



The smaller, the most delicious: Differences on vulnerability to predation between juvenile and adult of invasive ascidians

Clara B. Giachetti^{a,b,c,*}, Nicolás Battini^{a,b,c}, Karen L. Castro^{a,b,d}, Evangelina Schwindt^{a,b}

^a Grupo de Ecología en Ambientes Costeros (GEAC), Puerto Madryn, Chubut, Argentina

^b Instituto de Biología de Organismos Marinos (IBIOMAR-CONICET), Blvd. Brown, 2915, Puerto Madryn, Chubut, Argentina

^c Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (FCEyN, UBA), Argentina

^d Centro Regional Universitario Bariloche, Universidad Nacional del Comahue (CRUB, UNCo), Argentina

ARTICLE INFO

Keywords:

Ciona robusta
Ascidella aspersa
Fouling
Biotic resistance
Invasive species
Southwestern Atlantic

ABSTRACT

Ascidians are one of the most important taxa in fouling communities of artificial structures. These organisms can establish new populations and successfully invade new areas, spreading outside the region where they were initially introduced, due to their high reproductive and growth rates and their broad tolerance to environmental conditions. Predation pressure has also an influence on the colonization success of ascidians, with variations depending on their life stage, the fluctuations of the abundance of predators and their identity. Among these factors, the consequences of invasive and native predators on different life stages of invasive ascidians have been poorly evaluated. Thus, the goal of this work is to assess the survival of different life stages (juveniles and adults) of the two most successful invasive ascidians in the presence of different benthic predators in a cold temperate port. To evaluate this, we performed experiments offering juvenile and adult ascidians to different native and exotic benthic predators, under controlled aquarium conditions. We observed that juvenile ascidians were more vulnerable to predation than adults. Moreover, adult vulnerability differed between ascidian species probably because of different physical and chemical defences. Our results evidence the importance of evaluating the effects of predation through different stages of the life cycle of the preys, and the role of both native and exotic predators. Therefore, we hypothesize that juvenile ascidians depend on the use of different refuges, such as mussel beds and adult ascidians, to escape from predation until they reach a less vulnerable adult size. Understanding the differential effects of predation through different stages of life-history of the preys, and the interaction between native and exotic species helps to comprehend the causes of success in the establishment of invasive species.

1. Introduction

Ascidians are among the most successful invasive species (following the definition in Blackburn et al., 2011) colonizing new habitats and are one of the most common taxa in the fouling communities of port areas and artificial structures (Lambert and Lambert, 1998; Lambert, 2007; Zhan et al., 2015; Giachetti et al., 2020). Several features of the biology and ecology of ascidians facilitate their success in colonizing artificial structures. High reproductive and growth rates (Yamaguchi, 1975; Lambert, 2007; Zhan et al., 2015), and a broad tolerance to different environmental conditions, such as salinity, temperature, turbidity and pollution, allow ascidians to survive and reproduce in highly contaminated areas, like ports (Shenkar and Swalla, 2011; Pineda et al., 2012;

Osborne et al., 2018). Ascidians present a short-life lecithotrophic larvae with a restricted dispersal potential (Lambert, 1968; Zhan et al., 2015), thus vessels, through biofouling and ballast water, and other floating artificial structures, such as buoys and aquaculture devices, are the main dispersal vectors for these organisms (Zhan et al., 2015). In this way, with ports acting as the entrance point of exotic species, ascidians can establish new populations and successfully invade new areas, i.e. spreading regionally outside the location where they were originally introduced (Blackburn et al., 2011; Zhan et al., 2015). All these characteristics make ascidians an ideal model to study biological invasions (Lambert and Lambert, 1998).

Predation pressure can influence the colonization success of invasive ascidians in the new invaded areas, in terms of their abundance and

* Corresponding author. Blvd. Brown, 2915, Puerto Madryn, U9120ACD, Argentina.

E-mail addresses: claragia88@gmail.com, giachetti@cenpat-conicet.gob.ar (C.B. Giachetti).

dominance on the fouling communities. For example, in tropical and subtropical regions fish are active predators of colonial and solitary ascidians, reducing their abundance, the diversity of fouling communities and changing their species composition (Freestone et al., 2013; Kremer and da Rocha, 2016; Dias et al., 2020). Other studies also identified several benthic predators of solitary and colonial ascidians, such as sea urchins, sea stars, crabs, shrimps, sea slugs and other gastropods (Osman and Whitlatch, 2004; Nydam and Stachowicz, 2007; Epelbaum et al., 2009; Dumont et al., 2011; Giachetti et al., 2020; among others). At higher latitudes, benthic predators exert a significant predation pressure, inducing changes in cover, species composition and diversity of fouling communities (Dumont et al., 2011; Cheng et al., 2018; Giachetti et al., 2019, 2020; Leclerc et al., 2020). Indeed, benthic predators can also control population dynamics of invasive ascidians in artificial structures (Giachetti et al., 2019, 2020). Predation pressure fluctuates according to the seasonal variation of the abundance of predators (Cheng et al., 2018) or across the life stages of the ascidians (Osman and Whitlatch, 2004; Nydam and Stachowicz, 2007; Vieira et al., 2016).

