Mechanisms of biotic resistance across complex life cycles

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Summary

1. Biotic resistance is the ability of communities to inhibit the establishment, spread or impact of novel species. However, the interactions that underlie biotic resistance depend heavily on the contexts in which species interact. Consequently, studies of biotic resistance that consider single processes, patches, species or life-history stages may provide an incomplete picture of the capacity for communities to resist invasion.

2. Many organisms have multiphasic life cycles, where individuals can occupy distinct niches at different stages of the life history. Generally, studies of biotic resistance focus on interactions within a single life-history stage, and interactions at other life-history stages are overlooked. Here, we demonstrate that different mechanisms of biotic resistance occur across the life history and together limit the invasion success of an introduced marine invertebrate (*Ciona intestinalis*) in Northern California.

3. We tested the role of interactions (competition and predation) with the resident community in limiting the abundance of *Ciona* through experiments conducted on fertilization, larval survival, settlement, early postsettlement survival, and the survival of juveniles and adults.

4. Under some circumstances, *Ciona* became abundant in mid-successional stages and showed more rapid growth rates than a morphologically similar native species, *Ascidia ceratodes*. However, predators reduced *Ciona* abundance much more than that of *Ascidia* at several life stages. Furthermore, *Ciona* appeared to be a weaker competitor at the adult stage. Early life-history interactions with other sessile species at the fertilization, larval and recruit stages had modest to no effects on *Ciona* abundance.

5. The presence of biotic resistance mechanisms acting at multiple life stages, and potentially under different conditions, suggests that different components of biotic resistance interact to enhance the resident community's resistance to invasion.

Key-words: ascidian, epibenthic community, facilitation, larva, life histories, ontogenetic niche shift, predatory effects, species interaction

Introduction

The ability of a resident community to prevent or reduce the success of new colonizers is a crucial process influencing the distribution of biodiversity (Levine 2000; Byers & Noonburg 2003; Parker, Burkepile & Hay 2006; Pearson, Potter & Maron 2012). This process is known as biotic resistance and is characterized by negative interactions (such as competition and predation) between residents and the species attempting to colonize the community. Biotic resistance is rarely absolute and generally reduces the abundance, fecundity, reproductive output and/or spread of invasive species rather than completely preventing establishment (i.e. biotic containment rather than resistance sensu Levine, Adler & Yelenik 2004). The biotic resistance of a community therefore captures the cumulative effects of interactions between residents and

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colonizers at all stages of the invasion sequence and across multiple life-history stages.

Many organisms have complex life cycles, in which individuals undergo dramatic developmental transitions before reaching adulthood (Wilbur 1980). Accordingly, each life-history stage might be differentially susceptible to consumers, competitors and environmental variability (e.g. Boege & Marquis 2005). In addition, species interactions at one life stage can influence interactions at later stages (Osenberg, Mittelbach & Wainwright 1992; Vonesh 2005). Nonetheless, studies of biotic resistance are often restricted to interactions between residents and invaders at particular life-history stages (e.g. Hierro, Maron & Callaway 2005; Parker, Caudill & Hay 2007; but see Shea *et al.* 2005; Dangremond, Pardini & Knight 2010), thereby limiting our ability to accurately predict the probability of successful invasion.

Most studies of biotic resistance to date have focused on competition or predation among adults (e.g. Levine, Adler & Yelenik 2004; Parker, Burkepile & Hay 2006; Parker, Caudill & Hay 2007). The few studies that have investigated multiple mechanisms across life cycles have been in terrestrial plant systems and tend to find that competition is more intense at early stages, and herbivory at later stages (Levine, Adler & Yelenik 2004), although there are exceptions (Shea *et al.* 2005). It is therefore still unclear whether these mechanisms of biotic resistance operate similarly in other taxa and systems. For example, in marine invertebrates, it is the early life-history stages that are most vulnerable to predation (Osman & Whitlatch 2004), which leads to a very different prediction about the role of predation vs. competition across the ontogeny of an invader.

Variation in abundance at early life-history stages, whether the result of physical processes or biotic interactions, can leave lasting signatures that persist into the adult stages (Roughgarden, Iwasa & Baxter 1985; Levine 2000; Rius, Turon & Marshall 2009), even in the presence of counteracting processes later in life history (e.g. Levine 2000). Demographic models have been used as a way to reconcile how counteracting effects in different life-history stages influence population growth of invasive and native species (Shea & Kelly 1998; Parker 2000; Dangremond, Pardini & Knight 2010), usually based on observational data. Such longitudinal studies can enhance our understanding of the relative importance of biotic resistance mechanisms, but applying this approach might be hindered by logistical constraints in many systems. For instance, dispersal phases or small individuals can be intractable to manipulate or track in the field, while interactions among adults or sedentary stages might be equally difficult to assess under realistic conditions in the laboratory. Further, high mortality or low abundance at early life-history stages could lead to low power to detect effects at later life stages, even if they end up being important for final population size. A complementary approach to following a single cohort involves testing the effects of biotic resistance across the full life cycle using a series of independent experiments conducted on individuals collected from the same population at different life stages. While this approach makes comparisons of effects at different life stages difficult, it can narrow the focus of future efforts by identifying key stages where effects are relatively large and consistent. This may be particularly useful for species with life histories that make longitudinal studies intractable and for which demographic models are difficult to reliably parameterize.

