

NOTE

Periwinkle regulation: parasitism and epibiosis are linked

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ABSTRACT: The common periwinkle *Littorina littorea* is recognized as a keystone species in coastal habitats of the North Atlantic, and hence, interactions among factors influencing these snails' population dynamics are of ecological interest. Here, I address the relationship between 2 regulating forces, parasitism and epibiosis, in a Danish population of periwinkles serving as host to trematode parasites (*Himasthla elongata* and *Renicola roscovita*) as well as substrate for encrusting furoids (*Ralfsia* sp.). Results show a strong positive relationship between shell size (i.e. snail age) and epibiont coverage, but also a significant positive link between parasitism and epibiosis after statistical correction for shell size. Because epibiosis of periwinkles is controlled solely by mutual shell grazing, the relationship between parasitism and epigrowth likely stems from reduced grazing of infected snails' shells by conspecifics that are known to avoid parasitized individuals. Trematode parasitism may thus indirectly facilitate the community of epibionts and in turn reinforce fouling as a regulator of periwinkle populations.

KEY WORDS: *Littorina littorea* · Trematode infection · Fouling · Epiphyte · Facilitation

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INTRODUCTION

Widespread and often abundant, the common periwinkle *Littorina littorea* acts as a keystone species along North Atlantic shorelines (Lubchenco 1978, Bertness 1999). Through selective grazing, especially on ephemeral green algae, periwinkles effectively control the structure of the coastal plant community and in turn also the animal community relying on algae for food, shelter or substrate. Hence, in the quest to understand spatio-temporal patterns of biodiversity in coastal ecosystems, it is important to also unravel processes regulating periwinkle populations. Two such regulating forces, with importance not only for periwinkles but for coastal molluscs in general, are parasitism and epibiosis. For instance, periwinkles are commonly host to a range of parasitic flatworms (Trematoda) that, aside from castrating the snail host, also inflict direct parasite-induced mortal-

ity (Werdning 1969, Mouritsen & Poulin 2002). Similarly, heavy epibiosis, or fouling, by various animals and plants using the snail shell as a substrate may also significantly reduce the basibionts' (i.e. the snails) condition, fecundity and survival rate (Wahl 1996, Buschbaum & Reise 1999, Eschweiler & Buschbaum 2011). However, in order to determine the relative importance of regulating forces for population dynamics, it is of paramount importance to clarify whether the forces in question are entirely additive, compensatory or interact, for instance by reinforcement. In this respect, next to nothing is known about the relationship between parasitism and epibiosis in gastropods. Mouritsen & Bay (2000) provided evidence for a link between trematode infections and epiphyte growth in *Hydrobia* and *Biomphalaria* snails. In periwinkles, Buckland-Nicks et al. (2013) noted that individuals encrusted with coralline algae often also harbour a diverse community of endosym-

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bionts, including trematodes, but provided no data. Thieltges & Buschbaum (2007), on the other hand, found no relationship between trematode infection and fouling by barnacles in a Wadden Sea population of periwinkles.

Here, I provide evidence from a Danish population of periwinkles showing, for the first time, that the abundance of shell epiphytes and trematode infection is indeed linked.

MATERIALS AND METHODS

Common periwinkles *Littorina littorea* were collected mid-November 2014 east of Nappedam Marina, Kalø Bay, Denmark (56° 16' 35.5" N, 10° 29' 57.2" E). Here, the shallow shoreline is characterized by a mixed sand and gravel bottom with scattered rocks of various sizes supporting stands of fucoids. The tidal amplitude is approx. 20 cm superimposed a wind-generated amplitude of maximum 100 cm. Snails ($n = 244$) were obtained at low tide between the mean high water line and 20 m seaward, where the average (\pm SE) snail density reached 40.9 ± 5.4 ind. m^{-2} ($n = 16$; author's unpubl. data). Collected periwinkles were brought to the laboratory for determination of shell height, epibiosis cover and presence of primary trematode infections. Shell height (apex to aperture) was measured using an electronic caliper (± 0.1 mm). The cover of macroscopic epibionts on each of the collected periwinkle's shell was estimated in percent by 3 to 5 persons individually, and the average was used as the unit for analysis. Primary trematode infections (i.e. redial or sporocyst larvae), residing in the infected snails' gonad-digestive gland complex, were identified according to Werding (1969) during dissection under a stereomicroscope.