In ascidians, mortality caused by predators changes among species and across their life cycle, with post settlement stages, i.e. recruits and juveniles, being more vulnerable than adults (Osman and Whitlatch, 2004; Nydam and Stachowicz, 2007; Vieira et al., 2016). Mortality caused by predators can be direct, i.e. predators consume the juveniles (Vieira et al., 2016), or indirect, i.e. the juveniles are bulldozed from the substratum because of the displacement caused by mobile organisms (Nydam and Stachowicz, 2007). Once solitary ascidians reach their adult size, they can escape predation through different strategies, such as physical or chemical defences. For example, some species have sponges as epibionts that allow them to become cryptic, once the sponge is fully grown (Voultsiadou et al., 2010), while others present a rough and hard tunic with low palatability, as in the case of *Ascidella aspersa* which has a firm and cartilage-like tunic (Berrill, 1950; Tatián et al., 2010). In contrast, other species, such as *Ciona robusta*, also possess a cartilage-like tunic but considerably softer (Hoshino and Tokioka, 1967). Also, some ascidians have a low pH in their tunics, due to the presence of organic acids or other inorganic compounds stored in bladder cells in the tunic (Stoecker, 1980a; Pisut and Pawlik, 2002). These differences in physical and chemical defences among species may lead to different patterns of abundance in fouling communities, depending on predation intensity and the dynamics of the community of benthic predators, among other factors, such as differences in the population dynamics of each ascidians species.

Although the role of predation on the dominance of ascidians on fouling communities has been largely studied, the consequences of native and exotic predators on the invasive success of ascidians have been poorly evaluated (Epelbaum et al., 2009; Dumont et al., 2011; Rius et al., 2014). Native predators with a large abundance in the community, high feeding rate, and with a selective diet can exert strong biotic resistance against invasive preys (Skein et al., 2020). In addition, some exotic predators can reduce the colonization success or abundance of other exotic species (Collin and Johnson, 2014; Marraffini and Geller, 2015). Thus, both exotic and native predators should be considered when evaluating the biological interactions in a community, to understand and predict the differences among the colonization success of different ascidians species (Rius et al., 2014). Previous observations have shown that predators have a key role in reducing the dominance of invasive ascidians, but it remains unclear whether there are differences in predation across the life-stages of the ascidians (Giachetti et al., 2020). Our hypothesis is that the vulnerability of ascidians to predation is different between juveniles and adults, and between ascidian species. Therefore, the goal of this work is to assess the survival of juvenile ascidians and adult of the two most abundant invasive ascidians in the presence of different benthic predators in a cold temperate port. Specifically, we tested the mortality of juvenile and adult ascidians in presence of two native and two exotic benthic predators with two

aquarium experiments. We expect higher mortality rates and a larger number of predators in the case of juveniles compared to adults because of the lack of defences in juveniles, and that adults of *C. robusta* will present a larger vulnerability than *A. aspersa* because the different anti-predator strategies among both species.

2. Methods

2.1. Study area

This study was conducted in the Puerto Madryn port, situated within the Nuevo Gulf (Southwestern Atlantic, Argentina, 42°49' S; 65°04' W, Fig. 1). This port is characterized by calm and clear waters, and a semidiurnal tidal regime with an amplitude of approximately 5 m (Servicio de Hidrografía Naval, 2019). These characteristics facilitate the collection of the specimens used in the aquarium experiments and the estimation of ascidian and predator abundances.

In the study area, benthic predator communities are characterized by the presence of native sea urchins and sea stars, with *Arbacia dufresnii* and *Allostichaster capensis* being the most abundant, and several gastropods species (Rechimont et al., 2013; Souto et al., 2014). Among the exotic predators, the most abundant species are the sea slug *Pleurobranchaea maculata*, and the European green crab, *Carcinus maenas*, which are widely distributed along the Argentinean coast (Hidalgo et al., 2005; Torres and González-Pisani, 2016; Battini et al., 2019; Yorio et al., 2020). The ascidian fouling community is represented mainly by invasive or cryptogenic solitary and colonial species. Among the invasive solitary species, *Ascidella aspersa* and *Ciona robusta* are the most abundant, and were chosen as model species for this study (for a more detailed description of the fouling community see Giachetti et al., 2019, 2020).

2.2. Abundance of predators and ascidians in the port area

To evaluate the abundance of the potential predators and of *A. aspersa* and *C. robusta* in the port area, we performed a survey on the pilings of the pier. As the presence of ascidians depends on the availability of hard substrata and because the bottom of the port area is composed mainly by sand and small gravel, we measured ascidian and predator abundances only on the pilings. This survey comprised 12 subaquatic photoquadrats of 20 × 20 cm in each piling (n = 10) covering an area of 0.48 m² per piling, i.e. per replicate. Photographs were taken 2 m below mean low tide level, and to represent the whole fouling community of the piling, photographs were distributed in three vertical levels (4 photographs in each level, at least 1–2 m apart from each other and along the whole circumference). We chose the area of the photoquadrats according to the size of predators and ascidians observed in the area, all small species of a maximum length of 100 mm. Thus, we could observe several individuals of each species in each photoquadrat. We performed one survey within the day at the middle of each season approximately (summer, February 2018; fall, May 2018; winter, August 2018; spring, November 2018).

From each photograph we measured the abundance of both species of ascidians and all the potential predators (sea stars, sea urchins, crabs and sea slugs) by using the software Image J 1.52a (National Institute of Health, USA). In the case of ascidians, we quantified individuals growing on the pilings as well as those growing over other organisms.

2.3. Predation on adult and juvenile ascidians

We selected the potential predator species after a literature search of the diet items of the most common groups of benthic invertebrates in the study area. We selected the model species to use in trials considering their abundance in the study area and the feasibility to acclimate them to the aquarium conditions (Rubilar and Crespi-Abril, 2017; Battini et al., 2021) (Table 1). Moreover, we chose sea urchins and sea stars