Here, we examined how competition and predation at different life-history stages might combine to limit the invasion success of *Ciona intestinalis*, a marine invertebrate that has reached high abundances in many places outside its native distribution (Rius, Heasman & McQuaid 2011; Collin *et al.* 2013). Most experimental studies on invasion of marine communities have focused on the interactions (predation, disturbance, resource availability) that mediate the strength of competition during sessile stages (e.g. juveniles and adults; McDougall 1943; Osman 1977; Keough 1984; Stachowicz, Whitlatch & Osman 1999; Grey 2011). We broaden this approach to include all planktonic and benthic stages to examine how interactions throughout the life history combine to affect invasion success. To achieve this, we used an epibenthic system in Northern California, USA, into which *Ciona* has established and competes with an ecologically, developmentally and morphologically similar native, solitary ascidian – *Ascidia ceratodes*. This species is the dominant late-successional space-occupier in this system (Nydam & Stachowicz 2007; Edwards & Stachowicz 2011), and thus, we also examine how this resident species responds to potential limiting factors across the life history. Specifically, we quantify (i) growth rate of *Ciona* and *Ascidia*, (ii) the effects of competition between *Ciona*, *Ascidia* and other relevant native sessile species at fertilization, larval, recruit, juvenile and adult stages and (iii) predation by mobile species on larvae, new recruits and juvenile stages of *Ciona* and *Ascidia*. We expected that predation would be important in early life-history stages, as seen in other similar systems (Osman & Whitlatch 2004), but that such effects would decrease as individuals approached adulthood and reached a size refuge from predators. We also expected competition to reduce invader abundance at early stages (e.g. Levine, Adler & Yelenik 2004; Rius, Turon & Marshall 2009), but also at later stages due to space limitations and the resulting thinning (e.g. Guiñez & Castilla 2001). We compared the results of experiments at each life stage to identify the mechanisms that most likely result in the studied introduced species failing to achieve high biomass.

Materials and methods

STUDY SITE AND SPECIES

All experiments were conducted at the Bodega Marine Laboratory (BML) and the adjacent Bodega Harbour (38º19′20·16″N, 123º03′12·56″W). The organisms used for experiments were collected at

Spud Point Marina, the largest of three marinas in this 5 km² harbour. All field experiments were conducted at the same site. Solitary ascidians are often the dominant late-successional species in these systems (Jackson 1977; Nydam & Stachowicz 2007). In our system, this functional group is represented by two species, the introduced *C. intestinalis* and the native *A. ceratodes*, that are similar in gross morphology and reach a similar size as adults (*c.* 10–15 cm; see further details in Appendix S1, Supporting information). Common early successional species in these systems include *Botrylloides* spp. (colonial ascidians) and *Bugula neritina* (bryozoan; see details in Appendix S1, Supporting information). These species are all sessile postmetamorphosis and have free-swimming lecithotrophic larvae, which, under natural conditions, settle within hours to days of release. Although *Ascidia* and *Ciona* co-occur in Bodega Harbour, *Ciona* fails to produce the monospecific aggregations and associated ecological impact characteristic of its invasion in other geographical regions.

GROWTH RATES OF *CIONA* AND *ASCIDIA* IN THE ABSENCE OF PREDATORS

Larvae of the solitary ascidians *Ciona* and *Ascidia* were allowed to settle (see specific methods in Appendix S2, Supporting information) on separate circular plates and then grown in the field in the absence of competitors and predators to provide a baseline expectation for how each species would perform in the abiotic conditions of Bodega Harbour. These plates were made by cutting Petri dish lids (1 mm thickness, 50 mm diameter) to fit within the diameter of the Petri dish. After 24 h, all the settler positions were marked using a graphite pencil and the density of settlers equalized among replicates ($N = 10$ per dish and species). The lids were then attached at randomly assigned positions onto perspex backing panels (650 × 650 × 5 mm) with stainless steel bolts passed through a 6 mm hole drilled in the centre of the lid. The backing panels were protected by fine mesh (1 mm²) to restrict the access of potential predators (see Predation by Resident Species on Early Life Stages, below). However, the mesh did not prevent the settlement of other sessile organisms. This allowed for the assessment of growth in the context of natural settlement during the monitoring period. The caged panels were deployed from a floating dock at a depth of 2 m with the dishes facing downwards to reduce light and sedimentation. Growth was surveyed by taking photographs of the plates and then measuring individuals using the program ImageJ 1.45s (Wayne Rasband, National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij). The size of between 24 and 58 individuals of each species was measured after 5, 8, 10 and 14 weeks.

In addition to documenting growth rates of each species separately, we also recorded the relative abundance of *Ciona* and the morphologically similar *Ascidia* on unmanipulated artificial substrates throughout succession. Successional dynamics were tracked using 650 × 650 × 4 mm perspex panels ($N = 6$) deployed in the field in August 2011, a time of year when both species are known to settle (Nydam & Stachowicz 2007). The panels were randomly positioned on the western and eastern sides of the docks at a depth of 2 m and were positioned facing downwards to reduce the effects of light and sedimentation. Pictures of the panels were taken once the animals attained a visible size for which % cover measurements could be performed using image analysis (10, 12, 14, 16, 20, 23 and 35 weeks after deployment). Per cent cover was estimated using ImageJ. Long-term surveys

from previously collected data indicated that % cover of both *Ciona* and *Ascidia* was highest during fall and winter and that *Ascidia* achieved consistently higher abundance in late-successional communities (Fig. S1, Supporting information).

The remainder of the methods focuses on experimentally testing hypotheses for how interactions with native species at various life stages limit *Ciona* abundance. We organize these by the effect of resident species on *Ciona* at the different life stages shown in Fig. 1, starting with earliest life-history stages. In each section, we reference this figure, along with a letter that refers to the specific life stage(s) under consideration. To provide a means to compare effect sizes across the different potential biotic resistance mechanisms, we calculated a standardized effect size, Hedges' *g* (Borenstein *et al.* 2009) for each experiment. This allows comparisons of the magnitude of the effects across experiments that differ in the units in which they were measured.

