Importance of phenotypic plastic traits on invasion success: response of *Xenostrobus securis* to the predatory dogwhelk *Nucella lapillus*

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ABSTRACT: The ability of the invasive mussel *Xenostrobus securis* to activate defence mechanisms in response to the novel predatory dogwhelk *Nucella lapillus* was explored using field- and laboratory-based approaches. The importance of the origin of mussels was investigated in relation to different environmental conditions and levels of predation pressure (high and low). In the field, the responses of mussels were clearly asymmetrical: only individuals caged with dogwhelks at the site of high predation risk underwent phenotypical changes (stronger attachment, thicker shells and heavier adductor muscle). In contrast, shell growth was faster in mussels held in cages without dogwhelks at the high predation-risk site, suggesting trade-off patterns between growth and other energy-demanding actions. Nevertheless, *X. securis* activated inducible morphological defences without any detrimental effect on soft tissue growth (i.e. condition index). In the laboratory, the role of temperature on phenotypic responses of mussels exposed to dogwhelk was also evaluated. Mussels originally from the site of low predation risk showed a weaker response to the predator *N. lapillus*, probably because of difficulties in correctly identifying predator cues. At higher temperatures, mussels secreted stronger byssal threads regardless of their origin, while condition was poorer, shells thinner and gametogenesis activated more rapidly, particularly in the presence of dogwhelks. In summary, *X. securis* appears to be highly capable of activating protective mechanisms in marine environments within its geographical range of expansion through improved fitness.

KEY WORDS: Invasive mussel · Plastic traits · Anti-predatory actions · Temperature

INTRODUCTION

Predator–prey interactions and the evolution of adaptive traits are major ecological factors controlling the dynamics of populations, communities and ecosystems (Menge 1983, Freeman & Byers 2006). Such interactions are also important in novel communities arising as a result of the establishment of non-indigenous species (NIS) (Hines et al. 2009, Shinen et al. 2009). The success of NIS may primarily depend on the responses and eco-physiological plasticity of

organisms, which may in turn be mediated by factors such as predator–prey density, distribution limits, spatial and temporal scales of interaction, environmental conditions, feeding preferences and behaviour of predators and prey (Hines et al. 2009). Recent research suggests that the predator avoidance behaviour of many invertebrates may depend on experience (Turner et al. 2006). Indeed, unfed predators or predators feeding on prey that is unrelated to the target species usually induce weak behavioural and morphological responses in prey, while preda-

tors feeding on conspecifics induce strong responses (reviewed by Schoeppner & Relyea 2005). Studies of different taxa suggest that prey may lose their anti-predator behaviour in the absence of continued selection (Storfer & Sih 1998).

Predator-induced defence mechanisms are ecologically important forms of phenotypic plasticity whereby prey show adaptive morphological, behavioural or physiological shifts that increase their resistance to predation. The potential for inducible defence mechanisms to cause adaptive change over broad geographical and temporal scales has been reported to be of comparable magnitude to any temperature-related latitudinal effect (Trussell & Smith 2000). Common predator-induced responses include shell thickening in mussels and production of defensive spines in bryozoans (Freeman 2007). Other common responses in some mussel species include increased attachment strength and decreased clearance rates induced by risk cues (Naddafi & Rudstam 2013). Moreover, predator-induced changes may lead to lower fecundity or reproduction rates in individuals (Fässler & Kaiser 2008, Bourdeau 2010). These responses are often mediated by environmental factors such as temperature, which regulates the evolution of life history traits via energetic costs (Lass & Spaak 2003, Barbosa et al. 2014).

Chemical alarm responses (such as the release of infochemicals) represent a defensive strategy that is triggered by an evolved signalling substance released from a conspecific victim of predation (Leonard et al. 1996). Defensive responses of prey may be directly induced by predators (enemy avoidance kairomones; Kats & Dill 1998, Lowen et al. 2013) — even unfed predators (Trussell & Nicklin 2002, Freeman 2007) — as well as by other prey at the moment of attack, e.g. by conspecifics or closely related species (heterospecifics, as reported by Fässler & Kaiser 2008 for the first time in mussels). These mechanisms 'label' the predators so that they can be recognised by the prey (alarm pheromones; Smith 1992, Trussell & Nicklin 2002, amongst others). Both conspecific and heterospecific sources of chemosensory information are important for assessing predation risk as an alarm response (Hagen et al. 2002). Prey must ensure that signals are reliable (Harvell 1990) to prevent energetic losses or development of non-sense actions. There must therefore be a balance between the enhancement of defence mechanisms and fitness strategies, e.g. growth or reproduction, which may eventually lead to trade-offs (Hoverman & Relyea 2009). However, reduction in the ability of the prey to respond appropriately to predation pressure may

occasionally be an indirect consequence of a reduction in feeding or somatic growth due to predator signals rather than trade-offs (Bourdeau 2010).

Mussels are excellent target organisms for examining the mechanisms and costs of inducible defence responses because they are prevalent on intertidal rocky shores and rely on morphological and chemical defence actions to avoid predators (Leonard el al. 1999). The strong calcareous shell of mussels protects the soft body of the organism, and shell size and thickness are therefore the main factors involved in anti-predatory responses (Nagarajan et al. 2002). In the mussel *Mytilus edulis* Linnaeus, 1798, the presence of water-borne effluents from crabs and starfish modifies the protective tissues and behaviour of individuals, e.g. shell thickness and adductor muscle size and byssal tenacity (Leonard et al. 1999, Reimer & Harms-Ringdahl 2001, Fässler & Kaiser 2008). Moreover, *M. edulis* is capable of distinguishing different predators and expressing specific (inducible) defence mechanisms, although the eventual effectiveness of the mechanisms is asymmetrical, and therefore, the specific response to a cue from one predator species does not deter the other (Freeman 2007).

The black pygmy mussel *Xenostrobus securis* Lamarck, 1819, which, like *M. edulis*, belongs to the family Mytilidae, is an NIS native to Australia and New Zealand that has successfully invaded the Mediterranean Sea (Streftaris & Zenetos 2006) and the Atlantic coast of the Iberian Peninsula (Garci et al. 2007, Pascual et al. 2010, Adarraga & Martínez 2012). It was first reported in the Ría de Vigo (NW Spain) near the mouth of the Verdugo River in 2002 (Garci et al. 2007). Since then, it has gradually spread towards the middle part of the ría and into the nearby Ría de Pontevedra (Gestoso et al. 2012). It forms monospecific and mixed aggregations with the commercially important mussel *Mytilus galloprovincialis* Lamarck, 1819. Its success as an invasive species in the invaded area can be attributed to its ability to tolerate a wide range of environmental conditions, e.g. salinity fluctuations, and to a reduced biotic resistance by native communities, especially in the innermost areas of the Ría de Vigo (Babarro & Lassudrie 2011, Gestoso et al. 2012). However, in the outermost areas of rías, predation may be an important factor controlling the abundance of the invader (Gestoso et al. 2014).

