

2023-03

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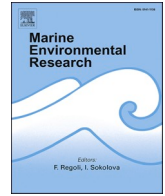
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10.1016/j.marenvres.2023.105941

Marine Environmental Research

Elsevier BV

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Epibionts provide their basibionts with associational resistance to predation but at a cost

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ARTICLE INFO

Keywords:

Associational resistance
Associational susceptibility
Predation
Epibiosis
Functioning
Interactions

ABSTRACT

Epibiosis is increasingly considered a survival strategy in space-limited environments. However, epibionts can create a new interface between its host, environment and potential predators which may alter predator-prey relationships and biological functioning. *Ex-situ* experiments investigated the potential costs and benefits of epibiont barnacles on mortality and feeding rate of the mussel, *Mytilus edulis*, and its predator, the whelk *Nucella lapillus*. Mussels with living epibiont barnacles suffered no mortality from whelk predation, but when barnacles were absent, mortality was ~21% over 48 days. Further comparisons revealed the structural complexity of barnacles provided mussels with protection from whelk predation, while the presence of living barnacles increased predator-prey encounters but led to predators targeting barnacles over mussels. Feeding trials revealed feeding rate increased by ~24% in mussels with living epibionts over mussels with dead or without epibionts, indicating potential costs of hosting epibionts. Our results show that epibionts provide important associational resistance for mussels against whelk predation but a potential cost to the mussel of hosting epibionts requiring increased energy acquisition. These findings advance our understanding of associational resistance derived from epibionts and serve to highlight the potential trade-offs affecting basibiont functioning while showing the importance of positive ecological interactions in ecosystem structure and functioning.

1. Introduction

Ecosystem engineers play a disproportionately important role in the structure and functioning of environments by regulating the availability of resources for other organisms (Jones et al., 1994). In the marine environment, autogenic ecosystem engineers like oysters, mussels and worms can aggregate providing critical habitat (Muller et al., 2021; Hawkins et al., 2020) and food resource (Hughes and Dunkin, 1984; Marsh, 1986; Reusch and Chapman, 1997; DeGaff and Tyrrell, 2004) for a wide range of taxa.

The habitat created by autogenic engineers can reduce physical stress for associated organisms (Bertness and Callaway, 1994). Extensive work on mussel beds, in particular, has shown aggregations can alter humidity and temperature (Ricciardi et al., 1997; Nicastro et al., 2012), attenuate wave action and reduce risk of dislodgement (Donker et al., 2013), or lower risk of mortality by reducing predator movement and foraging efficiency (Frandsen and Dolmer, 2002; Farrell and Crowe, 2007; Christensen et al., 2012; Knights 2012). Despite the creation of habitat by these engineers, in the intertidal, space is often a finite

resource such that occupancy of those habitats is strongly regulated by negative ecological interactions including pre-emptive competition for space and predation (Bertness and Leonard 1997). In the absence of predation, for instance, mussels can dominate competitive interactions with other species allowing them to monopolise primary substrata (Paine, 1974; Menge and Sutherland, 1976) when the role of positive interactions in structuring communities is increasingly emphasised (Bertness and Leonard 1997; Firth et al., 2020). One such positive interaction is epibiosis, wherein one species (the 'epibiont') uses another species (the 'basibiont') as habitat, with the basibiont often providing a refuge for the epibiont to escape negative interactions (Paine, 1974; Buschbaum, 2002). Indeed, epibiosis has long been considered a common solution to pre-emptive competition for space for many sessile organisms including hydrozoans, plants, and bacteria (Dayton, 1971; Suchanek, 1978; Wahl and Sonnichsen, 1992; Vergés et al., 2011), especially invasive species (Leonard et al., 2017; Firth et al., 2020). Whilst it is commonly assumed that these associations are negative for the basibiont (Wahl 1989), it is increasingly recognised that epibiotic relationships are complex networks of costs and benefits to both

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<https://doi.org/10.1016/j.marenvres.2023.105941>

Received 6 November 2022; Received in revised form 1 March 2023; Accepted 3 March 2023

Available online 4 March 2023

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epibiont and basibiont (Puccinelli and McQuaid 2021; Zardi et al., 2021; Firth et al. Accepted).

To date, there has been no research undertaken to assess the potential trade-offs of the negative and positive outcomes of epibiosis on a basibiont despite negative and positive outcomes being shown. For taxa like bivalve molluscs, on which epibiosis is commonly reported (e.g., Witman and Suchanek 1984; Laihonon and Furman, 1986; Buschbaum and Saier, 2001), epibiosis can become increasingly prevalent over time (Byers et al., 2006), the cost of which can include: erosion of the anti-fouling (periostracum) shell layer (Firth et al.); inducing the mussel (basibiont) to reallocate energy away from physiological parameters (e.g., growth) (Haag et al., 1993; Thieltges, 2005); reduced mussel mobility (Thieltges and Buschbaum, 2007; Johansson, 2010; Garner and Litvaitis, 2016); or compromised reproductive fitness (Wahl, 1989; Chan and Chan, 2005; Saier and Chapman, 2004). Contrasting this, others have argued for no negative effects for the basibiont (Laihonon and Furman, 1986) or even positive outcomes, such as inducement of byssal thread production that increases attachment strength and reduces the likelihood of wave dislodgement (Thieltges and Buschbaum, 2007; Johansson, 2010; Garner and Litvaitis, 2016). Most recently, Puccinelli and McQuaid (2021) suggest that the barnacle-mussel association in South Africa is amensalistic i.e. negative for barnacles, but neutral for mussels.

