

Vicious circle in the intertidal: Facilitation between barnacle epibionts, a shell boring polychaete and trematode parasites in the periwinkle *Littorina littorea*

David W. Thieltges^{*}, Christian Buschbaum

Alfred Wegener Institute for Polar and Marine Research, Wadden Sea Station Sylt, Hafenstrasse 43, 25992 List, Germany

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Abstract

We studied interactions between three organisms associated with a common gastropod of northern Atlantic shores, the periwinkle *Littorina littorea*: barnacle epibionts *Balanus crenatus*, a shell boring polychaete *Polydora ciliata*, and tissue invading trematodes which use the periwinkles as first intermediate host. Snails collected shortly after barnacle settlement with >50% cover of barnacles had significantly higher infestation of shell boring worms compared to unfouled snails, while trematode infestation was similar. The result was the same at two sites, and we conclude that the worm *P. ciliata* facilitates barnacle fouling on snails. The reverse was also the case. In an experiment with 14 weeks of exposure, snail treatments with barnacle epibionts had a significantly higher *P. ciliata* load than unfouled and cleaned snails. Again, trematode infestations were similar. The reciprocal positive interactions between barnacle epibionts and shell boring worms on snail houses is regarded as a case of facultative mutualism. On the other hand, for the snail basibiont, both barnacles and shell boring worms exert strongly negative effects by reducing fecundity, growth, and survival, resulting in a vicious circle for the snails. The combined effects of these associated organisms may rival in importance any competitive or predacious effects on the host *L. littorea*.

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1. Introduction

Epibiosis, i.e. species serving as substrate (basibiont) for other organisms (epibionts), is a common phenomenon and an important ecological issue in marine systems (Wahl, 1989). In molluscs and especially in gastropods, epibionts like barnacles have been described to exert multiple negative effects on their basibionts such as enhanced dislodgment by increased drag forces, shell destruction, reduced mobility, lower fecundity or even mortality

(Witman and Suchanek, 1984; Wahl, 1996, 1997; Buschbaum and Reise, 1999; Buschbaum and Saier, 2001).

Epibionts are not the only organisms associated with molluscs (Cheng, 1970; Lauckner, 1980, 1983). Shell boring polychaetes seek shelter in the calcareous substrate. Tissues of molluscs are often inhabited by parasitic trematodes utilizing molluscs as first intermediate hosts in their complex life cycles. These shell boring polychaetes and tissue inhabiting trematodes might interact with epibionts with the outcome being negative, neutral or positive.

In this study we investigated reciprocal effects of shell boring polychaetes and tissue inhabiting trematodes on

^{*} Corresponding author. Tel.: +49 4651 956 0; fax: +49 4651 956 200.
E-mail address: dthieltges@awi-bremerhaven.de (D.W. Thieltges).

barnacle epibionts and used the periwinkle *Littorina littorea* as a model host. *L. littorea* is one of the most common gastropods at North Atlantic shores (Bertness, 1999) with a diverse epibiont community (Wahl and Hay, 1995; Buschbaum and Reise, 1999). In our study area, epibionts of *L. littorea* are mainly barnacles with up to 70% of snails on lower intertidal mussel beds being strongly covered with barnacles (Buschbaum and Reise, 1999). Besides barnacles, the shell boring polychaete *Polydora ciliata* and six species of tissue inhabiting trematodes utilising the snail as first intermediate host are commonly associated with *L. littorea* (Dorsett, 1961; Werding, 1969; Lauckner, 1980; Warner, 1997).

It is likely that positive interactions occur between shell boring polychaetes or tissue inhabiting trematodes and barnacle epibionts. The holes excavated by *P. ciliata* might attract rough surface preferring barnacle larvae during settlement resulting in enhanced densities of barnacle epibionts. Barnacle epibionts on the other hand might enhance settlement by *P. ciliata* by providing rough surfaces resulting in higher infestation rates of this species. Trematodes might weaken the snail's natural defences (organic coating on shell surface, chemical defences etc.) against epibionts resulting in higher barnacle cover on infected snails. Barnacle cover on the other hand might attract trematode infective stages due to chemical cues or make snails more susceptible to trematode infections by weakening the snail's natural defences. In contrast, negative interactions are also likely: *P. ciliata* worms might push off barnacle larvae from the shells at settlement resulting in inhibition of barnacle epibionts. On the other hand, barnacle epibionts may prey on or otherwise prevent *P. ciliata* larvae from settling inhibiting worm infestation. Trematodes might make snails less attractive to barnacle epibionts resulting in a lower barnacle coverage. Barnacle epibionts on the other hand, may negatively affect trematodes by preying upon trematode larvae intending to infest the snail.

