

Fecundity limitation of *Calanus helgolandicus*, by the parasite *Ellobiopsis* sp.

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The effect of the parasite Ellobiopsis sp., on the fecundity of Calanus helgolandicus and Calanoides carinatus in the Bay of Biscay, was investigated in May 2003. An average of 6.8% of C. helgolandicus females were infected with Ellobiopsis sp., whereas none of the C. carinatus were found to be infected. An objective method of estimating gonad development was applied to quantitatively measure the effect of the parasitism on the reproduction of the copepod. Parasitism by Ellobiopsis sp. has the potential to reduce the fecundity of C. helgolandicus females.

INTRODUCTION

Numerous parasites are associated with copepods (Théodoridès, 1989), the most abundant metazoans in the sea and a key trophic link in pelagic food webs (Humes, 1994). Although parasitic infestations have shown their potential to regulate freshwater (Burns, 1985, 1989) and marine copepod populations (Théodoridès, 1989; Kimmerer and McKinnon, 1990; Shields, 1994), the consequences of parasitism for natural copepod communities have received little attention, and few investigations of copepod parasites have been carried out systematically (Skovgaard, 2005).

Only limited studies have been devoted to Ellobiopsids, a group of Protista that parasitizes Crustaceans, which has been associated with Dinoflagellates, although their systematic position remains uncertain (Théodoridès, 1989; Shields, 1994; Cavalier-Smith, 2003). The genus *Ellobiopsis* is a parasite of copepods (Théodoridès, 1989) and was first described in 1910 (Caullery, 1910). The *Ellobiopsis* life cycle (Jepps, 1937; Hovasse, 1952; Théodoridès, 1989; Shields, 1994) comprises a dispersion phase, a flagellated spore which settles presumably on or adjacent to a seta of an appendage of the host and then becomes ovoid or cylindrical whilst an attachment or sucking device develops (Fig. 1a and b). This sucking device penetrates through the host cuticle; when the body of parasite reaches a certain size, it becomes transversally septate, with two segments (Fig. 1c): the proximal or trophomere and the distal or gonomere. The gonomere

constitutes the reproductive body of the animal where spores are produced. Sporulation occurs as the gonomere becomes tightly constricted from the trophomere. After the dead parasite falls off (Fig. 1d), a channel remains as the last indication of previous infection (Fig. 1e).

Ellobiopsis sp. is known to cause epizootics in its host populations, which may result in fundamental changes in ecological communities (Shields, 1994). However, the impact of parasitism on marine copepod communities is difficult to assess (Ianora *et al.*, 1990). Difficulties in identifying a suitable number of parasitized individuals, together with the limitations of the methods to objectively measure the effect exerted on the host, are the main obstacles. Only recently have quantitative reproductive indexes, which allow the empirical prediction of egg production from preserved samples, been developed (Runge, 1987; Niehoff and Runge, 2003). The objective of this study was to test quantitatively the hypothesis that *Ellobiopsis* sp. has the potential to reduce fecundity in marine copepods, as has been suggested in previous works (Jepps, 1937; Wickstead, 1963).

METHODS

Zooplankton samples were collected during 22–25 May 2003 in a grid of 72 stations every 3 nautical miles that covered the southeastern corner of the Bay of Biscay (Fig. 2a). Samples were collected using vertical hauls of

