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Ectoparasites reduce scope for growth in a rocky-shore mussel (*Perna perna*) by raising maintenance costs

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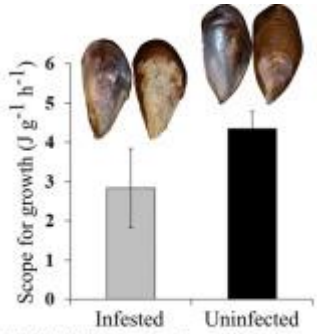
Abstract :

Endolithic cyanobacteria are ubiquitous colonisers of organic and inorganic carbonate substrata that frequently attack the shells of mussels, eroding the shell to extract carbon, often with population infestation rates of >80%. This reduces host physiological condition and ultimately leads to shell collapse and mortality, compromising the services provided by these important ecosystem engineers. While the ecological implications of this and similar interactions have been examined, our understanding of the underlying mechanisms driving the physiological responses of infested hosts remains limited. Using field and laboratory experiments, we assessed the energetic costs of cyanobacterial infestation to the intertidal brown mussel (Perna perna). In the field we found that growth (measured as both increase in shell length and rate of biomineralization) and reproductive potential of clean mussels are greater than those of infested individuals. To explore the mechanisms behind these effects, we compared the energy allocation of parasite-free and infested mussels using the scope for growth (SFG) framework. This revealed a lower SFG in parasitized mussels attributed to an energetic imbalance caused by increased standard metabolic rates, without compensation through increased feeding or reduced excretion of ammonia. Separate laboratory assays showed no differences in calcium uptake rates, indicating that infested mussels do not compensate for shell erosion through increased mineralization. This suggests that the increased maintenance costs detected reflect repair of the organic component of the inner nacreous layer of the shell, an energetically more demanding process than mineralization. Thus, parasite-inflicted damage reduces SFG directly through the need for increased basal metabolic rate to drive shell repair without compensatory increases in energy intake. This study provides a first perspective of the physiological mechanisms underlying this parasite-host interaction, a critical step towards a comprehensive understanding of the ecological processes driving dynamics of this intertidal ecosystem engineer.

Graphical abstract



Endolithic infestation reduces the size and the reproductive performance of intertidal mussels. This is mediated by a reduced scope for growth.

Highlights

► Endolithic parasites reduce the growth and reproductive capacity of marine molluscs. ► Shell erosion by endolithic cyanobacteria leads to lower scope for growth of intertidal mussels. ► Reduced scope for growth in parasitized hosts is caused by increased standard metabolic rates, without compensatory increases in energy intake. ► Energy budget models provide a means to decipher the underlying processes explaining sublethal effects of parasites.

Keywords : Bivalve, Energetic costs, Parasites, Shell boring endoliths

1. Introduction

Parasites are ubiquitous across terrestrial and aquatic natural systems, often with major effects on the development, performance and growth of host species (Mouritsen and Poulin, 2002). Because of their influence on population dynamics and community structure (Hatcher et al., 2012; Lafferty, 2013), there is considerable interest in the mechanisms by which parasites affect host organisms (Hatcher et al., 2012; Lafferty, 2013; Torchin et al., 2002, 2015).

Endolithic cyanobacteria are conspicuous parasites that colonize a huge range of organic and inorganic carbonate substrates, including the outer layers of mollusc shells (e.g., bivalves, Ndhlovu et al., 2019; Porter and Lingle, 1982), from which they derive carbon for use in photosynthesis, thus contributing to bioerosion (Garcia-Pichel et al., 2010; Marquet et al., 2013). Despite evidence of the negative effects of endolith infestation on the growth, reproduction, and survival of corals, molluscs, and other organisms (Garcia-Pichel et al., 2010; Gleason et al., 2017; Kaehler and McQuaid, 1999; Marquet et al., 2013; Shashar and Stambler, 1992), there is little information on the underlying physiological or energetic mechanisms that drive these effects. In the case of bivalves, internal trematode parasites can castrate the host or reduce growth (Calvo Ugarteburu and McQuaid 1998), while pinnotherid crabs that live between the valves can physically reduce the space for gonads to grow or interfere with the filtration and ingestion of food particles (Lafferty and Kuris, 2009). However, because they inhabit the outside of mollusc shells, the effect of endoliths on the host's physiology and condition can only be indirect (Flye-Sainte-Marie et al., 2009).

The negative effects of endoliths on the energy budget of their hosts would be especially problematic for species that normally experience energetically demanding conditions. As filter feeders, the energy intake of marine intertidal mussels is constrained to periods of high tide (Monaco and McQuaid, 2018). These sedentary organisms must also

regularly cope with extreme wave action, desiccation and thermal stress, all of which contribute to their relatively high baseline levels of physiological stress (Helmuth and Hofmann, 2001; Mouritsen and Poulin, 2002). The extra energy costs associated with repairing damage to the shell caused by endolithic cyanobacteria could limit the ability of intertidal mussels to build up the heat-shock response required to cope with normal low-tide conditions (Buckley et al., 2001; Hochachka and Somero, 2002; Somero, 2002). Furthermore, the combination of higher maintenance costs and environmentally induced stress (Sokolova et al., 2012) is expected to reduce the thermal tolerance of infested mussels. This could be critical because intertidal mussels often experience temperatures close to their thermal limits during low tide (Tagliarolo et al., 2016), with knock-on consequences for mussel populations currently experiencing the threat of climate change (Jones et al., 2010). A mechanistic understanding of the effects of endoliths on mussels is important at the community level because of their role as ecosystem engineers (Borthagaray and Carranza, 2007; Gutierrez et al., 2003). By aggregating into dense beds, mussels provide complex habitats for other benthic organisms that gain refuge from predators, protection from wave action, and reduced desiccation and thermal stress (Borthagaray and Carranza, 2007; Gutierrez et al., 2003; Jurgens and Gaylord, 2018).

