

# Behavioural and physiological responses of limpet prey to a seastar predator and their transmission to basal trophic levels

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## Summary

1. Besides the well-documented behavioural changes induced by predators on prey, predator-induced stress can also include a suite of biochemical, neurological and metabolic changes that may represent important energetic costs and have long-lasting effects on individuals and on the demography of prey populations. The rapid transmission of prey behavioural changes to lower trophic levels, usually associated with alteration of feeding rates, can substantially change and even reverse direction over the long term as prey cope with the energetic costs associated with predation-induced stress. It is therefore critical to evaluate different aspects and assess the costs of non-consumptive predator effects on prey.

2. We investigated the behavioural and physiological responses of an herbivorous limpet, *Fissurella limbata*, to the presence of chemical cues and direct non-lethal contact by the common seastar predator, *Heliaster helianthus*. We also evaluated whether the limpets feeding behaviour was modified by the predator and whether this translated into positive or negative effects on biomass of the green alga, *Ulva* sp.

3. Our experimental results show the presence of *Heliaster* led to increased movement activity, increased distances travelled, changes in time budget over different environmental conditions and increased feeding rate in the keyhole limpets. Moreover, additional experiments showed that, beyond the increased metabolic rate associated with limpet increased activity, predator chemical cues heighten metabolic rate as part of the induced stress response.

4. Changes in individual movement and displacement distances observed through the 9-day experiment can be interpreted as part of the escape response exhibited by limpets to reduce the risk of being captured by the predator. Increased limpet feeding rate on algae can be visualized as a way individuals compensate for the elevated energetic costs of movement and heightened metabolic rates produced by the predator-induced stress, which can lead to negative effects on abundance of the lower trophic level.

We suggest that in order to understand the total non-consumptive effect of predators in natural communities, it is necessary to evaluate not only short-term behavioural responses, but also the costs associated with the multiple interdependent pathways triggered by predator-induced stress, and determine how individuals cope with these costs in the long term.

**Key-words:** behaviour, energetic cost, non-lethal predator effects, physiology, predator-induced stress, trait-mediated indirect effects

## Introduction

Predators impact prey species not just through lethal (consumptive) effects but also through non-lethal (non-consumptive) effects, which occur when prey

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survives an encounter by modifying one or more traits following the detection of a predator (Werner & Peacor 2003; Miner *et al.* 2005; Peckarsky *et al.* 2008; Hawlena & Schmitz 2010a). A growing body of empirical studies has established that these interactions can have from very short to lifelong effects on the physiological, reproductive and overall performance of individuals (e.g. McNamara & Houston 1987; McPeck 2004; Steiner & Van Buskirk 2009; Thaler, McArt & Kaplan 2012), on the demography and distribution of the prey population (e.g. Boonstra, Krebs & Stenseth 1998; Sheriff, Krebs & Boonstra 2009; Madin *et al.* 2010; Aránguiz-Acuña, Ramos-Jiliberto & Bustamante 2011; Dee, Witman & Brandt 2012), on the structure, diversity and dynamics of communities (e.g. Turner & Mittelbach 1990; Schmitz, Beckerman & O'Brien 1997; Trussell, Ewanchuk & Matassa 2006a; Matassa 2010) and on the functioning of entire ecosystems (e.g. Hawlena & Schmitz 2010b). Certainly, recent reviews and syntheses of empirical studies underscore the widespread occurrence of non-consumptive predator effects in all major ecosystems and their far-reaching and, in many ways, unanticipated consequences (e.g. Werner & Peacor 2003; Miner *et al.* 2005; Preisser, Bolnick & Benard 2005; Creel & Christianson 2008; Preisser 2009; Hawlena & Schmitz 2010a; Boonstra 2013; Clinchy, Sheriff & Zanette 2013). But reviews also highlight the great complexity of the multiple interdependent and time-varying pathways of prey responses (biochemical, physiological, behavioural and morphological) triggered by predator-induced stress, which pose major challenges when attempting to assess the total effect of predators in natural communities.

The series of biochemical, neurological and metabolic changes undergone by individuals when facing predators has been termed the stress response or stress axis (Preisser 2009; Hawlena & Schmitz 2010a; Boonstra 2013). Experimental studies show that, beyond the potential loss of feeding opportunities commonly associated with predation risk, the stress response is itself energetically costly for the individuals. Rapid increase in metabolism, expression of heat-shock proteins (Rovero, Hughes & Chelazzi 1999; Barreto, Luchiaro & Marcondes 2003; Hawkins, Armstrong & Magurran 2004; Pauwels, Stoks & De Meester 2005) and changes in energy allocation to satisfy the energy demands of predator avoidance are common in vertebrate and invertebrate organisms (Angilletta *et al.* 2003; Preisser 2009; Steiner & Van Buskirk 2009; Hawlena & Schmitz 2010b). These altered energetic requirements can lead to reduced individual growth and/or reproduction and, consequently, to changes in demographic rates that can sometimes be even larger than the direct (lethal) effects of predation (Creel & Christianson 2008). Organisms may therefore cope with the energetic demands of stress by increasing resource consumption after the initial feeding inhibition, by increasing the proportion of carbohydrate-rich foods (McPeck, Grace & Richardson 2001; Hawlena & Schmitz 2010a) or by changing the allocation of the pre-existing energetic

reserves that could have otherwise been destined for growth and reproduction (Boonstra 2013; Clinchy, Sheriff & Zanette 2013). Recently, Thaler, McArt & Kaplan (2012) showed that elevated prey metabolism due to predation stress can also change the digestive efficiency of a prey caterpillar, which may also result in altered (and sometimes increased) feeding under predation risk.

In the context of ecological communities, one of the most important and most researched aspects of non-consumptive predator effects is their potential transmission to lower trophic levels (reviewed by Werner & Peacor 2003). A number of comparatively short-duration field and laboratory experiments have shown that rapid changes in prey behaviour induced by predators can have positive effects on the abundance of primary producers or other sessile basal species (Werner & Anholt 1993; Trussell, Ewanchuk & Bertness 2002, 2003; Dee, Witman & Brandt 2012). This frequently documented trait-mediated indirect effect (TMII) is interpreted as the natural result of the fundamental trade-off between feeding and the risk of being eaten (Werner & Peacor 2003; Preisser, Bolnick & Benard 2005). But trait-mediated indirect effects (TMII) on basal trophic levels do not have to always be positive (e.g. Skaloudova, Zemek & Krivan 2007; Griffin *et al.* 2011), and moreover, the direction of the net effect is expected to vary over time and therefore with the duration of the experimental observations (Steiner & Van Buskirk 2009; Thaler, McArt & Kaplan 2012). As indicated above, the high energy demands imposed by behavioural displays (e.g. escape) and by the predation stress itself can be compensated immediately, or at a later time, by increased feeding rates on basal resources, leading to negative TMII. For example, elevated metabolism in grasshoppers facing predation risk by spiders increased requirements for dietary digestible carbohydrate to fuel-heightened energy demands (Hawlena & Schmitz 2010b). Thus, assessing the total effect of predators on prey and on lower trophic levels within natural communities requires not only short-term measurements of behavioural changes and feeding rates, but a much better understanding of the physiological and energetic changes undergone by individuals, and how they cope with these changes in the long term (Creel & Christianson 2008; Boonstra 2013; Clinchy, Sheriff & Zanette 2013). As a first step towards this goal, in this study, we evaluate the behavioural and physiological effects of a seastar predator on a keyhole limpet prey known to recognize and quickly respond to predator cues by displaying active escape responses. Within the necessarily short duration (days) of a laboratory experiment, we also assess the potential for rapid transmission of predator effects to an algal species commonly consumed by limpets.