Epibiosis creates of a new interface between the basibiont (Laudien and Wahl, 2002) and its predator(s) that may alter predator-prey relationships (Wahl, 1997; Prinz et al., 2011) depending on the identity of the epibiont. For instance, epibiotic tunicates on mussels have been shown to reduce shore crab predation (Auker et al., 2014). Conversely, mussels fouled with algae experience no greater protection from predation (Wahl, 1997). Certain epibionts may therefore mediate predation risk as a result of changing a predator's interaction with the basibiont, for instance, by altering the tactile and/or chemical information of the prey (Farren and Donovan, 2007). Despite barnacles being common epibionts on mussels, their effect on predator-prey relationships between a predator and the basibiont has received limited attention. It has been suggested that epibiosis contributes to increased 'associational susceptibility' effects (Wahl and Hay 1995), whereby the epibiont heightens the 'attractiveness' of the basibiont as a result of chemical cues (Wahl, 1997) that can increase predator handling time (Enderlein et al., 2003). Alternatively, epibionts might instead provide 'associational resistance' (Laudien and Wahl, 2002; Laudien and Wahl, 2004; Thangarathinam and Chattopadhyay, 2020), instead reducing predation risk to the basibiont with the epibiont chemical cues masking the basibiont from potential predators (Laudien and Wahl, 2004), or by creating structural changes to the basibiont's predator-accessible surface that hinders predator feeding (Thangarathinam and Chattopadhyay, 2020).

We investigated the nature of the relationship between basibiont mussels, their barnacle epibionts and their whelk predators. The aim of this study was two-fold: (i) to test if epibionts provide associational susceptibility or associational resistance, mediating predator-prey interactions between the mussel *Mytilus edulis*, and its predator, the dog whelk *Nucella lapillus*; and, (ii) to identify if barnacle epibionts affect the functioning (feeding) of the mussel. To determine if barnacle epibionts provide associational resistance to *M. edulis* from *N. lapillus* predation we quantified the relative effects of barnacle epibionts on mussel mortality from whelks and change in whelk behaviour (specifically attachment frequency and handling time). Using a fully-crossed experiment, we determined where the structural and/or chemical features of an epibiont alter basibiont-predator interactions; and (iii) quantify change in the feeding rate of a basibiont with or without epibionts to determine if there is a physiological cost to the basibiont of hosting epibiont(s).

2. Materials and methods

2.1. Animal specimen collections

Mytilus edulis (mussels herein) between 20 and 25 mm and with

>50% epibiont barnacle coverage were collected from Queens Anne's Battery Marina, Plymouth (50.364111°N, 4.131500°W) in October 2020. Adult *Nucella lapillus* (whelks herein) ranging in size between 25 and 40 mm, and barnacle-covered rocks (>50% barnacle coverage) were also collected from Mount Batten, Plymouth (50.356125°N, 4.127642°W). The size ranges for mussels and whelks were chosen as they are considered the optimal size for predator-prey interactions (Hughes and Dunkin, 1984). Barnacles were a mixture of *Semibalanus balanoides*, *Chthamalus montagui*, and *Austrominius modestus* and are referred to simply as 'barnacles' herein. Although size can affect susceptibility to predation by whelks (Barnett, 1979), the barnacles here were of similar size and therefore considered to be equally susceptible to predation.

2.2. Habituation period

Dog whelks were placed in a storage tank containing UV-treated and 1 µm filtered seawater (temperature ≈ 15 °C, salinity ≈ 34, pH ≈ 8), then starved for 6 days to standardise their hunger levels (Hughes and Dunkin, 1984).

All mussels were cleaned to remove any other non-barnacle macroscopic epibionts (e.g., bryozoans, sponges, and tunicates) before being randomly assigned to one of four treatments: (1) mussels (M) with living epibiont barnacles adding both structural (S) and chemical complexity (C) to the basibiont (M + S + C); (2) mussels alongside living non-epibiont barnacles (i.e. barnacles were living on rock fragments held separately in a plastic container with holes to allow water exchange but prevent predator access) provided chemical complexity (+C) but not structural complexity (-S) to the basibiont (M-S + C) (3) mussels with euthanised epibiont barnacles (i.e. a seeker was used to remove all internal soft tissue leaving just the parietal callus cemented to the shell); providing structural but not chemical complexity to the basibiont (M + S-C); and (4) mussels with barnacles removed i.e., no structural or chemical complexity (M-S-C) (Fig. 1).