Growth rate in an organism is commonly used as an indicator of stress because it integrates major physiological responses, including the balance between the processes involved in energy acquisition and expenditure, which depend in part on the prevailing environmental conditions (Naylor et al., 1989; Widdows and Staff, 2006). A widely-used approach to decouple these processes is to estimate scope for growth, (SFG; Widdows and Staff, 2006). Scope for growth is the energy available for growth and reproduction after the requirements for maintenance have been fulfilled. It is estimated as the difference between the available energy that enters the organism and is transformed into tissue, and the energy

expended through respiration (Naylor et al., 1989; Widdows and Staff, 2006). Scope for growth has been successfully used to examine the influence of many environmental drivers (e.g., contaminants, temperature, ocean acidification, and food limitation) on the physiological condition of species (Brett, 1976; Naylor et al., 1989; Resgalla et al., 2007; Sarà and Pusceddu, 2008; Widdows and Staff, 2006). Here, SFG was used to assess the effects on marine mussels of sub-lethal stress due to cyanobacterial infestation of the shell.

Combined field and laboratory approaches were used to gain a deeper understanding of the effects of endolithic cyanobacteria on the physiological processes driving differences in fitness-related traits between non-infested (hereafter, 'clean') and infested intertidal brown mussels (*Perna perna*). Differences in growth, biomineralization, and reproduction rates under field conditions were examined first. Secondly, laboratory experiments were run to quantify energy allocation by infested and clean mussels by measuring the components of SFG (i.e., metabolism, ingestion, absorption efficiency, ammonium excretion). Thirdly, to test for compensatory responses to the shell erosion caused by endoliths, we compared calcification rates between clean and infested mussels. Finally, to ascertain if energy re-allocation due to infestation has an influence on the ability of mussels to tolerate rising temperatures, thermal tolerance was measured. We hypothesized negative effects of endolithic cyanobacteria on the field-measured growth and reproduction, and on the overall SFG. Particularly, we expected that higher energy maintenance costs would explain the lower SFG in infested mussels. Based on these expected responses, we also hypothesized higher calcification rates and lower thermal tolerance in infested mussels.

2. Materials and methods

Field and laboratory experiments were run using individuals of *Perna perna* collected on the southeast coast of South Africa. This species exists as two distinct genetic lineages that are largely allopatric, but co-exist along approximately 200 km of coastline in the study area

(Zardi et al., 2015). As these two lineages exhibit different physiological responses to environmental drivers (Tagliarolo and McQuaid, 2015), only individuals from outside the area of overlapping distribution were used. To determine the two levels of endolithic infestation considered here, infested and clean, we followed the criteria outlined by Kaehler (1999), Marquet et al. (2013), and Ndhlovu et al. (2019). The shells of clean mussels had intact periostracum with distinct outer striations, while those of infested mussels had damaged or partially missing periostracum, with the shell itself being pitted and brittle.

2.1 Field measurements of growth and reproduction

We measured the field growth rates of infested and clean mussels in two ways: (1) based on changes in shell length, and (2) changes in buoyant weight. The former represents a measure of whole-animal growth (McQuaid and Mostert, 2010), while the latter reflects shell biomineralization rates (Molina et al., 2005a). These growth experiments were conducted during the austral summer (November 2017 – February 2018) at Kidd's Beach (32º55'14.20''S, 27º29'18.00''E).

To measure *in situ* whole-animal growth rates, we marked clean and infested individuals (n = 45 per group; shell length mean ± SD = 36 ± 0.13 mm) in the field using paper tags superglued (Loctite, USA) to the posterior growing edge of the shell (McQuaid and Mostert, 2010). We marked haphazardly selected individuals that were within beds in the mid-mussel zone. We collected all marked individuals after 100 days and calculated the monthly growth rate as the difference between the initial (indicated by the tag) and final shell lengths measured using callipers (0.01 mm resolution).

To assess the effect of endolithic infestation on mussel shell biomineralization *in situ*, we measured rates of change in buoyant weight of infested and clean mussels. As the specific density of soft tissues is comparable to that of seawater, this method can effectively measure

changes in shell mass alone (Molina et al., 2005b). We used a 0.001 mg resolution balance (Sartorius, Germany) fitted with a hook from which mussels were hung while submerged in natural seawater. Clean and infested (n = 44 per group, shell length mean ± SD = 43 ± 0.16 mm) specimens were first taken to the laboratory, cleaned of any epibionts, individually marked using paper tags, and their initial buoyant weights measured. In the field, we placed mussels in 20 x 20 cm metal quadrats (n = 4, with 22 individuals per quadrat) attached to the rocks using rawl plugs and screws, covering the mussels with a canvas net (5 x 5 mm mesh size). This held the mussels in place until they could attach themselves naturally to the rocks. The mesh was removed after four weeks, when the mussels had become attached, and after a further 121 days, the mussels were returned to the laboratory and the buoyant weight was re-measured. Survival was 98%.