GENERAL LABORATORY METHODS

Large numbers of larvae from multiple species were required simultaneously to run the majority of the experiments (Fig. 1b–g). This required inducing artificial spawning (see specific spawning methods in Appendix S3, Supporting information) rather than simply collecting larvae in the field. The small size and mobile nature of early life stages limited field experiments to postsettlement processes. Reproductively mature adults were collected between June and November 2011, encompassing the timing of peak recruitment for most species (Nydam & Stachowicz 2007). The collected organisms were transported in insulated con-
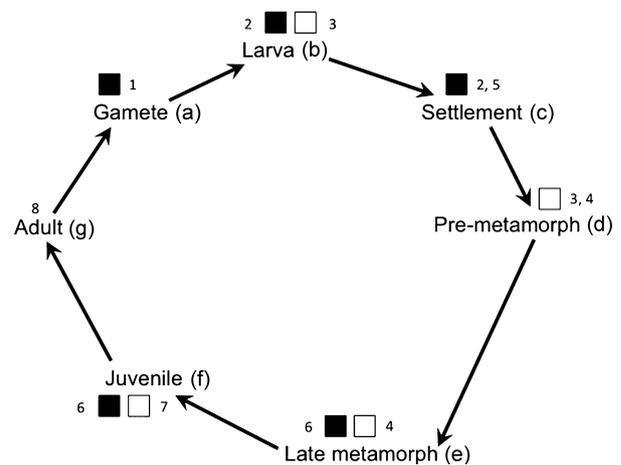

Fig. 1. Life cycle of the focal species (*Ciona intestinalis*) indicating the experiments performed to test the effects of resident species at a range of life-history stages. Filled boxes indicate analysis of interactions between *Ciona* and other space-holding sessile species, and open boxes when predatory effects were tested. Letters between brackets (a–g) refer to the different life-history stages, while numbers (1–7) refer to the experiments detailed below. 1. Gamete interaction (L); 2. Larval–larval or larval–recruit interactions (L); 3. Laboratory predatory experiments (L); 4. Predatory exclusion during early life-history stages (F); 5. Settlement preferences (L); 6. Postmetamorphic performance in presence of a colonial form (F); 7. Juvenile predatory experiments (F); 8. Adult survival and growth – % cover of adults and growth rates through time (F). Abbreviations indicate laboratory-based (L) or field-based (F) experiments.

tainers to the BML (*c.* 5 min journey) and kept in a tank with 20 L of constantly aerated sea water at a constant temperature of 16 °C. All manipulations and experiments were undertaken using filtered sea water obtained from the BML.

EFFECT OF *ASCIDIA* ON *CIONA* FERTILIZATION SUCCESS

Direct observations of *Ascidia* spawning at Spud Point showed that spawning can be synchronous and that gametes are released in large quantities. To test whether sperm interference or pre-emption by *Ascidia* could reduce the fertilization success of *Ciona* eggs (Fig. 1a), the approach used by Lambert (2000) was followed with some modifications (see details in Appendix S4, Supporting information). In brief, three treatments were used: (i) *Ciona* eggs exposed to *Ascidia* sperm first and then exposed to *Ciona* sperm, (ii) *Ciona* eggs only exposed to *Ciona* sperm, (iii) *Ciona* eggs unexposed to any sperm. The eggs and sperm of *Ciona* in treatments that involved both *Ascidia* sperm and no *Ascidia* sperm were of the same age at the time they were mixed. Cleavage in no sperm treatments was rare, but if it did occur, the run was terminated. Egg cleavage began *c.* 1 h after fertilization and as soon as the first four-cell cleavage stage was detected the proportion of cleaved eggs for each replicate was counted in order to determine fertilization rates. This experiment was repeated in three runs, and the % egg cleavage data (subject to logit transformation, Warton & Hui 2011) were analysed with exposure history considered as a fixed factor and run as a random factor in a mixed-model analysis of variance (ANOVA). This and all further statistical analyses were performed using R v.2.15 (R Development Core Team 2011).

EFFECTS OF HETEROSPECIFICS ON SETTLEMENT

Larvae of *Ciona* settled predominantly on downwards facing surfaces (see Results; methods in Appendix S5, Supporting information; Fig. 1b,c). To assess whether the presence of co-occurring heterospecific larvae affected settlement of *Ciona*, a combined additive and replacement series design was used. Two potential competitors with similar settlement preferences, namely *Ascidia* and *Bugula neritina*, were included. Single species treatments occurred at a range of densities: 8, 12 or 24 larvae per settlement plate; treatments with two species had 12 individuals of each species, and the full polyculture had eight individuals of each species. All possible combinations of densities and species were used, up to a maximum of 24 total individual larvae per replicate. Settlement success was measured after 24 h.

The effect of the presence of pre-existing con- and heterospecific settlers on settlement of larvae was examined using a similar array of species combinations in a replacement design. To produce these treatments, larvae were allowed 24 h to settle in all possible combinations of 1, 2 or 3 species. Any unattached larvae were then gently washed off. The number of larvae added to each dish was 48 for monospecific treatments, and 24 and 16 larvae per species for treatments involving 2 and 3 species, respectively. Since the density of settlers varied among replicates, they were thinned to ensure that total settler density did not initially differ among treatments. To these treatments, 24 larvae from a new fertilization/spawning event of the focal species were added creating conspecific or heterospecific interactions between larvae and 1-day-old settlers from which settlement success was measured

24 h later. This experiment was run three times, each time with a different one of the three studied species (*Ascidia*, *Ciona* and *Bugula*) as the focal. Four replicates per treatment were used in all experiments.

PREDATION BY RESIDENT SPECIES ON EARLY LIFE STAGES

Preliminary field outplants of uncaged recruits indicated massive mortality of solitary ascidians (near 100%), but virtually no mortality of colonial forms (i.e. *Bugula* and *Botrylloides*. Field and laboratory predator-exclusion experiments (Fig. 1b–d) were used to test whether predators might be responsible for the high mortality of solitary ascidians.

