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Impacts of copper contamination on a rocky intertidal predator-prey interaction

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Highlights

- Copper contamination increased mortality of a sessile prey.
- Copper decreased consumption of the prey by the predator.
- Predator-prey interaction strength was not affected by copper.
- Copper effects on predator-prey interactions may affect community dynamics

Abstract

Metal contamination can change ecological interactions with potential effects on community dynamics. However, understanding real effects of metals on biota relies on studies undertaken in natural conditions. Through a field experiment, we investigated the effects of copper contamination on the responses of a barnacle prey and its predator, the dogwhelk, and explicitly their interaction. Contamination increased barnacle mortality and reduced predation with no effects on interaction strength. This was because the higher mortality of the prey compensated for the lower consumption of the predator. Despite not affecting the interaction strength, these results suggest a decrease in energy flow in the trophic chain that may lead to important changes in community structure and ecosystem functioning. This study shows the importance of manipulative experiments designed to provide mechanistic insights into ecological interactions to better clarify the effect of stressors on the structure and dynamic of communities.

Key words: anthropogenic impacts, antifouling paints, pollution, predation, urban ecology.

1. Introduction

Metal contamination is a worldwide issue impacting natural systems. Historic and contemporary human activities have resulted in a general increase in metal contamination of aquatic environments, particularly in coastal regions (Bryan and Langston, 1992; Blackmore, 1998; Neto et al., 2006; McVay et al., 2018; Queiroz et al., 2018; Martinez et al., 2019) posing a threat to biodiversity through responses ranging from the individual to population and community-levels (Weis and Weis, 1996; Marsden and Rainbow, 2004; Roberts et al., 2008; Johnston and Roberts, 2009; Mayer-Pinto et al., 2010). Ultimately, metal contamination can impact ecosystem functioning with negative effects on goods and services essential for humans (Johnston et al., 2015; Hayes et al., 2018; Mayer-Pinto et al., 2020).

Among metals, copper is a ubiquitous contaminant of coastal environments (Stauber et al., 2005; Schiff et al., 2007; Marcelo-Silva and Christofoletti, 2019). In addition to the discharge of domestic sewage and industrial effluents, usage of antifouling paints is a main source of copper in aquatic ecosystems (Matthiessen et al., 1999; Biggs and D'Anna, 2012; Amara et al., 2018). After international restrictions on paints containing organotin, copper has become the main compound to combat biofouling. Such antifouling paints slowly release copper-based biocides to inhibit the adhesion and growth of fouling species (Thomas and Brooks, 2010). Although copper-based paints are considered a better alternative to organotin, investigations report toxic effects of copper compounds on organisms, including impairments in mobility, growth, reproduction and ultimately enhanced mortality (e.g. Brand et al., 1986; Weis and Weis, 1992, 1993; Real et al., 2003; Dafforn et al., 2011; Das and Khangarot, 2011; Amara et al., 2018). Despite the vast literature on the effects of copper contamination less is known on its impacts on ecological interactions.

The interaction between predator and prey is a fundamental ecological process shaping the dynamics of natural communities that is contingent on its strength (Paine, 1980; Menge, 1992; Menge et al., 1996). Interaction strength measures the per capita effect of one species on the abundance of another population (Paine, 1992; Berlow et al., 1999). Contaminants can thereby affect the strength of trophic interactions either through effects on the predator, the population of the prey or both (Rohr et al., 2006; Clements and Rohr, 2009; Saaristo et al., 2018). Previous research has shown that predators feed less on or avoid copper-contaminated food (Weis and Weis, 1992, 1993; Roberts et al., 2006), while waterborne copper exposure decreases feeding activity (Weeks, 1993; Lee and Johnston, 2007; Das and Khangarot, 2011; Warneke and Long, 2015). These results indicate changes in trophic interactions, with potential effects on ecosystem functioning as weakening top-down control and decreasing energy flow. Such evidence, however, is derived mostly from laboratory assays and may not represent real effects on natural communities.

The few studies done in natural field conditions have shown contrasting results to laboratory experiments with no effects of copper contamination on the feeding activity of a predator (Cartwright et al., 2006; Corte et al., 2017). Laboratory studies usually show strong consistent effects of metal contamination on the biota whereas field experimentation have found none, variable or weaker effects (Mayer-Pinto et al., 2010). Such contrasting results may occur because laboratory experiments fail to reflect the complexity of natural habitats, precluding extrapolations of results to nature (Underwood and Peterson, 1988; Underwood, 1995; Mayer-Pinto et al., 2010). In situ experiments have the advantage of testing causal relationship between biotic responses and stressors in natural varying conditions (e.g. Mayer-Pinto et al., 2011; McElroy et al., 2016; Corte et al., 2017). While the importance of laboratory approaches to investigating aspects of contamination is undeniable (e.g. Kwan et al., 2015; Warneke and Long, 2015), realistic field-based research is equally needed to understand contamination impacts in a more ecologically meaningful way (Underwood, 1995; Chapman, 2002).