An additional 20 periwinkles were collected to determine the wet weight of the encrusting phaeophytes relative to the wet weight of the periwinkle basibiont. Snails were individually blotted dry and the wet weight was determined. Then the encrusting brown algal epibionts were scraped off using a Stanley knife, and the snails were reweighed.

All statistical analysis were carried out in SPSS v.19.0 (Statistical Package for the Social Science). Student's *t*-tests were executed using separate variance estimates to account for heterogeneity of variance and, in case of epibiont cover, on arcsine-transformed data. In the multiple regression analysis, epibiont cover was rank-transformed to optimize linearity, and prerequisites regarding normality and homoscedasticity of residuals, absence of collinearity (variance in-

flation-factor) and absence of influential cases (Mahalanobis distance) were evaluated and confirmed. Aside from parasitism (infected or non-infected), periwinkle shell height was entered as a proxy for age in the multiple regression analysis. This is justified for both infection categories because trematode infections have very limited, if any, impact on the growth rate of periwinkles and in turn the size-age relationship (see Mouritsen et al. 1999). Note that the multiple regression approach does not clarify causality, and the analysis is limited by the set of predictors entered (here shell height and parasitism). Hence, unidentified predictors left out of the analysis may potentially change the statistical conclusion if entered.

RESULTS

Periwinkles *Littorina littorea* ranged between 16.1 and 26.6 mm (median = 21.0) in shell height. About 40% of the snails were infected by trematodes: 75 individuals (30.7%) were infected by *Himasthla elongata*, 16 (6.6%) by *Renicola roscovita* and 6 (2.5%) were infected by both species. Macroscopic epibionts almost exclusively consisted of encrusting phaeophytes (putative *Ralfsia* sp.; Fig. 1, insert photo), but occasionally a few barnacles *Semibalanus balanoides* and small rhodophytes were also found. Few snails were entirely free of these epibionts (1.6%), whereas a greater proportion (6.6%) attained epibiosis coverage exceeding 90%; median coverage was 43.0%. Epibiosis was a strong positive function of shell size (Fig. 1), suggesting time (snail age) to be a major determinant of epibiont coverage. However, parasitized snails also tended to cluster among the most heavily fouled individuals with no obvious pattern according to infection type (Fig. 1). Considering all infection types together, parasitized periwinkles supported 2-fold greater epibiont coverage than unparasitized (Fig. 2; Student's *t*-test on arcsine-transformed data: $t_{238.9} = 7.927$, $p < 0.0005$). Parasitized snails were on average also slightly larger (6.9%) than unparasitized (Fig. 2; Student's *t*-test, $t_{232.2} = 6.004$, $p < 0.0005$), and the relationship between parasitism and epibiosis may in part be due to a host size/age effect. Hence, to statistically separate the relative importance of size (as a proxy for age) and parasitism on epibiont cover, a multiple regression analysis was carried out, showing that (1) snail size and parasitism combined explain 46.5% of the variance in epibiont cover, and (2) both size and parasitism have an isolated (i.e. corrected for the influence of the other predictor) significant positive effect

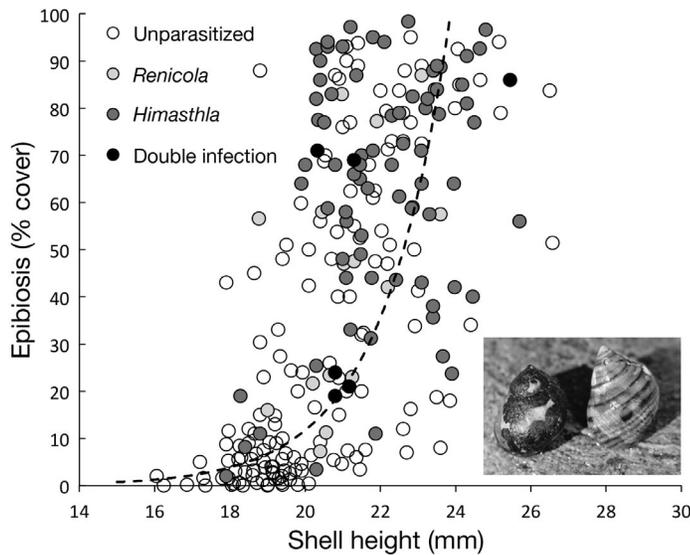


Fig. 1. Relationship between epibiosis (% cover) and periwinkle *Littorina littorea* shell height distributed according to infection status (unparasitized, *Renicola roscovita* infected, *Himasthla elongata* infected, and snails infected by both species of trematodes). An exponential trend line ($Y = 0.000178 \times e^{0.555X}$; $r^2 = 0.393$, $n = 244$, $p < 0.0005$) including all 244 data points is given (dashed line) although a linear fit is evenly applicable ($r^2 = 0.397$, $p < 0.0005$). Insert: 2 periwinkles. Unfouled (right) and heavily covered by encrusting brown algae, presumably *Ralfsia* sp. (left)

