

# A rhizocephalan (Crustacea; Cirripedia) infestation of the deep-sea galatheid *Munida sarsi* (Crustacea; Decapoda), the effects on the host and the influence of depth upon the host-parasite relationship

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(With 8 figures in the text)

Three species of rhizocephalan barnacle were found to be infesting 293 specimens of *Munida sarsi* Huus from the Porcupine Sea-bight (49–52°N, 11–14°W): *Tortugaster boschmai*, *Lernaeodiscus ingolfi* and, by far the most common, *Triangulus munidae*. Each species was found to have its own preferred abdominal segment site of emergence and particular effect on both male and female host pleopod structure. *Triangulus munidae*, however, caused a series of pleopod structure forms which led to the definition of a range of pleopod 'Types' for male and female hosts, including a peculiar masculinization of the female hosts' pleopods. The hormonal mechanisms behind these modifications are discussed.

Depth was found to have an influence upon the host-parasite relationship, there being both an increase in the percentage infestation of *M. sarsi* and a decrease in the effects of *T. munidae* on the host's pleopod structure with an increase in depth. At extremes of their bathymetric ranges, the host becomes more susceptible to infestation, while the effect of the parasite on its host breaks down.

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## Introduction

*Munida sarsi* Huus is the most northerly species of the galatheid genus *Munida* found in the North-East Atlantic, extending from the North Cape of Norway to the northern coast of Spain (Rice & Saint Laurent, 1986). It has been mainly recorded from depths between 200 and 800 m.

Three species of rhizocephalan parasite have been described infesting *Munida sarsi*: *Triangulus munidae* (Smith, 1906), *Lernaeodiscus ingolfi* Boschma, 1928 and *Tortugaster boschmai* Brinkmann, 1936. All three have also been found infesting *M. tenuimana* G.O.Sars and *M. rugosa* Fabricius (Høeg & Lützen, 1985).

The relationship between *Munida sarsi* and its three rhizocephalan parasites was studied by Brinkmann (1936) as part of a general study on the *Munida* from Norwegian fjords. He reported that each rhizocephalan species has a particular, discrete effect on the secondary sexual characters of the host munidid, namely a certain degree of modification of the pleopods of both male and female hosts. This paper is a further development of Brinkmann's observations, with the added influence of depth on the parasitization.

### Materials and methods

During the period 1978 to 1986, a total of 1933 *Munida sarsi* have been collected from the Porcupine Sea-bight (49–52°N, 11–14°W) between the depths of 205 and 815 m. Two types of sampling gear were used: the semi-balloon otter trawl (OTSB) complete with 'V' doors (see Merrett & Marshall, 1981) and the IOS epibenthic sled (BN) (see Rice, Aldred, Darlington & Wild, 1982). The specimens were fixed in 5% formalin solution and later transferred to 70% alcohol for preservation. They have been stored in the 'Discovery' collection at the Institute of Oceanographic Sciences Deacon Laboratories, Wormley, Surrey.

Two hundred and ninety three specimens infested by rhizocephalans were removed for further study. The following measurements were taken using either dial calipers or the graduated eyepiece of a Wild stereoscopic microscope. On *M. sarsi*: carapace length (CL) from the posterior margin of the right orbit to the posterior carapace margin. On Rhizocephala: maximum externa width from left to right. All measurements were made to the nearest 0.1 mm. Sex of the *M. sarsi* was determined by the position of the gonopores and the presence or absence of the first pair of pleopods. General pleopod structure could not be used reliably due to the modifications of their form caused by the action of the parasite. In addition, the abdominal segment (AS) position of the externa or scar on the host's abdomen and the state of modification of the pleopods by the parasitic infestation were noted.

For comparative purposes, the peculiar measurement of CL used by Brinkmann (1936) was also taken, this being from the notch between the main and right lateral rostral spine to the posterior edge of the carapace. A relationship to convert Brinkmann's carapace length (BCL) to the standard carapace length (CL) was obtained as:  $CL = 0.96 \cdot BCL - 0.51$ . Both measurements are in mm.

### Results

Three different rhizocephalan species were found to be infesting *Munida sarsi* in the Porcupine Sea-bight: *Triangulus munidae* (Smith), *Lernaeodiscus ingolfi* Boschma and *Tortugaster boschmai* Brinkmann. All these species were found on the *M. sarsi* population of Western Norway studied by Brinkmann (1936). The individual species and their effects on the external morphology of the host are detailed later.

In addition to those bearing externae of the three species, 43 specimens bore scars from a previous infestation. These scars are left behind when the externa either drops off after completing its life history, or is knocked off, especially during the sampling process or the resulting preservation. Several loose externae were found in the specimen jars, all of which were *Triangulus munidae*. From this fact, together with the frequency of *T. munidae* infestation and the structure of the scarred individuals' pleopods, it can safely be assumed that the great majority of the *M. sarsi*

TABLE I  
*Infestation of each sex of Munida sarsi by each species of rhizocephalan, plus scarred individuals*

Species	Sex	Total	No. Infested	% Infestation
<i>Triangulus munidae</i>	M	1059	113	10.67
	F	863	121	14.02
<i>Lernaeodiscus ingolfi</i>	M	1059	4	0.38
	F	863	1	0.12
<i>Tortugaster boschmai</i>	M	1059	7	0.66
	F	863	4	0.46
Scars	M	1059	20	1.89
	F	863	23	2.67

now bearing scars were once the hosts of *Triangulus munidae*. However, these squat lobsters with scars were dealt with separately for the purpose of analysis.

#### *Details of infestation*

##### *Degree of infestation by each species on Munida sarsi*

The whole sample was composed of 1059 males and 863 females, plus 11 individuals too small to sex which were therefore recorded as immature. A total of 293 *M. sarsi* were found to be infested (15.16%) and the breakdown of the infestation structure can be seen in Table I.

In the area studied, by far the most common rhizocephalan on *M. sarsi* is *Triangulus munidae*, forming 93.60% of the non-scar infestations, with *Lernaeodiscus ingolfi* and *Tortugaster boschmai* being represented by only five and 11 cases, respectively, out of 250 non-scar infestations.

Overall, females were infested significantly more than males ( $\chi^2$ ,  $P < 0.05$ ), with a total of 17.27% of females being parasitized, compared to only 13.60% of males. This is a reflection of the difference between the infestation of the sexes by the dominant species, *T. munidae*, which Brinkmann (1936) found also to infest females to a greater degree.