We carried out field and laboratory-based experiments to investigate how novel predators and environmental conditions affect the life history traits of the invasive *X. securis.* Specifically, we tested the effect of the predatory dogwhelk *Nucella lapillus*

Linnaeus, 1758, which is one the most abundant benthic predators on rocky shores of the inner areas of Galician Rías (Gestoso et al. 2014), although it is absent in areas characterised by low salinity. We carried out a transplant experiment between 2 locations that differed in predation pressure (absence versus presence of dogwhelks) and environmental conditions. We then carried out a laboratory experiment to evaluate how mussels completing their life cycle under different environmental conditions and predation pressure respond to water-borne cues from the dogwhelk *N. lapillus*. Because temperature is known to influence the balance of energy expenditure by organisms and thus life history responses (Broomhall 2004, Barbosa et al. 2014), we also investigated the role of temperature in shaping the responses of mussels.

MATERIALS AND METHODS

Study area and field experiment

The field experiment was conducted at 2 different locations in the Ría de Vigo (NW Spain) between the end of April and the end of July 2014: the inner location of Sampaio, at the mouth of the Verdugo river, and the outermost Cesantes, under a stronger oceanic influence (Fig. 1). The 2 locations differ in environmental conditions, predation pressure and abundance of the invader (Gestoso et al. 2014). At Sampaio, where the invader *X. securis* is most abundant (origin of the invasion process), the average salinity (19.05 ± 9.49, range 0 to 32.53, data reported as mean ± SD) and water temperature (mean 15.95 ± 3.90°C, range 8.36 to 23.73°C) are both lower than at the
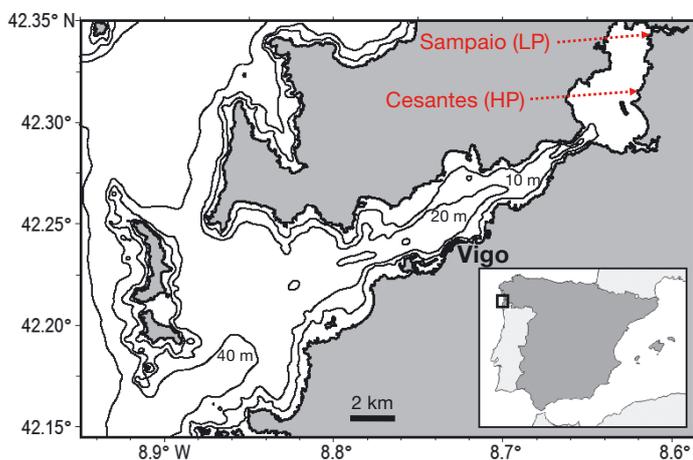

Fig. 1. The Ría de Vigo (NW Spain) showing the experimental sites where the mussels were collected (Sampaio [low predation, LP] and Cesantes [high predation, HP])

outer location (salinity: 29.4 ± 2.73, range 6.9 to 33.27; temperature 16.48 ± 2.76°C, range 11 to 22.34°C) (*in situ* 1 yr data obtained with Star Oddi mini DST CTDs), although the values vary widely due to the river influence and tidal cycles. Flow regimes also vary from 8 to 123.1 cm s^{-1} at Pontesampaio/sampaio and from 0 to 2.3 cm s^{-1} at Cesantes (Babarro & Lassudrie 2011). Pontesampaio/sampaio is also characterized by lower total particulate matter (TPM) and particulate organic matter (POM) contents (TPM: 1.98 ± 0.52 mg l^{-1}; POM: 0.78 ± 0.12 mg l^{-1}) and a lower chl *a* content (2.39 ± 0.61 µg l^{-1}) than at the outer location (TPM: 2.20 ± 0.72 mg l^{-1}; POM: 0.87 ± 0.24 mg l^{-1}; chl *a:* 3.24 ± 1.28 µg l^{-1}), where *X. securis* is less abundant. Pontesampaio/sampaio is characterized as an environment with a low predation risk (hereafter LP), whereas Cesantes, where benthic predators are abundant, is characterized as an environment with a high predation risk (hereafter HP). Although only few shell-drilling muricids and some fish have been reported as predators of *Xenostrobus* species in its native range (Morton & Leung 2015), diverse benthic species might prey upon it in the invaded area. The potential predators include the muricids *N. lapillus* and *Ocenebra erinaceus* Linnaeus, 1758, the crustacean *Carcinus maenas* Linnaeus, 1758, and fish of the families Gobiidae and Labridae (Filgueira & Castro 2011, Veiga et al. 2011, Gestoso et al. 2014).

We carried out a reciprocal transplant experiment to assess the effects of the physical environment and predation pressure on the physiological responses of the invader. A previous study reported that the physiological responses of *X. securis* were not affected by handling, caging or the biodegradable mesh used (Gestoso et al. 2014). Although the experimental design included Predation (presence [+] and absence [−] of dogwhelks) and Origin (LP and HP) as fixed factors, it was not fully orthogonal because dogwhelks do not occur naturally at LP.

Artificial mussel aggregations each comprising 8 individuals, were constructed on previously sanded PVC plates (14 × 14 × 0.5 cm). Similarly sized individuals (25.61 ± 3.09 mm of shell length) were collected from each location (LP and HP) and transported to the laboratory. The mussels were cleaned by removing biofouling and remains of byssal threads from the ventral margin and were labelled individually for later identification (by supergluing a piece of paper printed with a number to the shell). The mussels were placed on the PVC plates, which were held in the laboratory for 2 to 3 d, to enable primary attachment, before being situated in the field. A biodegrad-

A) Field

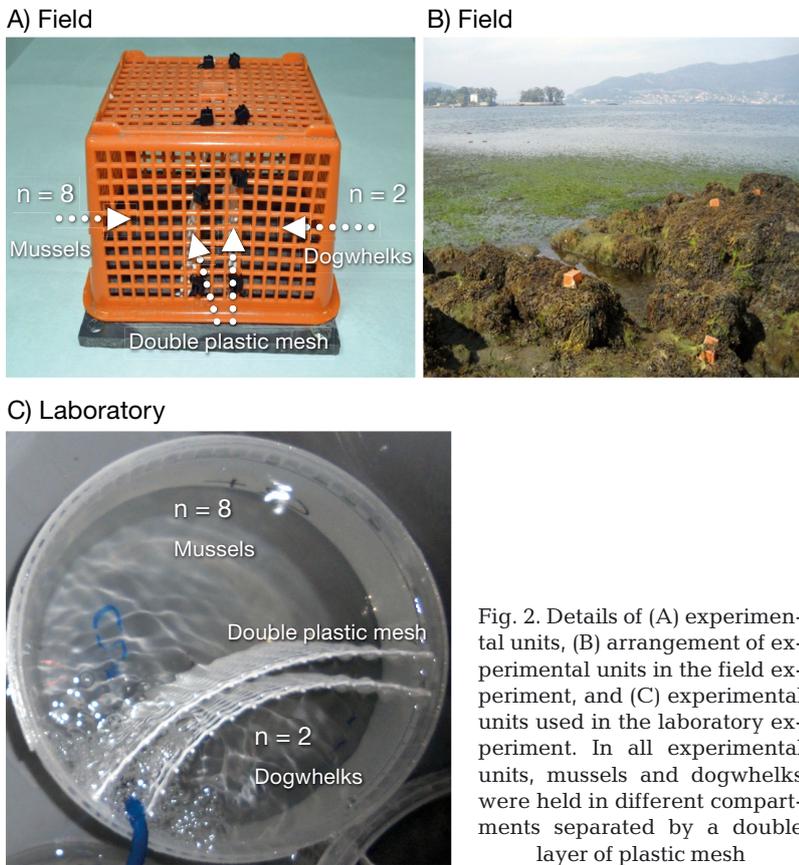**B) Field**

C) Laboratory

Fig. 2. Details of (A) experimental units, (B) arrangement of experimental units in the field experiment, and (C) experimental units used in the laboratory experiment. In all experimental units, mussels and dogwhelks were held in different compartments separated by a double layer of plastic mesh