We tested four null hypotheses regarding potential interactions between barnacle epibionts and infestation by *P. ciliata* or trematode parasites: 1) infestation by the shell boring polychaete *P. ciliata* does not affect the settlement of barnacles, 2) tissue inhabiting trematodes do not affect the settlement of barnacle epibionts, 3) barnacle epibionts do not affect the infection of snails by the shell boring *P. polydora* and 4) barnacle epibionts do not affect the infection of snails by tissue inhabiting trematodes. In the case that a null hypothesis is rejected, the interaction may be positive (facilitation) or negative (inhibition). To test hypotheses 1) and 2), we sampled and investigated snails with and without juvenile

barnacle epibionts shortly after barnacle settlement. To test hypothesis 3) and 4), we experimentally exposed snails that were barnacle-fouled, unfouled and cleaned (from barnacle cover) in the field for 14 weeks.

2. Materials and methods

2.1. Study area

Investigations were carried out in the northern Wadden Sea near the island of Sylt (North Sea, Germany) (Fig. 1). Tides are semidiurnal with a mean range of 2 m. Salinity remains close to 30 psu. Mussel beds (*Mytilus edulis*) cover about 3% in the intertidal zone and are the most favourable habitat for periwinkles *L. littorea* where they can reach densities of >2000 ind. m^{-2} (Buschbaum, 2000). For further information on the area see Gätje and Reise (1998).

2.2. Field survey

To investigate effects of shell boring *P. ciliata* and tissue inhabiting trematode presence on barnacle epibiont

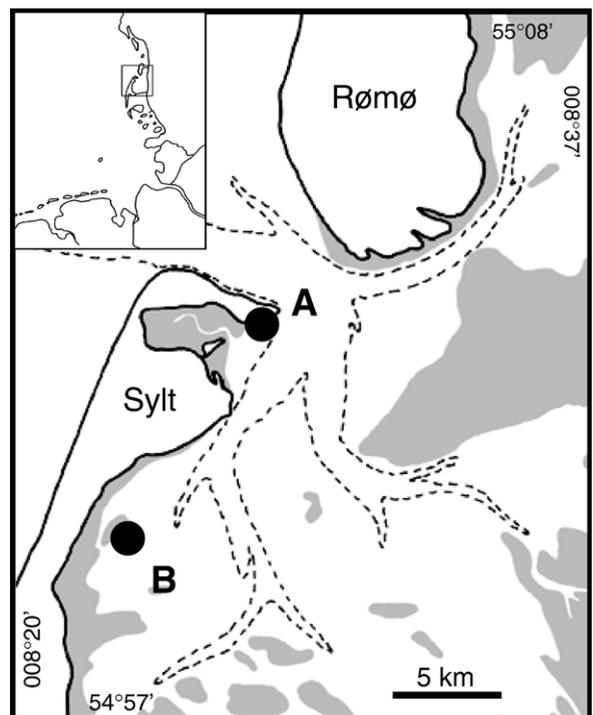


Fig. 1. Study area, the List tidal basin (North Sea, Germany). Black dots indicate mussel beds where investigations were carried out. Site A is located near the northern tip of the island of Sylt while site B is located in the inner part of the bight. Shaded areas indicate the intertidal.

settlement (hypotheses 1 and 2) we collected *L. littorea* from two mussel beds following the barnacle settlement period in May 2003 (Fig. 1). On each mussel bed, an unbiased sample of 100 individuals completely unfouled and 100 individuals fouled by barnacles (*Balanus crenatus*) with >50% shell coverage (Fig. 2) was obtained by repeatedly and randomly throwing a 1/8-m²-frame within an area of 20 m² in the lower intertidal (+50 cm mean low water level (MLWL) to MLWL) and collecting all snails with appropriate fouling and size. Snails fouled by early recruits of barnacles (<2 mm shell diameter) and no other epibionts were chosen to ensure that settlement had taken place within the preceding 2 weeks (Bertness, 1999). Since barnacles, *P. ciliata* and trematodes only occur in larger snails, individuals from 16 to 23 mm were collected. Snails >23 mm were not found at the sites. In the laboratory, snail shell height was measured with a calliper to the nearest 0.1 mm. After crushing the shell, body tissues and shells were investigated for *P. ciliata* and trematode (sporocysts/rediae) presence under a dissection microscope.