##### *Degree of infestation of each host size class*

The percentage infestation for each millimetre carapace length size interval was determined for *Triangulus munidae*, the sample sizes being too small to obtain valuable results for the two less common rhizocephalans if treated singly. The results (Fig. 1) show that there is a range of host size (11.0–13.0 mm) that is infested in higher proportions than the sizes both smaller and larger than this range. There is also some difference between the graph shapes for the sexes, the peak for females being more pronounced than that for males, falling quite rapidly after 13.0 mm, while the males remain relatively highly infested up to 14.0 mm.

The range of CL found over the whole sample was 4.0–34.0 mm, whereas the range of parasitization was only 8.0–19.0 mm, suggesting a maximum size of 19 mm when infestation can occur compared with the total sample in which individuals up to 34 mm CL were found. This suggests that there is in fact a size when *M. sarsi* does not become infested by the rhizocephalans

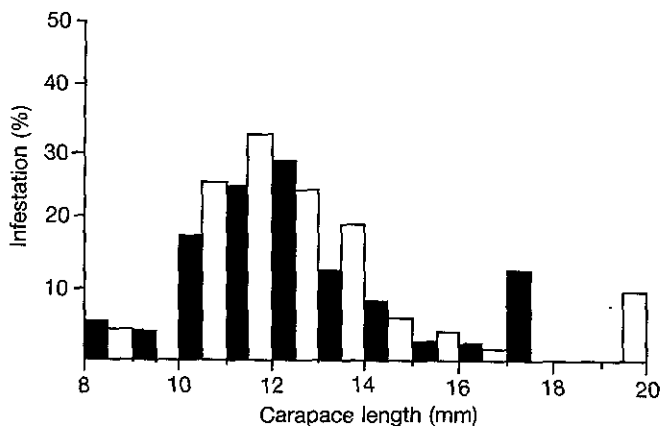


FIG. 1. Percentage infestation of each mm size class of *Munida sarsi* by *Triangulus munidae*.

N-values for each mm CL:

	8-0-	9-0-	10-0-	11-0-	12-0-	13-0-	14-0-	15-0-	16-0-	17-0-	18-0-	19-0-
Sex	8-9	9-9	10-9	11-9	12-9	13-9	14-9	15-9	16-9	17-9	18-9	19-9
Numbers of F	18	23	40	111	160	169	138	69	35	8	5	1
individuals M	24	36	48	80	111	147	149	131	105	67	52	25

Female (■); male (□)

studied here. The minimum size is a figure for individuals bearing externae, so actual infestation by the rhizocephalan cyprid could have occurred at a smaller size.

#### *Change in infestation levels at depth*

The specimens were divided into 100 m depth classes and the proportion at each depth that was infested by any of the three species, or bore the scars of infestation, was calculated. Figure 2 shows the results for *T. munidae*. However, no samples of *M. sarsi* have thus far been taken between 600–699 m, so there is an unfortunate gap in the data at this depth. Figure 2 reveals an unexpected rise in degree of infestation with depth, especially below 400 m. All *Lernaeodiscus ingolfi* were found between 400–499 m and the examples of *Tortugaster boschmai* encountered were all from the shallower depths (< 500 m).

From the data, depth ranges for each rhizocephalan species in the Porcupine Sea-bight can be stated:

<i>Triangulus munidae</i> :	243–793 m
<i>Lernaeodiscus ingolfi</i> :	455–490 m
<i>Tortugaster boschmai</i> :	243–490 m
Scars:	243–763 m
	(cf. <i>T. munidae</i> )

*Multiple infestations and site of externa emergence*

For each rhizocephalan species, there were hosts demonstrating multiple infestations, although three externae, for *T. munidae*, was the maximum observed. The other cases were of double externae (Table II). The more interesting multiple infestations are detailed below under 'Details of each rhizocephalan species.'

The frequency of emergence of each parasite species, plus scarred individuals, from each abdominal segment was recorded. Figure 3 shows the distribution for *T. munidae* and scarred individuals for comparison. By far the most common segment for *T. munidae* was AS3 and for *T. boschmai* this was AS6, as would be expected for this species (Høeg & Lützen, 1985). More details on the abdominal segment preference for each species can be found under the individual heading for the separate rhizocephalan species.

*Details of each rhizocephalan species and their effect on the pleopod structure of the host*

The rhizocephalans were identified using Høeg & Lützen (1985) and the basic features peculiar to each species are noted below, together with any new observations.

(a) *Tortugaster boschmai* (Brinkmann, 1936)

Asymmetrical, left side larger than right side, so the mantle opening, which is relatively small and thin, is displaced to the right-hand side. Externa body generally flat and often the edges are folded.

Size range observed: 1.2–11.9 mm (the latter undergoing ecdysis).

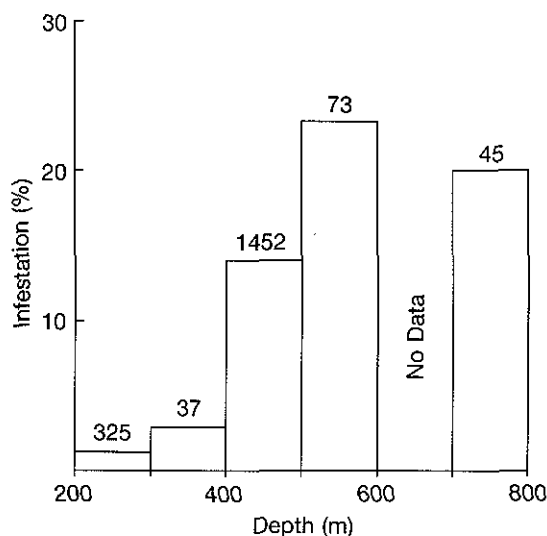


FIG. 2. Percentage infestation of *Munida sarsi* by *Triangulus munidae* at each 100 m depth interval. N-values for each depth interval on Figure.