For the first experiment, larvae of *Ciona* were allowed to settle on Petri dish lids separately and after 24 h, settler density was equalized among replicates and treatments. A single backing panel was used on which half of the plates were protected by fine mesh (1 mm^2) while the other half remained unprotected. Five replicates per treatment were placed randomly within the caged and noncaged sections of the backing panel. The survival of *Ciona* recruits was tracked for 3 weeks in the field. Since the caged and uncaged portions of the backing panel were adjacent to one another, panels under the cage were not expected to be subject to any other differences in environmental conditions except those caused by the cage. Plates were not individually caged and randomly interspersed as this would have created artefacts in which uncaged plates placed between two caged plates would have experienced at least partial exclusion of predators. Furthermore, individual plates were small enough that cages on each plate would have abraded some of the plate area, potentially injuring new recruits. As there is strong spatial variation in recruitment of potential competitors on substrates separated by <1 m (Edwards & Stachowicz 2011), which could affect mortality rates independent of predator treatment, individual plates were spaced *c.* 30 mm apart. As a comparison, we conducted an identical experiment with *Ascidia*.

Solitary ascidian recruits only survived within cages (see Results), and the high density of the caprellid amphipod, *Caprella mutica*, at the field site (up to 300 individuals per m^2, see Results) was suspected as being responsible for preying upon recruits in uncaged treatments. Larvae, 1-day-old recruits and 2-week recruits of *Ciona* were enclosed with two caprellids (maximum body length 10–15 mm) in a Petri dish (51 mm in diameter) with sea water and a lid (as above) for 24 h. To test larval predatory effects, 48 newly hatched larvae were pipetted into each Petri dish and both the number of larvae remaining and the number of settlers, which together provided a measure of total survival, were recorded. For 1-day-old recruits, the same methods as above were used to equalize starting density across treatments [mean initial settler density ± SE (total area = 6·17 cm^2); control = 15·4 ± 1·860, caprellid = 17 ± 1·304] and record settler survival. The 2-week-old recruits were obtained by growing 30 laboratory-settled individuals in mesh cages in the field. In addition, 24 larvae of each species were simultaneously offered to caprellids and consumption rates assessed. For all feeding experiments, survival after 1-day exposure to caprellids in the laboratory was compared with a no-predator control. An identical experiment was conducted for *Ascidia*. Abundance of *C. mutica* in the field was measured by deploying five PVC plates every 2 or 3 months for the time

period from May 2009 until September 2010, and these data were compared with seasonal patterns of *Ciona* abundance (see details in Fig. S1a, Supporting information) to assess potential seasonal variation in predation.

EARLY POSTSETTLEMENT COMPETITION

Although tests of competitive interactions between colonial ascidians or bryozoans and *Ascidia* at later life stages show that *Ascidia* is the competitive dominant (Edwards & Stachowicz 2011), competitive reversals could occur early in the life history when colonial ascidians are much larger than solitaries, leading to the reduced success of solitary ascidians (Fig. 1d–f). This possibility was tested with the largest colonial species in our system, *Botrylloides* and recruits of *Ciona*. To obtain *Botrylloides* settlers, preroughened polycarbonate sheets (2 mm thickness) were hung from backing panels in the field as outlined above. After a few days in the field, recruits attached themselves to the sheet. Pieces of the sheet that contained a single *Botrylloides* colony were then cut, and three of these pieces were glued to experimental surfaces using super-glue. These experimental surfaces (circular plates as above) were left in aerated sea water for 24 h to ensure that any deleterious chemicals from the glue had vanished. Following this, 48 larvae of the two solitary ascidians were placed into Petri dishes with lids either containing *Botrylloides* or not. Twenty-four hours later, the number of settlers on the lids was counted, then adjusted to equal densities and transported to the field. Settlement was adjusted as above (mean *N* individuals per plate ± SE, 31·6 ± 1·030, 38·4 ± 2·561; *Botrylloides* and Control treatment, respectively; *t*-test, $P > 0·05$). The same backing panels and mesh protection stated above were used to exclude predators. Survival of solitary species was assessed at week 2, 5, 8, 10 and 14. Organisms that had settled in the intervening period were not removed. Survival data (logit transformed) were tested using repeated-measures ANOVA where Petri dish was the unit of replication and treatment a fixed factor. The same experiment was conducted for *Ascidia*.

EFFECT OF PREDATORS ON JUVENILE SURVIVAL

To test whether older and larger *Ciona* and *Botrylloides* would be affected differently by predation in the field (Fig. 1f), cages from the Early Postsettlement Competition experiments were removed after 14 weeks and survival followed over the next 3 weeks. By this point, *Ciona* had reached length sizes of around 50 mm (see Results). The initial number of *Ciona* individuals per plate was 38 ± 5·5 and 31 ± 10.1 (mean ± SE) for treatments with and without *Botrylloides*, respectively.

Results

TEMPORAL FIELD ABUNDANCE PATTERNS AND GROWTH RATES

Across 6 years of monitoring, *Ascidia* was the dominant member of the epifaunal community. In both long-term panels (Fig. S1a, Supporting information) and single-year colonization sequences (Fig. S1b, Supporting information), *Ascidia* dominated late-successional stages (Fig. 1f, g). *Ciona*, when present, was never a dominant member of the late-successional community. More frequent and

detailed sampling of panels within a single year showed that *Ciona* can reach high abundance earlier in succession while *Ascidia* cover is still low, but that *Ascidia* eventually dominates (Fig. 2a). Among individuals that settled at the same place and time, *Ciona* grew much faster than *Ascidia*, generally being an order of magnitude larger over the first 14 weeks of life (Fig. 2b), despite similar initial offspring size (authors' pers. obs.).