2.3. Field experiment

To examine potential effects of barnacle epibionts on infestation by *P. ciliata* and trematodes (hypotheses 3 and 4) a field experiment was conducted on mussel bed A (Fig. 1). Fouled (with one year old *B. crenatus* >70% shell coverage) and unfouled snails of 16–20 mm were collected randomly within the same area of the mussel bed. Before the experiment, 100 fouled and unfouled snails were investigated for *P. ciliata* and trematode prevalence to estimate a baseline for infection levels. Barnacle cover on 120 fouled snails was carefully removed with a knife allowing us to create three treatments: 1) snails with barnacle epibionts (“fouled”), 2)



Fig. 2. Periwinkles (*L. littorea*) with (left) and without (right) barnacle epibionts shortly after barnacle settlement used for investigations.

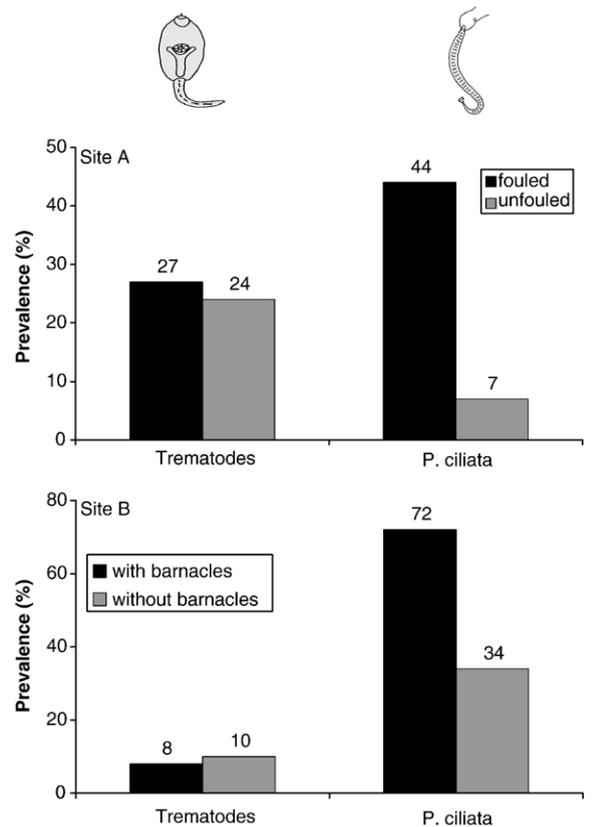


Fig. 3. Prevalence (%) of trematodes and *P. ciliata* in barnacle fouled and unfouled *L. littorea* on two mussel beds (*Mytilus edulis*). $n = 100$ each.

snails with barnacle epibionts removed (“cleaned snails”) and 3) snails without any natural epibionts (“unfouled”). 20 individuals of each treatment were put in a cage made of polyethylene (5 mm mesh size) of 25 cm diameter and 30 cm height together with blue mussels (*M. edulis*) of natural density. Cages were fixed with iron rods on the mussel bed in June 2003 in a completely random design near low water line. All three treatments were replicated six times. After 14 weeks of exposure the cages were emptied and snails investigated for *P. ciliata* and trematodes as described above.

2.4. Statistical analysis

Statistical analysis for differences in prevalence (% infected from total sample) of *P. ciliata* and trematodes between barnacle fouled and unfouled snails was performed using Chi-square tests (2×2 frequency tables). The field experiment was analysed using analysis of variance (one-way ANOVA). Post hoc calculations were carried out with Tukey HSD-test (Day and Quinn, 1989). Since Cochran’s-C-Test showed homogeneous

variances and data were normally distributed, non-transformed data were used for the analysis. Shell height of snails was compared between treatments using *t*-tests (field study) or one-way ANOVA (field experiment) following log-transformation of data to result in homogeneity of variance and normal distribution.

3. Results

Shortly after barnacle settlement, fouled *L. littorea* were found to be significantly more infested by *P. ciliata* than unfouled snails (Chi-square test, $p < 0.001$ for each mussel bed; Fig. 3). This was due to a 2–6 times higher infestation in fouled compared to unfouled snails. Significant differences in infestation by trematodes between barnacle fouled and unfouled snails were not detected at either site (Chi-square test, $p = 0.6$ for each mussel bed). There were no significant differences in snail shell height between fouled and unfouled snails at the two sites (*t*-tests, $F_{\text{Site A}} = 1.08$, $p_{\text{Site A}} = 0.30$; $F_{\text{Site B}} = 0.53$, $p_{\text{Site B}} = 0.47$). Parasites found in the snails were *Renicola roscovita*, *Cryptocotyle lingua*, *Himasthla elongata*, *Microphallus pygmaeus* and *Podocotyle atomon*.