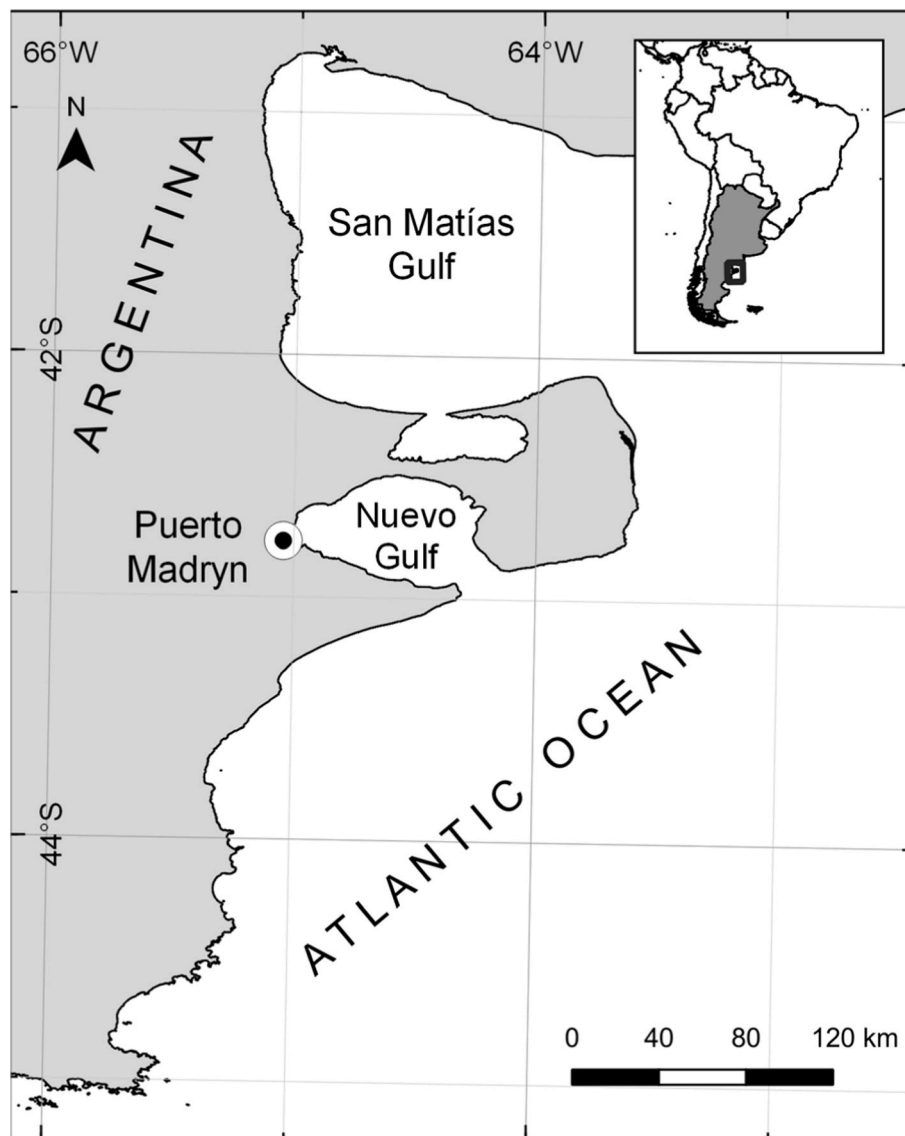


Fig. 1. Map of northern Patagonia, showing the location of Puerto Madryn port in the Nuevo Gulf and its location in South America (top right inset).

Table 1

Predators, control prey, lengths of starvation and experiment periods for each predator tested. Number of replicates between parentheses (in adult experiments the first value correspond to *A. aspersa* and the second one to *C. robusta* trials).

Group	Model species	Control prey	Starvation period	Adults experiment	Juveniles experiment
Sea urchin	<i>Arbacia dufresnii</i>	Green algae	2 days	7 days (n = 11/15)	6 days (n = 10)
Sea star	<i>Allostichaster capensis</i>	Mussels	9 days	10 days (n = 11/14)	5 days (n = 10)
Sea slug	<i>Pleurobranchaea maculata</i>	Open mussels	4 days	2 days (n = 10/10)	4 days (n = 10)
Crab	<i>Carcinus maenas</i>	Mussels	6 days	7 days (n = 10/10)	7 days (n = 10)

because of their key role in structuring benthic communities of the Southwestern Atlantic (Penchaszadeh and Lawrence, 1999; Castro et al., 2022; Laptikhovskiy et al., 2015), and the exotic sea slug *P. maculata* and the green crab *C. maenas* because of their abundance and wide distribution. At the aquarium, temperature and photoperiod were controlled to simulate natural conditions. Temperature varied according to the fluctuations of the sea surface temperatures throughout the year. Photoperiod was set in a 12:12 h light/dark cycle during all the year.

We collected individuals from one species of each group of predators in the port area using scuba diving (Table 1), and from both species of solitary adult ascidians (length >30 mm) carefully removing them from the pilings of the wharf. They were transported in containers filled with

sea water to the aquarium, acclimatized and fed *ad libitum* during seven days with control food, i.e. the principal item of their diets (Table 1). To collect juvenile ascidians, we suspended plastic 10 × 10 cm nets (1 × 1 cm mesh) between two pilings of the port, and we inspected them every two weeks. Once juvenile ascidians were visible, we removed the nets and transported them to the aquarium in insulated containers filled with sea water. Thus, here we referred as juvenile ascidians to individuals recently settled to the substratum (length <30 mm). We did not clean the plastic nets from other organisms, such as small algae and bryozoans, to avoid potential damage or loss of juvenile ascidians. However, we visually evaluated that the communities on nets were similar in the composition of species in the same trial. We fed juvenile and adult

ascidians with phytoplankton (Kent Marine PhytoPlex®) until the beginning of the experiments.