Here, we aimed to investigate the effect of copper contamination on the trophic interaction between key temperate rocky intertidal invertebrates: the dogwhelk *Nucella lapillus* and the barnacle *Semibalanus balanoides*. Dogwhelks are one of the main carnivores in intertidal habitats along rocky coastlines able to control prey populations and community structure (Menge, 1976). Suspension feeders, such as barnacles, are the most abundant prey, dominating extensive areas of the intertidal zone and playing an important role in nutrient cycling and benthic-pelagic coupling (Dame et al., 2001; Ostroumov, 2005). We conducted a field manipulation for 90 days to test the effect of copper contamination (delivered through the use of antifouling paint) on responses of prey (mortality) and predator (consumption rate, energy acquired and weight gain) and explicitly their interaction (per capita interaction strength). We evaluated responses cumulatively to understand overall effects of contaminant exposure and, in some cases, we also assessed temporal variability. We predicted higher mortality of adult barnacles exposed to copper contamination. We further predicted that copper would negatively affect consumption of barnacles by dogwhelks and that dogwhelks would consequently acquire less energy and less weight gain. Finally, we expected that interaction strength would be decreased by copper contamination (i.e. lower per capita effect of the predator on the prey population) owing to decreased consumption of prey.

2. Material and Methods

2.1. Experimental setup

A caging experiment was carried out in the rocky intertidal close to Cemlyn Bay (Wales, UK: 53.412877 N, -4.523239 W) for 90 days (between 19th April and 18th July 2019). A 2-way factorial design was used with the treatment factors being copper contamination (present, absent) and predator (present, absent). Predator exclusion treatments were used to quantify mortality of barnacles due to natural causes (no copper) or exposure to copper. In predator inclusion treatments barnacles were either exposed to predatory dogwhelks under natural conditions or under copper contaminated conditions. In this latter treatment barnacle mortality was potentially a function of both the direct effects of copper and the copper-modified behavior of the predator.

We haphazardly selected 32 plots of 20 x 20 cm occupied by the barnacle *Semibalanus balanoides* and at least two meters away from each other and assigned 8 plots at random to each of the 4 experimental treatments. All areas had a gentle slope (between 0 and 30 degrees). A perimeter was scraped clear around areas of 15 x 15 cm to deploy the cages. For the copper treatment, the paint was applied over the scraped area. All organisms other than this barnacle were removed from the plots. Cages (20 x 20 x 7 cm) made of stainless steel (⌀ = 1.3 mm, 13 mm mesh aperture) were fixed to the substrate. For the copper treated plots, two coats of light grey self-polish antifouling paint (EU-45 antifoul Ltd., UK) were applied in a 5-cm thick strip directly onto the rocky surface. The main component of this paint is dicopper oxide (40-50%), but it also contains hydrocarbons (20-25%), zinc oxide (5-10%) and rosine (5-10%) (Safety datasheet, Appendix A). To maintain the contamination of these treatments through time, extra paint coats were applied approximately every 30 days (days 0, 27 and 59). In predator inclusion treatments, three adult *Nucella lapillus* (shell length; mean ± SD: 28.90 ± 0.82 mm) were caged in each replicate, a density within the range found in natural areas elsewhere (Menge, 1976; Burrows and Hughes, 1989). During the whole experiment, only one dogwhelk died, which was replaced as soon as it was observed. Both initial barnacle density and dogwhelk size did not differ among treatments (Appendix B: Table S1, Figs. S1 and S2). Over the course of the experiment, we removed any detritus accumulated on the cages and no algae was observed growing on them.

2.2. Mortality of prey

The mortality of barnacles was quantified by counting the number of live barnacles in digital images at days 0, 27, 46, 59, 75 and 90. The number of barnacles consumed by dogwhelks was estimated as the difference between the number of dead barnacles in the presence of the predator and the average number of dead barnacles in the respective predator exclusion treatment (as in Menge et al., 2002; Sanford, 2002; Sanford and Swezey, 2008). At day 46 no images were taken for predator exclusion treatments; the number of dead barnacles in these treatments was estimated for each replicate at day 46 through polynomial equations (Appendix B: Table S2 and Figs. S3 and S4).