Mussels (M + S + C/M + S-C) and rocks (M-S + C) with living/dead epibiont barnacles had the percentage cover of barnacles over the mussel shell surface standardised to 50%. Both mussels and rocks were of similar size but percentage cover of barnacles on each mussel varied from 50% to nearly 100% on both valves. Therefore, to standardise

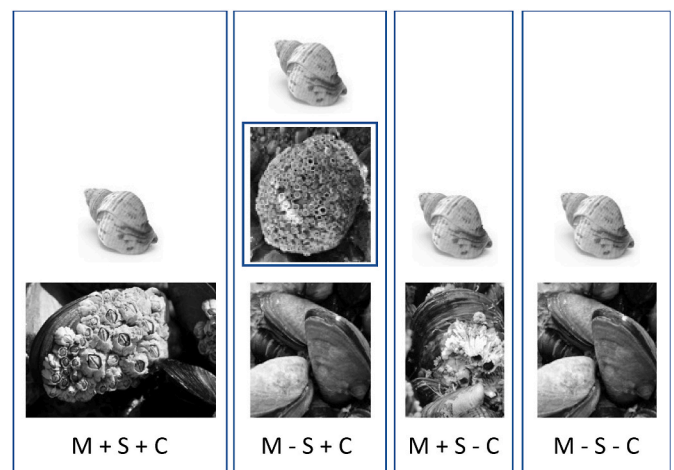


Fig. 1. Illustration of the four predator treatments used in the experiment. M + S + C = mussels with live barnacles as epibionts; M-S + C = mussels without epibionts and living barnacles on rocks (barnacle rocks were held in a plastic container with holes sufficiently large to allow water movement, but sufficiently small to prevent accessibility to predators. Barnacle abundance rock ~ barnacle abundance on mussels); M + S-C = mussels with dead epibionts but the parietal callus (calcium carbonate shell) remaining; and M-S-C = mussels with no barnacles.

potential ‘attractiveness’ of each mussel to a predator, individual barnacles were removed by hand using a seeker until barnacle percentage cover was ~50%. Barnacles on rocks used in the M-S + C treatment were handled in the same way as mussels.

Mussels were habituated in 24 × 400 mL experimental tanks containing filtered seawater (as above) for a two-week period. Each tank contained four mussels, with six replicate tanks per experimental treatment. Mussels were fed approximately ~3 mL (~150,000 cells) of mixed shellfish diet (Reed Mariculture, USA) three times a week. Photographs of individual mussels were also taken and ImageJ was used to count the number of barnacles (living/dead) on each mussel to enable us to quantify if epibionts are being preyed upon instead of the basibiont.

2.3. Epibiont influence on predator-prey relationship

After two weeks, a single whelk was randomly assigned to 12 of the experimental mussel tanks. These tanks represented a ‘+predator’ treatment. The other 12 tanks without whelks the ‘-predator’ treatment to test for background mussel mortality. Three tanks (replicates) were then assigned to each experimental treatment (Fig. 1) within each + predator/-predator group. The experiment was then run for 48 days, with the water in each tank replaced twice weekly and the mussels were fed using the same feeding regime described above.

Every 24 h, the number of dead mussels (mussel mortality), and whelk attachment (attached/unattached) to mussels was recorded. The cumulative number of days that whelks were attached to a mussel (‘handling time’) was also recorded. Photographs of barnacles on mussels were also taken and processed in ImageJ (<https://imagej.nih.gov/ij/index.html>) to calculate barnacle mortality. Barnacle mortality was easily observed as empty tests and a previous study (Gordon and Knights, 2018) has shown different barnacle species exhibit comparable mortality rates in the field. Mussels were considered dead if the mussel valves did not close after a direct physical disturbance was applied. In these instances, dead mussel(s) were removed and immediately replaced by mussel(s) of similar size (between 20 and 25 mm) and that had been held in identical environmental conditions in order to control for density. Replacement mussels were handled in the same way as described above with respect to the presence (percentage cover) or absence of living/dead barnacle epibionts depending on treatment.

2.4. Mussel feeding rate

At the end of the experiment, 3 mussels were randomly chosen from each treatment then starved for 24 h to first standardise their hunger levels (Rajesh et al., 2001). Individual mussels were then placed in beakers containing 297 mL of 2 µm-filtered seawater which was continually mixed using a magnetic stirrer (@400 rpm). Once mussels opened their valves (usually within 5 min of being placed in a beaker and varied between individuals), 3 mL of live phytoplankton prey (*Isochrysis galbana*) at a concentration of 10,000 cells/mL was introduced to each beaker. Every 2 min for 30 min, 1 mL of water was collected at random from the beaker resulting in 16 water samples per mussel. If mussels closed their valves, the chronometer would be stopped and only restarted once the valves had re-opened. The density of *I. galbana* in each water sample (n = 16) was determined using a haemocytometer. Feeding rate was then calculated ($\Delta[I. galbana]/\text{time}$) for each mussel based on regression models (see statistical analysis below).