Before starting the trials, we exposed each species of predator to a starvation period that varied in length between 2 and 9 days (Table 1). This period was previously determined as the time when no more faeces were visible after feeding the predators with control food. In the case of sea stars, as faeces were difficult to recognize, we evaluated different periods observing the signs of stress of animals, e.g. decolouration, inability to turnover (Giachetti, 2020; Rubilar and Crespi-Abril, 2017). We performed the trials in individual isolated covered 100 L tanks (79 × 44 × 29 cm). We chose isolated tanks to increase the independence among trials. Moreover, we maintained the tanks covered to avoid changes in salinity due to evaporation. Periodically (every 48 h), we partially (40–50%) renewed the water and assessed nitrite and nitrate concentration through a colorimetric test (®Sera GmbH). From previous experiments, we knew that these compounds were the ones that most affected the water quality. Each tank was subdivided into two equal compartments with a net of 2 × 2 mm mesh that allowed the passage of water but not the organisms. Each compartment was randomly assigned to one of two treatments: predation (P) and control (C).

In the case of juvenile ascidians, the predation treatment consisted of an individual of one species of predator with one plastic net to which juvenile ascidians were attached at the bottom of one compartment, while in the control treatment there was a similar plastic net with juvenile ascidians at the bottom of the other compartment, without any predator. We photographed each net at the beginning and at the end of the experiment, and we quantified the total number of recruits in each photograph to estimate the number of consumed juvenile ascidians. As we were interested in testing the survival of ascidians juveniles, which have similar defences among species contrary to adults that might present different defences among them (Stoecker, 1978; Stoecker, 1980a; Koplovitz and McClintock, 2011), we prioritized the survival of juveniles and decided not to identify to the species level. We also checked any notorious change in the cover of the algae and bryozoan growing in the plastic nests. At the end of the experiment, which lasted between 4 and 7 days depending on the predator species (Table 1), we fed predators with their control prey to confirm they were starved as with adult ascidians.

We performed the trials with adult ascidians following the same design used for juveniles, including the same potential predators and ascidians species (Table 1). The predation treatment consisted of four similarly sized individuals of the same species with a single individual of one predator species, while the control treatment consisted of four adults of the same ascidian species without predator. Having four ascidians per treatment allowed us to better represent the natural conditions, as ascidians settle in small groups, and to observe different consumption behaviours and marks. Control treatment allowed us to evaluate potential mortality of ascidians due to manipulation or stress during the experiment. At the end of the experiment, which lasted between 2 and 10 days depending on the predator species (Table 1), we registered the mortality of ascidians (i.e. either consumed or dead individuals) in each compartment. The experimental period length was previously defined in preliminary tests according to the feeding behaviour of each predator. When mortality was null, we fed predators with control food after the trials to confirm that the absence of predation was not due to stress or low levels of starvation. We discarded the trials in which the predators did not eat the control food following the experimental period.

2.4. Potential chemical defences

To analyse differences in the pH of the tunic of adult ascidians of both species, we measured its pH using analytical pH strips (EM ColorpHast), with a range of 0–14 and a resolution of 1 pH unit. We measured the pH of the tunic in two different ways following Pisut and Pawlik (2002). First, we removed adult ascidians from seawater, dried the excess of

water from the tunic surface and then placed the strips against the dry tunic during a few seconds. Then, to test for the presence of inorganic acids on bladder cells, we abraded the tunic with a stainless dissection probe, applying a pressure similar to that made during handwriting (Pisut and Pawlik, 2002), and we measured the pH on this surface. We repeated these measurements over 10 individuals per species.

2.5. Statistical analysis

We evaluated differences in the abundance of potential predators among sampling events (one per season) through generalized linear models (GLM). We performed a GLM with a Poisson distribution using *Sampling event* as a fixed factor. When dispersion index was >1.5, we used a Cornwall-Maxwell distribution (library *glmmTMB*; Magnusson et al., 2017) to incorporate the overdispersion to the model or performed a GLMM including the piling as a random factor (library *lme4*; Bates et al., 2015). We performed pairwise tests among levels of factor *Sampling event* using a Tukey test with a confidence of 95% using the library *lsmeans* in R (Lenth, 2016). We also used these analyses to compare the abundance of each species of ascidians among sampling events.

To analyse the effects of each predator on both adult and juvenile ascidians, we performed GLM analyses with a binomial distribution (library *stats* in R). We defined “success” as the number of recruits consumed or dead, and “fail” to those that survived. We considered a significant effect of predators in those cases in which $P < 0.05$. When dispersion index was >1.5, we included a random factor (i.e. the individual tank) to incorporate the overdispersion to the model. For trials with juvenile ascidians and sea slugs, the large number of zeros made impossible to analyse the data using GLMs, so we performed a Fisher’s exact test (library *stats* in R) comparing the number of trials with mortality >0 between treatment and control. To evaluate differences among the mean mortality caused by each species of predator on juvenile ascidians, when we found significant differences between treatment and control, we performed a pairwise *t*-test adjusting the p-values with a Bonferroni correction, and tested homogeneity of variance with a Levene test. If homogeneity cannot be met, a Welch approximation was used (library *stats*, R).

Regarding chemical defences on each ascidian species, we performed a *t Student* test for each species to compare the pH before and after damaging their tunic. We tested homogeneity of variance performing a Levene test. We performed all the analyses in R (R Core Team, 2020).

3. Results

3.1. Abundance of predators and ascidians in the port area

All groups of predators were present on the pilings. The abundance of sea urchins, sea slugs and sea stars did not vary within a year (GLM, Poisson distribution, sea urchins, $P = 0.331$; sea slugs, $P = 0.215$; sea stars, $P = 0.555$; $N = 10$ in all cases; Fig. 2). The abundance of crabs was higher during summer and fall (GLM, Poisson distribution, $P = 0.001$; $N = 10$; Fig. 2; Table 1 in Supplementary material). Regarding ascidians, the abundance of *Ascidella aspersa* was almost constant throughout the year, presenting a minimum during spring (mean abundance: $X \pm SE = 12.8 \pm 2.2$ individuals/m², $N = 10$), only significantly different to its abundance of during summer ($X \pm SE = 28.4 \pm 2.0$ individuals/m², $N = 10$; Fig. 3; Table 2 in Supplementary material). The abundance of *Ciona robusta* presented a maximum during fall ($X \pm SE = 50.8 \pm 10.8$ individuals/m², $N = 10$) and a minimum during spring ($X \pm SE = 4.6 \pm 1.4$ individuals/m², $N = 10$), whereas mean abundances of winter and summer did not differ between them (Fig. 3; Table 3 in Supplementary material).