mussels from HP with dogwhelks; (3) mussels transferred from LP to HP without dogwhelks; (4) mussels transferred from LP to HP with dogwhelks; (5) local mussels from LP without dogwhelks, and (6) mussels transferred from HP to LP without dogwhelks.

Shell thickness index (STI), specific growth rate of shell (SGR), mussel (byssal) tenacity in an aggregation (TEN), weight of the posterior adductor muscle (PAM), condition index (CI) and gonadal developmental stage (GS) were measured to test the effect of experimental treatments on mussel performance. Prior to the experiment, PAM (n = 20), CI (n = 12) and GS (n = 10) were also measured in some individuals in each population to evaluate the initial physiological status of mussels.

To evaluate growth, STI and SGR were measured in randomly selected and individually marked mussels of each experimental treatment at the beginning of the experiment (n = 32). STI was calculated as follows:

$$STI = 1000 \times \text{dry shell wt}/[L(H^2 + W^2)^{0.5} \times \pi/2] \quad (1)$$

where L, H and W are respectively the length, height and width of the shell (Freeman et al. 2009) measured with a digital vernier caliper (±0.1 mm). The immersed mass of each mussel was also obtained and converted to dry shell weight by using individual destructive regressions for each of the 2 mussel populations, i.e. 20 mussels per location (Palmer 1982). To estimate shell weight, mussels were sacrificed, the tissue was dissected out, and the shells were patted dry with paper towels and weighed on a Sartorius precision digital balance (±0.01 mg). After removing residues of organic material, the shells were dried in a muffle furnace at 100°C for 2 h to remove moisture.

SGR was calculated as follows:

$$SGR = \ln (\text{final length} / \text{initial length}) \times t^{-1} \quad (2)$$

where final length and initial length are the shell lengths at the end and beginning of the experiment, respectively, and t is the duration of the experimental period (90 d) in months (Christensen et al. 2015).

TEN of mussels in each aggregation was measured by connecting a single mussel to a spring scale (Digital Force Gauge DN431, 0.01 N resolution) with

able mesh was used to facilitate attachment of mussels to the plates. Plastic cages (14 × 12 × 8 cm) were used to exclude any other predators from the PVC plates to which the experimental animals were attached; the cages were divided into 2 equal compartments by a double layer of plastic mesh (1 × 1 cm) to prevent direct contact between the prey (mussels) and predators (dogwhelks). Eight mussels and 2 dogwhelks (29.04 ± 1.71 mm; apex-base length) were placed in separate compartments in each cage (Fig. 2A). The densities (ind. m^{-2}) were chosen on the basis of the natural densities of both species at Cesantes (*X. securis*: 8.47 ± 0.46, n = 60; *N. lapillus*: 1.67 ± 0.18, n = 60; results from 2011–2012 pooled data; data not shown). Once assembled, the experimental cage units (n = 4) were transported to the field and screwed to rocky platforms with stainless steel screws. The cages were randomly placed at the same height on the shore and separated by a minimum distance of ~1 m (Fig. 2B). Fouling was removed from cages every week, and the dogwhelks were replaced every 2 wk by others maintained in a reservoir tank in the laboratory and fed on *X. securis*.

The design included the following treatments: (1) local mussels from HP without dogwhelks; (2) local

the aid of custom-made forceps (see Babarro & Comeau 2014 for details of the procedure). Care was taken to avoid disturbing neighbouring mussels when dislodging one individual. Individuals that were immediately adjacent to those selected for dislodgement were not considered for trials if they had interconnected byssus threads. This restriction explains why sample sizes were variable and lower than the total number of individuals. Dislodgement measurements were made with wet mussels to prevent modification of the mechanical properties of the byssus. Attachment force (F) was normalized by mussel size in order to calculate TEN (N m^{-2}), as follows:

$$TEN = F / AP \qquad (3)$$

where AP is the projected area of the individuals pulled for dislodgement, approximately an ellipse obtained by the product of width and height values of shells (n = 13 to 15).

To determine PAM weight, the whole PAM of 4 mussels was removed with a knife and pooled into a single replicate (n = 4) to yield a sufficient amount of sample, which was then dried at 60°C for 48 h and weighed. PAM values were standardized to mussel shell area (mg cm^{-2}) and obtained from shell length, height and width values (see STI for shell area formula).

CI was calculated as follows:

$$CI = (DW_{tissue} / DW_{shell}) \times 100 \qquad (4)$$

where DW_{tissue} is the dry weight of the soft tissue, and DW_{shell} is the dry shell weight (Freeman 1974).

To determine GS, a piece of gonad from each individual and for each experimental condition (n = 10) was dissected and routinely processed for histology, i.e. fixed in Davidson formaldehyde for 24 h, dehydrated in an ethanol series, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin and eosin. Gonadal development stage was scored following a modified version of the scale proposed by Martínez-Castro & Vázquez (2012): resting, gametogenesis, maturity, spawning, post-spawning and exhaustion. When >1 developmental stage was evident within a single individual, the reproductive stage was assigned according to the stage observed in most follicles.

Laboratory experiment

A mesocosm experiment was carried out between the end of January and the end of April 2014 to evaluate the effects of predation and mussel origin on physiological performance of individuals and how these responses are shaped by temperature. The experimental design included Origin (LP and HP), Temperature (13 and 18°C) and Predation (presence [+] and absence [−] of dogwhelks) as orthogonal fixed factors.

Similar sized individuals to those used in the field experiment were collected from the same 2 locations and transported to the laboratory, where they were cleaned and marked following the same protocol described above. Eight individuals were placed in glass Petri dishes and allowed to establish primary attachment on a biodegradable mesh, which facilitated the final attachment. In this case, 3 l plastic containers (19 cm diameter × 13 cm depth) were used as experimental units (n = 4). The containers were divided into 2 compartments using a double layer of plastic mesh (1 × 1 cm), and the mussels in the glass Petri dish and 2 dogwhelks (when required) were placed in separate compartments (Fig. 2C). Experimental units were placed in 350 l PVC tanks inside an isothermal room and received light from above, with a 12 h light: 12 h dark photoperiod. The water temperature in the tanks was controlled using titanium heaters, and 2 levels were chosen to reflect mean temperature in the study area during late winter and early summer, respectively.