2.5. Statistical analysis

Six analyses were performed, all of which were conducted using R (R Core Team, 2021). All analyses included treatments with predators present only as no mussel mortality was observed in treatments without predators. Model summary data and contrasts are presented in Supplementary Tables.

1). A Linear mixed-effects (lme) model was used to test for

differences in mussel mortality (number of deaths) between epibiont treatments (fixed levels: 1–4 as described above) and tank (random factor) over time (days; continuous factor) and performed using the R package lme4 (Bates et al., 2015). The maximal model (AIC = 514.8) was as follows:

Maximal model = lme (mortality ~ treatment × time, random = tank).

A stepwise model reduction approach based on Akaike Information Criterion (AIC; Sakamoto et al., 1986) and likelihood ratio (performed using the function anova in R) was used to test the effect of model simplification on estimates. This indicated no significant effect of the random term, but a significant interaction between treatment and time (AIC = 567.2). Tukey post-hoc pairwise comparisons were performed using ‘glht’ in multcomp (Hothorn et al., 2008) to explore difference between treatment factor levels. A simple slope analysis in the interactions package (Long, 2019) was also used to investigate the effect of the coefficient of time on mussel mortality across different treatments (Bauer and Curran, 2005).

2). Logistic regression with binomial errors (logit link) was used to investigate the probability of whelk attachment (attached/unattached) in each treatment over time. The maximal model (AIC = 522.45) was as follows:

Maximal model = glm (attachment ~ treatment × time, family = binomial (link = logit))

Attempts to simplify the model using the stepwise approach described above led to a significant reduction in model fit (AIC = 555.84) therefore the maximal model was retained for analysis. Chi-square was used to test analysis of deviance for the generalised linear model fit. Tukey post-hoc pairwise comparisons (as above) were used to explore differences among treatments.

3). A generalised linear model (glm) using a Poisson log-normal (log-link) distribution followed by a chi-square test for analysis of deviance was used to investigate the interaction between mussel mortality (count of number dead) among treatments (see (1) above) depending on the cumulative time whelks spent attached to mussels. The maximal model (AIC = 1509.7) was the best fitting model determined by the model simplification procedure. Tukey post-hoc pairwise comparisons (as above) were used to explore differences among treatments.

4). A second simple linear model (lm) was used to investigate epibiont barnacle mortality on mussels (i.e. using the M + S + C treatment) and the cumulative frequency of whelk attachment.

5). A one-factor ANOVA and Tukey post-hoc pairwise comparisons were used to test for (i) difference in dog whelk handling time among epibiont treatments, and

6) Linear regression with Tukey pairwise contrasts were used to compare mussel feeding rates among experimental treatments.

3. Results

3.1. Predator-prey interactions: mussel mortality

There were significant differences in mussel mortality over time ($F_{7,580} = 509.5$, $p < 0.001$, Fig. 2) among all experimental treatments (*post hoc* tests, $p < 0.05$). Mussels without epibionts (+M-S-C) had significantly higher mortality than mussels with epibionts, experiencing ~4–5 deaths over 48 days. Mussels without epibionts but alongside living barnacles (on rocks; M-S + C) experienced significantly lower mortality of 2–3 deaths in mussels (*post hoc* tests, $p < 0.001$), and mussels covered with dead epibiont barnacles (M + S–C) fewer again with just 1 death (*post hoc* tests, $p < 0.001$). Mussels with living epibiotic barnacles (M + S + C) experienced no mortality over the course of the experiment (*post hoc* tests, $p < 0.001$, Fig. 2a). Mussels with living but