TABLE II

Number of single and multiple infestations of each rhizocephalan species on *Munida sarsi*, together with the expected number of multiple infestations of *Triangulus munidae* calculated using the poisson distribution. The total Chi-squared value obtained is not significant, the distribution of the externae being as expected

No. hosts with:	<i>Tortugaster boschmai</i>	<i>Lernaeodiscus ingolfi</i>	<i>Triangulus munidae</i>	Poisson Distribution	
				Expected	$\chi^2$
0 Externae	1922	1928	1699	1695	0.01
1 Externa	9	3	216	222	0.07
2 Externae	2	2	17	15	0.27
3 Externae	0	0	1	1	0.00
				Total = 0.35	

( $P > 0.9$ )

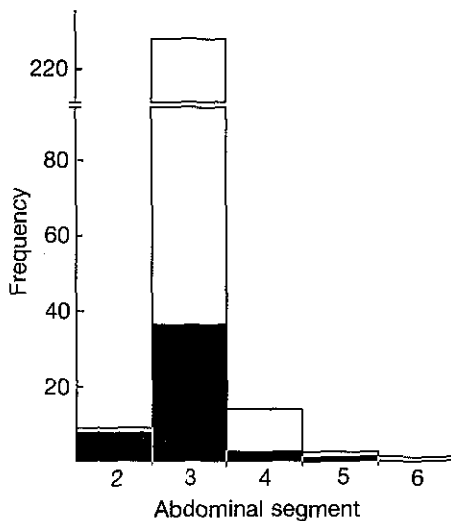


FIG. 3. Frequency of externae of *Triangulus munidae* (□) and scars (■) on the abdominal segments of *Munida sarsi*.

Originally reported emerging only from the sixth abdominal segment (Høeg & Lützen, 1985), but in this study one host was found to be carrying a single externa on AS5 and another bore a double infestation of *T. boschmai*, with a small externa (2.2 mm) on AS6, together with a larger body (5.7 mm) on AS5.

The parasite has no effect on the pleopod structure of either male or female hosts; both sexes show 100% normal pleopod structure (Fig. 4: a.i., b.i., c.i., d.i.; Fig. 5: a.i-iii.), supporting the observations of Brinkmann (1936).

Found only on shallower samples (< 500 m), Høeg & Lützen (1985) also reported it not found below 700 m (no data for 600–699 m in this study) and not common in the Porcupine Sea-bight (a new recorded locality for this species), with only 11 individual *Munida sarsi* being found infested (0.57%), seven males (0.66%) and four females (0.46%).

(b) *Lernaeodiscus ingolfi* (Boschma, 1928)

Perfectly symmetrical. The large mantle opening is central, with a protruding, swollen lip which is always open to some degree, usually extremely wide.

Size range observed: 1.5–9.6 mm.

Høeg & Lützen (1985) report only found singly, but two out of the five infestations were doubles. The single externae emerged one from AS3 and two from AS4, while the double infestations had externae on AS2+3 and AS2+4.

The effect on the pleopods of the host was constant, as was that of *T. boschmai*. However, in the male host, *L. ingolfi* induced a feminization of pleopods 3–5, the usual swimmeret Type developing into a more elongate and far more setose form (Fig. 4: c.ii., iii., d.ii.). This form is very similar to the effect produced on the sole female found parasitized by *L. ingolfi* (Fig. 5: b.i–iii.), all the pleopods being slightly degenerate and much less setose than those of a non-infested individual. The male copulatory appendages (pleopods 1 and 2) remained unaffected, as with *T. boschmai*.

Brinkmann (1936) found between 0.2 and 0.5% infestation of *L. ingolfi* on *M. sarsi* off the Norwegian coast and this compares favourably with the 0.26% infestation of the *M. sarsi* examined in this study from the Porcupine Sea-bight (a newly recorded locality for this species) and so suggests that *L. ingolfi* is not a particularly common parasite on this munidid.

All specimens of *L. ingolfi* were found in the one large sample taken over a depth range of 455–490 m.

(c) *Triangulus munidae* (Smith, 1906)

Asymmetrical body of externa, the right-hand side being larger than the left, causing the mantle opening to be displaced to the left-hand side. This opening is narrow and longitudinal, resembling a gash on the usually smooth externa surface.

Size range observed: 0.6–11.9 mm.

By far the most frequent rhizocephalan found on *M. sarsi* in the Porcupine Sea-bight (again a new locality for this particular species), with a total of 234 cases of infestation. This was composed of a 10.67% infestation in males and a 14.02% figure in females, confirming Brinkmann's (1936) statement that it occurs more frequently on female hosts, although the infestation in this study was considerably higher than that found by Brinkmann, that being between 1.4 and 8.6%.

The depth range for *T. munidae* was 205–795 m, increasing the depth range (70–600 m) noted by Høeg & Lützen (1985) for this species.

Externae were found on all abdominal segments from 2–6, adding segments 5 and 6 to those described as sites of emergence by Høeg & Lützen (1985). However, by far the most common site of emergence was AS3, especially the posterior part of the segment where it meets AS4, 90% of all externae being found on this segment. There were 216 cases with a single externa, 17 with two and only one triple infestation, all three being on AS3. This distribution does not deviate from the expected poisson distribution (Table II), indicating neither clumping nor attractant factors