The number of live barnacles at each time was also used for calculating the per capita interaction strength of the predator on the prey. We used Paine's (1992) per capita interaction strength index which is appropriate to test the magnitude effect of a consumer on a dominant prey. This index is calculated as (N–D)/(D*Y), where N ('normal' condition) represents the prey abundance at a given time in the manipulated presence of the predator; D ('deleted' condition) is prey abundance in the absence of the predator; and Y is the number of predators (as in Berlow et al., 1999). The index was calculated for each replicate at each time where predators were present. 'N' and 'D' were calculated using data of each sampling time. Because predator inclusion replicates were not paired with any particular predator exclusion replicate, the average background mortality at each time across all predator exclusion replicates was used to establish 'D'.

2.3. Weight gain and energy acquired by the predator

Before starting the experiment, the dogwhelks were brought to the laboratory, weighed (total wet weight) with a digital balance (0.0001 g) and marked with nail polish for later identification. Excess water was removed with absorbent paper before weighing. The same procedure was done at the end of the experiment and weight gain was calculated as the final minus initial weight.

To test the effect of contamination on the amount of energy acquired by the dogwhelks, we measured the size of consumed barnacles (empty shells; opercular length) (n = 57 to 94 per replicate) at the end of the experiment. Barnacle size was then converted into energy using an empirically derived equation that converts size into dry mass (Burrows and Hughes, 1990: $y = 0.0632x^{2.954}$). Finally, the estimated weight was converted into energy by means of an energetic conversion factor (22.55 J/mg: Wu and Levings, 1978) (as in Trussell et al., 2006, 2008). We averaged energy acquired per replicate and multiplied by the estimated per capita number of consumed barnacles.

2.4. Metal analysis

Copper concentrations were quantified in the tissues of predator and prey to ensure that the copper treatments truly represented contaminated conditions. Dogwhelks and barnacles were sampled (n = 3) before and at the end of the manipulation. Three dogwhelks of similar size range

(27-31 mm of shell length) were collected and their tissues were pooled for one replicate of metal analysis. Barnacle samples were collected by scraping a cluster of approximately 100 cm^2 area of individuals off the rock. At day 0, dogwhelks and barnacles were randomly sampled along the study area. At the end of the experiment, 3 random plots of each treatment were selected to collect predator and prey tissues for metal analyses. Dogwhelks were washed with distilled water. The soft tissue was then removed, dried in an oven (48h at 60°C) and macerated to a fine powder. The same procedure was applied for barnacles, but we used the whole animals (shell and soft tissue) due to their small size. Biological samples (50-100 mg) were sent to a private company (Campden BRI Ltd, United Kingdom: https://www.campdenbri.co.uk/) for metal analyses. Metals were quantified through ICP-MS (Inductively Coupled Plasma – Mass Spectrometry) operated with Helium collision cell. Zinc content was also quantified as it is present in the antifouling paint, but it did not differ among treatments for both predator and prey (Appendix B: Table S3).

2.5. Data analysis

All analyses were performed in R version 3.6.3 software (R Core Team, 2020) with packages ‘lmer’ (Bates et al., 2015), ‘glmmTMB’ (Brooks et al., 2017) and ‘GAD’ (Sandrini-Neto and Camargo, 2020). Figures were made using package ‘ggplot2’ (Wickman, 2016). Our experimental design allowed calculation of background mortality of prey in treatments where predators were absent. This was then used for estimating consumption of prey by the predator and calculating interaction strength. Responses were thereby tested separately for treatments with the predators absent and present. The effect of ‘treatment’ (antifouling paint or control) and ‘time’ on the mortality of barnacles in the absence of predator, per capita consumption of barnacles and per capita interaction strength (PCIS) were analyzed with (generalized) mixed modelling. For these models, ‘time’ was set as a factor to allow comparisons between treatments for each time. Models also considered ‘replicate’ as a random factor (varying intercept). For models of cumulative responses (mortality of barnacles in the absence of predator, per capita consumption and PCIS), we selected the best random structure of each model via AICc score to account for temporal dependence of replicates through time (as a continuous variable). Models were fitted with Gaussian (link identity), negative binomial (link log) or Poisson (link log) distributions and estimated through restricted maximum likelihood (REML). Post hoc comparisons, when pertinent, were done with package ‘emmeans’ using Satterthwaite estimation of degrees of freedom (Lenth, 2020). Assumptions of models were checked through visual inspection of residual plots. For negative binomial and Poisson models, validation of models was done in package ‘DHARMa’ (Hartig, 2020).

