Oecologia (2009) 159:873–882
DOI 10.1007/s00442-008-1256-y

CONSERVATION ECOLOGY - ORIGINAL PAPER

Non-lethal effects of an invasive species in the marine environment: the importance of early life-history stages

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Received: 27 January 2008 / Accepted: 3 December 2008 / Published online: 21 January 2009

Abstract Studies examining the effects of invasive species have focussed traditionally on the direct/lethal effects of the invasive on the native community but there is a growing recognition that invasive species may also have non-lethal effects. In terrestrial systems, non-lethal effects of invasive species can disrupt early life-history phases (such as fertilisation, dispersal and subsequent establishment) of native species, but in the marine environment most studies focus on adult rather than early life-history stages. Here, we examine the potential for an introduced sessile marine invertebrate (*Styela plicata*) to exert both lethal and non-lethal effects on a native species (*Microcosmus squamiger*) across multiple early life-history stages. We determined whether sperm from the invasive species interfered with the fertilisation of eggs from the native species and found no effect. However, we did find strong effects of the invasive species on the post-fertilisation performance of the native species. The invasive species inhibited the settlement of native larvae and, in the field, the presence of the invasive species was associated with a ten-fold increase in the post-settlement mortality of the native species, as well as an initial reduction of growth in the native. Our results suggest that larvae of the native species avoid settling near the invasive species due to reduced post-settlement survival in its presence. Overall, we found that invasive species can have complex and pervasive effects (both lethal and non-lethal) across the early life-history stages of the native species, which are likely to result in its displacement and to facilitate further invasion.

Keywords Fertilisation · Invasive species · Postmetamorphic performance · Settlement · Trait-mediated effects

Introduction

Invasive species can have a range of effects on native species; lethal effects are most commonly cited as the source of negative impacts on established assemblages (Ruiz et al. 1999; Strayer et al. 2006). For example, invasive species can prey upon native species, cause competitive displacement or modify local disturbance regimes (Mack and D'Antonio 1998; Snyder and Evans 2006). Whilst the impact of lethal effects on native species is becoming clear, the prevalence and role of non-lethal effects in species invasions has only recently started to be considered (e.g. Trussell et al. 2006). This is despite the recent recognition that non-lethal effects can have major impacts on the dynamics of communities (Trussell et al. 2003; Werner and Peacor 2003) and initial indications that introduced species can be a source of non-lethal effects (Nystrom et al. 2001; Pangle and Peacor 2006). In terrestrial plant systems, there is a growing recognition that invasive species can affect every phase of the life-histories of native species. For example, high densities of flowering invasives can disrupt the

Communicated by Geoffrey C. Trussell.

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pollination of native species resulting in lower seed production (Bjerknes et al. 2007). Invasives can also affect the dispersal syndromes of seeds, disrupting frugivore mutualisms that are crucial for the effective dispersal of native species (Christian 2001). Thus, the effects of invasive species can extend beyond simple competitive interactions during the adult phase: non-lethal effects disrupt the production and dispersal of native recruits, seriously exacerbating the effects of the invasive species. This is especially important for marine sessile organisms, for which ‘supply-side’ processes can be important determinants of population dynamics (Underwood and Keough 2001).

Many marine benthic organisms have been moved around the world’s oceans since ancient times by means of shipping (Carlton 1999), but the last century has seen a dramatic rise in the rate of introductions of alien marine species (Cohen and Carlton 1998; Mack et al. 2000). As a result, non-indigenous species have been moving beyond physical boundaries such as those created by ocean currents, and have spread worldwide (Wonham et al. 2001). The invasion of non-indigenous species is now regarded as one of the major threats to marine biodiversity and the number of studies examining the effects of marine invasive species has increased dramatically (Ruiz et al. 1997; Grosholz 2002; Galil 2007). Most studies examining the effects of invasive species in the marine environment have focussed on competitive displacement or predation as the major impact of the invasive species, and many have been restricted to examinations of the adult phase (but see Byers and Goldwasser 2001; Trussell et al. 2006). More recently, however, it has been recognised that invasive species in the marine environment can have strong indirect effects on native communities. For example, introduced species can change trophic cascades in marine foodwebs (Trussell et al. 2002, 2004; Kurle et al. 2008), reduce larval production (Gribben and Wright 2006) and change the behaviour (and hence distribution) of prey species (Trussell et al. 2003). These studies strongly suggest that marine invasive species have pervasive effects at a range of life-history stages and levels of community organisation in the marine environment.