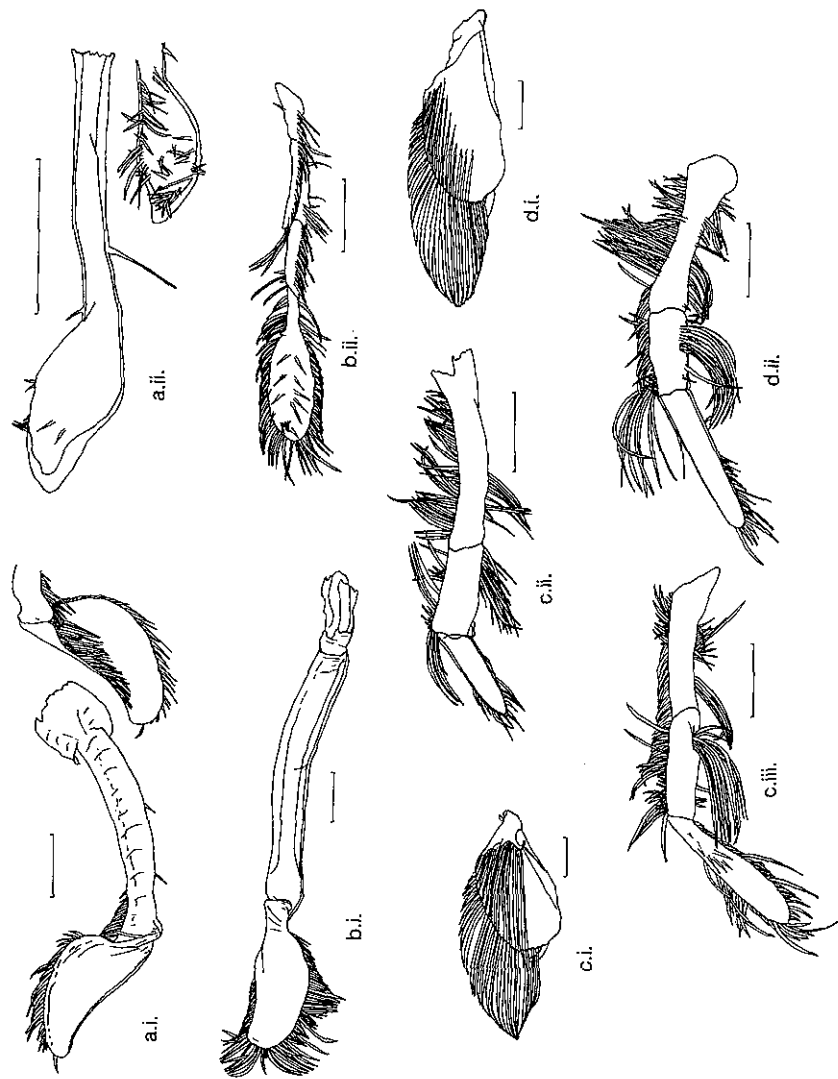


FIG. 4. Male pleopod Types:

	Pl. Number				
	1	2	3	4	5
Type 1	a.i.	b.i.	c.i.	c.i.	d.i.
Type 3	a.ii.	b.ii.	c.ii.	c.iii.	d.ii.
Type 4	a.i.	b.i.	c.ii.	c.ii.	d.ii.
Type 5	a.ii.	b.ii.	c.i.	c.i.	d.i.

All pleopods from LHS. a.i., b.i., c.i., d.i.: non-parasitized male *Munida sarsi*, CL 20.8 mm.  
 a.ii., b.ii., c.ii., c.iii., d.ii.: infested male *Munida sarsi*, CL 19.3 mm, externa of *Triangulus munidae*  
 on AS3.

Scale bars = 1 mm.



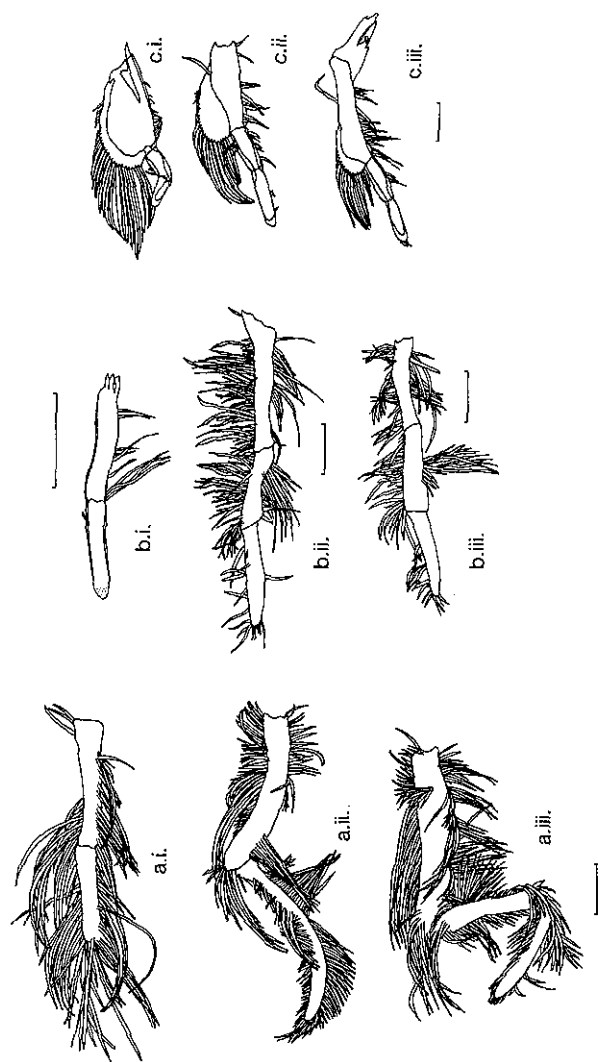


FIG. 5. Female pleopod Types:

	Pl. Number
Type 7	2
a.i.	3
a.ii.	4
a.iii.	5
Type 8	a.i.
b.i.	b.ii.
b.ii.	b.iii.
Type 9	b.i.
b.i.	c.i.
c.i.	c.ii.
c.ii.	c.iii.

All pleopods from LHS. a.i.-a.iii.: non-parasitized female *Munida sarsi*, CL 16.7 mm.

b.i.-b.iii.: infested female *Munida sarsi*, CL 14.4 mm, externa of *Triangulus munitidae* on AS3.

c.i.-c.iii.: infested female *Munida sarsi*, CL 13.9 mm, externa of *Triangulus munitidae* on AS3.

Scale bars = 1 mm.

influencing the settlement of *T. munidae* larvae on prospective hosts, nor greater than expected polyembryony or budding from a single infestation.

Brinkmann (1936) stated that the effect on the male hosts by *T. munidae* was a total degeneration of the copulatory appendages (pleopods 1 and 2) and a feminization of pleopods 3-5. However, the effect of the parasite on these secondary sexual characteristics seems to be more varied, with different pleopod structures for both male and female hosts being noted. The whole range of pleopod Types is described below.

#### *Pleopod Types observed on infested Munida sarsi*

##### (a) Males

*Type 1.* All pleopods unchanged by the presence of the parasite. The copulatory appendages 1 and 2 are relatively non-setose (Fig. 4: a.i., b.i.) and the pleopods 3-5 are the standard swimmeret Type found on unparasitized males (Fig. 4: c.i., d.i.).

*Type 2* (not illustrated). All pleopods are slightly affected, resulting in structures falling between Types 1 and 3. Pleopods 1 and 2 have diverged from the unparasitized form, with some reduction in size and change in the setae pattern, though considerably less degenerate than Type 3. Pleopods 3-5 show some elongation away from the swimmeret Type, but are small and lacking in setae compared to Type 3.