In the cage experiment significant differences in *P. ciliata* prevalence were observed between the three treatments (one-way ANOVA, $F_{2,15} = 38.30$, $p < 0.001$; Fig. 4). This was due to a 2–7 times higher prevalence of *P. ciliata* in fouled ($51.2 \pm 5.7\%$) compared to cleaned ($24.7 \pm 3.2\%$) and unfouled ($7.8 \pm 3.5\%$) *L. littorea* (Tukey–HSD: fouled–cleaned: < 0.001 ; fouled–unfouled: < 0.001 ; cleaned–unfouled: < 0.01). No statistical difference was observed in trematode prevalence (fouled: $12.4 \pm 2.2\%$; cleaned: $10.2 \pm 3.7\%$; unfouled: $7.8 \pm 3.5\%$) (one way-ANOVA, $F_{2,15} = 5.10$, $p = 0.61$) and mortality (fouled: $6.3 \pm 1.7\%$; cleaned: $1.7 \pm 1.1\%$; unfouled: $2.7 \pm 1.8\%$) (one way-ANOVA, $F_{2,15} = 2.25$, $p = 0.14$) of snails

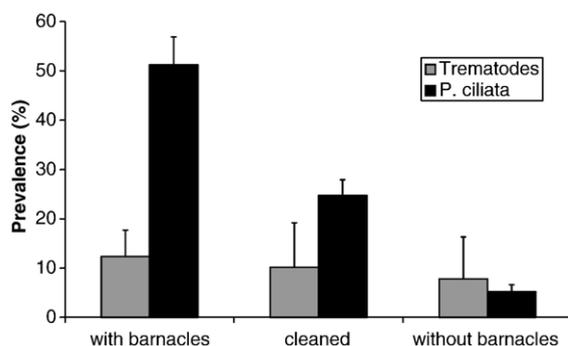


Fig. 4. Mean prevalence (%) ± SE of *P. ciliata* and trematodes in three experimental treatments of *L. littorea*. Fouled: Naturally barnacle fouled snails; cleaned: fouled snails with barnacle epibionts taken off; unfouled: naturally unfouled snails. $n = 6$ for each treatment.

between the three treatments. There was no significant difference in snail shell height between the three treatments (one way ANOVA, $F_{2,357} = 1.34$, $p = 0.25$). Trematodes found in snails were as above. At the beginning of the experiment, *P. ciliata* and trematode prevalence in fouled snails was 20% and 11%, respectively (unfouled snails: 8% and 9%).

4. Discussion

Our results demonstrate that barnacle epibionts and shell boring *P. ciliata* strongly facilitate each other while tissue inhabiting trematodes do not seem to interact with barnacle epibionts in *L. littorea*.

Infestation by *P. ciliata* facilitates the settlement of barnacles on *L. littorea*. Shortly after barnacle settlement, snails with barnacle epibionts present showed a higher infestation by *P. ciliata* than individuals without epibionts. Since only 2 weeks had passed between barnacle settlement and our investigation, the observed effects are due to the presence of *P. ciliata* before and not following the settlement of barnacles inferred from the mostly adult sizes of the spionid worms. Although not a controlled experiment, a facilitative process is likely: barnacle larvae are known to be attracted by rough, uneven surface textures at settlement (e.g. Crisp and Barnes, 1954) here provided by the holes and crevices created by *P. ciliata*. Larger barnacle epibionts were also found to be correlated with *P. ciliata* presence (Warner, 1997). However, Warner (1997) suggested this to result from barnacles facilitating *P. ciliata* settlement and not the other way round.

Trematode infestations did not correlate with barnacle settlement. We predicted that trematodes might weaken potential antifouling mechanisms of snails by interfering with the snail's metabolism resulting in a higher degree of fouling in trematode infected individuals. However, it is suspected that *L. littorea* has a lack of specific antifouling capacities (Wahl and Sönnichsen, 1992). This might explain our finding since there may not be a mechanism that can be influenced by trematode infestations.