The keyhole limpet *Fissurella limbata* (Sowerby) is a large (average 8 cm shell length) and common grazer species in the low rocky shore of central and northern Chile (Oliva & Castilla 1992). Individuals feed mostly on ephemeral green and corticated algal species (Santelices,

Vásquez & Meneses 1986) and can play an important role on the structure of algal assemblages (Oliva & Castilla 1986; Aguilera & Navarrete 2012a). The sun star, *Heliaster helianthus* (Lamarck), the most conspicuous seastar along south-eastern Pacific shores (Castilla & Paine 1987; Castilla *et al.* 2013), overlaps entirely in intertidal distribution with *F. limbata*. Seastars readily attack all fissure-lids they encounter in their foraging bouts, regardless of the time of day and at high or low tides (Navarrete & Manzur 2008; personal observations). Seastars sometimes engage in pursuits of limpets for 30–40 min (personal observations), which frequently end with the limpet escaping the reach of the seastar arms. This successful escape response probably explains the comparatively low frequency of *F. limbata* in the seastar diet (Navarrete & Manzur 2008; Barahona & Navarrete 2010), and the highly specific and quick escape response elicited by *H. helianthus* in 100% of limpet individuals in the field (Escobar & Navarrete 2011). We expect this active escape response to induce short- to long-lasting feeding inhibition (reduced feeding rate) in limpets, which depending on their magnitude may translate in positive effects on algal biomass. But if energy demands induced by predation stress are high, limpets may compensate by increasing feeding rates or by changing its diet, instead of diminishing individual growth (e.g. Trussell, Ewanchuk & Matassa 2006a). To simplify interpretation, in this study, we used only the preferred algal species consumed by limpets, green ulvoids (Aguilera & Navarrete 2012a), so that only changes in feeding rates were assessed. We also evaluated whether the predator-induced stress led to increased metabolic rate and therefore energy costs for individuals. Finally, we evaluated whether within the time frame of the experiment, predator-induced responses cascade down to produce changes in biomass of the algal species.

## Materials and methods

Experiments were conducted between April and May of 2011 at the Estación Costera de Investigaciones Marinas (ECIM), located in Las Cruces (33°30'S, 71°30'W), central Chile. All animals and algae used in experiments were collected from the low intertidal shore outside the ECIM marine reserve.

### NON-LETHAL EFFECTS OF *HELIAS-TER* ON *FISSURELLA* AND *TMII* ON MACROALGAE

Through a replicated laboratory experiment, we evaluated whether the predator *H. helianthus* had non-lethal effects on foraging (feeding rate), time allocation (resting, moving) and overall displacement (distance travelled) of the keyhole limpet *F. limbata*. In addition, the experiment examined whether non-lethal effects on this grazer species translated into measurable effects on the biomass of the green alga *Ulva* sp. The experiment was conducted in replicated 24-l tanks with a cement bottom plate provided with a crevice to offer a refuge for the limpet prey during simulated low tides. In the field, limpets rest inside crevices during daytime low tides, which apparently reduces temperature and desiccation

stress (Aguilera & Navarrete 2011, 2012b). To further reduce temperature stress and maximize the time individuals could forage, we conducted the experiment under an opaque roof that protected animals from direct solar radiation but maintained the natural daylight cycle.

Tanks were divided in two sections by a coarse plastic mesh that allowed free flow of water through the tank. A semidiurnal tidal regime was simulated during acclimation and throughout the experiment by emptying or filling the tanks every 6 h, recreating four environmental conditions (diurnal and nocturnal, high and low tides). Since predation attacks have been observed in day and night low tides, and apparently also occur under water during high tides (although waves inhibit *Heliaster* movement, Barahona & Navarrete 2010), the experiment considered all these conditions. In the section of the tank with the crevice, we included a single adult limpet (shell length 7.1–9.0 cm) and an algal transplant made with fresh *Ulva* sp. glued to a 10 × 10 cm acrylic plate. In the other tank section, we applied the predator treatment when corresponded. The water inlet of the tanks was located in the predator section and the outlet in the limpet section, ensuring water flow from the predator to the target limpet.

To control for algal autogenic changes in biomass and since limpet and predator exudates could affect algal growth (Peterson & Renaud 1989; Weidner *et al.* 2004), we also included an algal transplant (5 × 5 cm) on a side wall of the tank protected from limpet grazing. Algae for all transplants were collected in the field 2 days before the experiment, immediately attached to previously weighed acrylic plates and maintained in running sea water until experiment began. Right before the experiment started, algal transplants were spun in a salad spinner for *c.* 30 s to remove excess water and weighed in a precision scale. Limpets and predators were maintained food deprived, in separate 100L running seawater tanks for 7 days before starting the experiment to allow animals to acclimate and to standardize hunger level among individuals.

The experiment consisted of five predator treatments: (i) non-lethal non-feeding predator (+Hnl), in which we placed one *Heliaster* individual separated from the target limpet by the plastic mesh to prevent attacks, but allowing free flow of waterborne chemical cues. The predator was not fed for the duration of the experiment. (ii) Non-lethal feeding predator (+Hnlf), which was the same as above, but in this case, a second individual of *F. limbata* was introduced in the predator section of the tank. The *Heliaster* predator could then pursue, attack and consume the limpet. Eaten limpets were replaced with new ones. This allowed us to evaluate the effect of conspecifics predation on the behaviour of the target limpet. (iii) 'Lethal' *Heliaster* treatment (+Hl), in which the predator was placed within the limpet section of the tank. This treatment was not intended to evaluate consumptive effects of *Heliaster*, but to reproduce the limpet escape behaviour observed in the field, which in many cases involves long displacements after physical contact with the predator (Escobar & Navarrete 2011) and active persecution by the predator. If the attacks resulted in limpet death, the limpet was replaced with a new individual. (iv) Control without predator (–H), which allowed us to contrast limpet behaviour against the other treatments. Besides these four predator treatments we included: (v) a control treatment without limpet or predator (–H-L), which accounted for autogenic changes in algal biomass and allowed us to evaluate potential effects of effluents from prey or predator on algal growth. All treatments were replicated four times, and the experiment was run for 9 days, when limpets and predators were

removed and all algal transplants were weighed using the same procedure described above. All animals that were alive at the end of the experiment were returned to the field.

Continuous recording (24 h per day of experiment) of limpet and predator behaviour was obtained with webcams located above each tank. At night, infrared recording was aided by adding a dimmed red light above the tanks. Cameras were connected with computers using Active WebCam v.10.1<sup>®</sup> (Py Software, Berkeley, CA, USA), which allows for simultaneous continuous recording. Analysis of the video recordings was performed manually by one observer and consisted in recording the time limpets were moving or resting and the distance travelled. To be conservative, we considered that a limpet was moving when it actually changed its position and when it displayed a rotating movement to either side of the shell (a behaviour displayed by *F. limbata* when escaping from *Heliaster* in the field, Escobar & Navarrete 2011). All other behaviours (e.g. lifting of the shell from the substratum, movement of the head or extension of the cephalic tentacles) were registered as resting. Active feeding could not be unequivocally determined from the aerial view provided by cameras. Therefore, during the first 5 days of the experiment, we conducted direct observations to record limpet feeding behaviour once every 6 h (8:00, 14:00, 20:00 and 2:00). Because of the low number of feeding events, for the remainder of the experiment, we increased the frequency of observations to once every hour between 14:00 and 8:00 the next day, totaling 86 observations for each limpet during the 9 days of the experiment. Out of these 86 observations, we quantified the number of times each limpet was observed feeding. These discrete observations did not provide information on the time length of feeding events.