PRESETTLEMENT PROCESSES

The presence of *Ascidia* sperm (Fig. 1a) reduced the fertilization success of *Ciona*; however, this effect varied
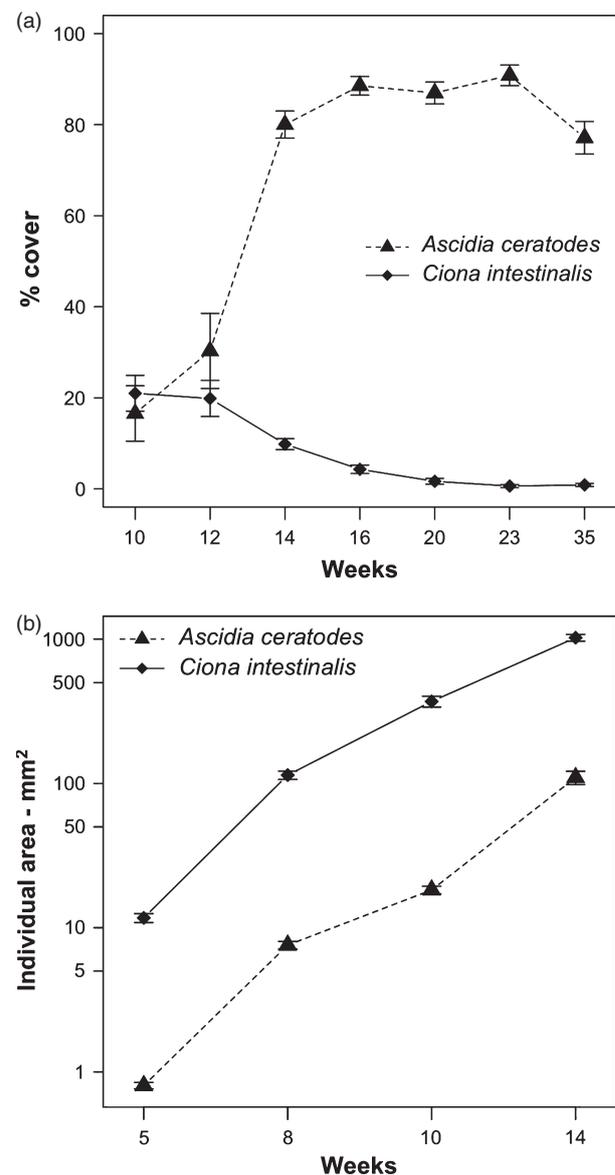
Fig. 2. Field abundance and size of two similar solitary ascidians: one introduced *Ciona* and one native *Ascidia*. (a) Colonization experiment showing mean % cover of each species over time since substrate was deployed; (b) mean size (note the logarithmic *y*-axis) measured as the total area occupied by each individual. Vertical bars denote standard error.

among experimental runs (run × treatment, $F_{4,72} = 6.144$, $P < 0.001$, Table S1, Supporting information. In the first run, the presence of *Ascidia* sperm reduced the fraction of *Ciona* eggs exhibiting cleavage by 35%, but in run 2 and 3, the effect was only 14% and 2%, respectively.

SETTLEMENT EXPERIMENTS

Ciona exhibited significant settlement preferences (loglinear models; log-likelihood = −63.700, LR $\chi^2 = 119.498$, d.f. = 2, $P < 0.001$) and settled predominantly on downwards facing surfaces (mean % settlement ± SE; *Ciona*, 60.417 ± 5.512). The remainder of larvae settled evenly between upwards facing and lateral surfaces. This settlement pattern was similar for other species tested (Appendix S6, Supporting information), indicating a potential for competition among larvae for settlement sites or among recent recruits for space. However, the initial larval or recruit assemblage composition (Fig. 1b,c) had no effect on the settlement success of *Ciona*, or the other species (one-way ANOVA for each species; $P > 0.187$ for all species).

EARLY POSTSETTLEMENT COMPETITION

The colonial ascidian *Botrylloides* did not affect the survival of *Ciona* (Figs. 1d–f and 3, Table S2, Supporting information). Although a marginal increase in survival of *Ciona* was detected when this species was with *Botrylloides* (Fig. 3), these differences were not statistically significant at α = 0.05 (Table S2, Supporting information). Survival of *Botrylloides* was 100% for all the surveyed weeks, except in week 8, in which a single *Botrylloides* died. *Botrylloides* also had no effect on *Ascidia* (see Appendix S6, Fig. S2, Supporting information).

EFFECTS OF PREDATION ON EARLY LIFE STAGES

Caging *Ciona* recruits dramatically increased survival relative to uncaged recruits, with 30–40% recruits surviving in cages and 0% in uncaged panels (Figs. 1d–f and 4).

Because all recruits outside cages died after 1 week, there was no variation among uncaged replicates and therefore statistics were unwarranted. We observed a similar effect of caging on *Ascidia* recruits (Fig. S3, Appendix S6, Table S3, Supporting information), although mortality was not as complete for *Ascidia* in uncaged treatments.

In the laboratory, caprellid amphipods reduced the survival of larvae (see Appendix S7, Supporting information) and 1-day-old recruits of *Ciona* (*t*-test; c. 70% mortality, $t = -3.333$, d.f. = 8, $P = 0.010$; Fig. 5). Survival of 2-week-old recruits was unaffected by caprellids (*t*-test; $t = -1.248$, d.f. = 8, $P = 0.247$; Fig. 5), but high variation among replicates in treatments, and an average 10% decline in abundance relative to controls, indicates that some caprellid individuals may be capable of consuming recruits of *Ciona*. Predation rates on *Ascidia* similarly declined with age (Appendix S7, Fig. S4, Supporting information), but *Ascidia* survival at the larval stage was twice that of *Ciona* (see further details in Appendix S7, Fig. S5, Supporting information). Caprellids were abundant seasonally during part, but not all of the time period that *C. intestinalis* was present in this system (Fig. 6). In particular, caprellids were abundant in the summer months, but absent fall through spring. *Ciona* was detected in our system during winter and spring as well as summer; thus, the effects of predation on *Ciona* abundance may be seasonally variable. *Ciona* adults were reproductively active from June until November (see also Nydam & Stachowicz 2007). The high variability among replicate samples in caprellid abundance indicates a possibility that predation may be spatially variable as well.