Barnacle epibionts facilitate infestation of snails by *P. ciliata*. After 14 weeks of exposure, snails with barnacle epibionts showed higher *P. ciliata* prevalence than con-specifics without epibionts. *P. ciliata* larvae are known to settle preferentially on uneven surfaces and scratches (Daro and Polk, 1973) and hence the crevices between the base of the barnacle shells and the snail shell surface may have made entering the gastropod shell easier for the polychaete. Indeed, direct observations confirmed that most *P. ciliata* holes occurred at the

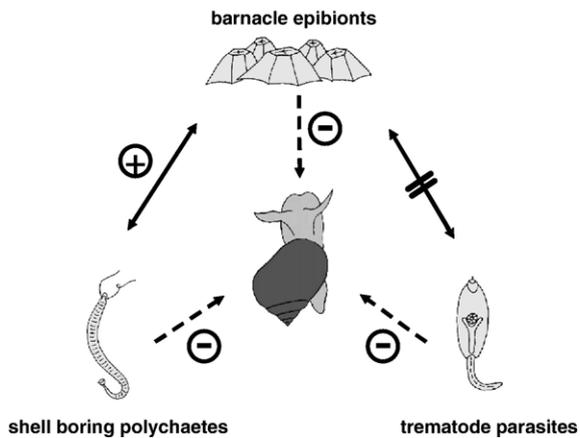


Fig. 5. Schematic illustration of the importance of facilitative processes in interactions between barnacle epibionts, shell boring polychaetes and trematode parasites. The facilitative interaction (+) between barnacle epibionts and shell boring polychaetes leads to a vicious circle since both exert negative effects (-) on the gastropod basibiont.

base of barnacle cones. A rapid colonization of experimental snails is highly likely: *Polydora* larvae are locally present all over the summer (Rodríguez Valencia, 2003) and have been shown to colonize experimentally exposed substrates within weeks (Hempel, 1957, 1960; Daro and Polk, 1973). The prevalence of *P. ciliata* in cleaned snails and unfouled snails was similar to the preload of *P. ciliata* of fouled and unfouled snails at the start of the experiment (20% and 8%, respectively). This indicates that no or only few additional infections occurred during the experiments in absence of barnacle epibionts. Hence, barnacle epibionts were responsible for the observed increase in *P. ciliata* infections.

Trematode prevalence was not affected by barnacle epibionts since there was no difference in prevalence in the three treatments after 14 weeks of exposure. Infective stages (miracidia) are present all over the year due to high abundance of seabird final hosts with high trematode loads in the area (Thieltges, unpublished data). Development of rediae and sporocysts in digenean trematodes takes several weeks and infections can be identified by careful observation even in early premature stages (Lo, 1995; Ataev et al., 1997; Graczyk et al., 2000). Hence, the duration of the experiment should have been long enough to detect any potential changes but of course it cannot be excluded that parasite accumulation in long lived hosts occurs too slowly to be detected within 14 weeks (Curtis, 1996). However, if there were a slightly positive effect in the long run, this may not be ecologically significant. Hence, we conclude that barnacle epibionts had no or a very weak effect on trematode prevalence.

Overall, the shell boring polychaete *P. ciliata* and barnacle epibionts strongly facilitate each other in *L. littorea* (Fig. 5). This reciprocal facilitation is an advantage for both species as they are provided with a better access to the substrate resource. This is an example of facultative mutualism (Stachowicz, 2001). *P. ciliata* and barnacle epibionts both exert strong negative effects on the basibiont *L. littorea*. Infestation by *P. ciliata* leads to a gradual destruction of the gastropod shell and a reduced resistance against shell breakage (Buschbaum et al., in press). This makes *P. ciliata* infested snails more vulnerable to predators like crabs (Buschbaum et al., in press) as has also been shown in *P. ciliata* infested bivalves (Ambariyanto and Seed, 1991). Barnacle epibionts have been shown to lower survival, growth and reproductive output in *L. littorea* (Wahl, 1996, 1997; Buschbaum and Reise, 1999). Considering these multiple negative effects, the observed facilitative process may be of high importance for *L. littorea* population dynamics. With *P. ciliata* and barnacle epibionts facilitating each other, interference competition with their snail hosts is strongly enhanced and results in a vicious circle for the snails. Hence, our findings are an example for a facilitative process (“+” interactions) between two associated organisms that results in strongly negative effects (“-” interactions) in the host (Fig. 5). In addition to single effects of epibionts (Buschbaum, 2000), predation (Rangeley and Thomas, 1987), competition (Fenske, 1997) and trematode infestations (Lambert and Farley, 1968; Lauckner, 1980; Huxham et al., 1993; Davies and Knowles, 2001), this study has shown that facilitative processes between epibionts and other snail associated organisms and concomitant indirect effects are also important biotic factors in *L. littorea* population dynamics.

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