#### PREY METABOLIC RATE UNDER RISK OF PREDATION

To test whether the risk of predation by *Heliaster* affected the basal metabolic rate of *F. limbata*, we measured oxygen consumption of keyhole limpets in the presence (+Exudate) and absence (−Exudate: Control) of waterborne chemical cues of *Heliaster*. To this end, limpets (shell length 5.5–7.7 cm) were collected in the field, their shells were thoroughly cleaned, and then, individuals were maintained food deprived in 40-l running seawater tanks to acclimate at 15 °C for 48 h. After acclimation, an individual limpet was randomly chosen and placed on an acrylic plate inside a 500-mL closed-circuit metabolic chamber filled with oxygen-saturated pure filtered seawater (0.45 µm) or with filtered exudates of *Heliaster*. Chambers were placed inside a temperature-regulated bath at 15 °C. Since *Heliaster* cues elicit escape responses in *Fissurella* (Escobar & Navarrete 2011), oxygen consumption can increase not only by alteration of basal metabolic rate (the stress response), but also as a result of increased movement. To separate these two effects, we measured oxygen consumption of limpets that were either allowed to move freely within the chamber or tied down to an acrylic plate using cable ties that impeded movement and then subjected to predator exudates or pure sea water. Four replicates were conducted for each treatment combination. To reduce manipulation stress, limpets were placed (tied or untied) on the plate for 30–40 min before introducing them into the metabolic chambers. Additionally, since most keyhole limpets are nocturnal (Aguilera & Navarrete 2011), we conducted separate day and night-time measurements. Exudates of *Heliaster* were obtained by immersing four individuals (24.0–29.0 cm in diameter) in 40-l tanks with filtered (1 µm)

sea water for 48 h. Water with exudates was then filtered to 0.45 µm to eliminate residues that could interfere with oxygen measures. Blanks for oxygen consumption or drift in the metabolic chambers were run for each measurement of limpet oxygen consumption, by measuring consumption rate inside the chamber immediately before the incorporation of each limpet during a 10-min period. These values were then subtracted from calculations. Oxygen measurements were conducted using a fibre optic oxygen meter Fibox 3 (PreSens<sup>®</sup>; Presens, Regensburg, Germany) that was calibrated at 0% and 100% of air saturation (using sea water saturated with nitrogen and air, respectively) before measurements began. Oxygen depletion was limited to a maximum of 40% air saturation within the chambers. We calculated limpet metabolic rate as µmol of oxygen consumed per unit of time (minutes) and per gram of body mass (Brante *et al.* 2003).

#### DATA ANALYSIS

To evaluate whether limpet activity varied significantly between day and night-time, between high and low tide, and among the different treatments, we calculated the frequency of individual limpets observed moving and the total time limpets moved each day under the different conditions. Frequency of individuals moving was averaged across the 9-day duration of the experiment for each environmental condition and treatment and compared using loglinear modelling, a generalized linear model extension of contingency tables (Agresti 1996). Daily activity time was expressed as the total time a given limpet moved in the experimental arena divided by the total time recorded for that experimental tank each day. Before statistical analysis, we plotted daily activity times against day of experiment for each individual replicate to determine whether there was a trend for limpets' daily activity to change during the course of the experiment. Since there were no apparent 'long-term trends' or drift in individual movement (see Results), we calculated the daily time activity of each individual throughout the experiment. Log-transformed data were analysed using a three-way ANOVA with daytime (night or day), tide (high or low) and predator treatment (four treatments) as fixed factors. In case of significant differences between treatments and absence of interactions, orthogonal planned contrasts tested: (i) whether the three treatments with *Heliaster* differed from the control without predator (−H), (ii) whether the 'lethal' predator treatment (+Hl) differed from the two non-lethal predator treatments (+Hnl and +Hnlf) and (iii) whether there was a difference between the non-lethal predator feeding on limpets (+Hnlf) from the non-lethal predator not feeding (+Hnl).

Similarly, the total distance travelled by an individual (in cm), measured from videos with the aid of a 3 × 3 cm grid, was calculated for each day of the experiment and then plotted against time to look for trends during the course of the experiment. In the absence of time trends (see Results), the daily average distance travelled by each individual throughout the experiment was calculated and analysed with a three-way ANOVA as described above for activity time. The same planned contrasts were applied in case of significance. Although distances travelled are not independent from the time individuals are observed moving, we believe they test sufficiently different aspects of limpet behaviour, and therefore, we did not correct significance levels.

Direct observations of feeding individuals (number of feeding events) were expressed as the total number of times (events) an individual was observed feeding on the algal transplant

throughout the experiment. Since no limpets were observed feeding under any of the environmental conditions in the –H predator treatment (see Results), we dropped this treatment from statistical comparisons. Moreover, since in the other predator treatments the limpets did not feed under some environmental conditions (see Results), we pooled feeding observations for each individual across all daylight and tide combinations and compared predator treatments using the log-likelihood chi-square test ( $\chi^2$ ) in a contingency table (Sokal & Rohlf 1981).

Before statistical comparisons of treatment effects on algal growth and because of inevitable small differences in initial algal biomass among replicates, we examined whether the rate of algal production/loss was related to initial biomass of the transplants by regressing the difference in algal wet weight (final minus initial biomass) against initial biomass. Since they were not strongly related (see Results), the simple difference between final and initial biomass was considered a good estimate of algal net growth/loss. To determine whether the effluents released by the predator and/or limpet (e.g. nutrients in the form of ammonium) had effects on algal biomass change rate (ABC), we compared, with a one-way ANOVA, the change in algal biomass (expressed as  $ABC_{NG} = (\text{final biomass} - \text{initial biomass})/\text{days/area}$ , in  $\text{g day}^{-1} \text{cm}^{-2}$ ) observed in the transplants protected from grazing deployed inside each aquarium, and the ABC rate measured in the no predator no limpet control treatment (–H–L). In case of significance, the following planned contrasts tested: a) whether the combination of sun star and limpet effluents affected algal growth (treatments +Hnl, +Hnlf, +HI against –H–L) and b) whether limpet effluents alone differed from control without effluents (–H vs. –H–L).

To evaluate the effect of predator treatments on algal biomass, we calculated algal biomass change per unit area of the transplants exposed to limpet grazing in each replicate ( $ABC_G$ ) and subtracted the algal ABC per unit area observed in the transplants protected from grazing ( $ABC_{NG}$ ). In this manner, differences in ABC rate among predator treatments could be solely attributable to limpet consumption. A one-way ANOVA was then used to compare the four predator treatments, followed by the same planned contrasts described above for the effects on daily activity time. Note that since we found no effects of predator or limpet effluents on ABC rates (see Results), another way to test the hypothesis of predator effects on algal biomass change is by using uncorrected ABCs observed in the transplants exposed to grazing and compare the four predator treatments and the control treatment with no predator and no limpet (–H–L). We preferred the test described above because of the more precise control of autogenic algal changes in each replicate.

Significance of treatment effects on limpet metabolic rates were assessed with a three-way ANOVA with *Heliaster* exudates (present or not), limpet mobility (tied or free to move) and daytime (day or night) as orthogonal fixed factors. Raw data were used for analysis because they conformed to normality and variance homogeneity assumptions.