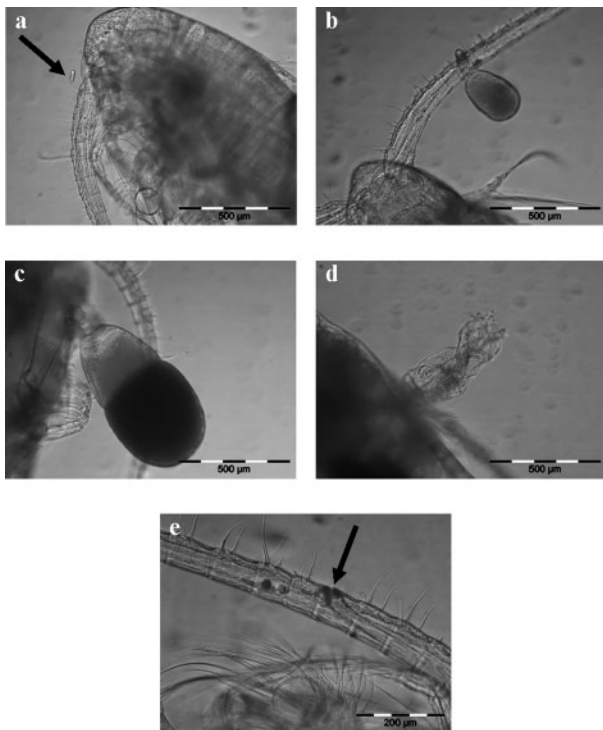


Fig. 1. Photographs (scale superimposed on each image) illustrating the life cycle of *Ellobiopsis* sp. on *Calanus helgolandicus* females. (a) Recently settled *Ellobiopsis* sp. (indicated by an arrow) projecting from setae on the antennule. (b) *Ellobiopsis* sp. with fully developed sucking device. (c) Reproductive stage of *Ellobiopsis* sp. showing the septation between trophomere and gonomere. (d) Empty body of dead *Ellobiopsis* sp. (e) Channel showing past infection by *Ellobiopsis* sp. (arrow shows the point of insertion).

a 150-µm PAIROVET net (0.25-m net diameter), lowered to a maximum depth of 100 m, or 5 m above the bottom at shallower stations. Net samples were preserved immediately after collection, with 4% borax-buffered formaldehyde.

Each sample was examined under a stereoscopic microscope and 30–35 females of *Calanus helgolandicus* and *Calanoides carinatus* were randomly sorted; all the females in the sample were sorted when the minimum number was <30.

Female gonad development stage (‘GS’) was determined following Niehoff and Runge’s (Niehoff and Runge, 2003) methodology; this classifies females, based on increasing gonad maturation, as GS1 (immature gonads, no developing oocytes in the diverticula), GS2 (immature gonads, one row of developing oocytes in the diverticula), GS3 (immature gonads, multiple layers of developing oocytes in the diverticula) and finally GS4 (mature gonads, oocytes undergoing final maturation; females predicted to spawn within 24 h).

Females with attached *Ellobiopsis* sp., of a minimum size of 75 and 50 µm for the major and minor axes,

respectively, were selected for detailed analysis of the parasite. This size criterion was established according to the comments of Jepps (Jepps, 1937) to avoid taking into account parasites without developed sucking device; these are not taking resources from the host, and so their survival is not assured. Parasites were classified according to the life cycle as ‘a’ (prior to the development of gonomere; Fig. 1b), ‘b’ (with gonomere; Fig. 1c) and ‘c’ (in the process of, or after, spreading of the spores; Fig. 1d). *Ellobiopsis* spp. on each female were counted; their points of insertion were determined, and major and minor axes were measured when the parasite was complete (‘a’ and ‘b’ stages). Finally, the volume of the stages measured was calculated assuming that of an ellipsoidal body.

Spatial incidence of the infection in the sampled area was determined using only stations with >20 females for each species: 58 stations of 72 (81%) for *C. helgolandicus* and 43 (60%) for *C. carinatus* fulfilled this condition.

RESULTS

A total of 2134 and 1706 females of *C. helgolandicus* and *C. carinatus*, respectively, were sorted for analysis. On average, 6.8% of *C. helgolandicus* females were infected with *Ellobiopsis* sp., whereas none of the *C. carinatus* were found to be infected. Day/night sampling (i.e. vertical migration) did not affect the percentage of females affected [7.2% during day (07:00 h–21:00 h) samples and 7% during night]. The highest infection incidence occurred at the oceanic stations (8.9% mean incidence at stations greater than 200 m depth compared with 4% at the remaining stations); maximum values of infection for a station reached 23%, whilst at only nine stations (of 58 with >20 females) was *Ellobiopsis* sp. absent (Fig. 2b).

The mean number of *Ellobiopsis* sp. on each parasitized female was 1.86, with a maximum of 7. The number of parasites attached to a female was not related to the total volume of parasite attached to it (Fig. 3). The points of attachment were, in decreasing order of prevalence: antennule (A1), maxillule (Mx1), maxilliped (Mxp), maxilla (Mx2), antenna (A2), mandible (Md), first pair of swimming legs (P1) and rostrum (R); there was the same incidence for the left and right side of the animal (Table I). Parasites with a major axis longer than 0.25 mm were able to develop the gonomere and reproduce (Fig. 4). Maximum volumes per parasite ranged from 0.05 mm³ (‘a’ stage) to 0.12 mm³ (‘b’ stage) with, respectively, 0.006 mm³ and 0.038 mm³ as the mean values.