The life-history of marine organisms suggests that any non-lethal effects of invasive species on the early life-history stages of native species are likely to be important. Most marine organisms are broadcast spawners, releasing eggs and sperm into the water column. Due to the high rate of sperm dilution, the fertilisation of eggs is rarely complete and fertilisation rates can range between 0 and 100% with mean rates of ~50% in many instances (Levitan and Petersen 1995; Yund 2000). Importantly, heterospecific sperm can disrupt fertilisation in broadcast spawners, resulting in lower fertilisation rates (Lambert 2000, 2001). This raises the possibility that marine invasive species could disrupt/reduce fertilisation success in broadcast spawners analogously to pollination disruption in terrestrial systems, although this possibility has not been explored. Similarly, marine invertebrate larvae sometimes avoid settling near dominant competitors (Grosberg 1981; Stoner 1994; but see Bullard et al. 2004). Given that marine invasive species can be competitively dominant (Reusch and Williams 1999; Piazzi and Ceccherelli 2002), one might expect that the larvae of native species reject settlement sites adjacent to invasive species. This non-lethal effect on the dispersal of native species is analogous to the disruption/reduction of frugivore mediated dispersal by invasive species in plants. This potentially important effect of invasive species in the marine environment has received less attention than other life-history stages. This is surprising given that the supply of new recruits into marine populations can have major influences on subsequent community structure (Underwood and Keough 2001) and the production of zygotes has the potential, at least, to limit population growth in broadcast spawners (Levitan 1995). Finally, mortality immediately following settlement can be intense in sessile marine organisms and can be a major determinant of adult distributions and abundance (Gosselin and Qian 1997). Given the ecological importance of the early post-metamorphic period, any influence that invasive species may have during this stage could have major implications for the population dynamics of native species.

Here we examine the effects of an introduced marine species (*Styela plicata*) on a native species (*Microcosmus squamiger*) across the early life-history stages, from fertilisation to larval settlement through to post-metamorphic performance. As both species coexist in the studied area (SE Australia), we wanted to explore the interactions between them. Given the potential for non-lethal and lethal effects to interact synergistically (e.g. Meyer and Byers 2005), we investigated both types of effects across different stages of the life-history. We chose solitary ascidians as our study organism as they are one of the major invasive groups in marine systems (Lambert 2007). We first examined whether the presence of heterospecific sperm from an invasive species reduced the fertilisation success of the eggs of a native species. We then examined the larval settlement responses of each species in the presence and absence of heterospecific and homospecific settlers. Finally, we examined the post-metamorphic survival and growth of both species in the presence and absence of heterospecific recruits in the field. We found strong, non-lethal effects on larval settlement and direct, lethal effects on post-metamorphic survival, as well as an initial reduction in growth, suggesting that this marine invasive species has the potential to dramatically change the population dynamics of native species.

Materials and methods

Study site and species

Microcosmus squamiger is native to Australia (Kott 1985; Rius et al. 2008) and occurs subtidally on artificial and natural substrata in sheltered areas where it can form dense populations (Kott 1985; and personal observation). *S. plicata* is considered an alien species in Australian waters (Hewitt 2002; Wyatt et al. 2005) and, although there is no available information about when and where exactly this species was introduced, it now successfully colonises shallow habitats in SE Australia (personal observation). Both species are solitary ascidians and they reach similar sizes (ca. 5–10 cm) as adults. At the Manly Marina (27°27′10″ S, 153°11′22″ E, Brisbane, Queensland, Australia), *S. plicata* is found inside the harbour attached to the floating pontoons while *M. squamiger* can be found only outside the harbour, with a small area at the entrance of the harbour where both species coexist (on the outermost pontoons). Reproductively mature *M. squamiger* and *S. plicata* were collected from these outer pontoons of Manly Marina between October and December 2006. They were then transported in insulated aquaria back to the laboratory (~45 min journey) and kept in a tank with 20 l constantly aerated seawater at room temperature.

General methods: production and settlement of larvae

To extract eggs and sperm for our experiments, we used standard protocols as described by Marshall et al. (2000) for strip spawning solitary ascidians. To produce pools of fertilised eggs, we used the sperm of three individuals and the eggs of one individual (both species are simultaneous hermaphrodites with an almost complete block to self fertilisation; M.R. unpublished data). We left the gametes in contact for 45 min and we then rinsed the sperm with filtered seawater and pooled the eggs from four individuals.