*Type 3.* All pleopods are severely affected. Pleopod 1 is reduced and markedly degenerate (Fig. 4: a.ii.), becoming even less setose in the process, though retaining its basic shape. Pleopod 2 is also reduced (Fig. 4: b.ii.), though more setose and morphologically developed towards the female form. However, the endopodite retains some resemblance of the original shaped male form. Pleopods 3-5 are feminized (Fig. 4: c.ii., iii., d.ii.), becoming elongate and setose with no trace of the swimmeret structure. However, this resembles the parasitized female state (Fig. 5: b.ii., iii.) rather than the more setose unparasitized mature female form (Fig. 5: a.ii., iii.).

*Type 4.* Pleopods 1 and 2 are unchanged, as in Type 1, but the pleopods 3-5 are feminized, as in Type 3.

*Type 5.* Pleopods 1 and 2 are totally affected, as in Type 3, but no change from the normal male swimmeret form of pleopods 3-5.

##### (b) Females

*Type 7.* Pleopods all represent the forms of a mature non-parasitized female, large and extremely setose (Fig. 5: a.i-iii.), unchanged by the presence of the parasite (only observed when infested by *Tortugaster boschmai*).

*Type 8.* All pleopods show a reduction in size and are considerably less setose than the unaffected form (Fig. 5: b.i-iii.). Pleopod 2 is greatly reduced and is practically free from setae (Fig. 5: b.i.).

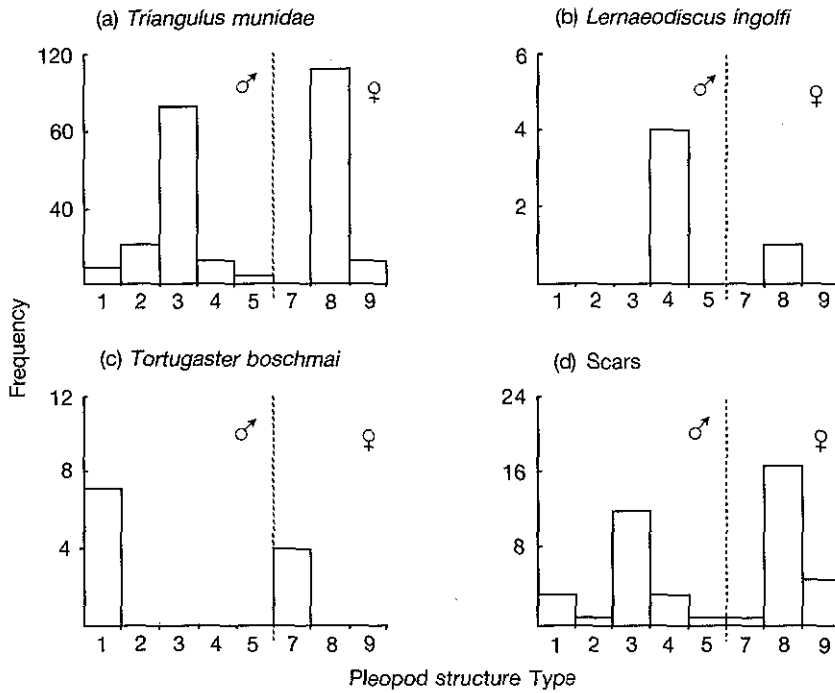


FIG. 6. Frequency of *Munida sarsi* pleopod structure Types induced by each rhizocephalan species, plus scars.

*Type 9.* A development towards the male unparasitized form of pleopods 3-5, the swimmeret structure emerging from the most proximal segment and the remainder of the original female pleopod form degenerating (Fig. 5: c.i-iii.). There is a decrease in masculinization posteriorly from pleopod 3. Pleopod 2 demonstrates the form of Type 8 (Fig. 2: b.i.).

Figure 6 shows the frequency distribution of the above pleopod Types for each rhizocephalan species, on both male and female hosts. This gives some indication of the main pleopod structures induced by each species of parasite, as outlined above, as well as the comparative frequencies of the effects caused by *Triangulus munidae*. Figure 6 highlights the single effects on each sex by both *Lernaeodiscus ingolfi* and *Tortugaster boschmai*, compared to the range of pleopod Types caused by *T. munidae*.

#### *Influence of other factors on host pleopod structure*

The variety of effects produced by *Triangulus munidae* on the pleopod structure of *Munida sarsi* may be influenced by other factors, such as size of the host, externa size, abdominal segment from which the externa erupts, and depth.

(a) *Depth*

Figure 7 shows the frequency of each pleopod structure Type of *M. sarsi* infested with *T. munidae* at each depth interval, converted to a percentage of all the parasitized individuals of each sex at the depth. This indicates a change in the composition of the pleopod Types from the form noted by Brinkmann (1936) caused by this rhizocephalan, Type 3, which is dominant in the shallower water (< 400 m), Fig. 7c, to the unaffected Type 1 structure in the deepest samples (Fig. 7a). Examples of Types 2, 4 and 5, the 'intermediate' Types, are to be found in the mid-depths (Fig. 7b, d, e).

With respect to the females, the unusual 'male-type' pleopods (Type 9) were only found in the deeper water (Fig. 7g), the shallower samples being composed entirely of the usual Type 8 structure induced by *T. munidae* (Fig. 7f).

(b) *Externa size*

Figure 8 shows the number of each mm size class of externa inducing the Type 3 male pleopod structure converted to a percentage of each of these size classes. It would appear from these results that hosts with smaller externae have the Type 3 form in lower proportions than the larger ones, the percentage of Type 3s again dropping for the very largest externae.

(c) *Host size and abdominal segment preference*

From the results (Figs 1 and 3), it is seen that *T. munidae* has both an abdominal segment and a particular range of host on which it is found most frequently. These 'preferred' criteria may influence to a small degree the pleopod structure of the host, particularly the site on the abdomen. However, the sample sizes at these extremes of both abdominal segment and host size are obviously so small that definite, significant statements cannot be made.

## Discussion

### *The form of the pleopods resulting from infestation by *Triangulus munidae**

*Triangulus munidae*, on infestation of *Munida sarsi*, influences the development of the host's pleopods and has the effect of producing a variety of pleopod Types (see above). However, some of these structures are not the expected forms usually induced by a rhizocephalan parasitizing a large decapod, such as anomurans and brachyurans.

The general pattern is a modification of males towards the ovigerous female form, such as the broadening of the abdomen in a variety of Brachyura (Veillet, 1945; Reinhard 1950; Hartnoll, 1967) and a development of the pleopods 3-5 to the setose female condition (Reinhard, 1956). In this study, this was demonstrated by the Type 3 pleopods of the infested males, which have been modified as expected, though there is some variation as discussed above.