There was a statistically significant difference ($P < 0.001$; Mann–Whitney test) in the gonad development stage, for

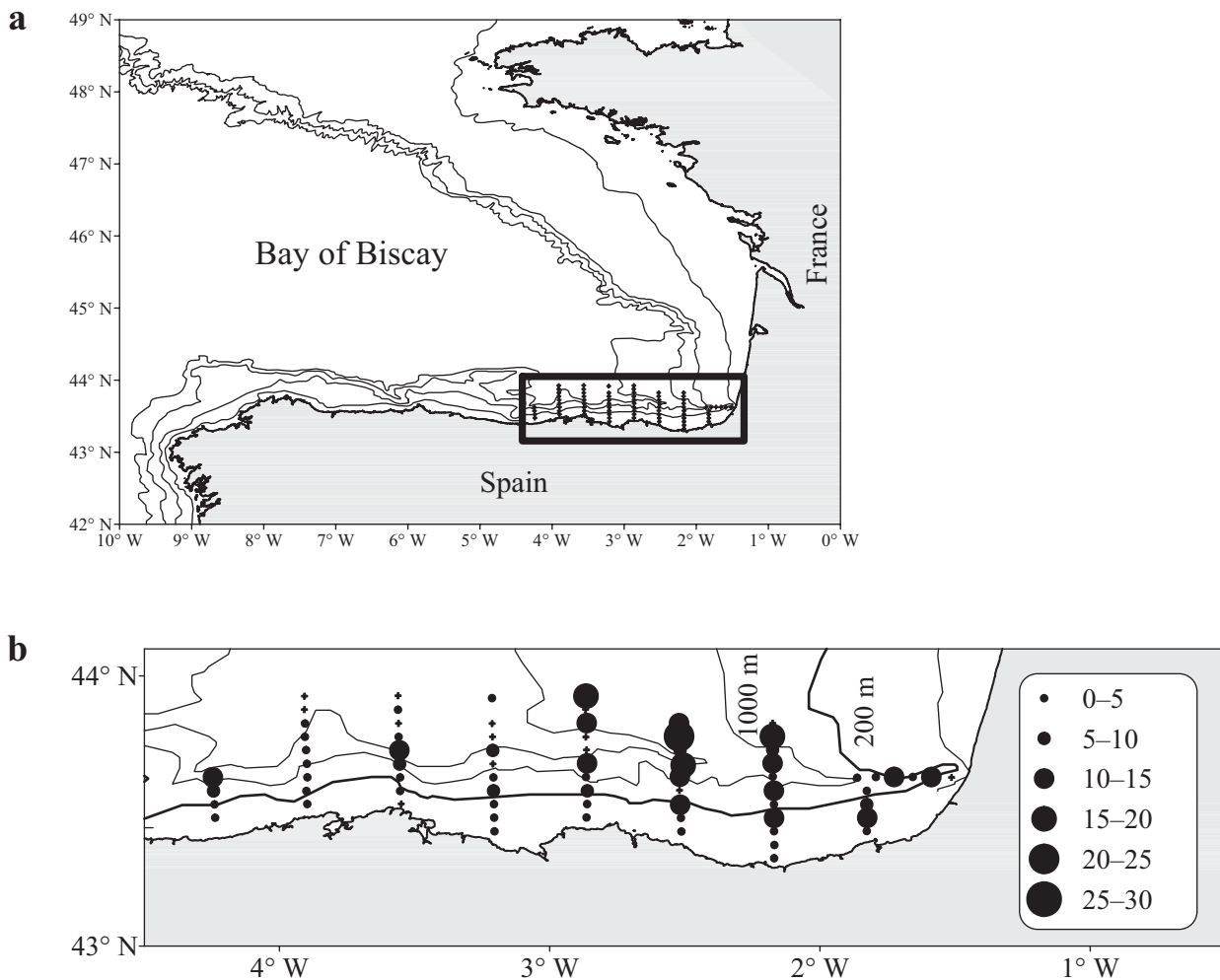


Fig. 2.(a) The Bay of Biscay showing the location of the sampled stations, together with the 100-, 200-, 1000- and 2000-m isobaths. (b) Detail of the sampled area with superimposed values for the incidence (%) of infection by *Ellobiopsis* sp. in *Calanus helgolandicus* females. The 200-m isobath is highlighted. Crosses indicate stations where incidence was not computed because <20 females were found. Note: for details, see text.