Prior to the experiment, mussels were acclimated at 13°C for 7 d, and the temperature was then either maintained or increased gradually (1°C d^{-1}) until reaching 18°C. Salinity of the seawater in the experimental units was maintained at 28 ± 0.2, and the seawater was renewed every 2 d. Dogwhelks, which were replaced in experimental units every 2 wk, were maintained in reservoir tanks under the same conditions as in the experimental treatments and were fed ad libitum with *X. securis.* Mussels in the experimental cage units were fed on a mixed diet composed of *Isochrysis galbana* clone T-ISO (40%), *Chaetoceros gracilis* (25%), *Phaeodactylum tricornutum* (25%) and *Rhodomonas lens* (10%); a ration of 3% of total tissue dry weight was supplied in 2 doses every 2 d coinciding with seawater renewal.

The STI, TEN, CI and GS were measured to evaluate the effects of the experimental treatments on performance of mussels (see 'Study area and field experiment' for a detailed description of the procedure). Prior to the experiment, TEN (n = 25), CI (n = 12) and GS (n = 20) were measured in some (n) individuals in each population to evaluate the initial physiological status of mussels. As in the field experiment, initial STI values were measured in randomly selected and marked individuals of each experimental treatment (n = 32).

Statistical analysis

Initial differences (i.e. status of mussels before field and laboratory experiments) in STI, TEN, PAM weight and CI from both locations were evaluated by 1-way ANOVAs (2-tailed tests) with Origin as a fixed factor.

In the field experiment, the response variables TEN, PAM and CI were analysed using distinct 2-way ANOVAs. Two-way ANOVA was first applied with Origin (LP and HP) and Exposure site (LP and HP) as fixed factors, excluding the effect of the dog-whelk presence. A second 2-way ANOVA was then applied with Origin (LP and HP) and Predation (presence and absence of dogwhelks) as fixed factors only for HP where dogwhelks occur naturally.

In the laboratory experiment, the response variables TEN and CI were analysed by 3-way ANOVAs with Origin (LP and HP), Predation (presence and absence of dogwhelks) and Temperature (13 and 18°C) as fully orthogonal fixed factors.

For the specific cases of STI and SGR, changes in these variables over time were evaluated by ANCOVAs with the same design as for the ANOVAs, but with initial STI and initial shell length values, respectively, as covariates. The interaction terms for each factor and the covariate were included in the design to test whether slopes of the regression lines were significantly different. No significant interactions, i.e. p-values > 0.05, with the covariates indicated homogeneity of slopes, and the analysis was re-run without considering interactions (McDonald 2009).

Normality and homogeneity of variances were examined respectively by the Shapiro-Wilk *W*-test and Levene's test. Data were transformed when necessary, and if heterogeneity persisted, rank transformation was used (Conover 2012). Significant differences between experimental groups were tested using *a posteriori* Tukey tests.

Gonadal stage was analysed by multinomial logistic regression. As the field experimental design was not fully orthogonal, the data were split into 2 datasets, and 2 separate analyses were carried out. The first analysis tested the effect of Origin on mussels at LP, and the second analysis tested the effect of Predation and Origin on mussels at HP. For the laboratory experiment, multinomial logistic regression was used to test the effect of the 3 factors: Origin, Predation and Temperature.

All analyses were performed using the STATISTICA 7.0 software, except for multinomial logistic regressions, which were performed with the multinom function from the multcomp package for R 2.12.1 (R Development Core Team 2010). All data are reported as means ± SD.

RESULTS

Field experiment

At the beginning of the experiment, the shells of the mussels from HP were 16% thicker ($F_{1,190} = 43.19$; p < 0.001) than those of the mussels from LP (Fig. 3A). The differences related to initial STI values lasted for the whole experimental period as the STI only differed significantly in relation to the origin of mussels (Table 1, Fig. 3A) when the effects of origin and exposure site were tested simultaneously. Both origin and predation had significant, non-interactive effects on the STI index of mussels at HP (Table 1). Mussels originally from HP had thicker shells (0.70 ± 0.10) than those transferred from LP (0.57 ± 0.06) at the end of the experiment (Fig. 3A). At the end of the experimental period, mussels also had thicker shells (6 to 7%) when exposed to predators and regardless of their origin (Fig. 3A).

The interaction between origin and site of exposure of mussels had a significant effect on SGR (Table 1, Fig. 3B). Mussels originally from LP grew faster (0.042 ± 0.012 mo^{-1}) than mussels from HP when transferred to HP (0.032 ± 0.002 mo^{-1}). Similarly, both origin and predation had a significant interactive effect on SGR of mussels at HP (Table 1). The SGR only increased significantly in mussels originally from LP and transferred to HP without dog-whelks (0.050 mo^{-1}; Fig. 3B). Although mussels grew, no significant variation in SGR was detected when mussels were transplanted from HP to LP (Fig. 3B).

TEN did not vary significantly with origin of mussels or exposure site (2-way ANOVA, Table 2) with values ranging from 6.38 to 7.99 (× 10^{-4}) N m^{-2} (Fig. 3C). In contrast, in mussels at HP, TEN was significantly affected by origin of mussels, but depended on the presence of predators (i.e. significant interaction Origin × Predation; Table 2, Fig. 3C). Byssus was stronger in mussels originally from LP transplanted to HP with dogwhelks (up to 11.32 × 10^{-4} N m^{-2}).