It is the effect on the female pleopods by the parasite that is particularly interesting, in relation to the actual structures resulting, the mechanisms by which these effects are induced, and the benefit of these pleopod forms to the parasite.

The result of an infestation of a female is usually hyper-feminization, but a reduction in the size and number of setae of the pleopods of parasitized females is not unknown, as in *Inachus*

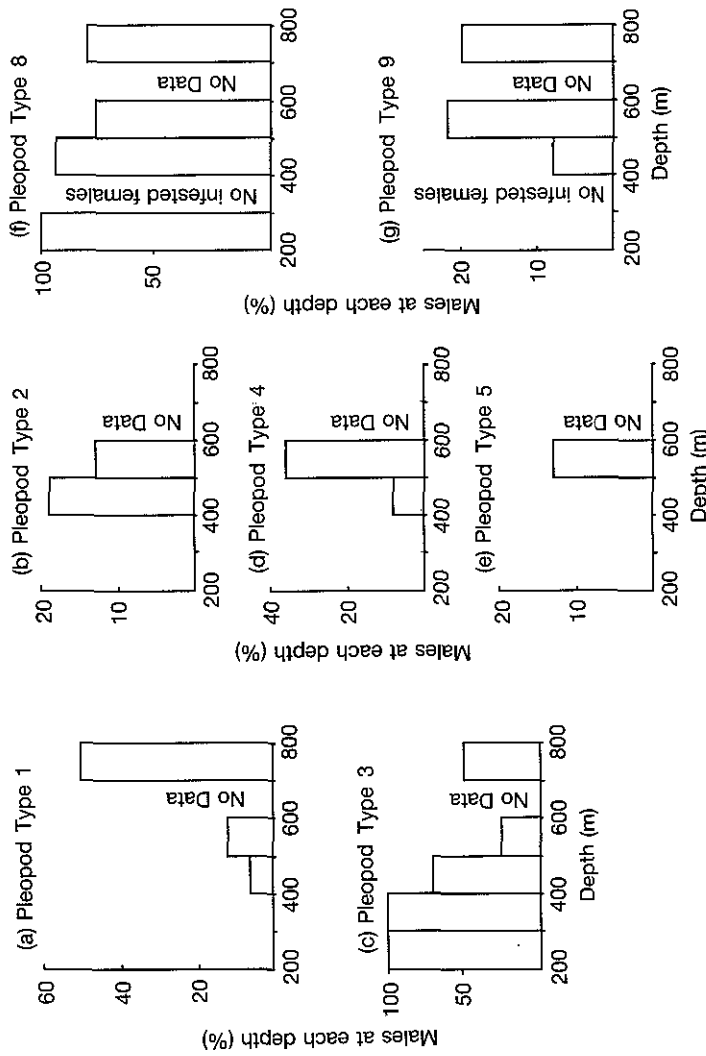


FIG. 7. Depth distribution of pleopod Types induced by *Triangulus munidae*. Percentage of population at each 100 m depth interval with each pleopod Type. (a)-(e): Males. (f)-(g): Females.

N-values for each depth interval infested by *Triangulus munidae*:

Depth interval (m)	200-299	300-399	400-499	500-599	600-699	700-799
N-values: M	1	1	99	8	No Data	4
F	3	0	104	9	No Data	5

*dorsettensis* (Smith, 1906, as *I. mauretanicus*), such reduced females resembling parasitized males, but not normal males (Reinhard, 1956). In this study, the majority of the females infested by *T. munidae* were of this reduced Type. Not one case of hyper-feminization was found. However, some of the parasitized females resembled normal males, pleopods 3-5 developing into the standard swimmeret form (Type 9). This Type, which was noted by Brinkmann (1936) in one individual (he was only sampling above 500 m), raises questions about the control of the development of the secondary sexual characters when under the influence of a rhizocephalan.

Various theories have been postulated to explain the effects on the host of parasitization by a rhizocephalan, but it would seem that Goldschmidt's theory of intersexuality (1931) is the most fitting in respect of the observations made in this study. The male and female sex substances produced as a result of the action of the gene in each individual decapod are delicately balanced, so Goldschmidt suggested that this balance between the M-gene's substance and the F-gene's substance is disrupted by the action of the parasite. The male form is the actively maintained state, the dominant M-genes activating the genes responsible for male morphogenesis, which in turn suppress those for the female state (Ginsburger-Vogel & Charniaux-Cotton, 1982). When the M-genes are absent, inactive or in small numbers, the genes for female morphogenesis can be expressed. However, if the dominant M-genes are in these small numbers, or their effect has been reduced due to some extraneous factor, such as parasitism, then this could lead to a state of intersexuality, a result of which would be the distinctive modifications of the secondary sexual characteristics seen in such infested Crustacea. The individual which up to the point of infestation was, for instance, male with dominant M-genes, would then have this balance destabilized, the amount of difference between the levels of the two sex substances then being insufficient to maintain the male state and the F-genes would start to have an influence. The result could be a feminization of the pleopods, the degree of modification depending on the level of influence of the F-substance, i.e. the amplitude of the difference between the levels of M- and F-substances in the body.

The mechanics of how exactly these levels are influenced by the rhizocephalan itself are more difficult to explain. Basically, either the parasite itself produces the substances and introduces them to the host's body, as conjectured by Reverberi (1944-45), or perhaps more likely the interna of the rhizocephalan causes the organs of the host to modify in such a way that either substances are prevented from being produced or are generated at a greater rate, or the genes themselves are affected.

In Malacostraca, the male sex-determining genes are known to be responsible for the development of the androgenic gland, the organ close to the vas deferens which has been shown to regulate male morphogenesis and spermatogenetic activity (Charniaux-Cotton, Zerbib & Meusy, 1966; Hoffman, 1968), i.e. the production site of the M-substances. So, in some species, the parasite has the effect of diminishing the influence of the M-genes by inhibiting the development of the androgenic gland. Therefore, the balance between the two types of sex-determining substances is affected, the F-genes become influential and the external result, as seen, can be the feminization of the pleopods of the male host.