To examine differences in reproductive output between clean and infested mussels, we used empirical relationships between gonad dry weight and shell length (Berry, 1978). We collected clean and infested mussels (n = 32 per group) ranging between 20 and 60mm shell length from the mid-intertidal zone at Brenton-on-Sea (34˚04'31.7" S, 23˚01'29.5" E). This sampling was done in January 2018, when the reproductive output of *Perna perna* is expected to be seasonally high (Zardi et al., 2007). Individuals were initially preserved in 70% ethanol. In the laboratory, we measured each individuals' shell length (0.01-mm resolution) and dissected out the somatic and gonad tissues. The gonad tissue was dried at 60 °C for 48 hours and weighed to the nearest 0.1 mg (Mettler Toledo, USA).

2.2 Laboratory estimates of calcification

To examine differences in shell deposition between clean and infested *Perna perna* under controlled conditions, we measured instantaneous calcification rates in the laboratory. Animals were collected in April 2018 from the mid-shore at Brenton-on-Sea and transported

to the laboratory (~ 4 hours) in an insulated cooler box fitted with ice packs and moist paper towels to maintain low temperature and high humidity. Mussels were cleaned of epibionts and placed in 20L tanks containing aerated, unfiltered natural seawater. Water was replaced daily to maintain quality and food levels. Tanks were kept in a controlled-environment room maintained at 20 ˚C under a 12h:12h light:dark cycle. The few individuals (< 2%) showing signs of stress-induced spawning were removed immediately. Animals were acclimated under these conditions for five days and then starved in 0.47-µm filtered seawater for 24 hours before the experiments to allow flushing out of faeces and pseudofaeces.

To measure calcification rates we used the alkalinity anomaly technique (Smith and Key, 1975), which assumes that total alkalinity decreases by two equivalents for each mole of $CaCO_3$ precipitated. For each condition (clean or infested) 12 animals were individually placed in seawater in a 500 ml beaker. Total alkalinity in each beaker was measured at time zero and after a one-hour period using a total alkalinity mini-titrator and pH meter (Hanas HI 84531, USA). Sample colouration was measured in a Genesys 20 spectrophotometer at 630 nm. Net calcification rates (G) were calculated using the following equation (Smith and Key, 1975; Tagliarolo et al., 2012): $G = - (\Delta TA \times v)/ 2 \times (\Delta t)$, where ΔTA is the change of total alkalinity during incubation ($\mu mol\ l^{-1}$), v is net bottle volume (L), and Δt is incubation time (h).

We also measured changes in ammonium, a waste product of molluscs (Bishop et al., 1983), to correct for changes in total alkalinity (Tagliarolo et al., 2012). After collection and 7 days of acclimation as above, individual clean and infested mussels (n = 12 per condition, shell length mean ± SD = 40 ± 0.17 mm) were placed in 220 ml chambers filled with filtered (0.47µm) sea water and incubated for 70 minutes. Water samples (1 ml) were collected at the beginning and at the end of the experiment. The production of ammonium was determined

using the phenol-hypochlorite method (Rosas et al., 2003). Sample colouration was measured at 630 nm, with milli-Q water used as a blank. Ammonium excretion was expressed in μmol NH_4 g $AFDW^{-1}$ h^{-1} and used to correct the measurements for total alkalinity.

2.3.1 Scope for growth

The scope for growth framework allows the estimation of the available energy for growth and reproduction based on the difference between the energy absorbed from the food consumed and energy lost via respiration (Bayne, 1976; Widdows and Staff, 2006). The quantification of the remaining energy, which is SFG, was done by measuring the energy budget components. We measured these energy budget components for clean and infested mussels in the laboratory, following Widdows and Staff (2006).

Animals (n = 12 for each group) were collected in April 2017 from the mid-shore at Brenton-on-Sea and acclimated as described for estimates of calcification. Very few individuals (less than 2%) showed signs of stress-induced spawning and these were removed immediately and the remaining individuals where acclimatised and used to measure all energy budget components. The same individuals were used for all energy budget component measurements following this sequence: feeding rate, absorption efficiency and standard metabolic rate.