PAM weight did not differ significantly between mussels from different locations at the beginning of the experiment (range of 0.64 to 0.67 mg cm^{-2}; Fig. 4A). After 3 mo in the field, differences in PAM weight were due to the exposure site condition (Table 2, Fig. 4A), and mussels from HP had heavier adductor muscles (0.75 ± 0.08 mg cm^{-2}) than those from LP (0.66 ± 0.04 mg cm^{-2}). Although the origin of mussels did not significantly affect PAM weight at HP, the presence of predators did have an effect (Table 2, Fig. 4A), with increments of 6% and 12% for mussels originally from HP and LP, respectively.
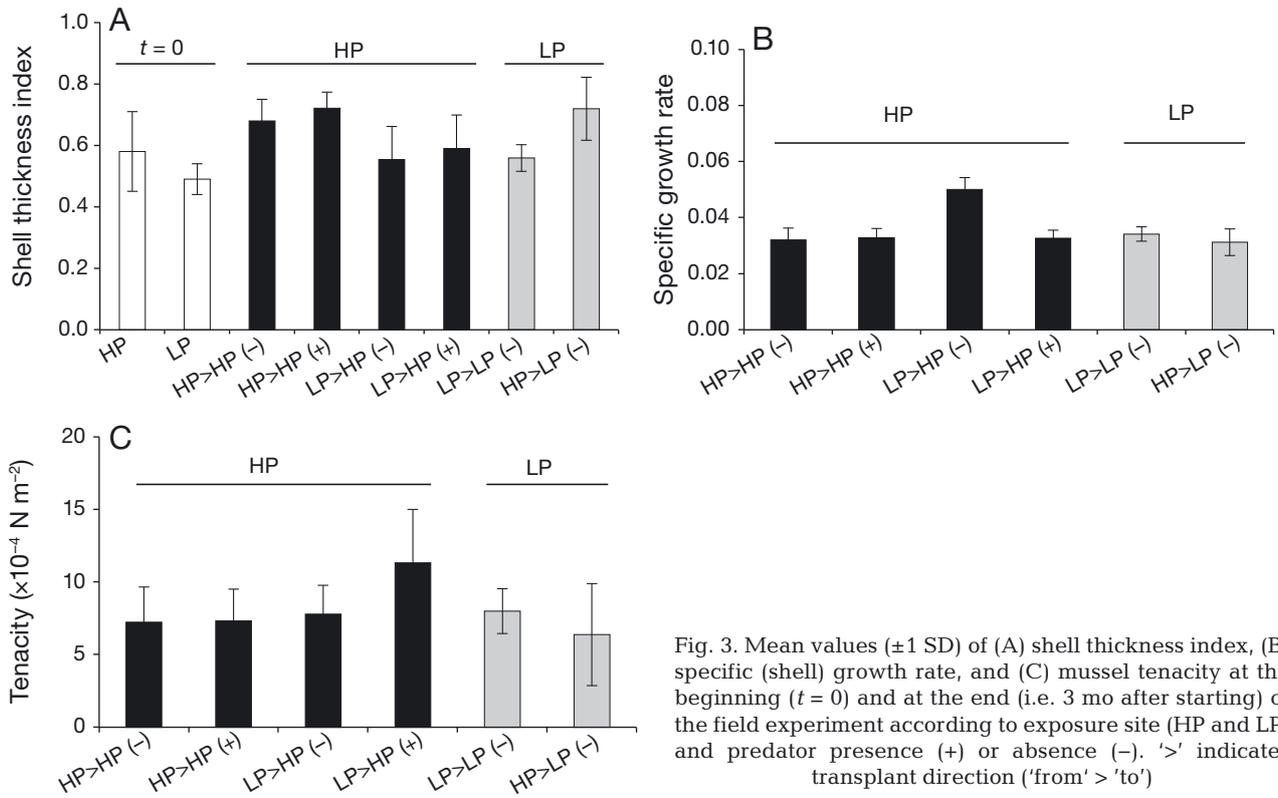
Fig. 3. Mean values (±1 SD) of (A) shell thickness index, (B) specific (shell) growth rate, and (C) mussel tenacity at the beginning ($t = 0$) and at the end (i.e. 3 mo after starting) of the field experiment according to exposure site (HP and LP) and predator presence (+) or absence (−). '>' indicates transplant direction ('from' > 'to')

CI differed significantly between mussels from the 2 locations at the beginning of the experiment ($F_{1,8} = 6.28$, p < 0.05), with higher values in mussels at LP (11.3% ± 1.10) than in mussels at HP (9.4 ± 0.76%; Fig. 4B). After 3 mo, CI values differed significantly depending on the origin of mussels and exposure site, but with no significant interaction (Table 2). Although the condition of all mussels originally from LP

(16.24 ± 3.10%) was better than that of mussels from HP (11.96% ± 2.35), at the end of experiment, CI was only higher in mussels from LP transferred to HP (Fig. 4B). The interaction between the origin of mussels and predation affected the condition of mussels at HP (Table 2, Fig. 4B). The condition of mussels originally from LP transferred to HP increased, especially in the absence of dogwhelks (~19.9%; Fig. 4B).

At the beginning of the experiment, mussels from both locations were at an advanced stage of maturation (Fig. 5) as most had all follicles filled with ripe gametes. The gametogenetic stage of mussels at LP did not differ significantly in relation to origin of mussels ($\chi^2 = 1.98$, df = 2, p = 0.371), although the percentage of individuals originally from LP that spawned was slightly lower than that of individuals transferred from HP (50% and 70%, respectively). In contrast, the gametogenetic stage of mussels at HP differed depending on the origin of mussels ($\chi^2 = 7.84$, df 3, p = 0.049; Fig. 5). The mussels originally from HP were at a more advanced stage of gametogenesis than the mussels transferred from LP.

Table 1. Field experiment. Results of ANCOVAs to determine the effect of initial ($t = 0$) shell thickness index (STI) and shell length values and (A) Origin and Exposure site and (B) Origin and Predation on STI and shell growth rate (SGR) at the end of the experiment. Values in **bold** are statistically significant (p < 0.05)

Factor	STI			SGR		
	df	F	p	df	F	p
A. Origin and Exposure site						
$t = 0$	1	88.56	**2.11 × 10⁻¹⁵**	1	147.45	**<1.00 × 10⁻¹⁸**
Origin (OR)	1	18.58	**3.80 × 10⁻⁵**	1	0.92	0.340
Exposure site (ES)	1	0.37	0.547	1	0.18	0.675
OR × ES	1	0.36	0.548	1	13.44	**3.86 × 10⁻⁴**
Error	99			106		
B. Origin and Predation						
$t = 0$	1	162.74	**<1.00 × 10⁻¹⁸**	1	184.07	**<1.00 × 10⁻¹⁸**
Origin (OR)	1	55.89	**2.41 × 10⁻¹¹**	1	0.68	0.412
Predation (PR)	1	9.16	**3.11 × 10⁻³**	1	2.34	0.129
OR × PR	1	1.55	0.215	1	5.39	**0.022**
Error	105			109		

Table 2. Field experiment. Results of 2-way ANOVA to determine the effect of (A) Origin and Exposure site and (B) Origin and Predation on byssal tenacity (TEN), posterior adductor muscle (PAM) weight, and condition index (CI) of the mussels. All analyses were subjected to log transformation prior to the analysis. Values in **bold** are statistically significant ($p < 0.05$)

Factor		TEN			PAM			CI	
	df	F	p	df	F	p	df	F	p
A. Origin and Exposure site									
Origin (OR)	1	3.95	0.053	1	2.94	0.112	1	15.70	**1.88×10^{-3}**
Exposure site (ES)	1	1.10	0.318	1	7.90	**0.015**	1	17.74	**1.21×10^{-3}**
OR × ES	1	1.97	0.212	1	0.15	0.708	1	3.36	0.091
Error	54			12			12		
B. Origin and Predation									
Origin (OR)	1	11.33	**1.44×10^{-3}**	1	0.81	0.385	1	13.53	**3.16×10^{-3}**
Predation (PR)	1	5.90	**0.018**	1	5.20	**0.040**	1	1.37	0.265
OR × PR	1	4.39	**0.041**	1	0.99	0.340	1	6.66	**0.024**
Error	52			12			12		

This trend was more evident in the presence of dogwhelks, although the differences were not significant (Fig. 5).

Laboratory trial

Shells of mussels originally from HP were thicker (~16%) at the beginning of the experiment ($F_{1,238} = 24.38$; $p < 0.001$; Fig. 6A). ANCOVA applied to the STI values at the end of the experiment highlighted the significant impact of origin and temperature as well as the interaction between the initial STI and both factors (Table 3, Fig. 6A). The shells were thicker at low temperature (0.53 ± 0.02) than at high temperature (0.50 ± 0.02). The shells of mussels from HP were thicker (values ranged between 0.51 and 0.57) than those of mussels from LP (values ranged between 0.46 and 0.50).