However, the effect on the pleopods is not constant, neither in respect of each individual's pleopod Type, nor in respect of the actual modification of the pairs of pleopods on one specimen. The general, Type 3, condition is for the first pair of pleopods to degenerate, while the other four pairs become more setose, elongate and diverge towards the female state. The reason that the first pair does not also follow such a pattern is that it does not come under the influence of the F-genes once the dominance of the M-genes has been diminished, this pair of gonopods being exclusively

male. Once there is no control from the M-genes on their structure, these appendages just degenerate, towards the female form where they are not present.

So, for males, the feminization appears to be caused by an inhibition of the androgenic gland by the ramifying interna of the rhizocephalan, allowing the F-genes to be expressed and so influencing the structures of those pleopods under the control of these female genes, the degree of modification depending on the extent of inhibition of the androgenic gland. This extent of inhibition can be used to explain the main Types of male pleopods observed:

*Type 1.* No degeneration of the androgenic gland at this stage, all pleopods are therefore unaffected due to the continual domination of the M-genes. There is no expression of the F-genes and so no feminization.

*Type 2.* Some influence from the parasite causing partial degeneration of the androgenic gland. M-genes have a decreased effect, allowing the F-genes to be expressed to some degree. The result is partial intersexuality, all pleopods showing some signs of feminization. Will probably lead to further degeneration of the androgenic gland and so to Type 3. A transition phase in the process of feminization.

*Type 3.* Androgenic gland degenerated, dominance of the M-genes lost and expression of the F-genes possible. Pleopods 2-5 come under the control of the F-genes, so becoming feminized. Pleopod 1 has no female control and so degenerates. The culmination of the parasitic feminization process.

These are the three main Types in the process of feminization of the pleopods of male *Munida sarsi* by *Triangulus munidae*, though Type 1 is also the effect caused consistently by *Tortugaster boschmai*. However, two other pleopod Types were recorded.

*Type 4.* Partial disruption of the functions of the androgenic gland by the rhizocephalan causes an upset in the balance of the two sex substances. The F-genes have some expression causing feminization of pleopods 3-5, though the influence of the M-substance is still sufficient to regulate the structure of the gonopods, pleopods 1 and 2. This, like Type 2, may be a transition stage, though the fact that this is the effect observed consistently on the pleopods by the other of the three rhizocephalans, *Lernaeodiscus ingolfi*, suggests that this may be an end result. It is feasible that the gonopods are under greater control by the M-genes, considering that they are the male copulatory appendages, and so in some cases would be affected by the parasitism at a further stage than the other three pairs of pleopods. These would in turn be under a high level of control by the F-gene, seeing that they are the egg-bearing appendages of the female munidid.

*Type 5.* A Type represented by only two examples in the whole sample. The peculiar degeneration of the gonopods without change of the other three pairs of pleopods seems to be incongruous in the context of what is known, which would suggest that, seeing as only two were found, another factor outside of the rhizocephalan may be involved. The pleopods may have been damaged in some way, or, more likely, development of the individual *M. sarsi* may have been retarded. Both individuals were very small and perhaps not fully mature before the infestation occurred.

If Goldschmidt's theory is applied to the infested females then the same type of parasitic activity should increase, in a similar way to the males, the relative amount of the F-substance, as concluded by Reinhard (1950). This would explain the other phenomenon associated with parasitized

females: the precocious development of the immature female abdomen into the mature adult structure in Brachyura. Examples of such observations are by Smith (1906) on *Inachus dorsettensis* (as *I. mauretanicus*), Pérez (1933) on *Pachygrapsus marmoratus* and Hartnoll (1967) on *Geograpsus lividus*. However, if this is the case, why do the pleopods of some such crabs and the pleopods of female-infested *Munida sarsi* also become reduced to the form observed? Studies by a variety of workers on ovarian hormones, such as Le Roux (1931a, b), Callan (1940) and Charniaux-Cotton (1952, 1953), have established that the sex substance that stimulates the abdomen to enlarge does not influence the development of the pleopods, these structures being under the control of another sex substance. Therefore, Reinhard (1950) suggested that the development of the pleopods is retarded due to the parasite disrupting the functioning of the ovary and so affecting the development of these specialized structures. This would therefore explain the 'normal' Type 8 parasitized female *Munida* pleopods observed in this study, the ovary being disturbed by the action of the ramifying interna, the control over the pleopods breaking down and the appendages degenerating to the Type 8 form. This leaves the unusual Type 9 structures; the masculinized pleopods of some infested females. This basic lack of ovarian control does not explain why the pleopods should develop towards the standard male swimmeret form, a process which suggests an influence of the M-genes or the resulting M-substance. The idea that perhaps these individuals are in fact males that have lost their first pair of pleopods and developed female genital openings without major changes to the pleopods 3-5, those most affected by the F-substance, is extremely unlikely and there is no evidence that this has occurred from a study of the internal structure.

As mentioned above, the androgenic gland is the site of production of the M-substance. Experiments have shown that implantation of this gland into juvenile, puberal or ovariectomized females results in some degree of masculinization, this first being demonstrated by Charniaux-Cotton (1954). Young *Orchestia gammarella* implanted with one or two androgenic glands become completely masculinized, their development and behaviour being that of a normal male. In decapods, the effect of implantation has not been so great, but external masculinization has been obtained by Charniaux-Cotton & Cazes (1979) with *Palaemonetes varians* and by Berreur-Bonnefant & Charniaux-Cotton (1965) with *Pandalus borealis*. Also, some effects have been noted in female *Carcinus maenas* and *Rhithropanopeus harrisi*, with a partial masculinization of their pleon (Charniaux-Cotton, 1958; Payen, 1969, 1975). In reference to these decapods, grafts were only successful on young females, so could it be more than coincidence that the majority of *Triangulus munidae* were found on the young adult munidids of CL between 11-14 mm, whereas no parasites were to be found on the larger specimens of greater than 20 mm CL? Reverberi (1944-45) suggested that the parasite itself may introduce a substance similar to the F-substance in order to initiate the feminization process observed in infested males, so the manufacture of an M-substance similar to that produced by the androgenic gland is not beyond the realms of possibility. Success in masculinization has been achieved by injection of an extract of the androgenic gland. Katakura, Fugimaki & Unno (1975) obtained a partial purification of a protein from 3000 pairs of male sexual organs (including the androgenic gland) from *Armadillidium vulgare*. This was injected into sexually active female *A. vulgare* which were externally masculinized at the ensuing moult. A later study with a more concentrated extract caused the development of testes, seminal vesicles and vasa deferentia in the injected females (Katakura & Hasegawa, 1983). In addition, Reinhard (1949) worked on the differentiation of sex in a bopyrid isopod parasite. He came to the conclusion that any female bopyrid already present on the host influenced the development of any further settling larvae by producing a masculinizing substance, so causing these larvae to develop into the dwarf males. Despite this, however, the introduction of such an M-substance by the rhizocephalan



seems unlikely as the benefit to the parasite of this action is unclear. All the same, this very fact suggests that this modification of the females is not an effect actively produced by the parasite, but rather an occasional by-product of the rhizocephalan's action. However, if this equivalent M-substance, or a substance that happens to influence the product of the F-genes and so may shift the character of the F-substance to that of the M-substance, were to be produced, then a young host may be necessary for the changes actually to occur.