The amount of copper in tissues of dogwhelks and barnacles before the experiment and in treatments after 90 days were compared with a 1-way ANOVA. Where heteroscedasticity was detected (Cochran’s test), we performed the analysis on transformed data. Post hoc SNK tests were applied to compare multiple means when pertinent. Per capita energy acquired and weight gain of the predator were compared between treatments through a Student’s t-test, after confirming homogeneity of variance (Levene’s test). Weight gain was averaged by replicate to avoid non-independence.

3. Results

Copper content in tissues of the barnacle *Semibalanus balanoides* increased slightly in control treatments (mean ± SD: 5.13 ± 2.25 $\mu g \cdot g^{-1}$) compared to background levels registered before the experiment (mean ± SD: 2.66 ± 0.40 $\mu g \cdot g^{-1}$). These values are typical of unpolluted areas (Reis et al., 2012). In the treatments where antifouling paint was applied copper concentrations (mean ± SD: 176.66 ± 37.85 $\mu g \cdot g^{-1}$) were 66 times higher than background levels (Fig. 1). Copper in tissues of the dogwhelk *Nucella lapillus* did not differ among treatments (Fig. 1; Appendix B: Table S3).

In the absence of predators, the mortality of the barnacle *Semibalanus balanoides* was higher in treatments where antifouling paint was applied than in those where it was not on days 59 and 75 of manipulation (Appendix B: Table S4, Fig. 2). On day 27, barnacle mortality was similar in both control and copper treatments likely due to disturbance from setting up the experiment. Further low mortality in the control treatment indicates that cage artefacts were probably negligible. Considering the whole experiment, the rate of barnacle mortality was higher in copper treatments, resulting in a mean cumulative number of dead barnacles two times that of the control (Table S4, Fig. 2).

Application of antifouling paint decreased the per capita number of barnacles consumed by the predator *Nucella lapillus* only on day 27 of manipulation. There were no differences between treatments on further days (Appendix B: Table S5, Fig. 3). This initial difference propagated through time resulting in lower consumption in copper treated plots over the 90 days (Table S5, Fig. 3). At the end of experiment, the mean number of consumed prey was reduced by ~20% in contaminated treatments.

Contrary to our expectations, per capita energy acquired by the predators after 90 days did not differ between treatments (Student's t-test: df = 11, t = -1.59, P = 0.13; Levene's test: F = 0.15, P = 0.70) (Fig. 4A), neither did weight gain (Student's t-test, df = 14, t = 0.44, P = 0.66; Levene's test: F = 4.52, P = 0.05) (Fig. 4B). Likewise, metal contamination did not affect per capita interaction strength (PCIS) of the predator *Nucella lapillus* on prey *Semibalanus balanoides* (neither at each time nor cumulatively) (Appendix B: Table S6, Fig. 5).

4. Discussion

This is the first study to investigate the effect of metal contamination on a well-known marine predator-prey system through field experimentation. Here we found that copper contamination via antifouling paint increased mortality of adult barnacles *Semibalanus balanoides* and reduced their consumption by the dogwhelk *Nucella lapillus*. Contamination did not affect the per capita interaction strength of the dogwhelk on the barnacles because the higher mortality of the prey compensated for the lower consumption of the predator resulting in a similar reduction in prey abundance. Despite not affecting the interaction strength, the effects of contamination on prey and predator might have important consequences for community structure and ecosystem functioning.

Exposure to antifouling paint in the field increased the copper content in barnacle tissues by more than 60 times, doubling the mortality. *Semibalanus balanoides* has long been known to bioaccumulate copper (Walker, 1977; Brown, 1982) and may do so to extreme levels. For example copper content in tissues of *S. balanoides* in a heavily polluted estuary varied between 2,496 and 21,800 $\mu g \cdot g^{-1}$ (mean 9,505 $\mu g \cdot g^{-1}$) (Morillo et al., 2005). We observed values after 90 days exposure to antifouling paint of 177 $\mu g \cdot g^{-1}$, which were much more similar to values observed on contaminated open coastlines (Rainbow et al., 1980; Reis et al., 2011). Therefore, the level of copper contamination produced by our experiment is representative of real pollution scenarios of coastal environments. Field experiments like ours are crucial for understanding real effects of pollution on biota, since laboratory assays tend to exaggerate pollution levels sometimes to unrealistic concentrations. In our experiment, exposure to copper led to an average reduction in barnacle abundance of 16.5% (min–max: 9.5–24.5%). This result reveals an important effect of this contaminant on adult populations at realistic exposure levels; previous evidence on the lethal nature of contaminants comes mostly from controlled laboratory assays on larval stages (e.g. Lang et al., 1980; Qiu et al., 2005). More importantly, this result shows that barnacle populations may decline, even though they can tolerate severe concentrations of metals. With declines in barnacle density, newly available open space might be occupied by invasive species (Piola and Johnston, 2008). Ultimately, decreases in barnacle abundance can potentially affect local ecosystem functioning via changes in nutrient cycling and benthic-pelagic coupling (Dame et al., 2001; Ostroumov, 2005).