## Results

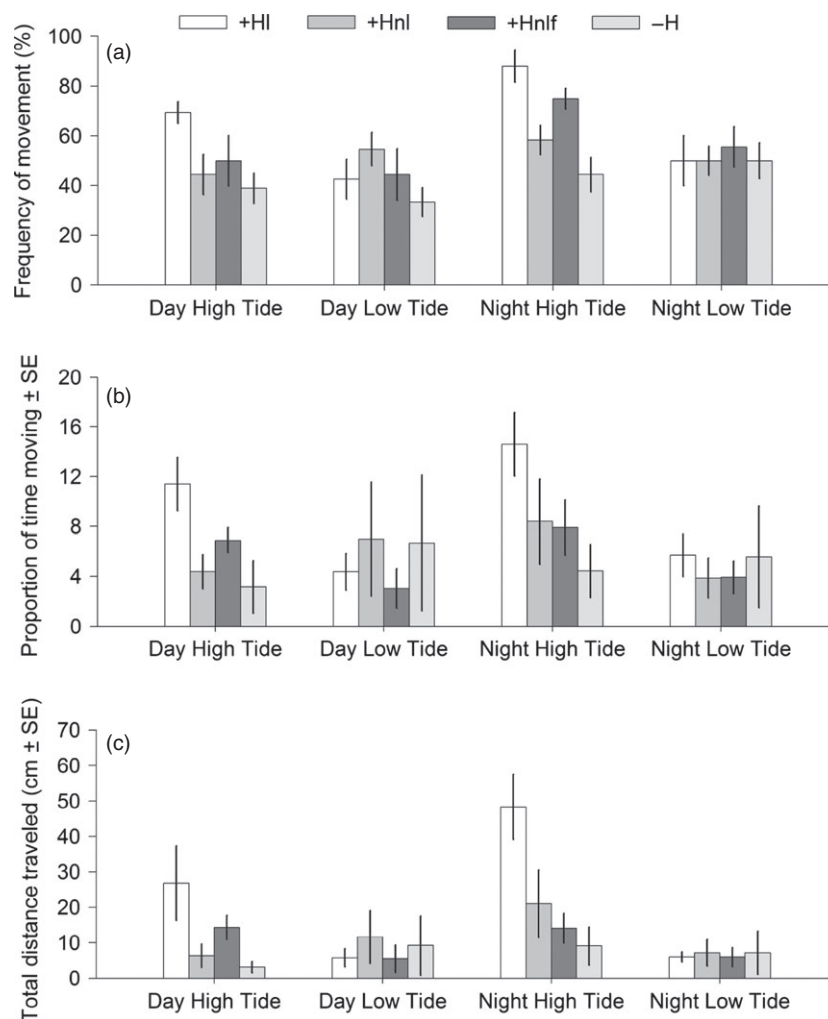
### NON-LETHAL EFFECTS OF *HELIASTER* ON *FISSURELLA* AND TMII ON MACROALGAE

The frequency of keyhole limpets observed moving everyday varied significantly among predator treatments, but

the effect depended on tidal condition (Fig. 1a, Table 1, significant treatment  $\times$  tide interaction). In the presence of a lethal *Heliaster* (+HI), most limpets were observed moving during night-time high tides (88%), and secondarily during daytime high tides (c. 70%), which contrasted sharply with the frequency of moving individuals under the same environmental condition but in the absence (–H treatment) of the predator (<50%). Moreover, in the presence of ‘lethal’ *Heliaster* or non-lethal *Heliaster* but with conspecific keyhole limpet prey (+Hnlf), the frequency of limpets moving was higher at high than at low tide, regardless of the day/night condition (Fig. 1a). Thus, in the presence of predators, individuals tended to be more active during high tides than when in the absence of predator cues. In general, more individuals moved in experimental treatments in which *Heliaster* was present (between 52% and 62% in +H treatments) than when it was absent (42% in the –H treatment, Table 1, significant predator treatment effect), and during high tides (58%) than low tides (42%), but these main effects must be interpreted with caution due to the significant interactions described above. Also more limpets moved during night (53%) than daytime (47%), a pattern that was consistent across predation risk treatments (Fig. 1a, Table 1).

We found no apparent long-term trends or ‘drift’ (i.e. signs of habituation) in individual behaviour (total time moving and distance travelled per day) throughout the duration of the experiment in the different predator and environmental condition treatments (Figs S1 and S2, Supporting information). Visual inspection of the proportion of time limpets moved, and the travelled distances over time suggested a more consistent day–night variability in the presence of ‘lethal’ *Heliaster* (+HI) than in the other treatments (Figs S1 and S2, Supporting information), but this was not consistent throughout the experiment. Therefore, for the following analyses, we averaged responses of replicate individuals throughout the duration of the experiment.

On average, the total time individuals were observed moving did not exceed 171 min per day, which corresponded to about 12% of the total recorded time, and thus, resting time represented more than 80% of individual limpet activity budget. Consistent with the frequency of individuals observed active, daily average activity showed that *F. limbata* spends more time moving at high tides, irrespective of day time (Fig. 1b; Table 2a), except for the limpets in the absence of *H. helianthus* (–H), which tended to move slightly more during low tide (Fig. 1b). The presence of a lethal *Heliaster* increased 3.6 times the daily average movement of the keyhole limpets during day high tides and by 3.3 times during night high tides in comparisons with controls without *Heliaster* (Fig. 1b). This large difference in time moving between the lethal predator and control treatments disappeared under the other two environmental conditions (Fig. 1b). However, ANOVA analysis (Table 2a) showed no significant interaction terms and only a marginally significant ( $P = 0.053$ ) overall effect of predation risk treatments.



**Fig. 1.** (a) Frequency of movement, (b) daily proportion of time moving ( $\pm$ SE) and (c) total distance travelled per day (cm  $\pm$  SE) for *Fissurella limbata* subjected to either a 'lethal' *Heliaster* (+HI), a non-lethal non-feeding *Heliaster* (+Hnl), a non-lethal feeding *Heliaster* (+Hnlf) and a control without *Heliaster* predator (-H) during day and night-time and for low and high tides.

**Table 1.** Loglinear analysis of the three-way contingency table for the frequency of individuals observed moving during day and night, ('daytime'), at high and low tides ('tide') and under the four different predator treatments [treatments: 'lethal' *Heliaster* (+HI), non-lethal non-feeding *Heliaster* (+Hnl), non-lethal feeding *Heliaster* (+Hnlf) and control without *Heliaster* predator (-H)]

Source	$\chi^2$	d.f.	<i>P</i>
Daytime	7.708	1	<b>0.006</b>
Tide	8.292	1	<b>0.004</b>
Treatment	12.975	3	<b>0.005</b>
Daytime $\times$ Tide	1.343	1	0.246
Daytime $\times$ Treatment	1.608	3	0.658
Tide $\times$ Treatment	11.331	3	<b>0.010</b>
Daytime $\times$ Tide $\times$ Treatment	1.809	3	0.613

Bold face indicates significance at  $\alpha = 0.05$ .

The maximum total distance travelled in one day by a keyhole limpet reached 2.88 m, and the average varied significantly among treatments. The presence of *Heliaster* significantly increased the distance travelled by *F. limbata*

in comparison with the controls without predator (-H; Fig. 1c; Table 2b; Planned Contrasts). For instance, distance travelled during night high tides in the lethal predator treatment was over five times higher than that in controls without predators, and distance travelled during day high tides was over nine times higher than that in controls (Fig. 1c). Although distance moved in the lethal *Heliaster* treatment (+HI) was more than twice that in the treatments with non-lethal *Heliaster* (+Hnl and +Hnlf), the difference was not statistically significant (Table 2b; Planned Contrasts,  $P = 0.065$ ). No overall significant difference in average distance travelled was observed between day and night-times, but limpets moved significantly longer distances during high tides than low tides (Fig. 1c, Table 2b).