By their nature, sex hormones tend to have similar chemical configurations, so a conversion of the F-substance by a chemical inadvertently released by the parasite is quite possible. If for some reason, such as an external factor, e.g. depth, the general workings and effect of the parasite on its host, has to some degree broken down, then this could be the result, hence the fact that all the Type 9 females were found at the extreme end of *Triangulus munidae*'s depth range.

#### *The influence of depth*

It was seen earlier that the distribution of the different pleopod Types was aligned with the bathymetric gradient, for males there being a decrease in the proportion of the Type 3 with depth. This involved an increase in the non-affected Type 1 pleopods in the deepest samples, with the intermediate stages becoming apparent in between the two (Fig. 7a-e). Likewise for the females, the Type 9 structures were absent in the shallower water (Fig. 7g). In addition to this change in pleopod structure with depth, Fig. 2 indicated an increase in the percentage infestation of *Triangulus munidae* with depth. So, in the deeper water, the rhizocephalan has a decreased effect on its host, but a greater proportion of the hosts are infested. This could be due to both *T. munidae* and *M. sarsi* being at the extremes of their depth ranges. Clearly, when an organism is at the limits of any physical range, the stress involved with the environmental parameters encountered, extremes of temperature, salinity, pressure, etc., will prevent the organism from achieving its optimum physiological state. This physiological weakness will in turn make them more susceptible to infections and generally make them less effective than individuals occupying more favourable conditions.

In the case of *Triangulus munidae* on *Munida sarsi*, both organisms are at the extremes of their ranges at below 700 m and so both become affected by the physical stresses of living in such an environment. *Munida sarsi*, being physiologically weakened, becomes more susceptible to infestation, hence the higher percentage of individuals found parasitized here than in the fitter populations at shallower depths. However, despite infesting their host to a greater degree, *T. munidae* is also influenced by being at the extreme of its bathymetric range, the result being that at depth it is unable to influence the development of its host as severely as it would if it were shallower. The Type 3 pleopod structure of males, the sign of the feminization required by the rhizocephalan for the munidid to protect the externa as if it were an egg mass, is therefore not achieved.

#### *The effect of externa size*

Figure 8 illustrates how the distribution of the pleopod Types was not spread evenly over the range of externa sizes, there being a development from the unaffected Type 1 to the feminized Type 3 as the externa grew in size. At first, this would seem peculiar, as it is known that in *Brachyura*, once the rhizocephalan becomes external, the crab ceases to moult (Reinhard, 1956; Hartnoll, 1967; Phillips & Cannon, 1978). However, the situation is different for anomurans as it is possible for them to undergo ecdysis despite the presence of an externa on the abdomen (Brinkmann, 1936;

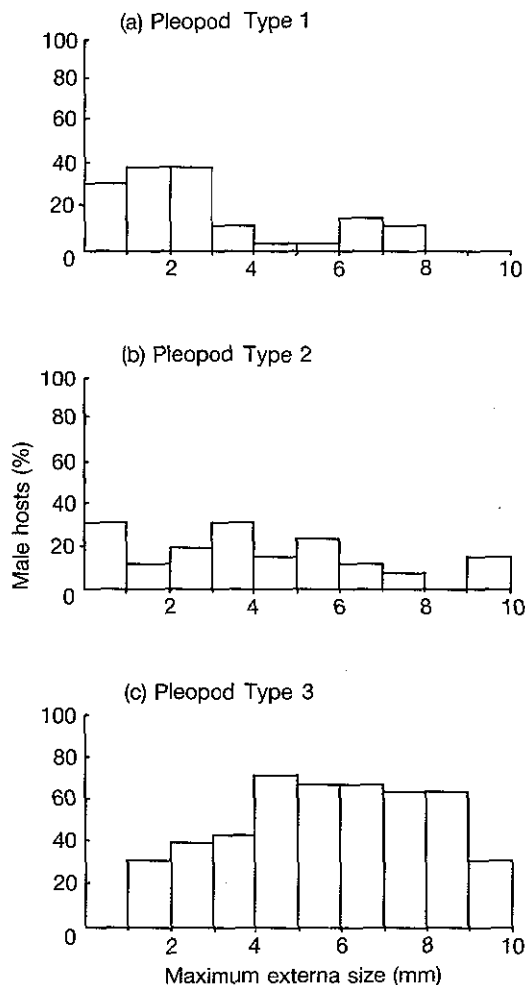


FIG. 8. The effect of externa size on the pleopod Type. Percentage of male *Munida sarsi* with pleopod Types 1-3 for each mm size class of *Triangulus munidae* externa. N-values:

Externa size (mm)	0.0-0.9	1.0-1.9	2.0-2.9	3.0-3.9	4.0-4.9	5.0-5.9	6.0-6.9	7.0-7.9	8.0-8.9	9.0+
Total numbers	3	10	5	9	16	19	22	16	8	6

Veillet, 1945; Høeg, 1982; Bower & Sloan, 1985) and so the modification of the pleopods could take place after the externa has emerged. This is backed up by the fact that no examples were found of an individual *M. sarsi* with parasitized Type pleopods, but neither externa nor scar.

If, as in brachyurans, the eventual inability to moult is due to mechanical rather than physiological factors (Brinkmann, 1936), then it is suggested that in galatheids it takes a certain size of externa before moulting is inhibited, if at all, and the development of the pleopods takes place when the externa has emerged, but is still in the immature growth phase.

The parasitization of *Munida sarsi* by *Triangulus munidae* demonstrates how an increase in depth, and so an increase in pressure and decrease in temperature, can affect the physiology and so the compatibility of deep-sea Crustacea, though how these factors, especially pressure, actually influence the internal workings of the crustacean is not yet clear.

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