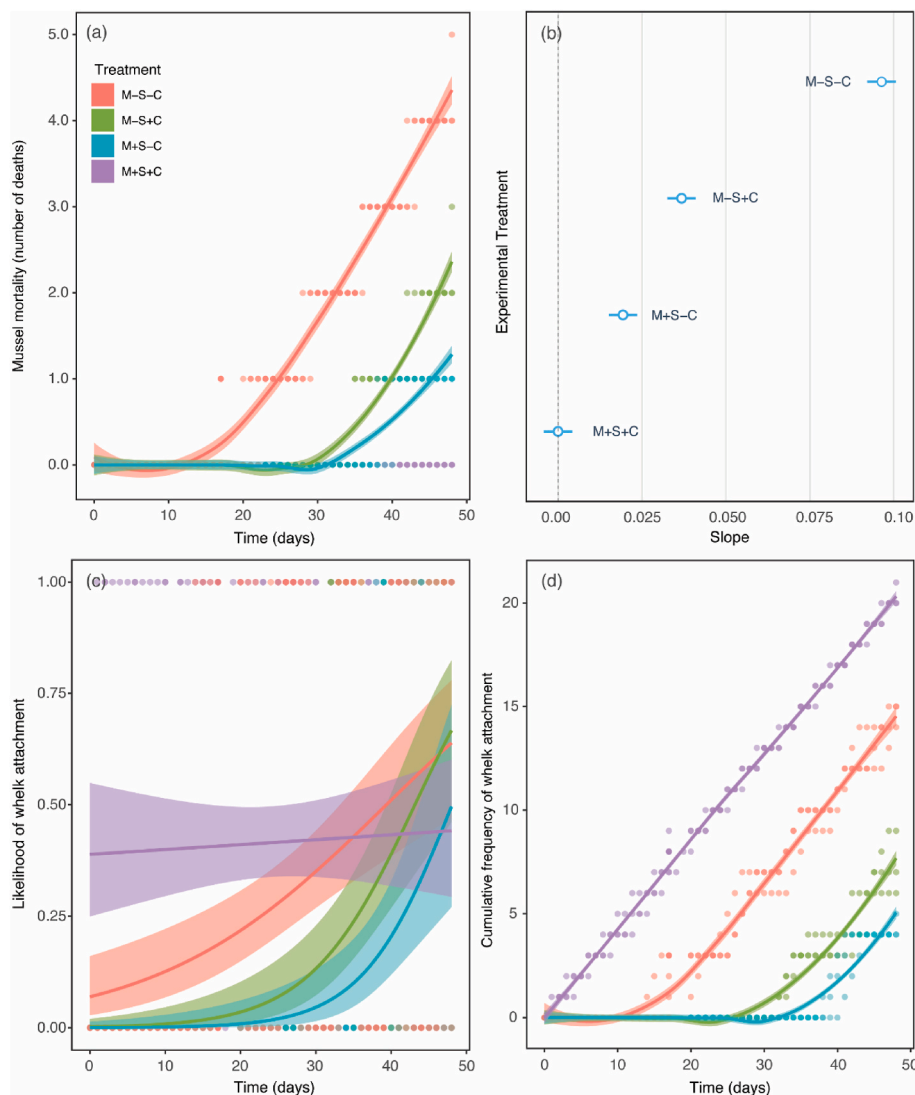


Fig. 2. (a) Mussel mortality over 48 days in epibiont treatments (colours separating treatments apply to all panels (a, c and d) as follows: red - mussels without barnacles (+M-S-C); green - mussels with live non-epibiont barnacles (M-S + C); blue - mussel with dead epibiont barnacles (M + S-C); purple - mussel with live epibiont barnacles (M + S + C)); (b) Simple slope analysis of mussel mortality against the coefficient of time in epibiont-free (M + S + C) mussels; (c) Probability of whelk attachment on a mussel basibiont over 48 days (0 = not attached; 1 = attached); and (d) Cumulative frequency of whelk attachment on to mussels over 48 days in four epibiont treatments.

non-epibiont barnacles (M-S + C) or dead epibiotic barnacles (M + S-C) survived for 30 days without mortality, whereas mussels without barnacles survived for 15 days without mortality (M-S-C) (Fig. 2a).

Simple slope analysis revealed a positive coefficient of time with mussel mortality in epibiont-free mussels (M-S-C) ($p < 0.001$), dead epibiont barnacles (M + S-C) ($p < 0.001$), and living non-epibiont barnacles (M-S + C) ($p < 0.001$, Fig. 2b), but no effect of predation on mortality over time in mussels supporting living epibiotic barnacles (M + S + C) ($p > 0.05$, Fig. 2b). Mortality rate in mussels without barnacles (M-S-C) was $\sim 2 \times$ faster than mortality in mussels with living non-epibiont barnacles (M-S + C), and $\sim 4 \times$ faster than mussels supporting dead epibiotic barnacles (M + S-C, Fig. 2b).

3.2. Whelk attachment

The probability of whelk attachment to mussels was significantly affected by treatment and time (logistic regression, $p < 0.001$, Fig. 2c). Time increased the probability of dog whelk attachment to the basibiont by $6.6\% \text{ day}^{-1}$ in mussels without barnacles (M-S-C) ($z = 4.320$, $p < 0.001$), $14.3\% \text{ day}^{-1}$ in mussels living alongside (non-epibiont) barnacles (M-S + C) ($z = 2.236$, $p < 0.05$), and by $16.8\% \text{ day}^{-1}$ in mussels supporting dead epibiotic barnacles (M + S-C) ($z = 2.037$, $p < 0.05$, Fig. 2c and d). For mussels supporting living epibiont barnacles (M + S + C), the probability of whelk attachment increased linearly over time at

a rate of $0.45\% \text{ day}^{-1}$ ($z = -3.179$, $p < 0.01$, Fig. 2c and d). Tukey's pairwise comparison revealed the likelihood of whelk attachment to mussels without barnacles present was significantly higher in comparison to mussels with living epibiont barnacles (M + S + C), dead epibiotic barnacles (M + S-C), and living with non-epibiotic barnacles (M-S + C) (Fig. 2c). There was no significant difference in the probability of whelks attaching to mussels with dead epibiotic barnacles (M + S-C) and mussels with living non-epibiont barnacles (M-S + C; $p > 0.05$, Fig. 2c).