Although feeding behaviour of the limpets was infrequent during the experiment (on average, three foraging events per capita out of the 86 observations made for each limpet, representing a 3.5% of the total observations), it occurred only in the presence of a lethal or

**Table 2.** Three-way ANOVA for (a) Proportion of time moving and (b) total distance travelled by *Fissurella limbata*. Factors daytime (night, day), tide (high, low) and predator treatment (‘lethal’ *Heliaster* (+Hl), non-lethal non-feeding *Heliaster* (+Hnl), non-lethal feeding *Heliaster* (+Hnlf) and control without *Heliaster* predator (–H)) were considered fixed. Orthogonal planned contrasts comparing levels of predator treatment are also presented

Source	d.f.	MS	F Ratio	P
<b>(a) Proportion of time moving</b>				
Daytime	1	0.582	0.851	0.361
Tide	1	3.902	5.716	<b>0.021</b>
Treatment	3	1.874	2.742	0.053
Daytime × Tide	1	0.061	0.089	0.767
Daytime × Treatment	3	0.015	0.021	0.996
Tide × Treatment	3	0.821	1.200	0.320
Daytime × Tide × Treatment	3	0.09	0.135	0.939
Error	48	0.684		
<b>(b) Total distance travelled</b>				
Daytime	1	1.580	1.569	0.216
Tide	1	11.414	11.337	<b>0.002</b>
Treatment	3	12.387	4.101	<b>0.011</b>
Daytime × Tide	1	0.958	0.952	0.334
Daytime × Treatment	3	6.327	2.095	0.113
Tide × Treatment	3	0.239	0.079	0.971
Daytime × Tide × Treatment	3	1.372	0.454	0.716
Error	48	1.007		
<b>Orthogonal planned contrast</b>				
Treatments with <i>Heliaster</i> vs. control without predator	1	8.769	8.711	<b>0.005</b>
‘Lethal’ predator treatment vs. non-lethal predator treatments	1	3.603	3.579	0.065
Non-lethal predator feeding on limpets vs. non-lethal predator not feeding	1	0.014	0.014	0.905

Bold face indicates significance at  $\alpha = 0.05$ .

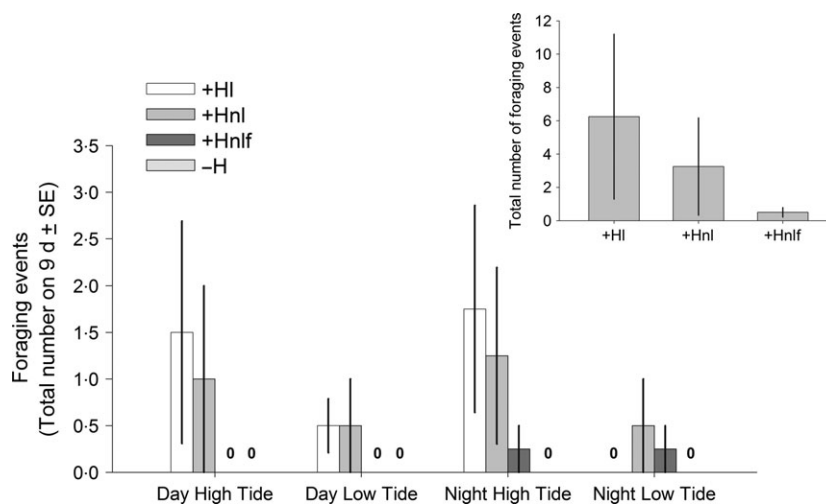
non-lethal *Heliaster* during night high tides and never in the absence of the predator (Fig. 2). Analysis with pooled feeding observations for each individual across all

daylight and tide combinations (see Data Analysis for details) showed that feeding was significantly higher when keyhole limpets were exposed to *Heliaster* physical contact than in the presence of a non-lethal *Heliaster* (Insert Fig. 2; log-likelihood chi-square test;  $\chi^2 = 18,812$ , d.f. = 2,  $P = 0.0001$ ).

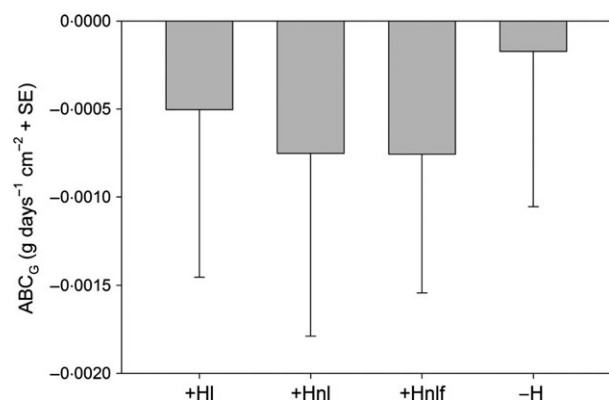
During the course of the experiment, the change in algal biomass ( $ABC_{NG}$ ) was not related to initial biomass of the transplant (Fig. S3, Supporting information), and therefore, the simple difference in weight during the experiment was considered a good estimate of algal growth. The change in algal biomass observed within the transplants protected from grazing ( $ABC_{NG}$ ) was not different from the control treatment (–H–L), suggesting that effluents released by predator and limpets had no effect on algal growth (one-way ANOVA;  $F = 1.612$ ; d.f. = 4, 14;  $P = 0.226$ ). Overall, algal biomass changes (quantified by  $ABC_G$ ) under the different predator treatments were markedly low and highly variable among replicates (see error bars Fig. 3), with a maximum decrease of  $c. 0.001 \text{ g day}^{-1} \text{ cm}^{-2}$ . Although limpet feeding behaviour was higher in the presence of *Heliaster* (Fig. 3), suggesting an increase in algal consumption under increased predation risk, algal biomass changes ( $ABC$ ) were not significantly different among treatments (one-way ANOVA;  $F = 0.008$ ; d.f. = 3, 11;  $P = 0.971$ ).

PREY METABOLIC RATE UNDER RISK OF PREDATION

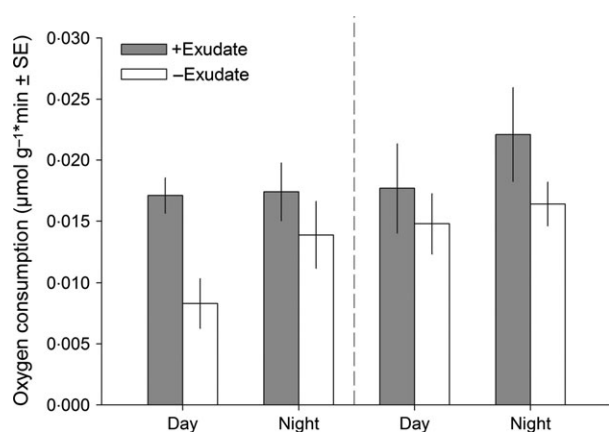
*Heliaster* predator chemical cues resulted in a significant increase in limpet oxygen consumption compared to controls without predator exudates (Fig. 4; Table 3). Oxygen consumption was also marginally higher when limpets were free to move within the metabolic chamber than when they were tied down ( $P = 0.071$ ; Fig. 4; Table 3).



**Fig. 2.** Total number of foraging events (±SE) performed by *Fissurella limbata* under different risk treatments during day and night-times and insert the total number of foraging events performed by *F. limbata* (±SE) pooling all daylight and tidal conditions for the predator treatments: ‘lethal’ *Heliaster* (+HI), non-lethal non-feeding *Heliaster* (+Hnl) and non-lethal feeding *Heliaster* feeding (+Hnlf).



**Fig. 3.** Algal biomass change ( $\text{g days}^{-1} \text{cm}^{-2} + \text{SE}$ ) corrected for autogenic changes (see Materials and methods for further details) subjected to either a 'lethal' *Heliaster* (+HI), a non-lethal non-feeding *Heliaster* (+HNI), a non-lethal feeding *Heliaster* and a control without *Heliaster* predator (-H).



**Fig. 4.** Oxygen consumption ( $\mu\text{mol g}^{-1} \cdot \text{min} \pm \text{SE}$ ) for *Fissurella limbata* in the presence (grey bars) and absence (white bars) of *Heliaster* waterborne chemical cues, for day and night-time and for tied and untied limpets.