To produce larvae, we fertilised eggs as above and then placed the developing embryos into an aerated beaker (containing ~500 ml filtered seawater) in a constant temperature cabinet at 20°C. In both species studied here, larvae hatch within 14 h of fertilisation. Afterwards, the larvae were pipetted out and placed in the experimental Petri dishes. We used pre-roughened 90 mm Petri dishes that had been maintained in aquaria with seawater for several days so that they could develop a biofilm that facilitates larval settlement (Wieczorek and Todd 1997). After 24 h, we gently rinsed the Petri dishes in seawater to remove any unattached larvae.

Experiment 1: does the presence of heterospecific sperm from an invasive reduce fertilisation success in a native?

We examined whether the prior exposure of *M. squamiger* eggs to *S. plicata* sperm affected subsequent fertilisation success. Eggs from a *M. squamiger* individual were split in three groups. The first group was a control (i.e. no exposure to *S. plicata* sperm), the second group was exposed to a ‘low’ concentration ($\sim 10^5$ sperm ml^{-1}) of *S. plicata* sperm and the third to a ‘high’ concentration ($\sim 10^7$ sperm ml^{-1}) of *S. plicata* sperm. Sperm concentrations were estimated using three replicate counts on a modified Fuchs-Rosenthal haemocytometer. *M. squamiger* eggs were exposed to *S. plicata* sperm in a final volume of 100 ml for 15 min, a period of time long enough to ensure that, if there was a glycosidase release from *M. squamiger* eggs, this release was completed (Lambert 2000), before being rinsed free of sperm in filtered seawater. The eggs were then placed in new Petri dishes and all the eggs of the three treatments (control, low and high) were exposed to *M. squamiger* sperm ($\sim 10^7$ sperm ml^{-1}) pooled from four individuals for 45 min. We then rinsed the eggs again in filtered seawater, placed them in a constant temperature cabinet at 20°C and allowed the embryos to develop for 14 h. We then assessed fertilisation success by counting the proportion of eggs that developed into unhatched embryos or hatched larvae relative to unfertilised eggs. We repeated this experiment for the eggs of three different individuals (i.e. three runs). To analyse the data, we first arcsine-square root transformed the data (which was estimated as the proportion of eggs fertilised). We analysed the data as an unreplicated block design where run was a random factor and exposure history was a fixed factor.

Experiment 2: does the presence of recruits affect settlement?

We were interested in whether the presence of heterospecific and homospecific recruits affected the settlement behaviour of both species. For each species, at the 14 h mark after fertilisation, we gently pipetted 40 larvae into new Petri dishes. We allowed them to settle (until 24 h mark) and then gently washed off any unattached larvae. We then introduced 40 homospecific or heterospecific larvae (depending on the treatment) from a new fertilisation event and counted how many of these new larvae had attached after 24 h. In these experiments, Petri dish was the unit of replication. The experiments using still water were the only reliable way to prevent the larvae from quitting the system and to quantify settlement rates of a controlled larval pool.

We examined the effect on settlement of pre-established recruits in all possible combinations: the effect of *S. plicata* recruits on *M. squamiger* settlement, of *M. squamiger* recruits on *S. plicata* settlement, of *M. squamiger* recruits on *M. squamiger* settlement and, finally, the effect of *S. plicata* recruits on *S. plicata* settlement (Table 1). In all of these experiments, we compared settlement in treatments consisting of Petri dishes with recruits to settlement in controls consisting of Petri dishes without pre-established settlers and we used the same number of control and treatment replicates. The number of runs and replicates, as well as the initial recruit densities in the treatment dishes, are listed in Table 1.

Because settlement was measured as the proportion of larvae that settled, we first arcsine-square root transformed the data. We analysed the effect of the presence of heterospecific recruits on settlement using a two-way, mixed model analysis of variance (ANOVA) where the experimental treatment was a fixed factor and experimental run was a random factor. When we examined the effect of *M. squamiger* recruits on *S. plicata* settlement, we found no interaction between run and treatment and, given that run explained little variance and was of no biological interest, it was omitted from the final model (Quinn and Keough 2002). For the effect of homospecific recruits for each species (one run only), we used a *t*-test to compare the experimental treatment with the control.

Experiment 3: does the presence of heterospecific recruits affect post-metamorphic performance?