After 14 weeks, we removed the cages from the Early Postsettlement Competition experiments and all *Ciona* disappeared (while *Ascidia* showed almost no mortality, see Appendix S6). This 'experiment' lacked any control (no animals were left caged); however, there had been virtually no mortality within the cages between weeks 5 and 14 (see Fig. 3), and that there were no remnants of dead tunics present as would have been the case if they had died as a result of something other than consumption.
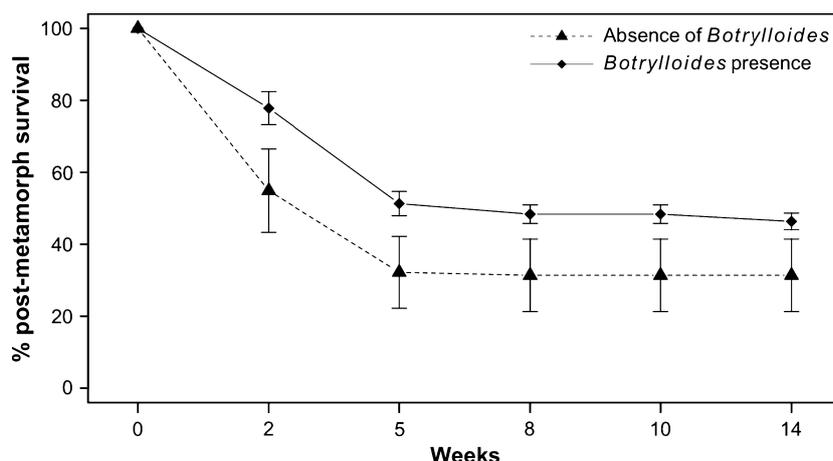

Fig. 3. Survival of recruits of *Ciona* in the presence or absence of recruits of *Botrylloides*, a potential early successional competitor. Vertical bars denote standard error.
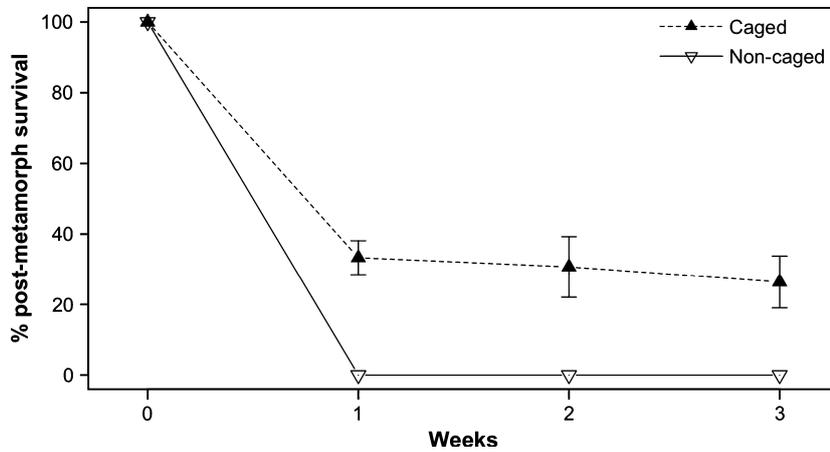
Fig. 4. Effect of predator removal (caging) on % survival of *Ciona* recruits in the field. Vertical bars denote standard error.
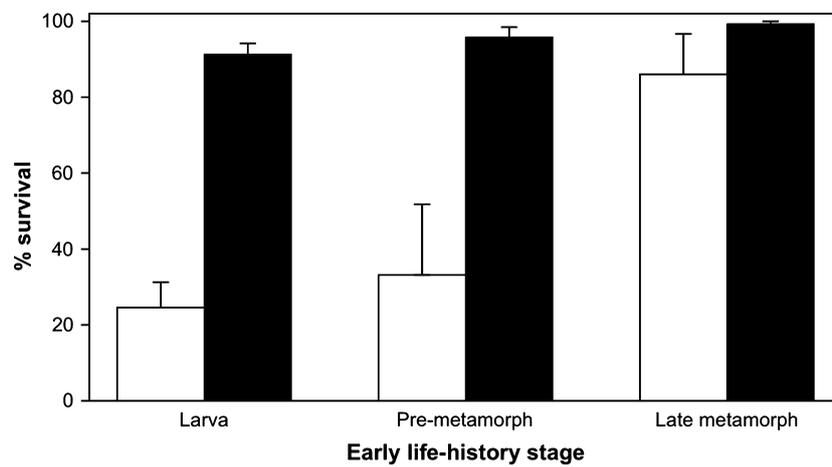

Fig. 5. Predation by caprellid amphipods on *Ciona* early life stages in the laboratory. Data show 24-h % survival of *Ciona* larvae, 1-day-old settlers and 2-week-old settlers (i.e. larva, premetamorph, late metamorph, respectively). Treatments that included caprellids are white bars and controls are in black. For the larval stage, % survival was calculated by adding attached settlers and both swimming and motionless larvae, but excluded unattached settlers, which were not considered viable. Vertical bars denote standard error.
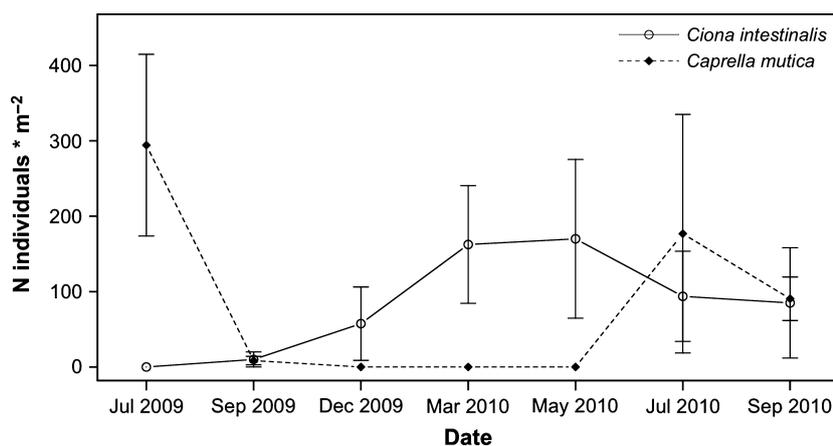

Fig. 6. Caprellid and *Ciona* abundance in the field for the time period of July 2009 until September 2010. Vertical bars denote standard error.