3.2. Predation on juvenile and adult ascidians

All predator species consumed juveniles, having a significant effect

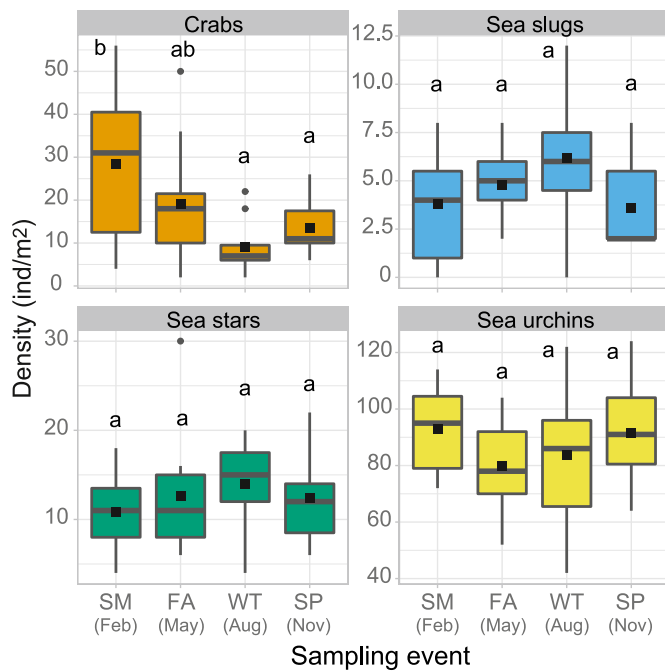


Fig. 2. Density of each predator found on the pilings at each sampling event (SM, summer; FA, fall; WT, winter; SP, spring). Different letters denote significant differences ($P < 0.05$). Note the different scales at Y axis. The superior and inferior ends of the box show the third (Q3) and the first (Q1) quartiles, respectively. The difference between them is the interquartile range (IQR), used to calculate the extreme lines of the box: from $Q3 + 1.5 \times IQR$ (superior) to $Q1 - 1.5 \times IQR$ (inferior). Inside the box, the black line represents the median of the data and the black square is the mean of the data (package *ggplot2*; Wickham, 2009).

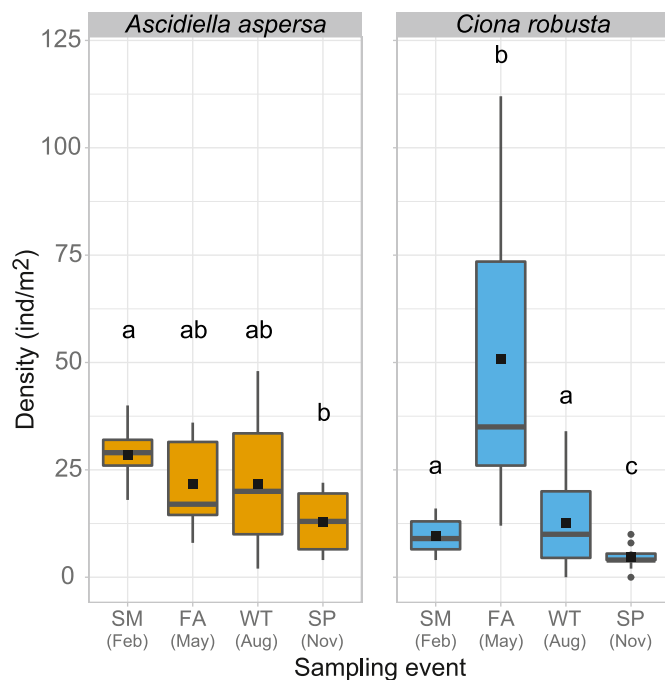


Fig. 3. Density of each species of ascidians found on the piling communities at each sampling event (SM, summer; FA, fall; WT, winter; SP, spring). Black squares: mean density. Different letters denote significant differences ($P < 0.05$). See Fig. 2 legend for box-plot references.

Table 2

Trials performed with each predator and each ascidians species: number of replicates (N), percentage of trials where dead/consumed adult ascidians were observed (occurrence of mortality), and P-value of the GLM model comparing treatment and control.

	<i>Ascidiella aspersa</i>			<i>Ciona robusta</i>		
	N	Mortality (%)	P-value	N	Mortality (%)	P-value
<i>Arbacia difresnii</i>	11	20	0.20	15	60	0.01
<i>Allostichaster capensis</i>	11	20	0.33	14	40	0.11
<i>Carcinus maenas</i>	10	40	1.00	10	20	1.00
<i>Pleurobranchaea maculata</i>	10	0	–	10	0	–

over their mortality (GLM, binomial distribution, $n = 10$, sea urchins, $\beta = 7.89$, $P < 0.001$; crabs, $\beta = 2.18$, $P < 0.001$; sea stars, $\beta = 2.17$, $P = 0.004$; sea slugs, Fisher's test, $P = 0.01$; Fig. 4). Among all predators, sea urchins were the ones that produced the largest mortality of juvenile ascidians ($76.3 \pm 10.5\%$ mortality (mean \pm SE); t -test, $P < 0.001$ in all the pairwise tests; Fig. 4). Indeed, they were the only group capable of completely excluding the juveniles from the plastic net (Fig. 5). The rest of the predators, unlike what occurred with adults, also caused a significant mortality of juveniles, and no differences were observed among species (mean \pm SE mortality: sea stars, $7.9 \pm 2.9\%$; crabs, $15.6 \pm 4.5\%$; sea slugs, $4.8 \pm 1.8\%$; Fig. 4). Although we did not identify each juvenile, we observed juveniles of different species on each net and did not notice any preference or a notorious survival of a particular juvenile.