We were interested in whether the presence of heterospecific recruits affected the subsequent performance of our two focal species. Thus we settled *M. squamiger* in the presence of *S. plicata* recruits and settled *S. plicata* in the presence of *M. squamiger* as described above. Controls consisted of Petri dishes in which larvae were settled in the absence of any pre-established recruits. We used eight replicates (i.e. Petri dishes) each per treatment and control for each species. The mean initial density of recruits in the *M. squamiger* experiment did not differ among treatments [mixed treatment mean was 16.625 (SD = 2.615) and the control was 19.375 (SD = 3.701); *t*-test, $t = -1.716$, $n = 8$, $P = 0.108$], and the same was found for the *S. plicata* experiment [mixed treatment mean was 20.375 (SD = 8.105) and the control was 14.5 (SD = 4.276); *t*-test, $t = 1.813$, $n = 8$, $P = 0.098$]. We marked all the settler positions in the Petri dishes, numbering them on the surface of the dishes using a pencil. We then drilled an 8 mm hole in the centre of each Petri dish. The dishes were transported to the field within ~45 min, in 20 l insulated containers. We attached the Petri dishes to a Perspex backing plate (500 × 500 × 8 mm) using stainless steel screws. The Petri dish positions were randomly assigned. We then hung the plates from the most external pontoon of the Manly harbour at a depth of 2 m (the dock floated at water level regardless of tide), facing down to reduce the effects of light and sedimentation (following Marshall et al. 2003a). For the experiment examining the effect of *S. plicata* recruits on the post-metamorphic performance of *M. squamiger*, we measured the survival of the *M. squamiger* settlers 1, 2, 5 and 10 weeks after being deployed into the field. We assessed survival as presence/absence of previously marked settlers on the Petri dish, a measure that is likely to reflect survival as reattachment to surfaces following removal is rare in ascidians (but see Edlund and Koehl 1998; Bullard et al. 2007). During each census of survival, we brought the Petri dishes back to the laboratory, assessed survival and removed any additional organisms that had settled in the intervening period. We also measured the size of recruits after 2, 5 and 10 weeks in the field by taking digital photographs of the diameter of the settlers with a camera attached to the dissecting microscope and connected to a computer. We subsequently measured the photographs using Image Pro (v. 5.1.0.12, Media Cybernetics; http://www.mediacy.com/) and we calibrated the measurements by taking a photograph using the haemocytometer grid.

For the experiment examining the effect of *M. squamiger* recruits on the post-metamorphic performance of *S. plicata*, we assessed survival only 1, 2 and 4 weeks after deploying the settlers in the field. This last experiment had to be halted after 4 weeks because the settlement plates were vandalised.

To analyse the survival and growth data, we used a repeated measures ANOVA where Petri dish was the unit of replication. Because survival was measured in proportions, we used arcsine-square-root-transformed data.

Table 1 Experimental treatments used to evaluate the effect on settlement of pre-established recruits using all combinations of *Styela plicata* and *Microcosmus squamiger* larvae and settlers

Treatment	Run	Number of replicates	Mean number of initial recruits	SD
S. plicata on *M. squamiger*	1	8	10.375	1.179
	2	12	18	1.243
M. squamiger on *M. squamiger*	1	12	14.667	1.437
M. squamiger on *S. plicata*	1	8	12.750	2.455
	2	4	13.5	2.255
S. plicata on *S. plicata*	1	4	20.25	3.351

SD standard deviation

Results

Experiment 1: does the presence of heterospecific sperm from an invasive reduce fertilisation success in a native?

Although the random factor run (=individual) was significant, reflecting differences in fertilisation rates among individuals, there was no significant effect of heterospecific sperm on the fertilisation success of the native species at either sperm concentration (Table 2), nor was there any trend for a negative or positive effect.

Experiment 2: does the presence of recruits affect settlement?

There was a strong effect of *S. plicata* recruits on the settlement of *M. squamiger* (Fig. 1a). Table 3 shows that there was a strong interaction between experimental run and the treatment of interest. Because the denominator for the F ratio to test the main effect is the $\mathrm{MS}_{\mathrm{interaction}}$, the *P* value for the main effect was not statistically significant. However, the direction of the effect of *S. plicata* recruits on *M. squamiger* settlement was consistently negative. The significant interaction was due simply to the size of this effect: in run 1, *S. plicata* had a ~3-fold reduction on *M. squamiger* settlement but in run 2, the effect was only a ~2-fold reduction. In contrast, the presence of conspecific recruits had no effect on the settlement of *M. squamiger* (*t*-test, $t = 0.425$, $n = 24$, $P = 0.675$; Fig. 1a).