**Table 3.** Three-way ANOVA on oxygen consumption rate of *Fissurella limbata* under the three treatments: limpet mobility (tied or untied), *Heliaster* chemical cues (+Exudate or -Exudate) and time of day (day or night), all considered as fixed factors

Sources of variation	d.f.	Mean square	F ratio	P
Limpet mobility	1	4.204E-06	3.562	0.071
<i>Heliaster</i> exudate	1	9.206E-06	7.799	<b>0.010</b>
Daytime	1	2.895E-06	2.452	0.130
Limpet mobility × <i>Heliaster</i> exudate	1	2.717E-07	0.230	0.636
<i>Heliaster</i> exudate × Daytime	1	1.417E-07	0.120	0.732
Daytime × Limpet mobility	1	3.811E-10	0.0003	0.986
Limpet mobility × Daytime × <i>Heliaster</i> exudate	1	1.373E-06	1.163	0.292
Error	24	2.800E-05		

Bold face indicates significance at  $\alpha = 0.05$ .

## Discussion

Our results show that the presence of chemical cues, as well as direct non-lethal contact with the seastar predator *H. helianthus*, can modify the behaviour of the keyhole limpet, *F. limbata*, inducing increased movement activity, changes in time budget under different environmental conditions and increased feeding rate. In the light of previous field studies that documented a specific and rapid (seconds) escape response of this limpet species following contact with the seastar (Escobar & Navarrete 2011), we interpret increased movement activity as part of the behavioural changes displayed by limpets to reduce the risk of predation. We further show that predator chemical cues induce a rapid increase in the metabolic rate of the prey, which cannot be explained by increased individual movement and therefore demonstrate the existence of an energy demanding predation stress response in limpets.

In our experiments, we observed three distinctive forms of limpet behavioural changes elicited by the seastar predator, which we associate either to the limpet escape response or to the predation stress response: (i) active escape of individuals from a pursuing seastar in the lethal predator treatment, (ii) increased movement activity, particularly during high tides, following the perception of predator cues in the non-lethal predator treatments and (iii) increased feeding events in the presence of the predator as compared to controls. It is clear that active escape from a pursuing predator is an adaptive response to reduce the risk of being captured and eaten. The response is readily displayed by animals in the field (Escobar & Navarrete 2011), and we have observed several occasions in which limpets escape the reach of a pursuing seastar, just like it occurred in the laboratory. Increased movement activity and distance travelled following perception of predator through chemical cues are probably also associated with the same escape response, in which limpets perceive the predator and flee. It is not clear, however, whether just increasing movement (and total displacement) when facing a predator signal, but not a pursuing predator, would actually reduce predation risk. The fact that the increased limpet movement in response to predator cues occurred during high tides (when covered by water) is an interesting pattern since *Heliaster* individuals move less when under water and waves intensify (Barahona & Navarrete 2010). Perhaps moving more when under water is advantageous in terms of reducing predation but not when they are exposed to air. Unfortunately, under water, observations in this high energy environment are extremely difficult, but further experiments could attempt to simulate wave activity and seastar foraging patterns in the laboratory.

A comparatively large number of short-term experimental studies have shown that the behaviour displayed by intermediate consumers (e.g. herbivores, smaller carnivores) to reduce the risk of predation, such as active escape responses, extensive use of refuges or movement inhibition, causes reduced feeding or even total starvation,



which in turn can result in positive effects on primary producers (Trussell, Ewanchuk & Matassa 2006a,b; Freeman 2006; McKay & Heck 2008; Matassa 2010 and see revisions by Werner & Peacor 2003 and by Schmitz, Krivan & Ovadia 2004). Our experiments showed that the presence of chemical cues or direct contact with *Heliaster* (lethal predator treatment) increased the frequency of feeding by limpets on green algae. In fact, limpets without predator cues (controls) did not feed at all during the course of the experiment. Recent studies suggest that increased feeding under predation risk might actually be more common than once thought (Hawlena & Schmitz 2010a; Thaler, McArt & Kaplan 2012). As we discuss below, the limpet feeding response is likely associated with increased energy demands produced by the heightened metabolic rate, and it is best understood as part of the series of neurological, biochemical and physiological changes that are initiated after the perception of a risk of being eaten by predators, which form part of the predator-induced stress response.

Predation-induced stress, leading to alteration of metabolism, digestive physiology, individual stoichiometry and reproductive investment, has been experimentally demonstrated in vertebrate and invertebrate prey (Rovero, Hughes & Chelazzi 1999; McPeck, Grace & Richardson 2001; Kagawa & Mugiya 2002; Pauwels, Stoks & De Meester 2005). Our laboratory experiments showed a rapid increase in limpet metabolic rate in the presence of predator chemical cues. This heightened metabolic rate was not a simple consequence of the increased limpet movement elicited by the predator and therefore demonstrated the existence of a metabolically costly predator-induced stress in this mollusc species. This guarantees that the predation risk response will be costly (Creel & Christianson 2008), even if costs of locomotion in response to predators are marginal, which might be the case when limpets are moving around during foraging bouts. Studies show that organisms may cope with the energetic demands of stress by increasing resource consumption, by increasing the proportion of carbohydrate-rich foods (McPeck, Grace & Richardson 2001; Hawlena & Schmitz 2010a) or by changes in allocation of the pre-existing energetic reserves that could have otherwise been destined for growth and reproduction (Boonstra 2013; Clinchy, Sheriff & Zanette 2013). In our experiments, we had only one type of food, and therefore, limpets could not choose to consume algae with different carbohydrate content or with higher assimilation efficiency to compensate for higher energy demands. Limpets could therefore only increase feeding rate to cope with the energy demands of predation-induced stress. The experiments were also too short in comparison with typically low limpet growth rates to be able to detect changes in growth/reproduction of individuals under the different predator treatments, as shown for instance by Trussell, Ewanchuk & Matassa (2006a) for *Nucella lapillus* in response to crab predators. Recently, Thaler, McArt & Kaplan (2012) showed that

elevated prey metabolism due to predation stress can also change the digestive efficiency of the tobacco hornworm caterpillar, which could also result in altered (and sometimes increased) feeding under predation risk. Further experiments should therefore evaluate assimilation efficiency in limpets under varying predator treatments to determine whether changes in digestive physiology can contribute to increased limpet feeding rates under predation risk.

The rapid stress responses induced by predators can be instantaneous and reversible (Steiner & Van Buskirk 2009), or have long-lasting consequences (Boonstra, Krebs & Stenseth 1998). For example, Steiner & Van Buskirk (2009) found that tadpoles immediately increased oxygen consumption in the presence of predatory dragonfly larvae, consistent with a 'fight-or-flight' response. But over longer time, after 3 weeks of predator exposure, tadpoles lowered oxygen consumption, reducing the cost of the predator defence on growth, and therefore, the authors argue that metabolic costs alone might not explain the trade-off between growth and predator avoidance (Steiner & Van Buskirk 2009). However, it is unclear whether such a long-term chronic exposure to predator cues, which is necessary to produce decreased oxygen consumption, does occur under natural conditions. Boonstra, Krebs & Stenseth (1998) found that predator-induced stress affected several physiological parameters in hares (e.g. hormone levels, body condition), which led to reduced reproduction following population decline. In our study, experiments to measure oxygen consumption lasted only few hours. We could not assess temporal variation in the physiological response, and therefore, we cannot evaluate its contribution to the total cost of the predator-induced response displayed by keyhole limpets. But since *F. limbata* behaviour did not show signs of 'habituation', the increased respiratory costs required for the increased movement were sustained for the duration of the experiment. The costs of locomotion are expected to be high, particularly in the wave-exposed environment where animals have to avoid dislodgment by waves, which would help explain why limpets spend most of the time (>80%) resting in crevices (see also Aguilera & Navarrete 2011). In our experiments, 'untied' limpets were confined to the small chambers, but yet they consumed more oxygen than 'tied' limpets (although non-significantly so). In all, we interpret the feeding activity in limpets as a way limpets cope with the higher energy demands induced by seastar predators. Further studies evaluating the direct costs of locomotion, as well as long-term metabolic changes associated with the stress response, are necessary to better understand the total non-lethal effect of predators on limpets.

