anomura, Galatheididae). — J. crust. Biol.
- COSTLOW, J.D. 1965. Variability in larval stages of the blue crab *Callinectes sapidus*. — Biol. Bull. mar. biol. Lab. Woods Hole, **128**:58-66.
- CRIALES, M.M. 1985. Untersuchungen zur Larvalentwicklung von *Crangon crangon* L. und *Crangon allmanni* Kinahan (Decapoda, Natantia, Caridea). — Dissertation University of Kiel, Kiel, FRG: 223 pp.
- CRIALES, M.M. & ANGER, K. 1986. Experimental studies on the larval development of the shrimps *Crangon crangon* and *C. allmanni*. — Helgoländer Meeresunters., **40**:241-265.
- DIAZ, H. & EWALD, J.J. 1968. A comparison of the larval development of *Metasesarma rubripes* (Rathbun) and *Sesarma ricordi* H. Milne Edwards (Brachyura, Grapsidae) reared under similar laboratory conditions. — Crustaceana Suppl., **2**:225-248.
- DIAZ, H. & BEVILACQUA, M. 1986. Larval development of *Aratus pisonii* (Milne Edwards) (Brachyura, Grapsidae) from marine and estuarine environments reared under different salinity conditions. — J. Coast. Res., **2**:43-49.
- . 1987. Early developmental sequences of *Aratus pisonii* (H. Milne Edwards) (Brachyura, Grapsidae) under laboratory conditions. J. Coastal Res. **3**,63-70.
- FINCHAM, A.A. 1977. Larval development of British prawns and shrimps (Crustacea: Decapoda: Natantia). 1. Laboratory methods and a review of *Palaemon (Palaeander) elegans* Rathke 1837. — Bull. Br. Mus. nat. Hist. (Zool.), **32**:1-28.
- . 1979. Larval development of British prawns and shrimps (Crustacea: Decapoda: Natantia). 2. *Palaemonetes (Palaemonetes) varians* (Leach, 1814) and morphological variation. — Bull. Br. Mus. nat. Hist. (Zool.), **35**:163-182.
- GORE, R.H. 1979. Larval development of *Galathea rostrata* under laboratory conditions, with a discussion of larval development in the Galatheididae (Crustacea Anomura). Fish. Bull. U.S. **76**(4), 781-806.
- GORE, R. H. & SCOTTO, L.E. 1982. *Cyclograpsus integer* H. Milne Edwards, 1837 (Brachyura, Grapsidae): the complete larval development in the laboratory, with notes on larvae of the genus *Cyclograpsus*. — Fish. Bull. U.S., **80**:501-521.
- HARTNOLL, R.G. 1964. The freshwater grapsid crabs of Jamaica. — Proc. Linn. Soc. Lond., **175**:145-169.
- . 1965. Notes on the marine grapsid crabs of Jamaica. — Proc. Linn. Soc. Lond. **176**:113-147.

- HAYNES, E. 1979. Description of larvae of the northern shrimp, **Pandalus borealis**, reared in situ in Kachemak Bay, Alaska. — Fish. Bull. U.S., **77**:157-173.
- MELO, G.A.S., 1984. Taxonomia, padrões distribucionais e ecologia dos Brachyura (Crustacea, Decapoda) do litoral sudeste do Brasil. — Ph.D. Thesis, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil: 215 pp.
- SANDIFER, P.A. & SMITH, T.I.J. 1979. Possible significance of variation in the larval development of palaemonid shrimp. — J. Exp. Mar. Biol. Ecol., **39**:55-64.
- WEHRTMANN, I. 1989. Seasonal occurrence and abundance of caridean shrimp larvae at Helgoland, German Bight. — Helgoländer Meeresunters., **43**:87-112.
- YANG, W.T. 1971. The larval and postlarval development of **Parthenope serrata** reared in the laboratork and the systematic position of the Parthenopinae (Crustacea, Brachyura). — Biol. Bull. mar. biol. lab. Woods Hole, **140**:166-189.