S. plicata settlement was lower in the presence of *M. squamiger* recruits and the size of the effect was more consistent among experimental runs (Table 3; Fig. 1b). The non-significant interaction term allowed us to test a reduced model in which both treatment and run proved highly significant. Again, we found no effect of homospecific recruits on *S. plicata* settlement (*t*-test, $t = 0.159$, $n = 8$, $P = 0.879$; Fig. 1b).

Table 2 Analysis of variance (ANOVA) examining the effect on fertilisation success of pre-exposing *M. squamiger* eggs to *S. plicata* sperm

Source	*df*	MS	*F*	*P*
Experimental run	2	0.083	16.44	0.012*
Heterospecific sperm	2	<0.001	0.07	0.931
Error	4	0.005		

Note that the model is reduced after testing for a non-significant interaction between run and the treatment of interest

* $P < 0.05$

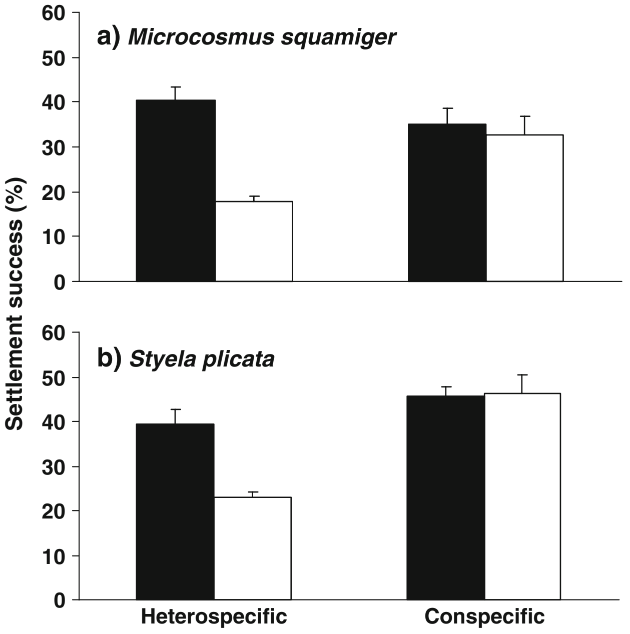

Fig. 1 Experiment 2: effect of *Styela plicata* and *Microcosmus squamiger* recruits on the settlement success of **a** *M. squamiger* and **b** *S. plicata*, pooling data from all runs. *Shaded bars* indicate controls (no established recruits), *open bars* established recruits, +SE

Table 3 ANOVA examining the effect of settled heterospecific recruits on the settlement of both *M. squamiger* and *S. plicata* larvae

Source	*df*	MS	*F*	*P*
Effect of *S. plicata* on *M. squamiger*				
Treatment	1	0.741	6.55	0.237
Experimental run	1	0.011	1.04	0.313
Treatment × experimental run	1	0.113	11.18	0.002*
Error	36	0.010		
Effect of *M. squamiger* on *S. plicata* settlement				
Treatment	1	0.212	17.79	<0.001*
Experimental run	1	0.098	8.25	0.009*
Error	21	0.012		

* $P < 0.05$

Experiment 3: does the presence of heterospecific recruits affect post-metamorphic performance?

The proportion of *M. squamiger* recruits surviving in the field decreased over time. The presence of *S. plicata* had a strong negative effect on the subsequent survival of *M. squamiger* in the field (Fig. 2a). After 10 weeks in the field, the mean proportion of *M. squamiger* that had survived was ~33% in the absence of *S. plicata* but was <5% in the presence of *S. plicata*. This difference in survival appeared to be driven by the initial responses of the two treatments; there were large differences in survival after the first week, and they persisted through time (Table 4).