At the beginning of the experiment (i.e. 1 wk under experimental conditions), TEN was only significantly affected by temperature, with an increase at high temperature ($F_{1,43} = 9.25$, $p < 0.01$ and $F_{1,49} = 7.91$, $p < 0.01$ for LP and HP, respectively; Fig. 6B). At the end
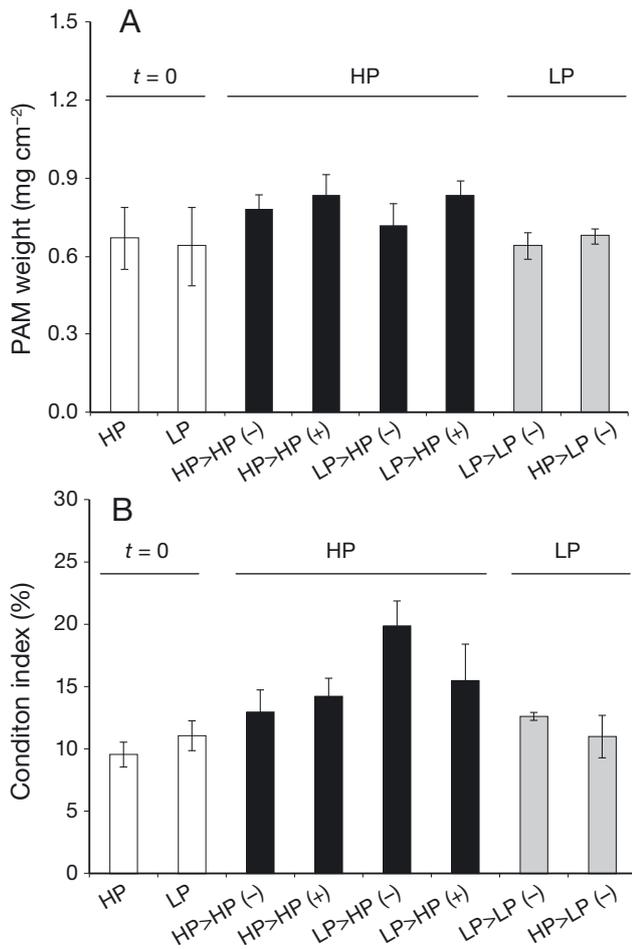

Fig. 4. Mean values (±1 SD) of (A) weight of posterior adductor muscle (PAM) and (B) condition index of mussels at the beginning ($t = 0$) and at the end (i.e. 3 mo after starting) of the field experiment according to exposure site (HP or LP) and predator presence (+) or absence (−). '>' indicates transplant direction ('from' > 'to')
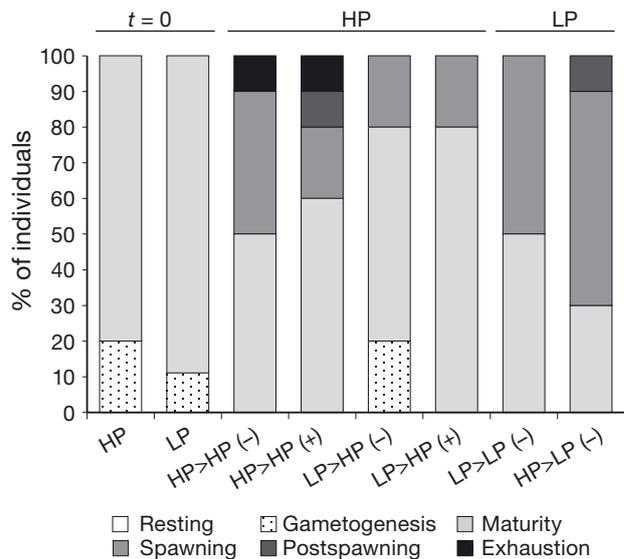

Fig. 5. Gonadal stage of mussels (n = 10) in the field experiment at the beginning of the experiment ($t = 0$) and in relation to exposure site (HP or LP) and predator presence (+) or absence (−). '>' indicates transplant direction ('from' > 'to')
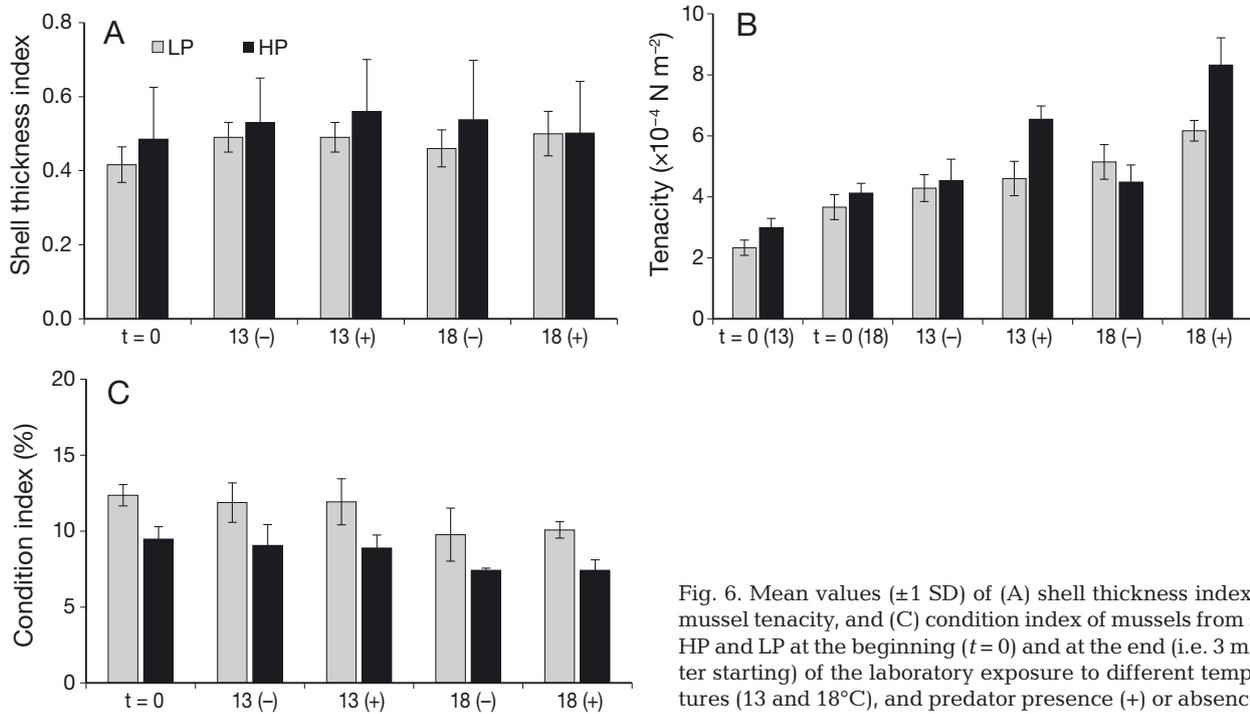
Fig. 6. Mean values (±1 SD) of (A) shell thickness index, (B) mussel tenacity, and (C) condition index of mussels from sites HP and LP at the beginning ($t = 0$) and at the end (i.e. 3 mo after starting) of the laboratory exposure to different temperatures (13 and 18°C), and predator presence (+) or absence (−)

of the experiment, TEN varied significantly with temperature (Table 3), but also with origin of mussels, although depending on the presence of predators (i.e. significant interaction Origin × Predation (Table 3, Fig. 6B). TEN increased by up to 21% in mussels exposed to high temperature. Moreover, TEN in mussels originally from HP increased, but only in the presence of dogwhelks (up to 65% higher).