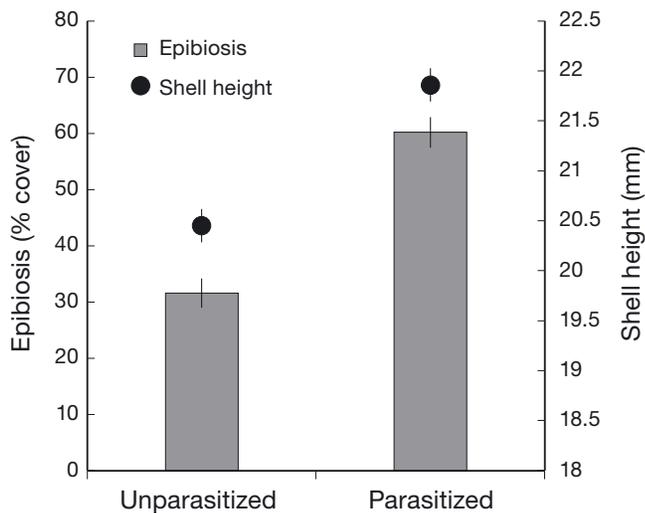


Fig. 2. Mean (\pm SE) % cover by epibionts and mean (\pm SE) shell height (mm) of unparasitized ($n = 147$) and parasitized (all infection types combined, $n = 97$) periwinkles *Littorina littorea*. The difference in mean values between unparasitized and parasitized snails is statistically significant for both epibiosis (Student's t -test on arcsine-transformed data: $t_{238.9} = 7.927$, $p < 0.0005$) and shell height (Student's t -test, $t_{232.2} = 6.004$, $p < 0.0005$)

Table 1. Summary statistics of the multiple regression evaluating the influence of parasitism (presence/absence of trematode infection; all types combined) and periwinkle *Littorina littorea* shell height on rank-transformed epibiosis (% cover) as the dependent variable. In the case of the 2 predictors, the r^2 column gives the squared partial correlation coefficient, i.e. the proportion of variance explained when the influence of the other predictor has been corrected for

Predictors	F -ratio	t	r^2	p
Parasitism		4.729	0.085	<0.0005
Shell height		11.194	0.342	<0.0005
Total regression	$F_{2,241} = 104.785$		0.465	<0.0005

on fouling (Table 1). Of the 2 predictors, size was clearly the most important, explaining 4-fold more variance than parasitism.

The demonstrated linkage between epibiosis and trematode infection was driven mainly by *H. elongata* as the most abundant trematode species. Indeed, running the above analyses solely including *Himasthla*-infected snails result in similar conclusions qualitatively, quantitatively and statistically (data not shown). Whereas analyses including solely *Renicola*-infected and double infected snails, respectively, showed similar trends, sample sizes ($n = 16$ and 6) provided insufficient statistical power for establishing significant relationships.

Although heavily fouled (median coverage: 65.0%, range: 50 to 95%), the encrusting phaeophytes covering each of the 20 additionally collected periwinkles (median shell height: 26.1 mm, range: 21.4 to 30.0 mm) represented only 3.7% (median) of the combined epibiont and basibiont wet weight (range: 1.6 to 11.9%).

DISCUSSION

Results showed that the size of the periwinkle *Littorina littorea* basibiont was a major determinant of the degree of fouling by encrusting brown algae in the studied population. This is likely an age-effect following from the fact that development of epibiosis is a time-consuming process, especially when involving slow-growing algal crusts such as those in focus here (Wahl 1989, Dethier & Steneck 2001, Kain 2008). However, when size was corrected for, trematode infection also emerged as a significant isolated factor related to algal coverage: infected snails tended to be more heavily fouled than uninfected. This is the first demonstration of a link between epibiosis and parasitism in periwinkles. Because cause and effect can-

not readily be separated in regression analyses, there are 2 main interpretations of this linkage: epibiosis facilitates parasitism, or parasitism facilitates epibiosis.