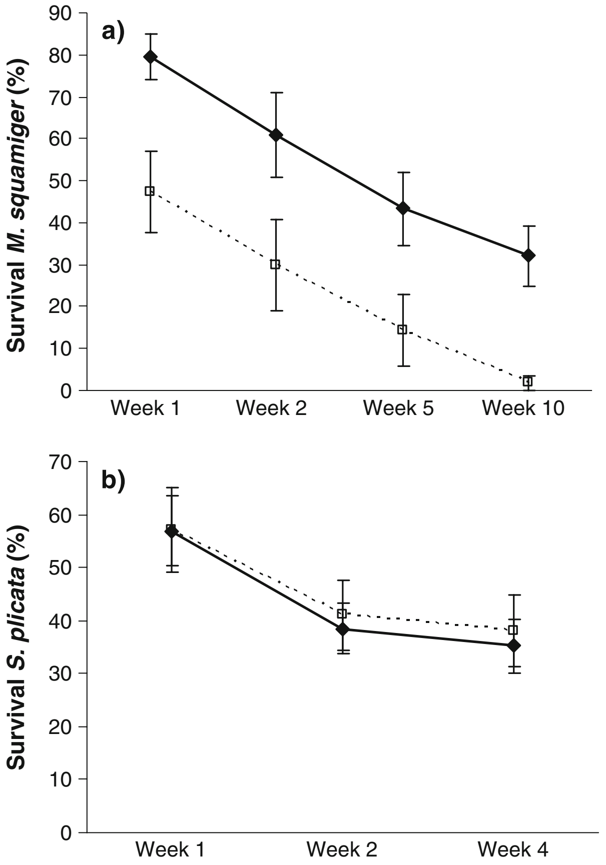

Fig. 2 Experiment 3: impact of heterospecific recruits on post-metamorphic survival (mean + SE) in the field of **a** *M. squamiger* and **b** *S. plicata*, in the presence of heterospecific recruits (*dotted line with squares* mixed), and in their absence (*solid line with diamonds* control)

Table 4 Repeated measures ANOVA examining the effect of the presence of one species on the survival of the other in the field

Source	*df*	MS	*F*	*P*
Effect of *S. plicata* on *M. squamiger*				
Between subjects				
Treatment	1	3.683	14.70	0.002*
Error	13	0.250		
Within subjects				
Time	3	1.137	34.69	<0.001*
Time × treatment	3	0.032	0.97	0.417
Error	39	0.033		
Effect of *M. squamiger* on *S. plicata*				
Between subjects				
Treatment	1	0.005	0.05	0.823
Error	14	0.088		
Within subjects				
Time	2	0.217	20.48	<0.001*
Time × treatment	2	0.001	0.098	0.907
Error	28	0.011		

* $P < 0.05$

In contrast to the effect of *S. plicata* on *M. squamiger*, the presence of *M. squamiger* had no effect on the subsequent survival of *S. plicata* after 4 weeks in the field (Table 4; Fig. 2b).

It was impossible to photograph all *M. squamiger* recruits from the Petri dishes, owing to the fact that some had settled in the corner of the dish and thus reliable measurements with photographs were not possible. However, a large proportion of individuals were successfully photographed (2nd week: mixed 72.72%, control 50.53%; 5th week: mixed 66.66%, control 93.85%; and 10th week: mixed 100%, control 83.33%). In the second week of the experiment, the *M. squamiger* recruits in presence of *S. plicata* were significantly smaller than those in the controls but this difference disappeared after 5 weeks (Table 5; Fig. 3). After 10 weeks, no statistical comparisons were possible as there was only one remaining *M. squamiger* recruit in the mixed treatment.

Discussion

The presence of the invasive ascidian *Styela plicata* affected a number of crucial life-history stages in the native ascidian *Microcosmus squamiger* and, overall, a combination of lethal and non-lethal effects of the invasive may synergise to exclude *M. squamiger* from its native habitat. These results further expand our understanding of how sublethal effects of invasive organisms affect natives, and reaffirm the importance of such effects during early life-history stages.

We found no effect of *S. plicata* sperm on the fertilisation success of *M. squamiger* eggs. In previous studies (Lambert 2000, 2001), homologous and heterologous sperm were mixed, while in our experiment we washed the eggs before exposure to homologous (*M. squamiger*) sperm. In this way we excluded the possible negative effects of sperm competition. As a result, we restricted our observation to whether or not exposure to the sperm of the invasive was affecting fertility of the native eggs. In light of our results, we found that *S. plicata* neither activate *M. squamiger* eggs nor interfere with subsequent egg activation. The lack of interference of *S. plicata* on fertilisation of *M. squamiger* eggs may be because the two species are not closely related and thus sperm recognition proteins are highly divergent. Alternatively, given that these species live sympatrically, there may have been a strong positive selection on sperm-egg recognition proteins to reduce costly hybridisation (Byrd and Lambert 2000; Veen et al. 2001; Harper and Hart 2005). It would be interesting to repeat our experiments in populations that are not sympatric but in our populations it appears that the invasive species does not interfere with the fertilisation success of the

Table 5 Repeated measures ANOVA examining the effect of the presence of *S. plicata* on the size of the *M. squamiger* in the field