3.3. Whelk attachment and mortality

Rate of mussel mortality increased with the number of whelk attachments in instances where mussels were living without barnacle epibionts (M-S-C) ($z = 12.892$, $p < 0.001$), with dead epibiont barnacles (M + S-C) ($z = 5.127$, $p < 0.001$), and with living non-epibiont barnacles (M-S + C) ($z = 5.304$, $p < 0.001$, Fig. 3a), but at different rates between treatments. Whelk attachment was $\sim 1.5 \times$ greater in mussels without barnacle epibionts (M-S-C) than on mussels with living non-epibiont barnacles (M-S + C), $\sim 3 \times$ greater than on mussels with dead epibiont barnacles (M + S-C). This corresponded with a 2-fold increase in mortality in M-S-C over mussels with living non-epibiont barnacles (M-S + C) and a 5-fold increase over mussels with dead epibiotic barnacles (M + S-C) (Fig. 3a). For mussels supporting living epibiont barnacles (M + S + C), mussel mortality was zero despite whelks attaching

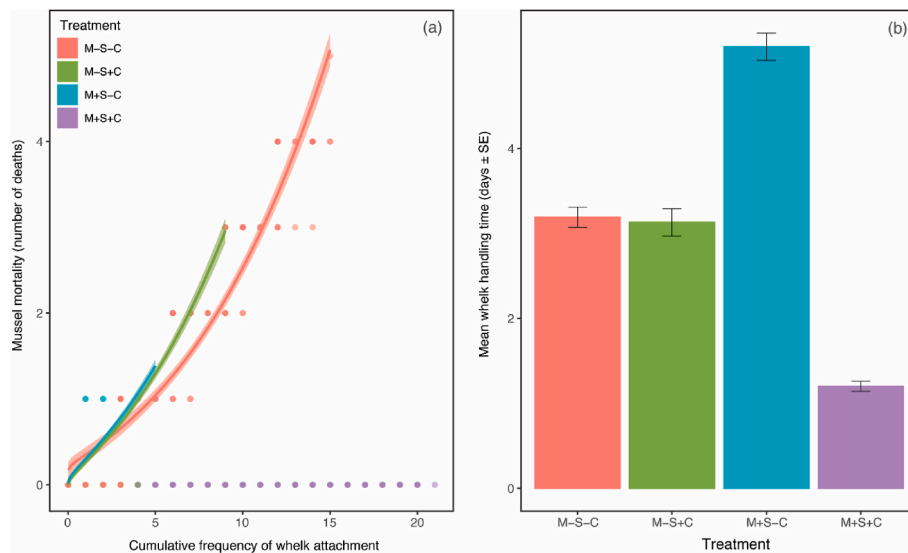


Fig. 3. (a) Relationship between frequency of whelk attachment and mussel mortality by experimental treatment; and (b) Mean (\pm SE) handling time (days) of whelks on (red - mussels without barnacles (M-S-C); green - mussels with live non-epibiotic barnacles (M-S + C); blue - mussel with dead epibiotic barnacles (M + S-C); purple - mussel with live epibiotic barnacles (M + S + C)).

on >20 occasions over 48 days ($z = -0.001$, $p > 0.05$, Fig. 3a), but ~15 epibiotic barnacles died per basibiont mussel (M + S + C).

3.4. Handling time

The presence of barnacle epibionts significantly increased the whelk's handling time of the mussels ($F_{3,41} = 264.1$, $p < 0.001$). Average handling time of mussels with dead epibiotic barnacles (M + S-C) was ~4 days longer than for mussels with living epibiotic barnacles (M + S + C; $p < 0.001$, Fig. 3b), and ~2 days longer for mussels with dead epibiotic barnacles (M + S-C) over mussels with living non-epibiotic barnacles (M-S + C; $p < 0.001$) or mussels without epibionts (M-S-C; $p < 0.001$, Fig. 3b). There was no significant difference in handling time of mussels between mussels with living non-epibiotic barnacles (M-S + C) and mussels without barnacle epibionts (M-S-C; $p > 0.05$, Fig. 3b).

3.5. Epibiont-basibiont interaction: feeding rate

Mussel feeding rate was significantly affected by the presence of barnacle epibionts ($F_{3,56} = 56.4$, $p < 0.0001$). Mussels supporting living epibiotic barnacles (M + S + C) consumed algae significantly faster than mussels without dead epibionts (M + S-C) or no epibionts (M-S + C/M-S-C) (Fig. 4); feeding rate in these 3 treatments was not significantly different (post-hoc tests, $p > 0.05$). Maximum rates of algal consumption occurred in the first 10 min before rates began to decline. The maximum feeding rate in the M + S + C treatment was ~24% faster (16.1×10^4 cells/min) than in M + S-C, M-S + C and M-S-C treatments (combined treatment mean = 12.9×10^4 cells/min) (Fig. 4).