To measure feeding rates, we first put individual mussels in 4500 ml glass beakers containing 0.47-μm filtered seawater and an initial concentration of a commercial algae paste (Brightwell Aquatics, Phytogreen-M, USA) at a concentration of 0.43 mg ml^{-1}. Preliminary trials indicated no pseudo-faeces production at this concentration. To ensure particles remained in suspension, we used a magnetic stirrer placed away from the mussel. Animals were acclimated for 15 minutes until the valves were open and pumping had resumed. We collected two 500 ml samples 15 minutes after the algal concentration was added and again

after 1.5 hours. Two mussel-free beakers were used as controls. Pre-weighed dry and ashed glass fibre filters (GFC, Merck Millipore, 0.47 μm) were used to filter all samples. We measured the ash-free dry weight of these samples after drying for 24 h at 60 ˚C and burning for 4 h at 450 ˚C. We calculated the feeding rate (mg h^{-1}) based on the difference between initial and final weight of algal paste (Petersen et al., 2004; Sarà and Pusceddu, 2008), and converted this to energy equivalent units, assuming 1 g of algae paste = 20.043 J based on product specifications.

Assimilation efficiency (AE) was determined based on the ratio of organic matter absorbed to organic matter ingested (Conover, 1966) as: $AE = F - E/[(1 - E) \times F]$, where F is the ash-free dry weight/dry weight ratio of the food, and E is the ash-free dry weight/dry weight ratio of the faeces. Faeces were siphoned off six hours after the feeding rate measurements using a 10 ml pipette, and treated with ammonium formate solution that was isotonic to sea water. This was done to remove excess seawater salts from the faecal samples (Berry and Schleyer, 1983). The samples were dried at 60 ˚C for 24 hours and weighed, and subsequently burnt at 450 ˚C for four hours and re-weighed. To gather enough material from faeces for analysis, measurements from four mussels were pooled together, with two groups of four mussels used from each treatment. To calculate the energy absorbed by mussels, we multiplied feeding rate by assimilation efficiency.

Standard metabolic rates were measured for individual mussels using a closed-system respirometry protocol (Sarà et al., 2008). Prior to the experiment, mussels were placed in 0.47 μm filtered sea water for 24 hours to ensure they were in a post-absorptive state. Animals were then transferred to water-tight glass chambers (220 ml) filled with oxygen-saturated filtered seawater. Each chamber had an optical oxygen sensor attached to the inside, which allowed the measurement of oxygen concentration with minimal disturbance to the mussel. A

magnetic stirrer was used to mix the water inside the chamber. Mussels were acclimated for 15 minutes before measurements began, after which oxygen concentration was recorded every 5 minutes for 70 minutes using an optical sensor (PreSens, Fibox 3, USA). The measurements were done in a controlled-environment room set to 20 ˚C, and during the dark phase of the day-night cycle to prevent photosynthesis by endolithic cyanobacteria on the infested mussels. We used two mussel-free chambers as controls. At the end of each trial, mussels were removed from their chambers and the volume of water measured. We calculated the oxygen consumption rate (mg l^{-1} h^{-1}) based on the difference between initial and final concentration and the volume of water in the chamber (Widdows and Staff 2006). All oxygen consumption measurements were corrected to standard body size measurements (soft-tissue dry weight) using allometric scaling and then converted to energy equivalents (J g^{-1} h^{-1}) assuming that 1 mg of O_2 = 14.1 J (Brey, 2001).

2.4 Lethal temperature

Mortality of clean and infested mussels across a range of air temperatures naturally experienced on rocky shores (23, 26, 28.8, 31.7, 34.8, 37.5, 38.7, 39.8 and 40.5 °C) Monaco and McQuaid, 2018) was examined by simulating a 2-hour low tide period in a chamber which was placed inside a temperature controlled water bath (Grant TXF200-R4, UK). Groups of 10 infested and 10 clean mussels were subjected to each temperature treatment simultaneously, following a temperature ramping protocol. For the first hour, temperatures were raised gradually from an initial temperature of 20 °C to the treatment temperature and maintained at that temperature for another hour. Mussels were then removed from the controlled environmental chamber and placed in aerated sea water for 24 hours to recover at 20 ˚C, after which mortality was assessed. We estimated LT_{50} (the temperature at which 50 % of the mussels died) using logistic regression models coded in R (R Core Team 2017). LT_{50} values and 95 % confidence intervals for each treatment were estimated and the difference

between treatments was then determined based on the overlap between the confidence intervals.

2.5 Data analysis

We used one-way analyses of variance (ANOVAs) to test the effect of infestation level (fixed factor, two levels) on field growth and calcification rates, as well as overall SFG and each of its components (feeding rate, standard metabolic rates). One-way analysis of covariance (ANCOVA) was used to compare the reproductive output of infested and clean mussels (fixed factor, two levels), with shell length as a co-variate. Reproductive output data were $\log_{10}$-transformed to meet the assumptions of a parametric test. These tests were done using Statistica 13 (Statsoft, USA), with an α level of 0.05.

3. Results

3.1 Growth and reproduction

Clean mussels exhibited significantly higher whole-body growth rates than mussels infested by endolithic cyanobacteria (ANOVA: $F_{(1, 88)} = 15.003$, $p < 0.001$, Fig. 1A). The biomineralization of clean mussels, as measured by the change in buoyant weight, was also faster than that of infested individuals ($F_{(1, 86)} = 44.881$, $p < 0.001$, Fig. 1B). In the laboratory, the rate at which $CaCO_3$ was absorbed by mussels did not differ significantly between the two groups (ANOVA: $F_{(1, 22)} = 1.483$, $p = 0.236$, Fig. 1C).