We observed active feeding behaviours in three of the four predators (crabs, sea urchins and sea stars), but only sea urchins caused a significant mortality to adults of *C. robusta* (GLM, binomial distribution, $n = 15$, $\beta = 1.19$, $P = 0.01$; Table 2; Fig. 6), consuming ascidians in 73% of the trials. Sea urchins were able to damage the tunic of *A. aspersa*, but those injuries did not cause the death of the adult ascidians, and we only observed mortality in 27% of the trials (GLM, binomial distribution, $n = 14$, $\beta = 1.45$, $P = 0.20$; Table 2; Fig. 6). Regarding crabs, the analyses showed underdispersion due to the absence of mortality in the control treatments regardless of the probability distribution, preventing us to make further conclusions. However, the absence of mortality in the control suggests that some predation from crabs occurred for both species of ascidians. In fact, we observed mortality of ascidians in 20% of the trials with *C. robusta* ($n = 10$) and in 40% of the trials with *A. aspersa* ($n = 10$). Finally, sea stars were capable of injuring the tunic of *A. aspersa* and caused mortality in 27% of the trials ($n = 11$) and killing individuals of *C. robusta* in 57% of the trials ($n = 14$). However, they did not cause significant mortality on any of the species (GLM, binomial distribution: *A. aspersa*, $\beta = 1.15$, $P = 0.33$; *C. robusta*, $\beta = 0.91$, $P = 0.11$; Table 2).

3.3. Potential chemical defences

The pH of the dry tunic of both species of ascidians was close to neutral. The tunic of *A. aspersa* showed a mean pH of 6.4 ($SE \pm 0.16$, $N = 10$), whereas *C. robusta* showed a tunic pH of 6.3 ($SE \pm 0.15$, $N = 10$). However, when tunics were abraded simulating an attack from a predator, *A. aspersa* turned significantly more acidic (paired t -test, $t = -16.45$, $P < 0.001$), with a mean pH value of 2.6 ($SE \pm 0.16$, $N = 10$), contrasting with *C. robusta*, which maintained a similar pH (paired t -test, $t = -1.09$, $P = 0.288$) with a mean value of 6.1 ($SE \pm 0.1$, $N = 10$).

4. Discussion

Our results showed that ascidians are more vulnerable to predation during the early stage of their life cycle, with a larger number of predators that could feed on them and led to a higher mortality compared to adults. Native and exotic predators caused a high mortality on juvenile

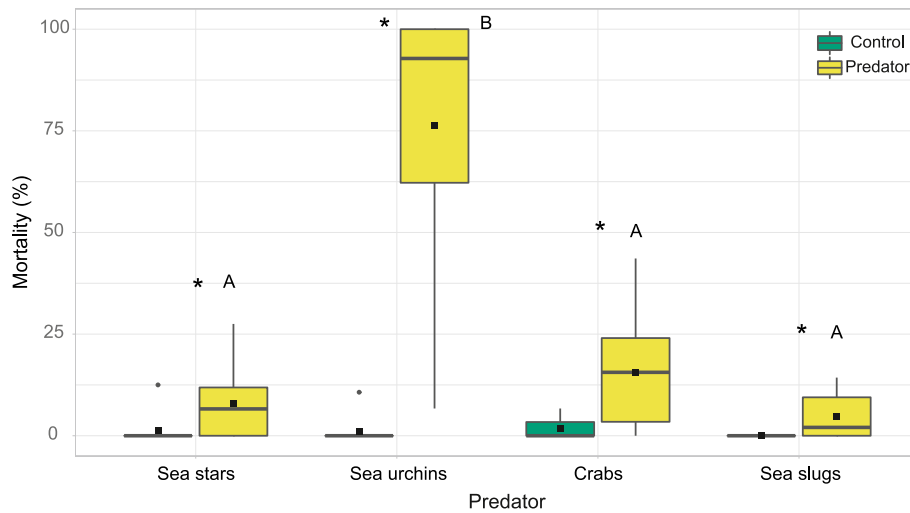


Fig. 4. Mortality (%) of juvenile ascidians in the absence (control) and presence of each predator (predator, N = 10 for each predator species). Asterisks indicates significant differences between treatments for each predator. Different letters indicate significant differences among the mortality (%) caused by each type of predator. Black squares: mean mortality (%). See Fig. 2 legend for box-plot references.

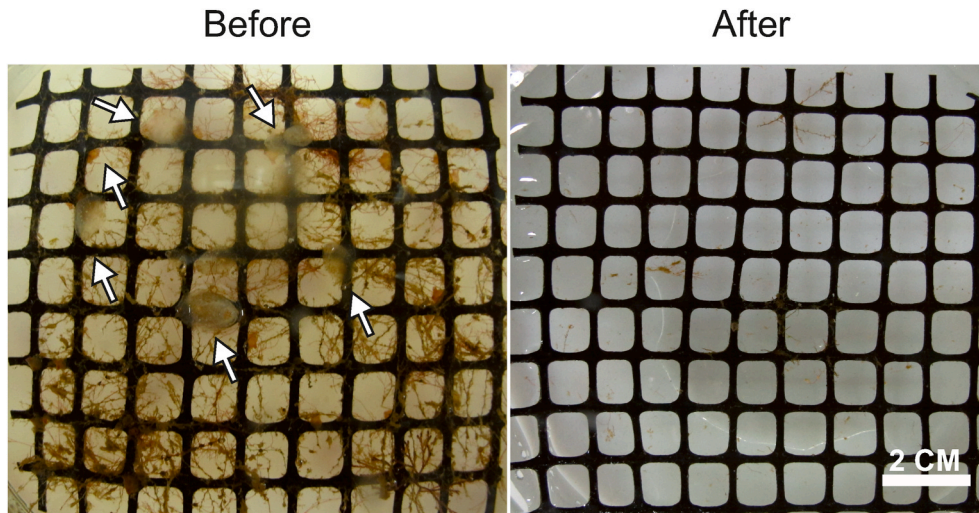


Fig. 5. Example of the plastic nets used with sea urchins before and after the trial. It shows how this predator can exclude completely juvenile ascidians from the net. Arrows mark some of the juvenile ascidians on the net.