In contrast to barnacles, *Nucella lapillus* did not increase copper concentrations in their tissues, despite feeding on highly copper-contaminated barnacles for three months. This suggests that dogwhelks are able to regulate copper content, balancing intake and elimination rates (Langston et al., 1998; Rainbow, 2002). Nevertheless, exposure to contamination decreased consumption of prey early in the experiment (day 27). Such initial effects were maintained over time resulting in approximately 20% less barnacles eaten by the end of the experiment. Such a decrease in consumption could be caused by impairments of chemosensory abilities of predators in detecting prey (Lürling and Scheffer, 2007; Das and Khangarot, 2011) or on decision-making during feeding (i.e. handling, drilling) (Warneke and Long, 2015). Regardless of mechanism, it happened only early on the experiment and then the predators acclimatized to the contaminated condition. An alternative mechanism would be the behavior of actively avoiding contaminated habitats (Medina et al., 2005; Araújo et al., 2016). If so, dogwhelks could have tried to move away from the contamination source instead of feeding during early exposure.

A lower energy transfer from prey to predator would be expected with less consumption, and thereby reduced predator growth (e.g. Weis and Weis, 1993). In our experiment, however, we did not detect any effect of treatments on energy acquired or weight gained. Our estimates of energy acquired by the predator was based on size of consumed prey, assuming energy available in barnacle tissues is the same regardless of contamination status. This may however be a false assumption, because handling metal contamination can decrease energy reserves (Sokolova et al., 2012). Therefore, it is possible that dogwhelks feeding on contaminated barnacles indeed acquired less energy. The lack of any observed difference in weight gain, may reflect limited time in which differences could accrue. In both treatments the average weight gain of dogwhelks was low possibly as a consequence of caging. Dogwhelks typically move to refuges under stressful conditions (e.g. increased wave exposure or temperature) (Burrows and Hughes, 1989), but experimental caging prevented this behavior. It is worth noting that the energetic demand to metabolically cope with metal contamination could potentially reduce energy available for other response variables not measured in this study (Calow, 1991; Sokolova et al., 2012). For example long-term exposure to copper may negatively affect population size of dogwhelks by reducing reproduction capacity (e.g. Real et al., 2003; Das and Khangarot, 2011). Potential shifts in predators' density or traits, in turn, could impact top-down regulation of communities (Fleeger et al., 2003; Clements and Rohr, 2009; Saaristo et al., 2018). Such possible effects of copper on dogwhelks may, however, be contingent on seasonal changes of feeding behavior. For example, reduced feeding activity of *Nucella lapillus* in winter (Bayne and Scullard, 1978) could decrease potential effects of copper or the costs to cope with contamination. Also, it is important to note that our experiment was conducted at only one location. Replication of experiments across a range of physical conditions (e.g. wave exposure, productivity) will address the potential interaction of such factors with contamination in affecting predator-prey interactions.

5. Conclusion

With increasing urbanization on the coast it is important to elucidate how pollution affects patterns and processes of ecosystems (Todd et al., 2019). Our work contributes to the understanding of effects of contamination on ecological processes under natural conditions, providing important information for the management of coastal habitats (Underwood, 1995; Mayer-Pinto et al., 2010). Measurements of the strength of ecological interactions are an essential cornerstone to understanding and forecasting community dynamics (Wootton and Emmerson, 2005; Novak and Wootton, 2010). Although our results did not show changes in interaction strength due to contamination, we found important effects on the structure of the prey population and the behavior of the predator that can otherwise affect community structure and ecosystem functioning via other mechanisms. This study shows the importance of evaluating processes involved in the strength of ecological interactions to better clarify how stressors affect the structure and dynamic of communities.

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Data accessibility

Data archived in figshare (DOI: 10.6084/m9.figshare.13136195).

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Figures captions

Fig. 1. Copper content in dry tissues of the barnacle *Semibalanus balanoides* and the dogwhelk *Nucella lapillus* in the field experiment. bef = before; ctrl = control; AP = antifouling paint. Large and small symbols are mean ± SE and raw data, respectively.