The effect sizes of all experiments are reported in Table 1. Because experiments were conducted in different arenas and for different lengths of time, we consider only general patterns of effect sizes among processes. The results showed that predation had a stronger negative effect on *Ciona* than competition across the life cycle.

Discussion

Our work highlights the importance of studying biotic resistance mechanisms across multiple life-history stages that together limit invasion success. *Ciona* had faster growth rates than *Ascidia* (Fig. 2b) and was abundant in

mid-successional assemblages (Fig. 2a); had we only looked at this stage, or used medium-term experiments, we might have concluded that *Ciona* would come to dominate our ecosystem. However, *Ciona* was more affected by predation across several life-history stages (Fig. 5, see Results for *Ascidia* in Fig. S4, Supporting information) and was never the late-successional dominant (Figs. 2a and S1, Supporting information). Biotic resistance effects occurred at several stages of the life history (Table 1), but the mechanisms varied with predation being important between larval and juvenile stages, while competition appeared most important during fertilization and the adult stage. Below we discuss these mechanisms and speculate why some regions may be more resistant to invasion by *Ciona*. Our findings suggest that research considering a single or few life-history stages can lead to incorrect predictions about the impact or success of an introduced species.

As early as the fertilization stage, *Ascidia* sperm potentially interferes with the fertilization of *Ciona* eggs. Heterologous sperm can induce glycosidase release in ascidian eggs, which produces an early block to polyspermy that reduces fertilization (Lambert 2000). So in areas where *Ascidia* are abundant and *Ascidia* spawn at the same time as *Ciona*, this process could reduce *Ciona* fertilization success. However, we found the magnitude of negative effect of *Ascidia* sperm on *Ciona* fertilization differed among runs, possibly due to intraspecific variation in the sensitivity to heterospecific sperm. Thus, the importance of prefertilization biotic resistance is difficult to assess in this instance.

Larval behaviour can reduce settlement near competitive dominants (Grosberg 1981; Rius, Turon & Marshall 2009), so it was surprising that *Ciona* larvae did not alter settlement behaviours in response to the presence of heterospecific larvae or settlers. However, the lack of shared co-evolutionary history between these species means that selection for such avoidance may only be recent. Alternatively, if *Ciona* occupies an earlier successional stage and can reach reproductive maturity prior to being excluded by other competitors, then such selection might be weak, explaining the lack of avoidance.

Predators had a large and consistent negative effect on *Ciona* during the larval to the juvenile stages. A surprising result was to find that caprellid amphipods, which are commonly known to be periphyton scrapers or suspension feeders (Caine 1977), actively preyed upon ascidian larvae and settlers in the laboratory. Caprellids occur at very high densities in the field at our site, and their exclusion resulted in large increases in survival of solitary ascidians. Mesopredators are known to prey upon newly settled ascidians in other regions (Osman & Whitlatch 1995), producing major community shifts that reduce the abundance of non-native species (Osman & Whitlatch 2004). Caprellids consumed the larvae and settlers of both species intensely, although they exhibited a preference for *Ciona* over *Ascidia* (see Appendix S7, Fig S5, Supporting information). Although our observations require further testing, it appeared that recent *Ciona* settlers are more weakly attached to the substrate than *Ascidia* (authors' pers. obs.), and thus might be more easily removed by caprellids. As several-month-old juveniles, only *Ciona* juveniles disappeared in the field when cages were removed, despite the fact that at this time, *Ciona* were larger than *Ascidia*. At this stage, predators such as crabs or fishes likely were responsible, and the greater thickness of *Ascidia* tunics relative to *Ciona* could play a role, although we cannot rule out a contribution from chemical or other defences. The general greater susceptibility of the non-native species to a suite of resident generalist predators seen here is consistent with findings for plant–herbivore interactions (Parker, Burkepile & Hay 2006). In our case, the fast growth rate of the exotic relative to the native suggests that predator susceptibility could be due to a growth vs. defence trade-off.

The high cover that solitary ascidians reached on some panels suggests that at least some individuals survive predation, perhaps due to spatial or temporal variation in predation or predator swamping. However, *Ciona* abundance gradually declined while *Ascidia* abundance increased and the latter appeared to out-compete the former. While we did not conduct *Ascidia* removal experiments to test this hypothesis explicitly, other such experiments identify *Ascidia* as a competitive dominant in this region (Nydam & Stachowicz 2007; Edwards & Stachowicz 2011). Although the mechanisms by which *Ascidia*

Table 1. Summary of the results divided by life stage and biotic resistance mechanism. The Hedges' *g* (±SE), which is an unbiased estimate of effect size (Borenstein *et al.* 2009), is provided for each experiment. Competitive interactions were tested among organisms at the same life-history stage

Life-history stage	Effect of resident species on *Ciona*	
	Competition	Predation
Gamete	Reduced fertilization ($g = 0.755 \pm 0.278$)	NT
Larva	No effect ($g = 0.185 \pm 0.616$)	Reduced survival ($g = 5.278 \pm 1.311$)
Settlement	No effect ($g = 0.594 \pm 0.633$)	Reduced ($g = 1.904 \pm 0.712$)
Premetamorph	NT	Reduced survival ($g = 2.766 \pm 0.504$)
Late metamorph	No effect ($g = 0.939 \pm 0.294$)[a]	No effect ($g = 0.713 \pm 0.593$)
Juvenile	No effect ($g = 0.939 \pm 0.294$)[a]	NT
Adult	Potentially reduced survival[b]	NT