invasive ascidians by direct consumption when compared to adult ascidians, regardless of the species. Although we did not identify juveniles to species level, we observed a decrease on the vulnerability to predation once they reached their adult size, as adult ascidians of both species showed less mortality and damage by predation. Indeed, only the native sea urchin could cause significant mortality to adults of *Ciona robusta*. The difference in mortality among species might be explained by differences in the tunic pH and hardness between both species. *Ascidella aspersa*, not only has a harder tunic, but also became acidic when abraded, which can be detrimental to predators with grazing feeding behaviours such as sea urchins, sea stars, sea slugs and crabs.

Previous experiments showed that ascidians thrive in the absence of benthic predators in the fouling communities of the study area (Giachetti et al., 2020), and this work confirms that juvenile predation is an important driver of the decrease in their abundance. The high abundance of ascidians in fouling communities suggests that there may be other factors allowing juvenile ascidians to escape predation. For example, ascidian recruits could find refuge against predation among native mussels, as *Aulacomya atra* and *Mytilus* spp., which are dominant in these fouling communities (Giachetti et al., 2019). In addition,

ascidian recruits generally settle close to their parents and usually the settlement is faster in the presence of adults (Rius et al., 2010). This favours a gregarious distribution of the individuals, which may decrease the predation on the recruits, and aide ascidians to avoid the biotic resistance exerted by benthic predators (Skein et al., 2020). The role of predators, causing direct or indirect mortality on juvenile ascidians, was also observed in port areas of California and Brazil where they had a large effect on the composition and cover of the fouling community (Nydham and Stachowicz, 2007; Vieira et al., 2012). In fact, predation on juvenile ascidians can indirectly affect competition for space (Nydham and Stachowicz, 2007) driving the patterns related to the dominance of species in the community in the long term (Osman and Whitlatch, 1996).

When ascidians finally reach a certain adult size, they present physical and/or chemical defences against predators, such as size, hardness of the tunic and/or acidic secretions that could explain their abundance on fouling communities (Dayton, 1971; Jackson, 1977; Stoecker, 1978; Stoecker, 1980a; Koplovitz and McClintock, 2011). Besides the potential physical defences, such as the thickness and hardness of its tunic, we observed that adults of *A. aspersa* are able to release acids from their tunic as a response to physical damage. Indeed,

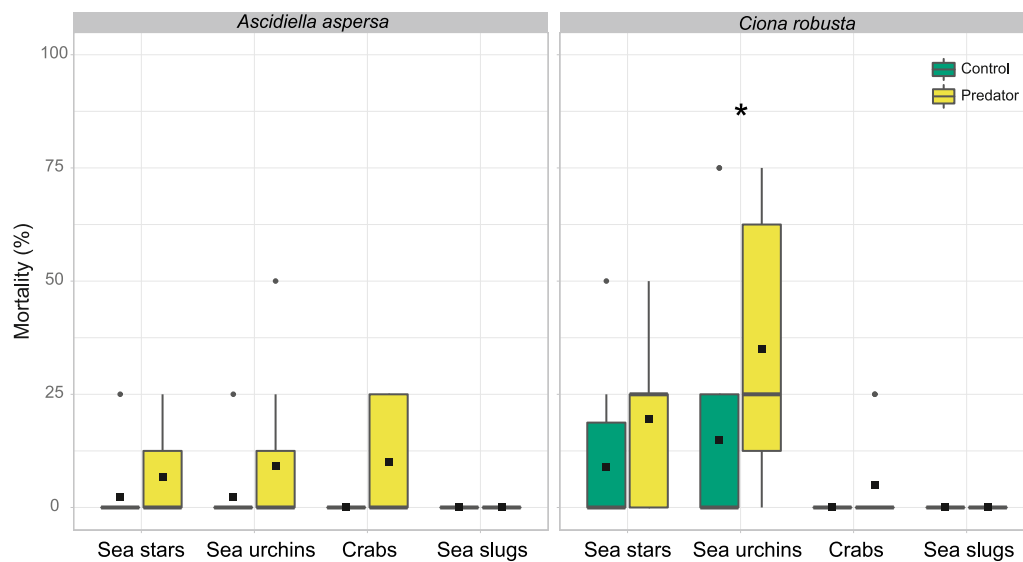


Fig. 6. Mortality (%) of adult ascidians in the presence of each predator. Asterisks indicates significant differences between treatments for each predator. Black squares: mean mortality (%). See Fig. 2 legend for box-plot references.