For both clean and infested mussels, gonad dry mass increased with shell length (ANCOVA: $F_{(1,61)} = 138.862$, $p < 0.001$), and no interaction between these groups was detected ($F_{(1,60)} = 3.9$, $p = 0.053$). We found that gonad dry mass was higher in clean than infested mussels ($F_{(1,61)} = 82.806$, $p < 0.001$, Fig. 1D).

3.2 Scope for growth and temperature tolerance

We found no significant effect of infestation on the amount of ammonium excreted (ANOVA: $F_{(1, 22)} = 0.191$, $p = 0.666$, Fig. 2A) or on individual feeding rates (ANOVA: $F_{(1, 18)} = 1.832$, $p = 0.38$, Fig. 2B). There was only a minor difference in mean absorption efficiency between the two groups: infested = 0.54 and clean = 0.52. Standard metabolic rates were substantially and significantly higher for infested than clean mussels ($F_{(1, 18)} = 218.154$, $p = 0.001$, Fig. 2C). Infested mussels exhibited average maintenance costs of 6 J g^{-1} h^{-1} compared to an average of 4.1 J g^{-1} h^{-1} for clean mussels. Scope for growth was also significantly higher for clean than infested mussels ($F_{(1, 18)} = 19.055$, $p < 0.001$, Fig. 2D), indicating more energy available for growth and/or reproduction in clean mussels.

The prediction that infested mussels would have lower lethal limits than clean mussels was not supported. LT_{50} estimates for clean and infested mussels were similar, at 38.23˚C and 38.08˚C respectively (Fig. 3), while overlap of the LT_{50} 95% confidence intervals between the two groups indicated no significant difference.

4. Discussion

Endolithic cyanobacteria are extremely widespread in marine species (Gektidis et al., 2007; Golubic et al., 2005; Mao Che et al., 1996; Porter and Lingle, 1992), frequently influencing host fitness (Flye-Sainte-Marie et al., 2009; Glynn, 1997; Kaehler, 1999). Here, we demonstrate that endoliths, although attacking only the external shells of mussels, compromise host fitness through a causal link between the infestation and raised metabolic costs.

Our field experiments showed that endolithic infestation reduces whole-animal growth and reproduction, generally agreeing with previous studies comparing fitness-related traits between clean and infested hosts (Kaehler and McQuaid, 1999; Marquet et al., 2013).

Our experiments also revealed significant reduction in shell production with a net loss of shell mass, as baseline shell deposition in infested individuals is counterbalanced by the erosion due to endolithic tunnelling (Garcia-Pichel et al., 2010). This effect could be compensated by boosting of mineralization rates (Kaehler and McQuaid, 1999), but our laboratory measurements of calcium carbonate uptake by the host showed no difference between clean and infested individuals. This lack of compensation indicates that reduced shell growth in the field can be entirely explained by the boring action of endoliths.

Infested mussels also showed significantly reduced SFG and measurement of the various energy budget components indicated that this was the result of increased maintenance costs. Similarly, when infected by brown ring disease bacteria, the standard metabolic rate of Manila clams (*Venerupis philippinarum*) can double, thus reducing their scope for growth (Flye-Sainte-Marie et al., 2009). In both clams infected by the brown ring disease bacteria, and mussels infested by endolithic cyanobacteria, the parasite's activity can degrade the host's shell. Because shell production is an important contributor to somatic maintenance in bivalves (Sanders et al., 2018), we expected that the difference in standard metabolic rates between clean and infested mussels would be associated with increased rates of $CaCO_3$ deposition, but our data did not support this interpretation. Explaining the higher maintenance costs of infested mussels requires a more detailed examination of the biochemical intricacies of shell building (Zhao et al., 2017). The main layers of bivalve shells, nacreous and prism, are composed of inorganic $CaCO_3$ crystals ('bricks') and an organic matrix ('mortar') that holds them together (Jacob et al., 2008). Shell production is an important contributor to higher metabolic rates and the higher metabolic rates of infested *Perna perna* may be explained primarily by the increased cost of building the organic matrix of the nacreous layers (which we did not measure) rather than the energetically less demanding cost of increased mineral deposition (Palmer, 1983, 1992). The fact that endolithic cyanobacteria can

compromise the immune system of the host (Kaehler and McQuaid, 1999; Zardi et al., 2016), may also contribute to raising metabolic rates. Parasite infestation can drive alterations to the rate of food intake (Palmer 1992), but endoliths pose no mechanical constraints on the feeding activity of *Perna perna* and, as in the case of trematode parasites (Calvo-Ugarteburu and McQuaid, 1998), infested and clean individuals showed no significant differences in ingestion rates or assimilation efficiencies. Thus, there is an overall increase in metabolic loses, with no compensatory change to energy acquisition.