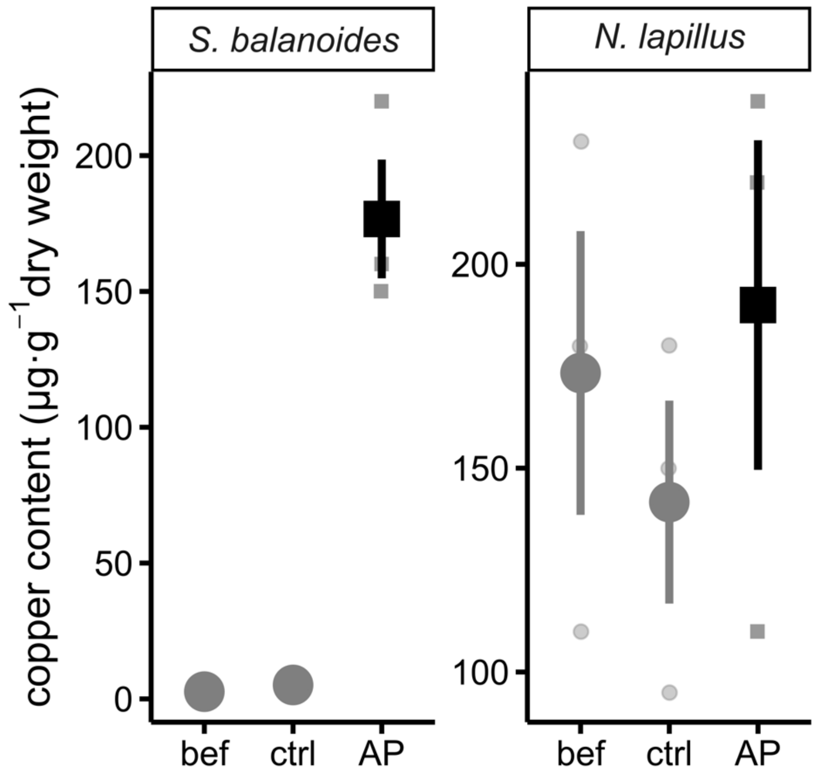
Fig. 2. Number of dead barnacles *Semibalanus balanoides* (in each time and cumulative) in the treatments with or without the application of antifouling paint (AP or control) in the absence of the predatory dogwhelk *Nucella lapillus*. Large and small symbols are mean ± SE and raw data, respectively; lines and shaded areas represent model predictions ± SE. Where present, significant difference between treatments within time are shown (***$P < 0.001$).

Fig. 3. Estimated number of consumed barnacles (in each time and cumulative) by the predatory dogwhelk *Nucella lapillus* in the treatments with or without the application of antifouling paint (AP or control). Large and small symbols are mean ± SE and raw data, respectively; lines and shaded areas represent model predictions ± SE. Where present, significant difference between treatments within time are shown (**$P < 0.01$).

Fig. 4. (**A**) Energy acquired and (**B**) weight gain of the predatory dogwhelk *Nucella lapillus* in the treatments with or without the application of antifouling paint (AP or control) after 90 days of experiment. Large and small symbols are mean ± SE and raw data, respectively.

Fig. 5. Per capita interaction strength (PCIS) (in each time and cumulative) of the predator *Nucella lapillus* on the barnacle *Semibalanus balanoides* in the treatments with or without application of antifouling paint (AP or control). Large and small symbols are mean ± SE and raw data, respectively; lines and shaded areas represent model predictions ± SE.