some ascidians have inorganic acids in bladder cells on the tunic (Goodbody, 1975; Stoecker, 1978, 1980a), which are released when the tunic is damaged as a defence mechanism against predators, modifying their palatability (Stoecker, 1980b). Interestingly, adults of *A. aspersa* were never consumed completely. Although the sea urchin *Arbacia dufresnii* and the sea slug *Pleurobranchaea maculata* consumed part of the epibenthic microalgae present on the surface of the tunic, both predators released the individuals of *A. aspersa* once the tunic was partially damaged without killing the individual. All these results strongly suggest that the invasive *A. aspersa* presents chemical defences against predators, through the secretion of acidic substances. Contrary to *A. aspersa*, *C. robusta* has a softer tunic (Berrill, 1950; Tatián et al., 2010) and a neutral pH even after damage. These characteristics likely allowed native sea urchins to consume completely the individuals of *C. robusta*, including the adults. This was not observed for other predators, suggesting that other factors might be affecting these interactions. Because of their generalist diet, crabs and sea slugs could be consuming other common organisms, such as mussels, sea anemones and polychaetes in the natural fouling communities (Grosholz and Ruiz, 1996; Battini et al., 2021). Interestingly, the green crab *Carcinus maenas* co-exists with, and consumes *C. robusta* in the Northern Hemisphere (Carver et al., 2003). Thus, we expect that the interactions between both species might change through time, as it was observed with other exotic species that delayed on recognizing certain species as preys (Epelbaum et al., 2009; Papacostas and Freestone, 2019). Nevertheless, *A. dufresnii* and *C. maenas* caused mortality on juvenile ascidians, suggesting that native and exotic predators may play a key role in the control of the abundance and spread of these invasive ascidians.

Previous studies in Puerto Madryn port showed that the effect of predation on the cover and diversity of fouling communities, and on the abundance of invasive ascidians did not vary within a year (Giachetti et al., 2019). In the present study, we found that the abundance of predators did not vary temporally or spatially in a way that explains the abundance patterns observed for invasive ascidians. While *A. aspersa* presented a similar abundance along the year, *C. robusta* showed a peak during fall and a minimum during winter. This could be explained by a differential predation on larvae too. Chemical defences against predators present in the adults might translocate to eggs and prevail in the larvae, in form of nutritional reserves provided by the parents (Young and Bingham, 1987; Lindquist et al., 1992; Pisut and Pawlik, 2002). In addition, sea urchins, main consumers of adult *C. robusta* and juveniles of both species of ascidians, presented a particularly large abundance on

pilings throughout the year. The sea urchins *A. dufresnii* are known to present plasticity in their diet, being generalists and omnivorous with a tendency to carnivory or herbivory according to the availability of faunal or algal items (Penchaszadeh and Lawrence, 1999; Newcombe et al., 2012; Gianguzza and Bonaviri, 2013; Castro et al., 2022). Predation pressure of sea urchins on ascidians in pilings and rocky shores might be lower than we observed in our experimental aquarium conditions, firstly because of a lower density ratio ascidians/sea urchins in natural conditions, and secondly because of the generalist diet of *A. dufresnii*. This could partially explain why invasive ascidians are not totally excluded from the fouling communities of pilings; despite the mean abundance of sea urchins on the pilings was 87 individuals/m², i. e. the highest of the four predators considered in this study. Nevertheless, although we observed that predators cannot exclude or limit the range of invasive ascidians, these and previous results showed that they exert biotic resistance through the regulation of the abundance of ascidians on fouling communities (Giachetti et al., 2020; Skein et al., 2020).

It is important to remark that other processes besides predation might affect the abundance and colonization patterns of both invasive ascidians. Interspecific competition during different life stages affects the colonization success of an invasive species over other invasive (Rius et al., 2009, 2014). For example, some characteristics of the reproductive cycles of *A. aspersa* and *C. robusta* could led to competition among gametes and/or larvae, e.g. share similar maturity peaks and release their gametes at the same moment (Giachetti, 2020). Both species present mechanisms to avoid hybridization and eggs can block their membrane in presence of male gametes regardless the species, reducing the fertilization rates (Lambert, 2000). Moreover, larvae can avoid settle near of individuals of other species. Thus, in the presence of highly competitive species, larvae spend more time searching for an adequate substrate to settle, consuming more nutritional reserves and reducing the odds of a post-metamorphosis success (Rius et al., 2009).

Our results showed that both native and invasive benthic predators negatively affect the survival of ascidians, mainly driven by predation on the early stages of the life cycle. Predation on juveniles might explain the low abundance of ascidians in fouling communities of artificial structures associated with benthic predators compared to suspended structures (Giachetti et al., 2020). However, once ascidians reach a certain adult size, they possess physical and chemical defences against predation, and they may facilitate the survival of juveniles settled among adults. In this work, we evidenced the importance of evaluating both

native and invasive predators, as their effect on the success of invasive species might be additive (Marraffini and Geller, 2015). Differential effects of predation through different stages of the life cycle of prey, along with particular characteristics of native predators (abundance, type of diet, etc.) are key to provide a better understanding of the potential of native communities to exert biotic resistance reducing the abundance or avoiding the establishment of exotic species (Rius et al., 2014; Skein et al., 2020). This type of studies contributes to comprehend the interactions between native and exotic species, the causes of success in the establishment of invasive species, and add understanding of these biological interactions on sessile communities of rocky shores outside port areas.

Availability of data and material

Under request.

Funding

This study was funded by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET-PIP 20130100508) and by Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT-PICT, 2016-1083) to ES. CBG, NB and KLC were supported by doctoral fellowships from CONICET.

CRediT authorship contribution statement

Clara B. Giachetti: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Nicolás Battini:** Investigation, Writing – review & editing, Methodology. **Karen L. Castro:** Writing – review & editing. **Evangelina Schwindt:** Conceptualization, Funding acquisition, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We are grateful to R. Vera, N. Ortiz, F. Irigoyen and F. Quiroga for their assistance in SCUBA diving and field work. We also thank the Puerto Madryn Port Administration (APPM) for permission to work inside the port area. We are thankful to T. Rubilar and L. Epherra for their advice in the maintenance of sea urchins and sea stars in the aquarium, to M. Moris and A. Gorosito for their assistance in the experimental aquarium. We also thank to M. Tatián and A. Bortolus for their helpful advice throughout the work. We are grateful with the Editor D. Baird and two anonymous reviewers for their valuable comments during the reviewing process. This study is part of first author's Doctoral Thesis at Universidad de Buenos Aires, Argentina.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecss.2022.107810>.

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