4. Discussion

4.1. Do barnacle epibionts provide associational resistance or associational susceptibility?

Whelks consumed between $2 \times$ and $5 \times$ more mussels without barnacle epibionts than those with barnacles present nearby (M-S + C) or as epibionts (M + S + C) respectively. This suggests that (i) barnacle epibionts provide important associational resistance for mussels against whelk predation, and (ii) greatest protection from predation occurs when barnacles are directly attached to the mussel shell. However, mussel feeding increased when live epibionts were present on their shell

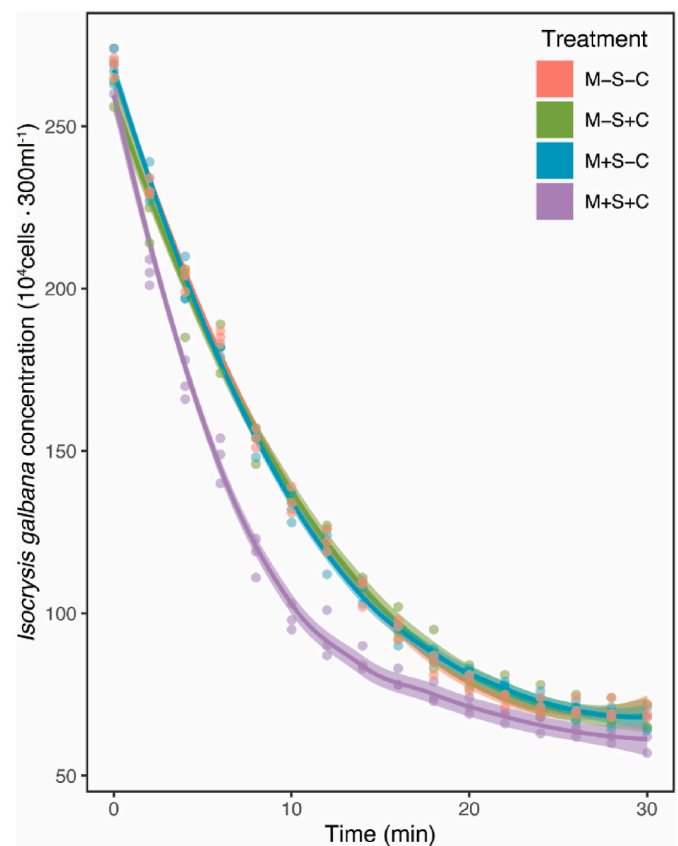


Fig. 4. *Isochrysis galbana* concentration (starting concentration: 10^4 cells per 300 mL) measured at 2 min intervals over 30 min in experimental treatments: epibiont-free mussels (M-S-C), mussels with live non-epibiotic barnacles (M-S + C), mussel containing dead epibiotic barnacles (M + S-C), and mussel with live epibiotic barnacles (M + S + C).

over all other scenarios suggesting a trade-off between no epibiosis increasing the risk of predation, and epibiosis reducing predation risk but requiring upregulation of feeding. Consequently, the epibiont-basibiont relationship may be neither commensal or antagonistic, but

mutualistic if food resources are sufficient to offset increased feeding requirements. Our findings related to mortality are in congruence with those of [Laudien and Wahl \(2002, 2004\)](#) who showed that fouled mussels (i.e., with epibionts) exhibited lower mortality from starfish predation than those without epibionts but in contrast to others finding that epibionts confer associational susceptibility (or ‘shared doom’; [Wahl and Hay, 1995](#)) to the basibiont as a result of increased prey ‘attractiveness’ ([Wahl, 1997](#); [Laudien and Wahl, 2002](#)). It is therefore not as yet clear if there is a general rule under which epibiosis confers associational resistance or susceptibility, and instead, interactions appearing to be idiosyncratic or system-specific.

4.2. The mechanistic effect of barnacle epibionts on the predator-prey relationship

Our results suggests that the structural protection of the epibiont confers greater protection to the basibiont than its chemical signature alone, but living epibionts provide the greatest protection to the basibiont indicating epibiosis directly influences the handling of prey and predation success of predators. Previous studies have suggested that associational resistance can be provided by epibiont(s) via chemical and/or structural means ([Bloom, 1975](#); [Forrester, 1979](#); [Laudien and Wahl, 2004](#)); although this is the first time that their roles have been fully differentiated. Longer handling time, an important element in optimal foraging theory, can elicit a higher energetic cost to the predator ([Kitchell et al., 1981](#); [Stephens and Krebs, 1986](#)). Here, mortality in mussels with structural protection (+S) was reduced by up to 4 × when epibionts were present indicating lower predation success for the whelk. [Bloom \(1975\)](#) showed a similar effect of sponges as epibionts on mussels and their role in impeding predatory starfish adhesion. If barnacles were also living (+C) they conferred some associational resistance, reducing mortality by 2–3 × in comparison to the treatment with structural complexity but with dead barnacles (i.e. (M + S–C); Tukey pairwise comparison - $p < 0.05$). [Laudien and Wahl \(2004\)](#) showed that chemical cues can reduce mortality, and may be an important component in ‘masking’ prey from predators, suggesting preferential prey selection by predators based on chemical signatures.