Figure 1



S. balanoides
N. lapillus
copper content (µg·g⁻¹dry weight)
200
150
100
50
0
bef
ctrl
AP
200
150
100
bef
ctrl
AP

Figure 2

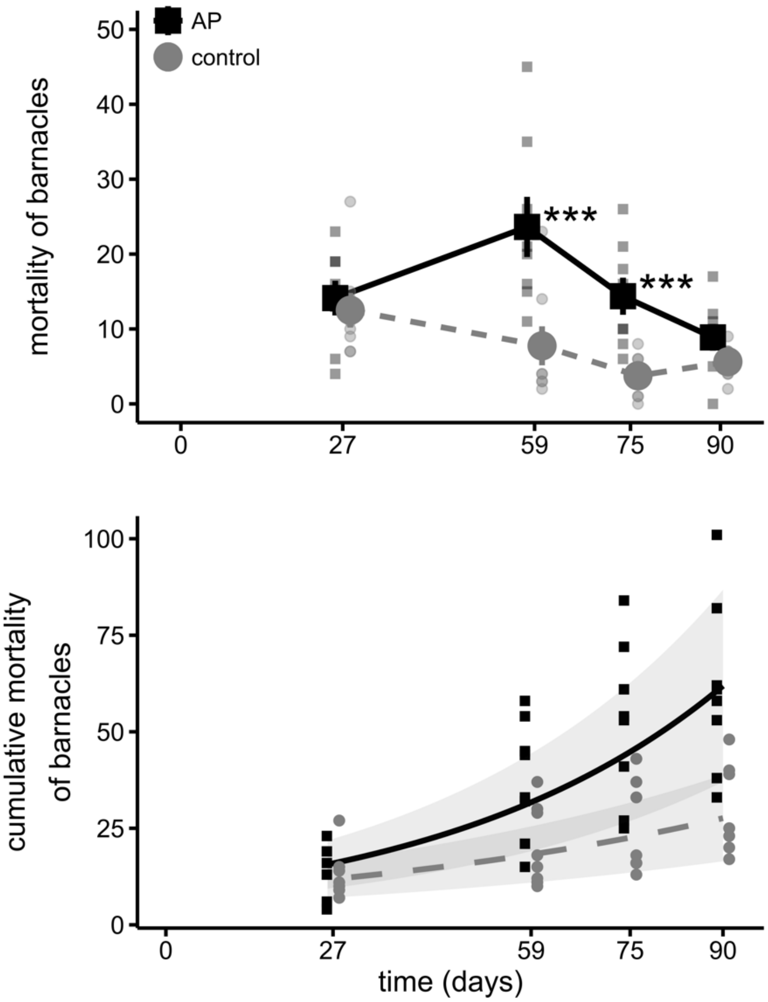
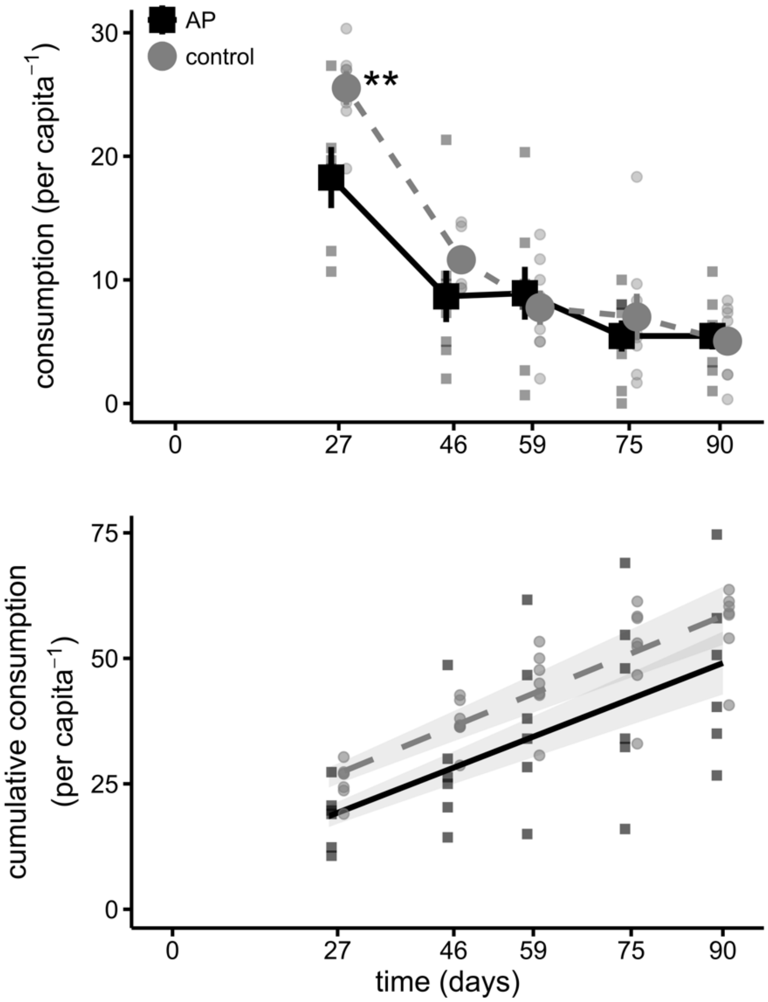

AP
control
mortality of barnacles
***
***
cumulative mortality of barnacles
time (days)

Figure 3



AP
control
**
consumption (per capita^{-1})
30
20
10
0
0
27
46
59
75
90
cumulative consumption (per capita^{-1})
75
50
25
0
0
27
46
59
75
90
time (days)