NT, not tested.
[a]The results were obtained from the same experiment (Fig. 3) and analysed together.
[b]Not explicitly tested, but see both short- and long-term field observational patterns suggesting competitive exclusion of *Ciona* by *Ascidia* (Figs. 2a and S1b, Supporting information), despite faster growth of *Ciona* (Fig. 2b).

dominance is achieved are unknown, adults of *Ascidia* generally occupy a larger amount of primary space than *Ciona* because of their lateral attachment to the substrate at early life stages and thus they may be more difficult to displace. Another plausible explanation for the successional shift observed on long-term plates is that predation at the adult stage affects *Ciona* more than *Ascidia*, but this remains to be tested.

Despite our findings of multiple biotic resistance mechanisms across the complex life cycle of *Ciona*, resistance was not absolute as *Ciona* is still present. A possible explanation is that high propagule pressure (via the arrival of allochthonous propagules through shipping or from the resident *Ciona* adults) produces enough individuals to compensate the negative effects of biotic resistance and is able to maintain the population. Another mechanism by which competitive exclusion might be prevented involves regular disturbance (Margalef 1963; Connell 1978). Observations of old panels in Bodega Harbour revealed large aggregates of *Ascidia* detach when they become too heavy or fouled by epibionts (authors' personal observation), as seen in other gregarious organisms (Stachowicz *et al.* 2002). This process is unrelated to predation and might facilitate persistence of *Ciona*. Furthermore, if recruitment of *Ciona* coincides with times of reduced *Ascidia* recruitment (and low predator abundance), this could help facilitate coexistence, as occurs for competitively inferior bryozoans in this system (Edwards & Stachowicz 2011). Similarly, in terrestrial ecosystems, the performance of invaders is constrained by both biotic and abiotic factors and the interaction of these factors determines invasion success (Going, Hillerislambers & Levine 2009).

There is a growing recognition of the importance of analysing the consequences of ecological processes across multiple life-history stages (Grosberg & Levitan 1992; Boege & Marquis 2005), and as shown here, invasion biology studies could also benefit from adopting such an approach. Our results on effect sizes (Table 1) indicate that predation and to a lesser extent competition have strong negative effect on *Ciona* and likely contribute to the failure of *Ciona* to dominate Bodega Harbour assemblages. A true assessment of the relative importance of these mechanisms for *Ciona* population size and whether mechanisms interact synergistically or antagonistically await further experiments. However, it seems likely that given the considerable temporal and spatial variation in *Ascidia* recruitment (Edwards & Stachowicz 2011) and caprellid abundance (Fig. 6) that both mechanisms contribute to the biotic containment of *Ciona*. As a result, this species fails to dominate Bodega Harbour in the way that has been observed elsewhere. Indeed, it is possible that predation and competition play complementary roles that enhance the resident community's resistance to *Ciona* invasion more than either would alone. In San Francisco Bay, located just *c.* 70 km south of Bodega Harbour, *Ascidia* is rare due to fluctuating salinities associated with seasonal freshwater influx (Chang

2009), but the fast growing *Ciona* periodically reach very high abundances (Blum *et al.* 2007). Contrary to what we found in Bodega Harbour, caprellid abundance in San Francisco Bay, and also in Tomales Bay (a site close to Bodega Harbour), peaks during fall and early winter, and is extremely low during spring and summer (A.L. Chang, pers. comm.). The lack of ecologically similar resident competitors or reduced predator abundance in San Francisco could both contribute to reduced biotic resistance and increased abundance of *Ciona* there and elsewhere in the world where *Ciona* becomes dominant (Rius, Heasman & McQuaid 2011; Collin *et al.* 2013). Definitive answers await comparative experiments conducted across the life history of these and other potential invaders in distinct biogeographical regions. Most mechanisms of biotic resistance are far from absolute (Levine, Adler & Yelenik 2004), perhaps in part because they are spatially and temporally variable. Future work should consider this possibility and conduct factorial experiments, as well as fecundity estimates per capita at each life-history stage, to understand the relative importance of different biotic resistance mechanisms across the life history and ultimately, to evaluate their role in population growth and persistence.

Data accessibility

Data deposited in the Dryad repository: http://dx.doi.org/10.5061/dryad. 82j1g.

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Supporting Information

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

Fig. S1. % Cover of *Ascidia* (filled circles) and *Ciona* (open circles) in Spud point marina.

Fig. S2. Survival of *Ascidia* in the presence or absence of *Botrylloides*.

Fig. S3. Effect of predator removal on % survival of *Ascidia* recruits in the field.

Fig. S4. Caprellid experiment after 24-h showing % survival of *Ascidia* larvae, 1-day old settlers and 2-week old settlers (i.e. larva, pre-metamorph, late metamorph respectively).

Fig. S5. Prey-choice experiment after the 24-h mark showing % survivors (pooling numbers of settlers and larvae together) of *Ascidia* (white bars) and *Ciona* (black bars).

Table S1. Mixed model ANOVA examining the effect on fertilization success of pre-exposing *Ciona* eggs to *Ascidia* sperm.

Table S2. Repeated measures ANOVA examining the effect of the presence of *Botrylloides* on solitary ascidians.

Table S3. Repeated measures ANOVA examining the effect of predatory exclusion on *Ascidia*.

Appendix S1. Characteristics of the studied species.

Appendix S2. Settlement methods.

Appendix S3. Spawning methods.

Appendix S4. Fertilization experiment methods.

Appendix S5. Settlement preferences in the absence of other species.

Appendix S6. Results of the experiments conducted with *Ascidia*.

Appendix S7. Comparing predatory susceptibility of *Ascidia* and *Ciona*.