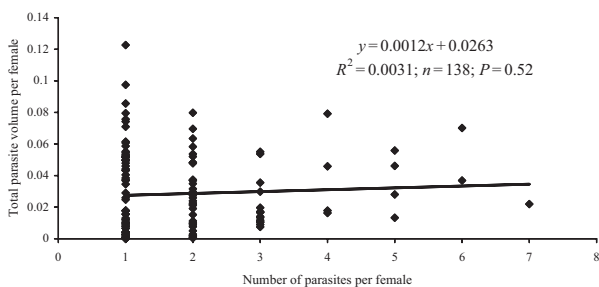


Fig. 3. Relationship between the computed volume of parasite per female (mm^3) and the number of *Ellobiopsis* sp. Note: females with parasites in the sporulation process ('c' stage, see text) were not included, as measuring the volume was not possible.

parasitized and non-parasitized females. The percentage of females with no developing oocytes, in both the anterior

and posterior diverticula of oviducts (GS1 females; Niehoff and Runge, 2003), reached 75% in the parasitized females; in the non-parasitized population, this represented only 6% of the cases (Fig. 5). Otherwise, females with oocytes undergoing final maturation (GS4 females) comprised only 12.8% of the parasitized population; in contrast, this proportion was 55.6% in the non-parasitized animals (Fig. 5).

For parasitized females, the incidence of the GS4 stage decreased with an increasing volume of parasite attached (Fig. 6).

DISCUSSION

Of the two co-occurring populations of copepods selected for the study, only *C. helgolandicus* was infected by *Ellobiopsis* sp. These results confirm the host

Table I: Distribution of attachment points for *Ellobiopsis* sp.

Point of insertion	Number of <i>Ellobiopsis</i>	Left	Right	Incidence (%)	Prevalence
R	1	1	0	0.31	8
A1	112	57	55	34.25	1
A2	29	13	16	8.87	5
Md	14	8	6	4.28	6
Mx1	70	32	38	21.41	2
Mx2	35	16	19	10.7	4
Mxp	63	33	30	19.27	3
P1	3	0	3	0.92	7
P2	0	0	0	0	
P3	0	0	0	0	
P4	0	0	0	0	
P5	0	0	0	0	
F	0	0	0	0	
O	0	0	0	0	
Total	327	160	167	100	

A1, antennule; A2, antenna; F, furca; Md, mandible; Mx1, maxillule; Mx2, maxilla; Mxp, maxilliped; O, others; P1, first pair of swimming legs; P2, second pair of swimming legs; P3, third pair of swimming legs; P4, fourth pair of swimming legs; P5, fifth pair of swimming legs; R, rostrum. Points of insertion are shown, together with the number of parasites attached to them, discriminating between the left and the right side of the animal. Incidence is expressed as the percentage of total parasites for each point of insertion.

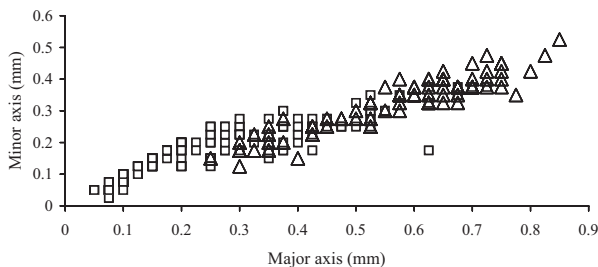


Fig. 4. *Ellobiopsis* sp. shape curve, with minor plotted against major axis lengths, resulting from combining all the measurable parasites in the present study: stages 'a' (squares) and 'b' (triangles) (see text for further explanation).

specificity of *Ellobiopsis* sp. that has been suggested in previous studies (Wickstead, 1963; Hoffman and Yancey, 1966). The reasons for this association remain undetermined (Shields, 1994), but different biological or behavioural factors could be involved. The difference in infection between *C. helgolandicus* and *C. carinatus* is unlikely to be due to spatial factors because although *C. carinatus* does have a more neritic distribution, both species overlap in areas where *C. helgolandicus* is infected.