Figure 4

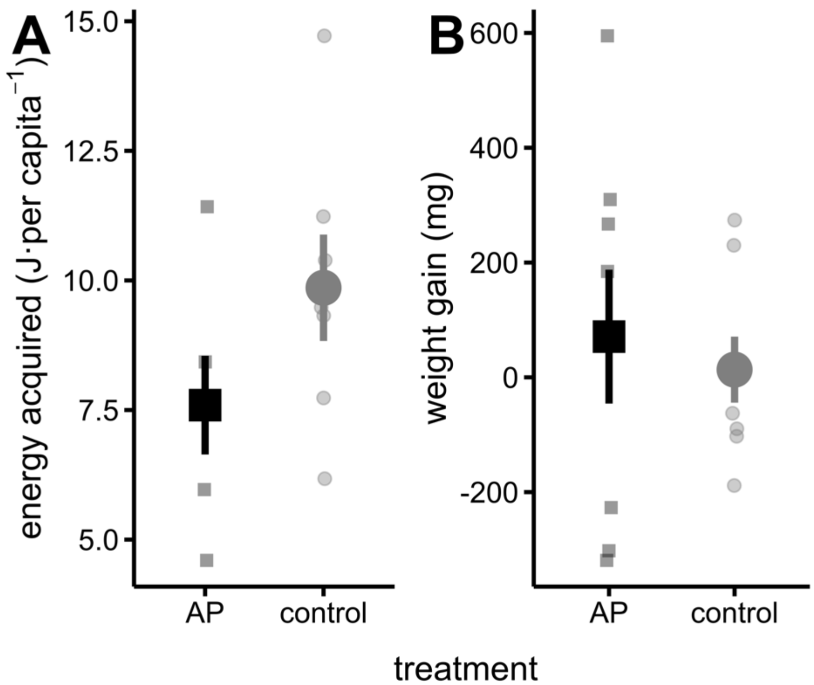
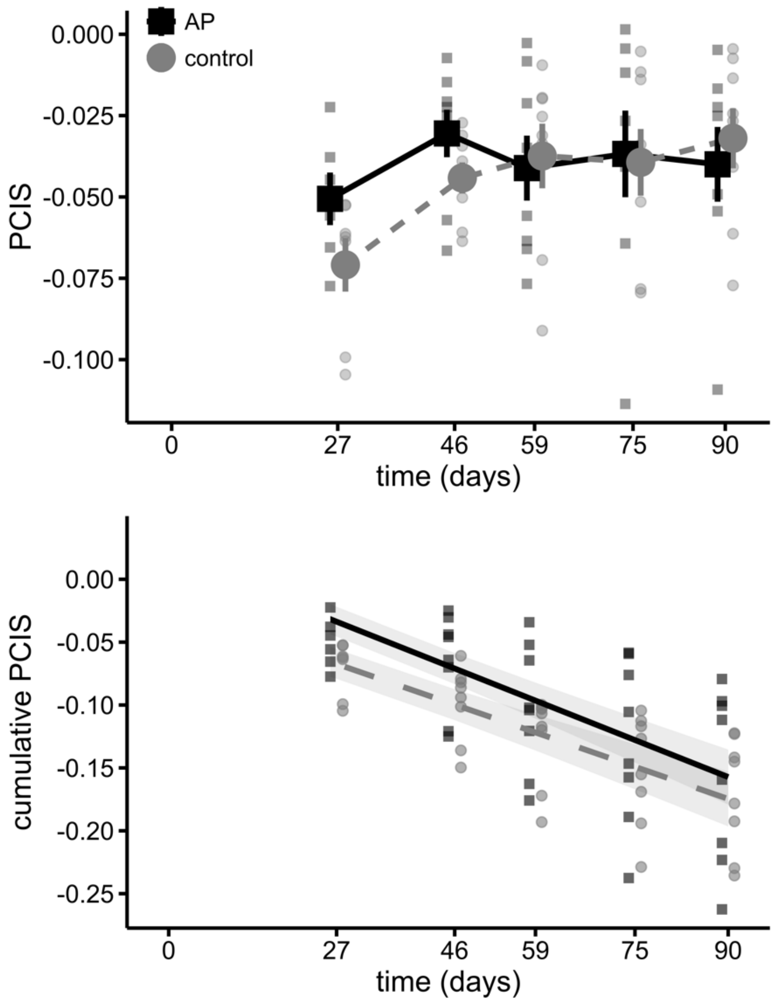

A
B
energy acquired (J·per capita⁻¹)
15.0
12.5
10.0
7.5
5.0
weight gain (mg)
600
400
200
0
-200
AP
control
AP
control
treatment

Figure 5



AP
control
0.000
-0.025
-0.050
-0.075
-0.100
PCIS
0
27
46
59
75
90
time (days)
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
cumulative PCIS
0
27
46
59
75
90
time (days)