We also hypothesized that an indirect consequence of higher energy demands for parasitized mussels would degradation of their ability to cope with heat stress (Buckley et al., 2001; Hochachka and Somero, 2002; Somero, 2002). Our estimates of lethal temperatures, however, did not differ between clean and infested mussels, suggesting no differences in their physiological sensitivities to future warming or their capacity to activate a heat-shock response to combat the effect of increasing temperatures (Buckley et al., 2001; Hochachka and Somero, 2002; Schneider, 2014; Somero, 2002). Interestingly, because endolithic cyanobacteria increase the reflectivity of solar radiation by the shell, thus reducing body temperature during low-tide when temperatures are stressful, these parasites can even provide a service to their hosts (Gehman and Harley, 2019; Zardi et al., 2016). Together, these earlier biophysical studies and our assessment of the impact of endolithic cyanobacteria on mussel thermal tolerance suggest a net positive effect in the face of short-term thermal stress. Of course, this ignores the detrimental direct impacts on growth, reproduction, and longevity that determine individual fitness.

5. Conclusion

By complementing insights from field manipulative experiments to quantify the effects of infestation on growth and reproduction with laboratory assessments of scope for growth, our study provides a first look at the physiological mechanisms underlying the

negative effects of endolithic cyanobacteria on host fitness. For intertidal molluscs, the challenge brought by these parasites augments the realised costs of inhabiting habitats subject to extreme variability in food availability and thermal stress (Helmuth and Hofmann, 2001). This has secondary consequences in the case of mussels as they are critical habitat-forming species that directly enhance local biodiversity (Kent et al., 2017; Norling and Kautsky, 2008). The influence of endolithic cyanobacterial species as parasites on mussels is important as it affects other species that are dependent on mussels as habitat forming species. This study provides a springboard for future studies to focus on the potential impacts posed by parasites not just on their hosts, but also on the communities they support, highlighting the indirect cascading influence of parasites when they infest ecological engineers. The physiological performance of such ecological engineering species is further challenged by climate change-driven warming and ocean acidification (Gazeau et al., 2007; Melzner et al., 2011; Rodolfo-Metalpa et al., 2011; Stumpp et al., 2011; Sunday et al., 2017). Because stressors of biotic and abiotic origin can be gauged from the basis of individual energy budgets (Flye-Sainte-Marie et al., 2009; Monaco and McQuaid, 2019; Sokolova et al., 2012), our results can directly inform future predictive models that integrate these processes.

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Figure legends

Figure 1: Energy acquisition and expenditure in *Perna perna* mussels. (A) Mean (±SD) growth rate (mm $month^{-1}$) of infested and clean *Perna perna* mussels (n = 45) measured *in situ*. (B) Mean (±SD) change in buoyant weight for infested and clean mussels (n = 44). (C) Linear regression between shell length and gonad dry mass (n = 32). (D) Mean (±SD) change $CaCO_3$ absorption for infested and clean mussels (n = 12). Error bars represent standard deviation between clean and infested samples. ** $p < 0.05$; *** $p < 0.001$.

Figure 2: Energy acquisition and expenditure in *Perna perna* mussels. (A) Mean (±SD) ammonium excretion for infested and clean *Perna perna* mussels (n = 12). (B) Mean (±SD) feeding rates of infested and clean *Perna perna* mussels measured in situ. (C) Mean (±SD) metabolic rates for infested and clean mussels (n = 10). (D) Mean (±SD) scope for growth of infested and clean mussels (n = 10). Error bars represent standard deviation between clean and infested samples. ** $p < 0.05$; *** $p < 0.001$.

Figure 3: Survival probability as a function of air temperature for *Perna perna*. Logistic regression fits for clean (blue) and infested (red) mussels, along with 95% confidence intervals (grey shaded areas). Points are off-set slightly on the x-axis.