Although positive trait-mediated indirect effects of carnivores on primary producers are widely reported, and commonly explained as the natural consequence of the fundamental trade-off between feeding and the risk of being eaten (Werner & Peacor 2003; Preisser, Bolnick & Benard 2005), negative TMIs have also been reported, although as

a result of prey habitat shifts (e.g. Trussell, Ewanchuk & Matassa 2006a; Dee, Witman & Brandt 2012). Skaloudova, Zemek & Krivan (2007) showed that increased predation risk either by introduction of a caged predatory mite (*Phytoseiulus persimilis*) or by the predator cues led to an increase in walking activity and plant damage inflicted by the two-spotted spider mites *Tetranychus urticae*. But authors attributed this result to the short duration of their observations (10 min) and speculated that over a longer time, the effect of predatory cues should be attenuated (see also Oku, Yano & Takafuji 2004). In our experiments, behavioural effects on limpets lasted over the 9-day duration of experiments. Therefore, considering the demonstrated ecological effects of fissurellid limpets on algal assemblages (Oliva & Castilla 1986; Aguilera & Navarrete 2012a), we expect that increased feeding rate will translate into negative effects of green algal biomass in the rocky shore. In our experiments, differences in algal biomass loss across treatments did suggest a negative effect of seastars on lower trophic levels, but the results were not statistically significant, mostly because the large variability in individual algal biomass across the replicates (see error bars Fig. 3). Further experiments must improve the precision of the measurements of algal biomass and, at the same time, offer algal fronds in a manner that best mimic algal availability to limpets in the field. At any rate, our results show that there are no positive effects of limpet or seastar exudates on algal growth, and therefore, increased limpet feeding implies that the TMII of *Heliaster* on *Ulva* sp. cannot be positive.

Despite the rich history of experimental studies on predation conducted in rocky shores around the world, we are just beginning to uncover the short- and long-term effects that predators can have in their communities through inducing stress responses in their prey. Short-duration behavioural experiments, which largely document positive effects of carnivore predators on basal trophic levels, have increased our awareness of non-lethal effects. But much more research is needed to understand the mechanisms of predator-induced stress in invertebrates and, particularly, to determine how individuals cope with stress in the long term before we can anticipate the total non-consumptive effect of predators in natural communities.

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## References

Agresti, A. (1996) *An Introduction to Categorical Data Analysis*. John Wiley and Sons Inc, Hoboken, New Jersey, 357 pp.

- Aguilera, M.A. & Navarrete, S.A. (2011) Distribution and activity patterns in an intertidal grazer assemblage: influence of temporal and spatial organization on interspecific associations. *Marine Ecology Progress Series*, **431**, 119–136.
- Aguilera, M.A. & Navarrete, S.A. (2012a) Functional identity and functional structure change through succession in a rocky intertidal marine herbivore assemblage. *Ecology*, **93**, 75–89.
- Aguilera, M.A. & Navarrete, S.A. (2012b) Interspecific competition for shelters in territorial and gregarious intertidal grazers: consequences for individual behaviour. *PLoS One*, **7**, e46205.
- Angilletta, M.J., Wilson, R.S., Navas, C.A. & James, R.S. (2003) Trade-offs and the evolution of thermal reaction norms. *Trends in Ecology and Evolution*, **18**, 234–240.
- Aránguiz-Acuña, A., Ramos-Jiliberto, R. & Bustamante, R.O. (2011) Experimental evidence that induced defenses promote coexistence of zooplanktonic populations. *Journal of Plankton Research*, **33**, 469–477.
- Barahona, M. & Navarrete, S.A. (2010) Movement patterns of the seastar *Heliaster helianthus* in central Chile: relationship with environmental conditions and prey availability. *Marine Biology*, **157**, 647–661.
- Barreto, R.E., Luchiari, A.C. & Marcondes, A.L. (2003) Ventilatory frequency indicates visual recognition of an allopatric predator in naive Nile tilapia. *Behavioural Processes*, **60**, 235–239.
- Boonstra, R. (2013) The ecology of stress: a marriage of disciplines. *Functional Ecology*, **27**, 7–10.
- Boonstra, R., Krebs, C.J. & Stenseth, N.C. (1998) Population cycles in small mammals: the problem of explaining the low phase. *Ecology*, **79**, 1479–1488.
- Brante, A., Fernández, M., Eckerle, L., Mark, F., Pörtner, H.-O. & Arntz, W. (2003) Reproductive investment in the crab *Cancer setosus* along a latitudinal cline: egg production, embryo losses and embryo ventilation. *Marine Ecology Progress Series*, **251**, 221–232.
- Castilla, J.C. & Paine, R.T. (1987) Predation and community organization on Eastern Pacific, temperate zone, rocky intertidal shores. *Revista Chilena de Historia Natural*, **60**, 131–151.
- Castilla, J.C., Navarrete, S.A., Manzur, T. & Barahona, M. (2013) The South American sunstar *Heliaster helianthus*. *Asterioidea: The Biology and Ecology of Starfish* (ed. J.M. Lawrence), pp. 153–160. The Johns Hopkins University Press, Baltimore, Maryland.
- Clinchy, M., Sheriff, M.J. & Zanette, L.Y. (2013) Predator-induced stress and the ecology of fear. *Functional Ecology*, **27**, 56–65.
- Creel, S. & Christianson, D. (2008) Relationships between direct predation and risk effects. *Trends in Ecology and Evolution*, **23**, 194–201.
- Dee, L.E., Witman, J.D. & Brandt, M. (2012) Refugia and top-down control of the pencil urchin *Eucidaris galapagensis* in the Galápagos marine reserve. *Journal of Experimental Marine Biology and Ecology*, **416–417**, 135–143.
- Escobar, J.B. & Navarrete, S.A. (2011) Risk recognition and variability in escape responses among intertidal molluscan grazers to the sunstar *Heliaster helianthus*. *Marine Ecology Progress Series*, **421**, 151–161.
- Freeman, A. (2006) Size-dependent trait-mediated indirect interactions among sea urchin herbivores. *Behavioral Ecology*, **17**, 182–187.
- Griffin, J.N., Butler, J., Soomdat, N.N., Brun, K.E., Chejanovski, Z.A. & Silliman, B.R. (2011) Top predators suppress rather than facilitate plants in a trait-mediated tri-trophic cascade. *Biology Letters*, **7**, 710–713.
- Hawkins, L.A., Armstrong, J.D. & Magurran, A.E. (2004) Predator-induced hyperventilation in wild and hatchery Atlantic salmon fry. *Journal of Fish Biology*, **65**, 88–100.
- Hawlena, D. & Schmitz, O.J. (2010a) Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. *The American Naturalist*, **176**, 537–556.
- Hawlena, D. & Schmitz, O.J. (2010b) Herbivore physiological response to fear of predation alters ecosystem nutrient dynamics. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 15503–15507.
- Kagawa, N. & Mugiya, Y. (2002) Brain Hsp70 mRNA expression is linked with plasma cortisol levels in goldfish (*Carassius auratus*) exposed to a potential predator. *Zoological Science*, **19**, 735–740.
- Madin, E.M.P., Gaines, S.D., Madin, J.S. & Warner, R.R. (2010) Fishing indirectly structures macroalgal assemblages by altering herbivore behavior. *The American Naturalist*, **176**, 785–801.
- Matassa, C.M. (2010) Purple sea urchins *Strongylocentrotus purpuratus* reduce grazing rates in response to risk cues from the spiny lobster *Panulirus interruptus*. *Marine Ecology Progress Series*, **400**, 283–288.
- McKay, K.M. & Heck, K.L. Jr (2008) Presence of the Jonah crab *Cancer borealis* significantly reduces kelp consumption by the green sea urchin