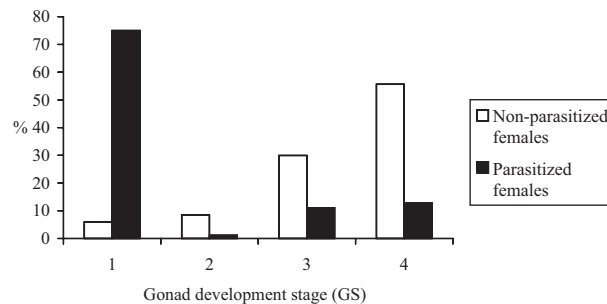


Fig. 5. Comparison ($P < 0.001$; Mann–Whitney test) of the degree of gonad development, expressed as percentage of the different gonad maturation stages (see text for further explanation), for parasitized (filled bars) and non-parasitized (empty bars) *Calanus helgolandicus* females.

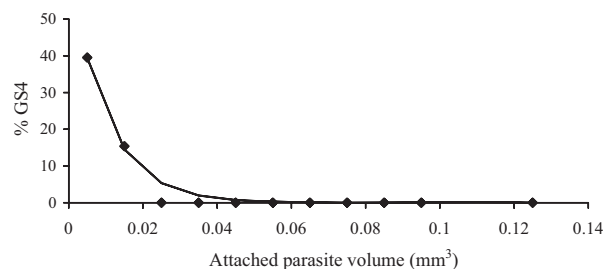


Fig. 6. Relationship between the probability of spawning within 24 h, expressed as percentage of GS4 stage following Niehoff and Runge (2003) (left axis) in parasitized *Calanus helgolandicus* females (fitted curve: $y = 65e^{-100x}$) and the attached parasite volume (mm^3). Females were grouped according to the attached volume of parasite in 11 classes, to permit the calculation of % GS4; females with parasites in the sporulation process ('c' stage, see text) were not included, as measuring the volume was not possible.

In our samples, individuals of *Pseudocalanus elongatus*, *Ctenocalanus vanus* and *Temora longicornis* were infected with *Ellobiopsis* sp. However, whether the parasite in these copepods is the same species or not is currently under discussion (Boschma, 1959; Shields, 1994). The size and the presence of one gonomere suggest that the *Ellobiopsis* studied in the present investigation corresponds to the description of *Ellobiopsis chattoni* (Caullery, 1910; Shields, 1994).

The incidence of infection of *C. helgolandicus* in our study (Fig. 2b) is comparable with that found in the limited published literature on the topic (Table II). Although 6.8% is a considerable value for infection, this is a conservative estimate, because only parasites with a minimum size of 75 and 50 μm for major and minor axes, respectively, were counted. Whilst the mechanism of infection for other parasites of copepods remains undetermined (Kimmerer and McKinnon, 1990; Skovgaard, 2005), the preferred location of *Ellobiopsis* sp. (Table I) suggests that they are captured

Table II: Reported prevalence of *Ellobiopsis* sp., in copepod populations, expressed as the percentage of infected individuals

Host	Location	Reference	Prevalence (%)	Volume ratio
<i>Calanus finmarchicus</i>	Loch Striven, Scotland	Marshall <i>et al.</i> (1934)	0.3	No data
<i>Undinula vulgaris</i>	Zanzibar Channel of Africa	Wickstead (1963)	26	1/8–1/10
<i>Metridia longa</i>	Kachemak Bay, Alaska	Hoffman and Yancey (1966)	5–22.4	No data
<i>Undinula vulgaris</i>	Bay of Bengal, India	Santhakumari and Saraswathy (1979)	8.3	No data
<i>Calanus finmarchicus</i>	Norwegian Sea	Timofeev (2002)	15	No data
<i>Calanus helgolandicus</i>	Bay of Biscay	Present study	6.8	1/5–1/6

The column 'volume ratio' shows the relationship between the maximum parasite volume and the mean host volume.

during the feeding activity of the animal. Calanoid copepods are known to create currents with their cephalic appendages that, along with the foraging movement, guide the surrounding particles from the top of the animal to the mouth area (e.g. Koehl and Strickler, 1981; Malkiel *et al.*, 2003). This movement corresponds well with the described pattern, as the antennule becomes the first exposed part and also has the highest incidence, whereas the swimming legs carry few if any parasites.