Source	*df*	MS	*F*	*P*
Treatment	1	5.65	2.79	0.1336
Error	8	2.03		
Within subjects				
Time	1	281.31	179.86	<0.0001*
Time × treatment	1	9.57	6.12	0.0385*
Error	8	1.56		

* $P < 0.05$

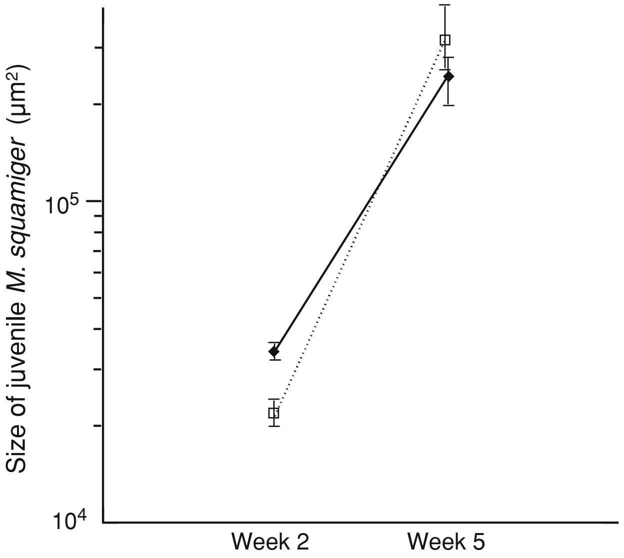

Fig. 3 Experiment 3: size of *M. squamiger* juveniles (mean ± SE) after 2 and 5 weeks in the field, in the presence of *S. plicata* (*dotted line with square* mixed), and in their absence (*solid line with diamond* control). Note the log scale on the *y*-axis

native species. In contrast, the effects of the invasive on the post-fertilisation performance of the native species were more dramatic.

Inhibition of settlement by superior competitors has been demonstrated in a number of marine invertebrates (e.g. Grosberg 1981; Young and Svane 1989; Davis et al. 1991) but its prevalence remains in debate (Bullard et al. 2004). In our system, both species avoided settling in the presence of the other but only one species had a significant, negative effect on post-metamorphic performance. The reason for the negative effect of *M. squamiger* on *S. plicata* settlement remains unclear, but may be due to a general avoidance response of ascidian larvae (e.g. Stoner 1994). Regardless, the effect of each species on settlement of the other suggests that species recognition at settlement is acting in these two species, even if *S. plicata* seems to be a relatively recent introduction to Australian waters (Wyatt et al. 2005).

The inhibition of settlement of native larvae in the presence of the exotic is analogous to the disruption of dispersal syndromes in plants whereby the presence of an invasive species reduces the effective dispersal of native propagules. However, in our study, the effect of inhibiting settlement may have a number of additional, potentially dramatic consequences (Elkin and Marshall 2007). Inhibiting settlement essentially forces larvae to continue to search for alternative suitable habitat and this increase in searching time carries a number of direct and indirect costs. Mortality while dispersing in the water column can be extremely high and thus any native larvae that are inhibited from settling by invasive recruits may experience higher rates of mortality than they would in the absence of the invasive (Morgan 1995). Furthermore, in species with non-feeding larvae such as the ascidians and other marine organisms, increasing the duration of the larval phase can result in reduced performance after metamorphosis—larval swimming is costly and reduces the level of reserves available for post-metamorphic survival and growth (Wendt 1998; Maldonado and Young 1999; Marshall et al. 2003b; Pechenik 2006). Thus, the post-metamorphic performance of native settlers may be lower in places where the invasive species is more common and inhibits settlement. Overall then, the inhibition of native larval settlement by invasive recruits may negatively affect native populations in three ways: decrease settlement directly, increase planktonic mortality and decrease post-metamorphic performance. Previous work has shown that native species change their behaviour (and thus their distribution) in response to invasive predators (Trussell et al. 2002, 2003). Our findings suggest that competition from invasive species can also drive changes in the behaviour of native species.