- Strongylocentrotus droebachiensis*. *Marine Ecology Progress Series*, **356**, 295–298.
- McNamara, J.M. & Houston, A.I. (1987) Starvation and predation as factors limiting population size. *Ecology*, **68**, 1515–1519.
- McPeck, M.A. (2004) The growth/predation risk trade-off: so what is the mechanism? *The American Naturalist*, **163**, E88–E111.
- McPeck, M.A., Grace, M. & Richardson, J.L.M. (2001) Physiological and behavioral responses to predators shape the growth/predation risk trade-off in damselfishes. *Ecology*, **82**, 1535–1545.
- Miner, B.G., Sultan, S.E., Morgan, S.G., Padilla, D.K. & Relyea, R.A. (2005) Ecological consequences of phenotypic plasticity. *Trends in Ecology and Evolution*, **20**, 685–692.
- Navarrete, S.A. & Manzur, T. (2008) Individual- and population-level responses of a keystone predator to geographic variation in prey. *Ecology*, **89**, 2005–2018.
- Oku, K., Yano, S. & Takafuji, A. (2004) Nonlethal indirect effects of a native predatory mite, *Amblyseius womersleyi* Schicha (Acari: Phytoseiidae), on the phytophagous mite *Tetranychus kanzawai* Kishida (Acari: Tetranychidae). *Journal of Ethology*, **22**, 1090–1112.
- Oliva, D. & Castilla, J.C. (1986) The effects of human exclusion on the population structure of key-hole limpets *Fissurella crassa* and *F. limbata* on the coast of central Chile. *Marine Ecology*, **7**, 201–217.
- Oliva, D. & Castilla, J.C. (1992) Guía para el reconocimiento y morfometría de diez especies del género *Fissurella* Bruguière 1789 (Mollusca Gastropoda) comunes en las pesquerías y conchales indígenas de Chile Central y Sur. *Gayana, Zoología*, **56**, 77–108.
- Pauwels, K., Stoks, R. & De Meester, L. (2005) Coping with predator stress: interclonal differences in induction of heat-shock proteins in the water flea *Daphnia magna*. *Journal of Evolutionary Biology*, **18**, 867–872.
- Peckarsky, B.L., Abrams, P.A., Bolnick, D.I., Dill, L.M., Grabowski, J.H., Luttbegg, B. *et al.* (2008) Revisiting the classics: considering non consumptive effects in textbook examples of predator-prey interactions. *Ecology*, **89**, 2416–2425.
- Peterson, C. & Renaud, E. (1989) Analysis of feeding preference experiments. *Oecologia*, **80**, 82–86.
- Preisser, E.L. (2009) The physiology of predator stress in free-ranging prey. *Journal of Animal Ecology*, **78**, 1103–1105.
- Preisser, E.L., Bolnick, D.I. & Benard, M.F. (2005) Scared to death? The effects of intimidation and consumption in predator–prey interactions. *Ecology*, **86**, 501–509.
- Rovero, F., Hughes, R.N. & Chelazzi, C. (1999) Cardiac and behavioural responses of mussels to risk of predation by dogwhelks. *Animal Behaviour*, **58**, 707–714.
- Santelices, B., Vásquez, J. & Meneses, I. (1986) Patrones de distribución y dietas de un gremio de moluscos herbívoros en habitats intermareales expuestos de Chile Central. *Monografías Biológicas*, **4**, 147–171.
- Schmitz, O.J., Beckerman, A.P. & O'Brien, K.M. (1997) Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. *Ecology*, **78**, 1388–1399.
- Schmitz, O.J., Krivan, V. & Ovadia, O. (2004) Trophic cascades: the primacy of trait-mediated indirect interactions. *Ecology Letters*, **7**, 153–163.
- Sheriff, M.J., Krebs, C.J. & Boonstra, R. (2009) The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. *Journal of Animal Ecology*, **78**, 1249–1258.
- Skaloudova, B., Zemek, R. & Krivan, V. (2007) The effect of predation risk on an acarine system. *Animal Behaviour*, **74**, 813–821.
- Sokal, R.R. & Rohlf, F.J. (1981) *Biometry*. W.H. Freeman & Co., New York, New York, 859 pp.
- Steiner, U.K. & Van Buskirk, J. (2009) Predator-induced changes in metabolism cannot explain the growth/predation risk tradeoff. *PLoS One*, **4**, e6160. Doi: 10.1371/journal.pone.0006160
- Thaler, J.S., McArt, S.H. & Kaplan, I. (2012) Compensatory mechanisms for ameliorating the fundamental trade-off between predator avoidance and foraging. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 12075–12080.
- Trussell, G.C., Ewanchuk, P.J. & Bertness, M.D. (2002) Field evidence of trait-mediated indirect interactions in a rocky intertidal food web. *Ecology Letters*, **5**, 241–245.
- Trussell, G.C., Ewanchuk, P.J. & Bertness, M.D. (2003) Trait-mediated effects in rocky intertidal food chains: predator risk cues alter prey feeding rates. *Ecology*, **84**, 629–640.
- Trussell, G.C., Ewanchuk, P.J. & Matassa, C.M. (2006a) The fear of being eaten reduces energy transfer in a simple food chain. *Ecology*, **87**, 2979–2984.
- Trussell, G.C., Ewanchuk, P.J. & Matassa, C.M. (2006b) Habitat effects on the relative importance of trait- and density-mediated indirect interactions. *Ecology Letters*, **9**, 1245–1252.
- Turner, A. & Mittelbach, G. (1990) Predator avoidance and community structure: interactions among piscivores, planktivores, and plankton. *Ecology*, **71**, 2241–2254.
- Weidner, K., Lages, B.G., da Gama, B.A., Molis, M., Wahl, M. & Pereira, R.C. (2004) Effect of mesograzers and nutrient levels on induction of defenses in several Brazilian macroalgae. *Marine Ecology Progress Series*, **283**, 113–125.
- Werner, E.E. & Anholt, B.R. (1993) Ecological consequences of the trade-off between growth and mortality-rates mediated by foraging activity. *The American Naturalist*, **142**, 242–272.
- Werner, E.E. & Peacor, S.D. (2003) A review of trait-mediated indirect interactions in ecological communities. *Ecology*, **84**, 1083–1100.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Fig. S1.** Temporal variation in the daily proportion of time moving ( $\pm$ SE) for limpets exposed to (a) ‘Lethal’ *Heliaster* (+Hl), (b) non-lethal non-feeding *Heliaster* (+Hnl), (c) non-lethal feeding *Heliaster* (+Hnlf) and (d) control without *Heliaster* (–H) during day (white dots) and night-time (black dots).

**Fig. S2.** Temporal variation in the daily displacement (cm  $\pm$  SE) of limpets exposed to (a) ‘lethal’ *Heliaster* (+Hl), (b) non-lethal non-feeding *Heliaster* (+Hnl), (c) non-lethal feeding *Heliaster* (+Hnlf) and (d) control without *Heliaster* (–H) during day (white dots) and night-time (black dots).

**Fig. S3.** Linear regression analysis (with 95% CI) of algal biomass change (ABC) on the initial biomass of *Ulva* sp. transplants.