The lack of correspondence between the number of parasites and the total parasite volume per female (Fig. 3) suggests that the parasite-carrying capacity per female is limited. Such a limitation is probably related to energy resources for parasite feeding and the need to ensure that the female remains alive to complete the life cycle of the parasite. The data presented in Fig. 4 support this idea, by showing that the development of the gonomere ('b' stage; Fig. 1c) is independent of the dimensions acquired, once a minimal size threshold is reached; this coincides with a shift in the shape, from a more spherical body to a more ellipsoidal one. This process allows the successful reproduction of the parasite when low resources are available (either due to the drainage of the nutritional source or due to competition for resources in multiple infections), by decreasing the future spore production.

Although parasitized *C. helgolandicus* were feeding (i.e. relatively full guts were observed), the degree of gonad development of the infected population was significantly reduced when compared with the non-infected population (Fig. 5). The percentage of females predicted to spawn within 24 h (GS4 females; Niehoff and Runge, 2003) was more than four times higher in the non-infected population; this shows that infection due to *Ellobiopsis* sp. reduces maturity index in female *C. helgolandicus* by preventing the normal development of gonads. The same conclusion was reached by Jepps (Jepps, 1937) and Wickstead (Wickstead, 1963), based

on the observation of gonads of female *Calanus finmarchicus* and *Undinula vulgaris* parasitized by *Ellobiopsis chattoni*. However, in these earlier studies, no objective method of estimating gonad development was used, whilst the magnitude of the infection was not quantitatively measured. Based on this pathology, *Ellobiopsis* sp. is defined as a parasitic castrator rather than a true parasite (Kuris, 1974).

As females with an attached volume of parasite exceeding 0.02 mm³ never reach the GS4 stage (Fig. 6), with the medium size of a fully developed parasite ('b' stage) being 0.038 mm³, it can be concluded that all infected females will stop laying eggs as the parasite evolves. A reduction in fecundity of ~6.8%, for the parasitized *C. helgolandicus* population in the Bay of Biscay, can thus be assigned to parasitism by *Ellobiopsis* sp. A 6.8% increase in the early-stage mortality is not a negligible value; as such, it should be taken into account when estimating the mortality of a population, because it implies a significant reduction in the recruitment rate. Although the recruitment rate is initially relatively high, on average only 2.7% of the eggs survive to the first larval stage in the congeneric *C. finmarchicus* (Ohman and Hirche, 2001). Assuming the same survival for *C. helgolandicus* and adding the effect of parasitism by *Ellobiopsis* sp. (100% mortality of eggs, for 6.8% of females), the survival would decrease to 2.5%. Although apparently negligible, small reductions in survival in the early, most abundant, development stages have important effects on population dynamics. Furthermore, the castration of a host may have a more important effect on the host population than does the death of the afflicted host (Kuris, 1974). If parasitized females mate, the effects on the copepod population may be considerable; this is not only because infected females do not produce eggs, but also because mating with a sterile female is bound to reduce the overall potential for the successful mating of healthy males (Skovgaard, 2005).

The increase in mortality of the *C. helgolandicus* population assigned to *Ellobiopsis* sp. is underestimated; this is not only because the incidence of infection was computed only for parasites over a size threshold, but also because the observed parasitism of males and copepodites was not computed. Parasitized copepodites and males of *C. helgolandicus* were observed during this study, but the effect was not quantified; however, moulting cessation and feminization of males are common consequences in marine crustaceans infected by parasitic castrators (Shields, 1994). Several males were selected for the observation of gonads: size reduction of both seminal vesicle and developing spermatophore sac was noted in parasitized individuals, when compared with non-infected animals. Similar findings were reported by Mauchline (Mauchline, 1966) in male euphausiids parasitized by Ellobiopsids.

The genus *Ellobiopsis* is widespread, on a global basis, having been reported infecting different copepod species from the Arctic to the Indian Ocean (Shields, 1994). The significant effect on copepod fecundity, as measured in the present study, has identified the need for further research, to properly evaluate the impact of parasitism on copepod population dynamics.

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