The presence of *S. plicata* in the field increased the juvenile mortality of *M. squamiger* by 10-fold. In addition, we found a significantly reduced growth of *M. squamiger* in mixed treatments compared to the controls in the second week. This trend was not maintained in the following weeks, which is perhaps unsurprising as the densities of *M. squamiger* in the mixed treatments declined dramatically over those first weeks and high levels of variation among the few survivors prevented a meaningful comparison. Although the reason for the decreased survival and growth of the native in the presence of invasive needs to be further investigated, we consider that there are three (non-mutually exclusive) mechanisms for the negative effect of invasive species on the survival and growth of the native species: competition for food, allelopathy or indirect effects mediated by third species. We favour the first hypothesis, *S. plicata* may be a better competitor for food than *M. squamiger* and thus *M. squamiger* may have had higher mortality and reduced early growth due to starvation. Conversely, the presence of pre-established *M. squamiger* had no effect on post-metamorphic performance of *S. plicata*. Given that water flow rates were reasonably low at the study site, it is possible that a better competitor could deplete the local abundance of food in the boundary layer above the plates. Competition for space seems unlikely due to the small size of the recruits during the first weeks, and it might have been important only in the last weeks of the experiment when the animals have grown enough to physically interact. However, the most drastic reduction in survival and growth of the mixed treatments in comparison to the control treatments occurred in the first few weeks. It is interesting in the sense that, in the experiment in which we analysed the effect of *M. squamiger* recruits on *S. plicata* performance (and found no effect), the pre-established *M. squamiger* themselves experienced high mortalities (similar to those in the experiment with pre-established *S. plicata*, data not shown). In other words, the presence of *S. plicata* affected the survival of *M. squamiger* even if the recruits of the latter arrived before and were already in place.

While we believe that the most likely source of the effect of *S. plicata* on *M. squamiger* survival in the field was competition, we must also consider other potential explanations. Allelopathic effects of invasive species on natives have been found in some studies (Schenk 2006; Figueredo et al. 2007), and in our study the interaction of the two species might induce the production of waterborne allelopathic metabolites in the introduced species that could reduce both survival and growth of the native. An alternative mechanism for the negative effect of the invasive on the native species in the field is that there are indirect effects via a third organism. For instance, the presence of the invasive may increase predation on the native species but leave the invasive unaffected. While such a scenario does not explain the early differences in growth, it may still explain the differences in survival. In our experiments, the experimental plates were hanging from the pontoon, which excluded benthic predators, but fish could, for example, still access the experimental individuals. Although this scenario seems unlikely, carefully designed predator exclusion experiments that do not interfere with food supply would be necessary to rule it out. Regardless of the underlying direct or indirect mechanisms, our study joins a growing list showing that the presence of marine invasive species is likely to result in the reduced abundance of local biota (Bando 2006).

The effects of *S. plicata* on the settlement and survival of *M. squamiger* and the reciprocal effects of *M. squamiger* on *S. plicata* settlement have some interesting implications for the dynamics of invasion in this system. We suggest that the presence of the native incumbent inhibits invasion by *S. plicata*. However, if a disturbance clears space for *S. plicata* to settle, then they will outcompete any newly settled *M. squamiger* and furthermore will inhibit recolonisation by the native. We also found that the presence of *S. plicata* recruits did not reduce *S. plicata* settlement success suggesting that initial invasion will not interfere with further arrivals. Previous studies have shown that both disturbance and prior invasion facilitate further invasion (Crooks 2002; Altman and Whitlatch 2007); here we provide one potential mechanism for such an effect. While our results appear to be a classic case of a priority effect (sensu Almany 2003), interestingly, this effect is not mediated by resource limitation; there was ample space for larvae to settle (only ca. 0.01% of the Petri dish surface is occupied by pre-established settlers), they are simply inhibited from doing so. Whether propagule pressure can reach levels that overwhelm the ‘biotic resistance’ of the community associated to *M. squamiger* (e.g. Hollebone and Hay 2007) remains unclear but, at least initially, the presence of the native species appears to inhibit the invasion by the introduced species (Osman and Whitlatch 1995), which can have significant effects at different spatial scales (Stachowicz et al. 2002).

Overall, we found a mixture of lethal and non-lethal effects of the invasive species on the native species. These effects may lead to the invasive species outcompeting the native species whenever space becomes available. This study suggests that invasive species can have significant non-lethal and lethal effects on early life-history stages of native species in the marine environment. Further experiments comparing settlement success in the presence or absence of invader recruits in water flow devices (see Butman et al. 1988), as well as experiments assessing the interaction during adult phases will provide further understanding of the interactions between invasive and native sessile marine invertebrates.

Acknowledgments We are grateful to B. Galletly and A. Crean for assistance in the field. M.R. was supported by a travel grant from the Spanish “Ministerio de Educación y Ciencia” during his stay at the University of Queensland. This project was funded by projects DPO666147 of the Australian Research Council, and CTM2007-66635 and PIE 200730I026 of the Spanish Government. This work was carried out in accordance with the laws of Australia.

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