At the beginning of the experiment, CI differed significantly between mussels from both locations ($F_{1,6} = 25.99$, p < 0.01; Fig. 6C), and the condition of mussels from site LP was better (12.36 ± 0.70%) than that of mussels from site HP (9.27 ± 0.81%). The differences due to origin of mussels were maintained after the experimental period (Table 3, Fig. 6C), with values ranging from 10 to 12% and from 7.4 to 8.9% for mussels from LP and HP, respectively. CI was higher at low temperature (10.40% ± 1.70) than at high temperature (8.60 ± 1.40%) (Table 3, Fig. 6C).

Table 3. Laboratory. Results of (A) ANCOVA to determine the effect of the initial shell thickness index (STI) ($t = 0$) as covariate as well as Origin, Temperature, and Predation on STI and (B) 3-way ANOVA for tenacity (TEN) and condition index (CI) of the mussels with the same factors. STI was rank transformed prior to the analysis. TEN and CI were log transformed. Values in **bold** are statistically significant (p < 0.05)

A. ANCOVA	——— STI ———		
Factor	df	F	p
$t = 0$	1	78.05	**4.41×10^{-16}**
Origin (OR)	1	4.57	**0.033**
Temperature (TEMP)	1	15.06	**1.40×10^{-4}**
Predation (PR)	1	0.30	0.584
OR × TEMP	1	0.01	0.793
OR × PR	1	0.08	0.933
TEMP × PR	1	2.52	0.116
OR × TEMP × PR	1	0.94	0.334
OR × $STI_{t=0}$	1	5.47	**0.020**
TEMP × $STI_{t=0}$	1	10.67	**1.27×10^{-3}**
Error	206		

B. Three-way ANOVA	——— TEN ———			——— CI ———		
Factor	df	F	p	df	F	p
Origin (OR)	1	2.27	0.135	1	48.06	**3.61×10^{-7}**
Temperature (TEMP)	1	4.98	**0.028**	1	20.22	**1.49×10^{-4}**
Predation (PR)	1	18.31	**4.30×10^{-5}**	1	0.04	0.864
OR × TEMP	1	1.20	0.275	1	1×10^{-4}	0.994
OR × PR	1	5.14	**0.025**	1	0.16	0.688
TEMP × PR	1	1.15	0.287	1	0.10	0.755
OR × TEMP × PR	1	0.04	0.843	1	0.03	0.869
Error	99			24		

Mussels collected from both locations were at the end of the gametogenetic cycle as almost all of them were already spent (Fig. 7), and a new reproductive cycle started during the experimental period. The presence of dogwhelks affected maturation of the mussels, although the effect varied with temperature (i.e. Temperature × Predation interaction, $\chi^2 = 12.46$, df = 5, p = 0.028; Fig. 7). Gametogenesis occurred faster at the higher temperature, particularly in the presence of dogwhelks (i.e. more mussels were spawning or already spent) than at the lower temperature. These patterns were not affected by mussel origin.

DISCUSSION

Although we are aware that the results of this study would be more robust if we had replicated populations, it was not possible in practice within Ría de Vigo, especially at the mouth of the river, because both environmental and biotic conditions varied at a very small spatial scale. Furthermore, there is no other river mouth in the ría with similar density of *Xenostrobus securis*. The best alternative as a complement to the field survey was to carry out a mesocosm experiment in which individuals from the 2 distinct populations were maintained under controlled

Fig. 7. Gonadal stage of mussels (n = 10) in the laboratory experiment at the beginning of the experiment ($t = 0$) and in relation to temperature (13 and 18°C) and predator presence (+) or absence (−). The data from HP and LP were pooled as neither the origin nor any interaction with this factor was significant

conditions and exposed to different temperatures and predation pressure during a period of 3 mo. The results of the mesocosm experiment pointed in the same direction than those of the field survey and suggested that the origin of mussels is an important factor influencing phenotypic responses. To mitigate potential pseudo-replication problems and draw more accurate conclusions, future studies including mussel populations from other areas outside the Ría de Vigo that experience similar abiotic and biotic conditions and present similar pattern of invasion by *X. securis*, e.g. Ría de Pontevedra, would be necessary.

The non-indigenous mussels were clearly capable of activating phenotypic responses to predation risk after 3 mo of exposure, although they had not been exposed to the predator signals in their original habitat (i.e. the population at LP). The field transplant experiment revealed asymmetry in relation to the impact of transplant direction as the most significant changes occurred in the mussels transferred to HP, where the oceanic influence was stronger. The presence of dogwhelks induced development of protective tissues in mussels, e.g. greater TEN and, to a lesser extent, thicker shells and heavier PAM, but with different effects depending on the origin of the mussels. Similar predator-induced phenotypic responses (i.e. enhanced attachment strength, shell thickening and heavier PAM) have previously been reported for other mussel species (Leonard et al. 1999, Lowen et al. 2013). In the laboratory, exposure of mussels to higher temperature caused an increase in TEN, but STI and CI were significantly lower than in mussels maintained in colder water. Gametogenesis occurred faster at higher temperature in the presence of dogwhelks.

Of all response variables considered here, mussel tenacity most clearly illustrates the plasticity linked to the origin of mussels in both field and laboratory experiments. Byssus secretion represents a relatively short-term response of individuals and can be activated by different abiotic and biotic factors in 6 to 50 h (Côté 1995, Cheung et al. 2004, Shin et al. 2008). Mussels originally from HP may have reacted to dogwhelks as an important predator in their original habitat based on recognition experiences, as confirmed in the laboratory experiment. In contrast, other responses such as changes in PAM weight and shell thickness (involving calcium carbonate deposition and energy uptake allocated toward soft tissues) may take longer to be modified significantly than byssus secretion. This difference may partly explain the smaller magnitude of differences in PAM and STI between mussels in the presence and absence of
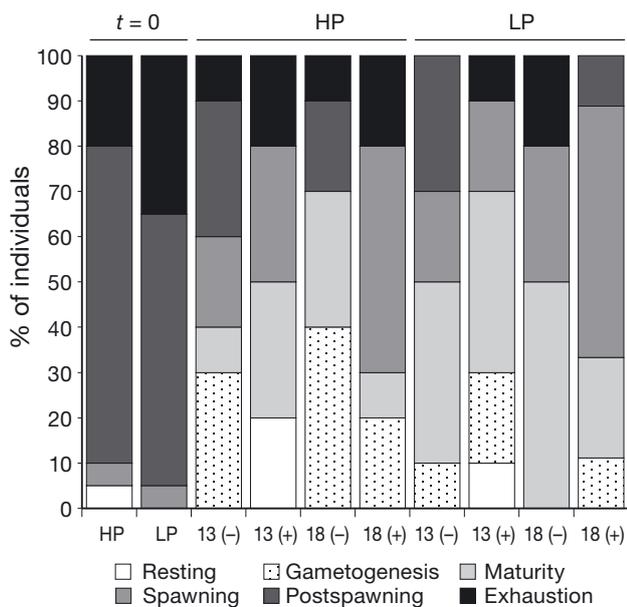
dogwhelks (6 to 17%) in both (laboratory and field) experiments. Mussels are able to recognize and differentiate between predator species and to apply different types of phenotypic plasticity (Reimer & Harms-Ringdahl 2001). In the present study, the increase in PAM weight may represent a minor response that would not counteract the most common type of attack used by dogwhelks (see also Freeman 2007), i.e. drilling holes, although it could confer mussels with a general strategy to respond to predation risk in a novel marine environment because dogwhelks can also feed on mussels through the gap between valves (Ebling et al. 1964). In the case of shell thickness, the initial differences between mussels from both populations, which lasted until the end of the experiment, may have minimized or masked any potential response to dogwhelk presence. The abundance of native predators in the wild (see Caro & Castilla 2004, Babarro & Abad 2013) and environmental factors such as wave exposure, temperature and salinity may have accounted for the initial variation in shell properties across locations (Dickinson et al. 2012).