Can epibiosis facilitate parasitism? Heavy fouling by epibionts is known to pose detrimental effects on gastropod basibionts. For instance, fouling by oysters and barnacles significantly reduced mobility and fecundity of the sandy shore snail *Batillaria zonalis* (Chan & Chan 2005), and similar effects, in addition to reduced growth and survival rates, were reported from common periwinkles *L. littorea* overgrown by barnacles and stands of macroalgae (Wahl 1996, Buschbaum & Reise 1999, Eschweiler & Buschbaum 2011). These effects are believed to follow from the greater weight of fouled snails and/or the greater hydrodynamic drag the epibionts poses on their substrate organism, markedly increasing energetic cost of movements and attachment. Hence, in this way energy-drained, stressed snails may express a less effective internal defence system, making them more susceptible to parasite infections. However, in the mentioned investigations the weight of involved epibionts exceeded several-fold those of the basibiont. In contrast, the weight of the smooth encrusting phaeophyte epibionts involved in the present study (Fig. 1) reached a mere few percent of the basibiont weight, and are therefore unlikely to have weakened their basibionts to an extent that made them more susceptible to infection.

Can parasitism facilitate epibiosis? Trematode infections interfere with a multitude of physiological and biochemical processes within their molluscan intermediate host (Lauckner 1980, Galaktionov & Dobrovolskij 2003, Arakelova et al. 2004, Tunholi et al. 2011), likely affecting any chemical anti-fouling mechanism launched by the host as well. Such parasite-induced impairment has been suggested to operate in the only other demonstration of a linkage between epibiosis and parasitism in molluscs (Mouritsen & Bay 2000). However, the common periwinkle lacks typical anti-fouling mechanisms, chemical measures included, which rules out this possibility in the present case (Wahl & Sönnichsen 1992). Instead, periwinkle fouling appears to be governed by their population density in that individual snail's epigrowth is controlled by mutual grazing on the shells (Wahl & Sönnichsen 1992). Thus, well-developed epibiosis is found at low snail densities whereas fewer fouled specimens occur at high population densities. The abundance of periwinkles at the present study site ($>40 \text{ ind. m}^{-2}$) is well within the range of densities where epibiosis is markedly regulated by mutual grazing (see Wahl & Sönnichsen 1992), sug-

gesting that the fouling of the periwinkles collected here was suppressed by such intraspecific behavioural interactions. Interestingly, however, trematode infected periwinkles are generally avoided by conspecifics due to the snails' ability to detect the parasites cercarial dispersal stages in their vicinity (Erlandsson & Kostylev 1995, Davies & Knowles 2001). For instance, periwinkles are highly reluctant to cross mucus trails of conspecifics contaminated by these larval parasites (Davies & Knowles 2001). The 2 trematode species commonly infecting European periwinkles, *Himasthla elongata* and *Renicola rosco-vita*, not only use the snails as a first intermediate host but also a second intermediate host, forming metacercarial cysts in the mantle and gill tissue (Werding 1969, Lauckner 1980). It is therefore adaptive for periwinkles to avoid parasitized conspecifics emitting the infective cercariae. Together, these lines of evidence lead to the intriguing but highly parsimonious interpretation of the observed positive linkage between epibiosis and trematode parasitism: infected periwinkles actively avoided by conspecifics are not grazed upon, in turn causing epibionts to settle and develop unchecked on their shells.

That parasitism may indirectly facilitate fouling of periwinkles has ecological implications in situations where the snail population supports more detrimental epibionts than the encrusting brown algae involved here (see 'Introduction'). Because both trematode infection and heavy epibiosis is highly stressful to the snails, the combined negative impact of these processes may push a group of infected, and therefore increasingly fouled, individuals over the edge. Hence, some populations of periwinkles may become regulated to a larger extent than the isolated effect of parasitism and epibiosis otherwise predicts. Because the periwinkle is a keystone species structuring coastal communities through its selective grazing activity (see 'Introduction'), reinforced regulation of the snail population is bound to impact the surrounding community of plants and animals. Moreover, as long as the snails are alive, the parasite–epibiosis interaction will also facilitate the community of epibionts, particularly in sedimentary coastal ecosystem where nonbiogenic hard substrate is a limiting resource. Molluscan shells acting as basibionts for epiphytes and fauna significantly facilitate species richness in the community (Wahl 1989, Gribben et al. 2009, Thyrring et al. 2013, Thomsen et al. 2016). This is particularly evident when the substrate is less heavily grazed upon (Wernberg et al. 2010), underlining that parasitism in this way may indirectly boost the biodiversity of coastal habitats.

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