Figures

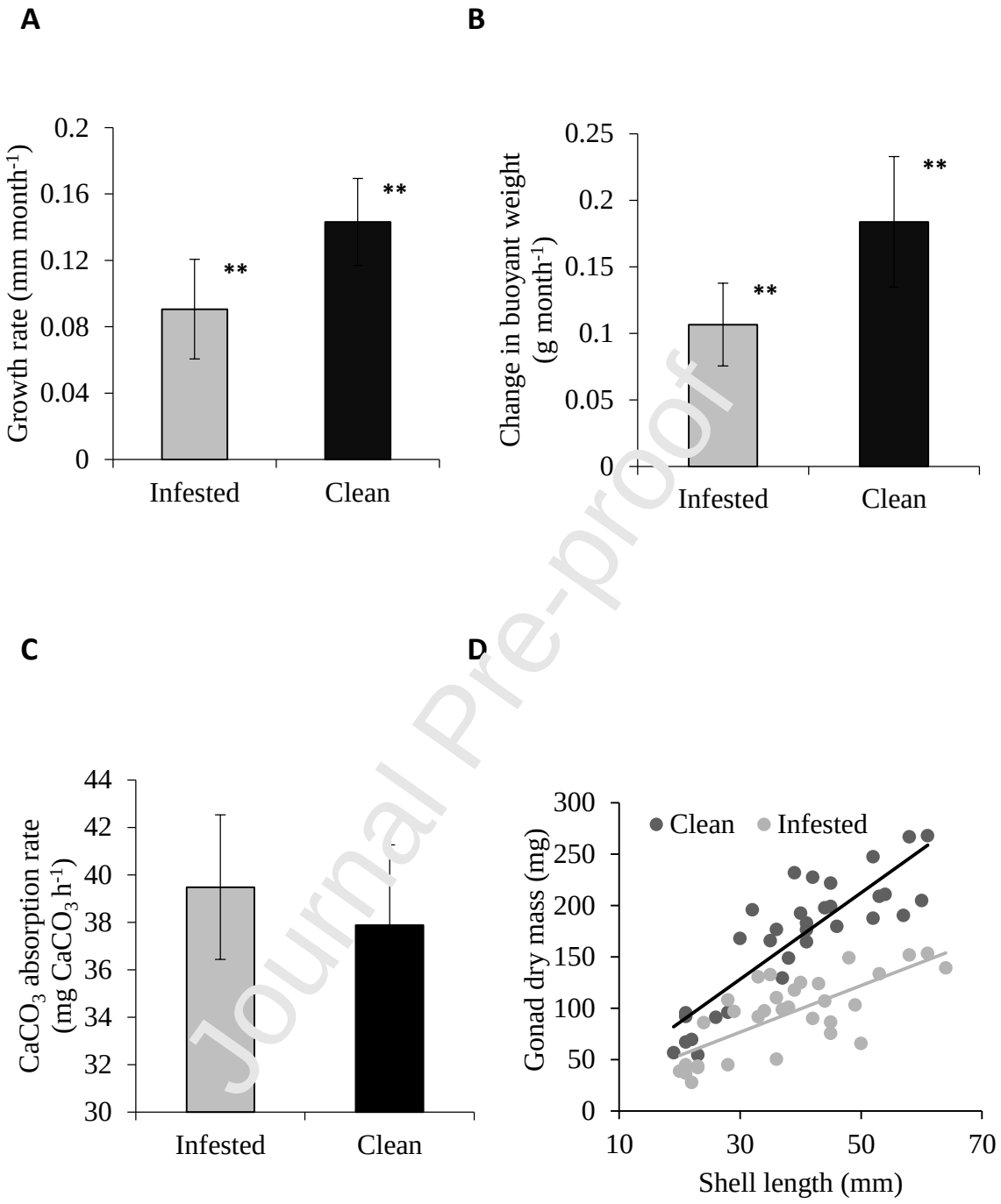


Figure 1

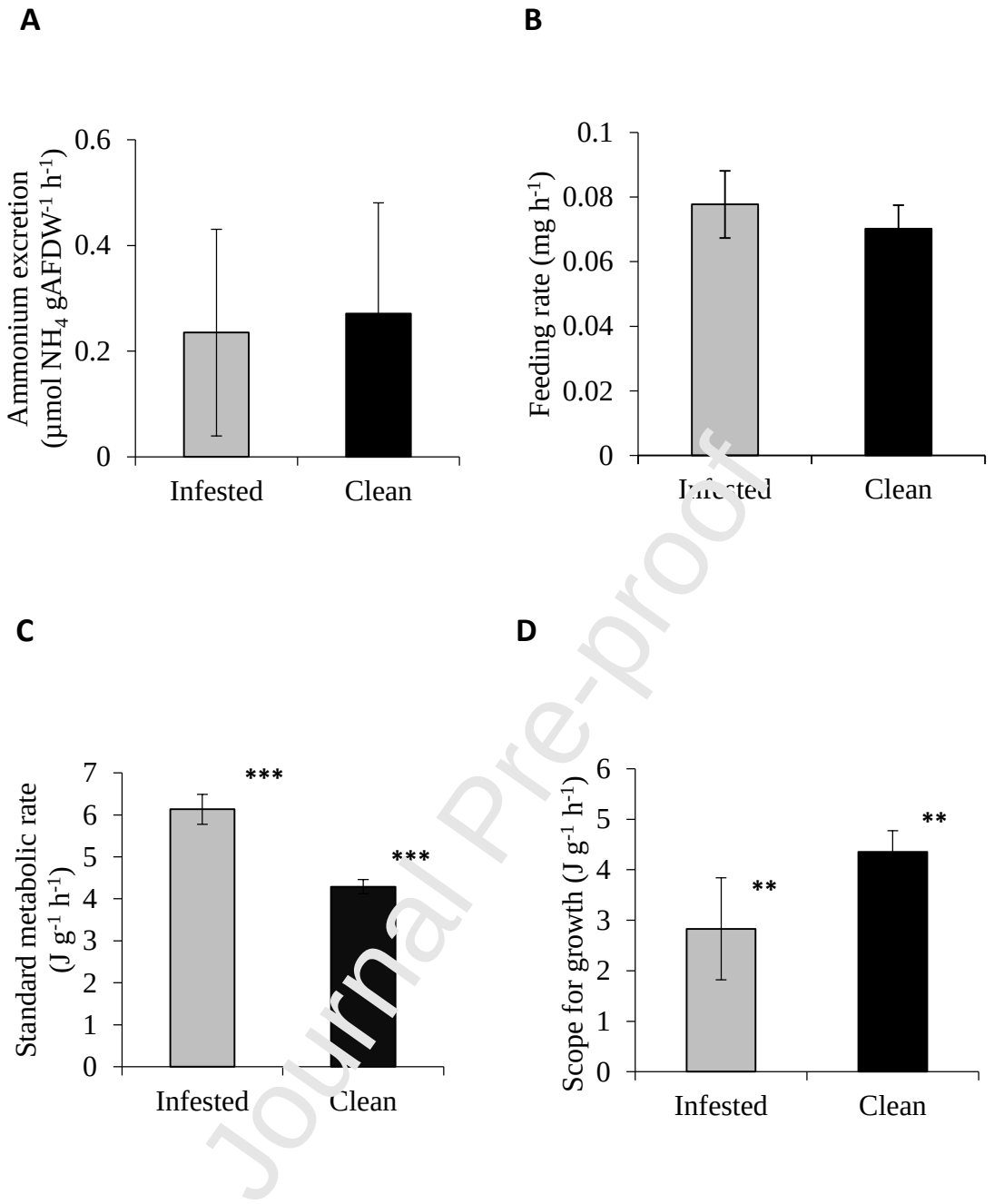


Figure 2

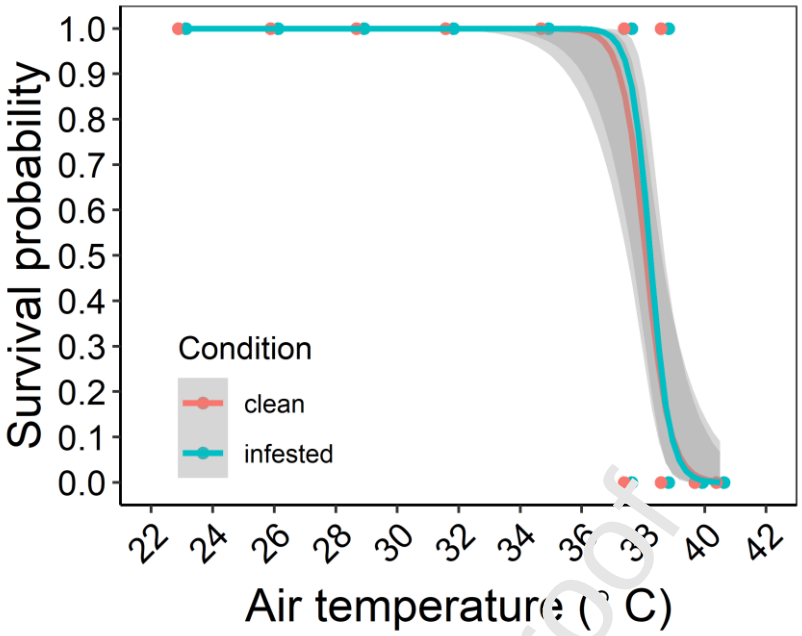


Figure 3

Author contributions

CJM and CDM conceived the ideas

CJM and AN designed the methodology;

AN and CJM collected the data;

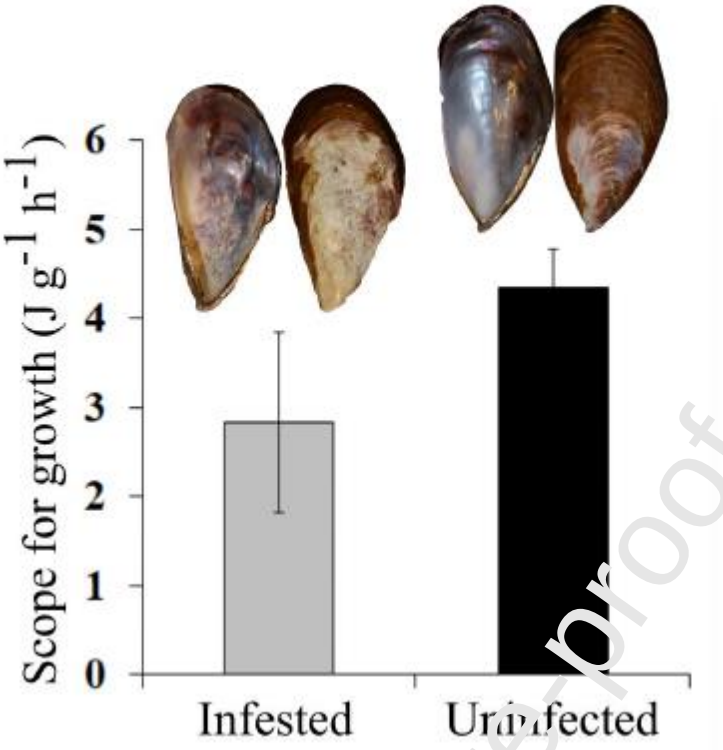
AN and CJM analysed the data;

CDM led the writing of the manuscript, AN and CJM contributed.

All authors contributed critically to the drafts and gave final approval for publication.

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.



Endolithic infestation reduces the size and the reproductive performance of intertidal mussels. This is mediated by a reduced scope for growth.

Graphical abstract

Highlights

- Endolithic parasites reduce the growth and reproductive capacity of marine molluscs.
- Shell erosion by endolithic cyanobacteria leads to lower scope for growth of intertidal mussels.
- Reduced scope for growth in parasitized hosts is caused by increased standard metabolic rates, without compensatory increases in energy intake.
- Energy budget models provide a means to decipher the underlying processes explaining sublethal effects of parasites.