As with any other trait, there is a cost associated with activation of inducible defences (Harvell 1990, Trussell & Smith 2000). Induced predatory responses (especially TEN) in mussels transferred from LP were made at the expense of growth of shells and soft tissues (i.e. condition index), which only increased significantly in the absence of dogwhelks at HP. Thus, our findings support the notion of a trade-off between energy allocated to byssus production and growth, as previously found for other mussel species (Garner & Litvaitis 2013). Indeed, byssus production constitutes a substantial cost for some mussel species and may require up to 44% of total carbon and 21% of total nitrogen uptake (Hawkins & Bayne 1985). Moreover, mussels from HP had thicker shells together with a poorer condition (i.e. CI) at the beginning of the experiments. The reduction in somatic growth may be the result of a direct trade-off between tissue growth and shell thickness in response to higher predation pressure at HP. Nevertheless, reduced somatic growth in response to predation risk may also be a consequence of reduced or even suppressed feeding, rather than a direct trade-off associated with production of thicker shells (Smee & Weissburg 2006, Bourdeau 2010). Our results indicated a direct (active) physiological response of *X. securis* to predation risk as the most likely underlying mechanism for the following reasons: (1) mussels originally from LP transferred to HP did not fully exploit their growth potential in the presence of dogwhelks; (2) in

the presence of dogwhelks, TEN, PAM weight and shell thickness increased in the transplanted animals, as did soft tissue weight, i.e. condition (Fig. 4B), which would be only plausible under optimal feeding or physiological rates (see Paige 1992); and (3) there was no relationship between the increase in linear shell growth and shell thickening, which suggests no direct constraints on energy investment in both shell characteristics.

The fact that the origin of mussels was an important factor explaining phenotypical responses, especially in the laboratory experiment, is consistent with previous findings (Trussell & Nicklin 2002, Turner et al. 2006). In this study, mussels originally from LP did not respond significantly to the presence of kairomones, i.e. signals emanating from the predator itself. In contrast to other mussel species like *Mytilus edulis*, which shows poor phenotypic integration with distinct predation cues (Freeman et al. 2009), the invader showed inducible changes specifically activated in the presence of dogwhelks and not disrupted by cues from other potential predators in the surroundings (Filgueira & Castro 2011, Gestoso et al. 2014). The ability of *X. securis* to respond to (new) predation risk would be extremely important for warning other conspecifics, with an eventual impact on other species of the community and their interactions. In the study area, dogwhelks seem to prefer to prey on *M. galloprovincialis* rather than on the invader (Gestoso et al. 2014), and, consequently, alarm cues may have emanated not only from conspecifics but eventually also from heterospecifics, with the magnitude depending on feeding preferences (Fässler & Kaiser 2008). Although the responses of *X. securis* reported here may have depended on experience (i.e. results of laboratory tests), other factors such as responses to conspecific and heterospecific cues may have made some contribution, according to the field results. Further research with replicated populations from distinct environments previously exposed or not exposed to dogwhelks would be necessary to draw more accurate conclusions.

Temperature, as a key parameter that regulates physiological and behavioural responses of organisms (Barbosa et al. 2014), had a significant impact on TEN, shell thickness and soft tissues of mussels, independently of the predator presence. The increase in TEN with increasing temperature was accompanied by a faster gametogenetic cycle, which may support the hypothesis that tenacity increases after spawning events in mussel displaying low reproductive activity (Carrington 2002). In contrast, STI and CI

values were higher at low temperature. These results are surprising given that thinner shells are commonly secreted at lower temperature because calcium carbonate saturation decreases and dissolution rates increase with decreasing temperature (Trussell & Smith 2000). Clearly, the lower condition index reported at high temperature can be explained by the fact that >80% of the population was spawning or already spent. Because the mussels used in the laboratory experiment were collected in winter, we can also hypothesize that the sudden increase in temperature up to 18°C may have caused metabolic adjustments to optimized fitness (Barbosa et al. 2014). In addition, exposure to higher temperature may have increased the energy demands in mussels (see Mackenzie et al. 2014), while food availability was maintained constant in both temperature treatments. In contrast to the other physiological responses, gametogenesis was interactively affected by temperature and predation. Indeed, thermal conditions can affect reproductive traits of individuals in response to predation risk (Barbosa et al. 2014). The fact that most of the response variables were not interactively affected by temperature and predation can have different explanations. At 18°C, dogwhelks were observed laying egg capsules on the walls of containers throughout most of the experimental period. Reproduction may have negatively affected the production of kairomones because of the associated cost, e.g. dogwhelks begin to forage optimally only after 2 to 3 wk of reproduction (Gosselin & Bourget 1989), with significant consequences on perception of the predator. Alternatively, because kairomones released from predators may decompose over time, it is possible that the degradation process was faster at the higher temperature, i.e. 18°C (see Lass & Spaak 2003).

In conclusion, the cost of constitutive defences and the variability in predation pressure in estuarine areas favour the development of inducible defence mechanisms in *X. securis*. The study findings also demonstrate that the environment with the strongest marine influence colonised by the invader offered natural resources that allowed individuals to activate inducible defences without compromising growth (e.g. of soft tissues). However, the activation of protective responses of mussels to the presence of predators came at a cost, as indicated by the observed trade-off between shell growth and TEN. The fact that the origin of mussels helped to explain individual responses indicates that the invader is able to adapt and respond to new environments. The ubiquity and magnitude of predator-induced changes suggest that phenotypic plasticity plays an important role in determining the invasiveness of an NIS in new invaded habitats and thus in shaping marine communities. Further studies integrating the topics of biological invasions and phenotypic plasticity are urgently needed for accurate assessment of the invasion risk associated with other species.

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