Although structural and chemical properties appear important determinants of predation success in general, the anti-predator resistance conferred by them waned over time. This change could be due to the whelks becoming increasingly hungry over the course of the experiment, possibly indicating reduced selectivity in their prey preference over time as hunger increases ([Perry, 1987](#); [Vadas et al., 1994](#)). The likelihood of whelk attachment was dependent on experimental treatment, with attachment occurring latest (>33 d) on mussels with dead barnacle epibionts (+M + S–C), but occurring immediately on treatments where live epibiont barnacles were present, suggesting active selection of barnacles by whelks over mussels. This selection was reinforced by time to attachment onto mussels living with non-epibiont barnacles of ~25 d. Importantly, however, mussel mortality was zero when barnacles were present as live epibionts, and extremely low (1 death) when barnacles were present but dead (M + S–C). This finding is at odds with the long-standing consensus that mussels are favoured over barnacles by whelks ([Hughes and Drewett, 1985](#)) although it should be noted that this earlier study was conducted in the field rather than in a laboratory. Previous studies have suggested that a complex array of potential drivers of behaviour might occur in the field that lead to differential emergence of behavioural response to those observed in the laboratory ([James et al., 2019](#); [Knights et al., 2012](#)), although such experiments are valuable for disentangling lone effects. Indeed, our results here suggests that the reduced handling time associated with feeding on barnacles might override the potential nutritional value of feeding on a larger mussel. [Thangarathinam and Chattopadhyay \(2020\)](#) recently showed that if sufficient food is available to a predator, then prey with epibionts are less likely to be the subject of predation due to the higher energetic cost required to consume the fouled prey. The choice of prey of a predator is

likely the outcome of a number of choices, including consideration of prolonged handling time presenting a greater risk to the predator from predation themselves ([Rovero et al., 1999](#)), or selecting prey with lower defences and lower risk to the predator e.g., avoiding byssal thread trapping ([Davenport et al., 1996](#); [Farrell and Crowe 2007](#); [Johansson, 2010](#)).

4.3. Influence of barnacle epibionts on mussel feeding rate

Barnacle epibiosis led to increased feeding rate in the basibiont mussels; a novel finding in mussels, but previously observed among copepods supporting ciliate epibionts ([Puckett and Carman, 2002](#)). Several previous studies have suggested that ‘carrying’ epibionts may be energetically demanding resulting in additional costs for the basibiont (e.g. [Okamura, 1986](#)). These costs for mussels may be manifested as an increase in byssus thread production to reduce the heightened risk of dislodgement by waves ([Thieltges and Buschbaum, 2007](#); [Johansson, 2010](#)), reduced mussel growth ([Haag et al., 1993](#); [Buschbaum and Saier, 2001](#); [Thieltges, 2005](#)) or change in reproductive success ([Wahl, 1989](#); [Chan and Chan, 2005](#)). Interestingly, feeding rate increased when mussels supported living epibionts, but not when supporting dead epibionts or when living in the presence of non-epibiont barnacles, which might suggest an ability of the mussels or barnacles to determine if an epibiont/basibiont is living, dead or non-biological (e.g. rock) and the proximity of a potential competitor (e.g. [Peterson and Andre, 1980](#)), perhaps through detection of chemical cues ([Atema, 1995](#)). This increased feeding rate may point toward an adaptive behaviour undertaken by the mussel, perhaps in an attempt to increase food consumption to sustain its biological functions as epibionts have been shown to create drag affecting feeding success ([Wahl, 1996](#)). Sharing a home with another filter feeder may lead to greater physiological costs (as above) rather than creating direct competition for food ([Buschbaum and Saier, 2001](#)) despite both mussels and barnacles feeding on similar sized particles ([Thieltges and Buschbaum, 2007](#); [Johansson, 2010](#); [Hunt and Alexander, 1991](#)). Alternatively, increased feeding might suggest that the epibiont allows the mussel to be more ‘bold’ as it is at lower risk of predation, thereby allowing them to feed more often ([Naddafi et al., 2007](#); [Antol et al., 2018](#)). Nevertheless, the increased feeding rate when mussels support living epibionts suggests a trade-off between increased survivorship from predation (behaviourally-derived associational resistance) but an increased energetic cost as a result of the epibiont.

5. Conclusion

This study reveals that barnacle epibionts provide behaviourally-derived associational resistance to mussels from whelk predation. We show that the structural features of the barnacle confer stronger associational resistance compared to the chemical signature alone, but when in combination (i.e., live epibionts), maximal protection for the basibiont is achieved. The diminishment of anti-predator resistance over time suggests that availability of resources for the predator undoubtedly alters the strength of predator-prey interactions, but in a natural environment where food resources are likely less limited, then epibiosis is a potentially important associational defence for mussels from predation.

CRedit authorship contribution statement

Sam Downes: Investigation, Formal analysis, Writing – original draft. **Louise B. Firth:** Conceptualization, Writing – review & editing, Supervision. **Antony M. Knights:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors wish to thank the School of Biological and Marine Sciences for financial support and in-kind technical support for this project, especially from Marie Palmer.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2023.105941>.

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