



# Alien vs. predator: influence of environmental variability and predation on the survival of ascidian recruits of a native and alien species

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**Abstract** Drivers of successful introduction of exotic species remain a major headline in marine invasion biology. We ran two experiments aiming to disentangle the effects of abiotic factors like contaminants and the effect of predation on recruits' survival of one native and one alien ascidian species. A feeding experiment allowed us to monitor microscale variation of generalist fish predation, which varied significantly within a marina. We also monitored the in situ survival of lab-grown ascidians at three locations within the marina, half with predator cage exclusion. The survival of the native *Ciona intestinalis* was conjointly highly influenced by location and caging. We were able to identify a link between predation intensity exerted by mobile generalist macropredators

and *C. intestinalis* survival, whereas none of the measured contaminants accounted for site variability of survival. The non-indigenous *Styela clava* had significant higher survival and biomass when uncaged, suggesting a positive effect of predation for this species. The natural in situ recruits of *C. intestinalis* showed higher biomass when caged and may have competed with lab-grown *S. clava*. Our results suggest that generalist fish predation may play a crucial role in the success of non-indigenous species due to facilitation through competitive release.

**Keywords** Competitive release · Facilitation · Biotic resistance · Invasive species · Predation · Ascidians

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## Introduction

With increasing globalization and interconnectivity of trade among countries, accidental and voluntary species introductions have multiplied with the use of trade ships, a trend that is predicted to further increase in coming years (Levine and D'Antonio 2003; Seebens et al. 2016; Carrasco et al. 2017). The ecological consequences of the spread of non-indigenous species (NIS) can be drastic, with some invaders completely restructuring ecosystems, potentially leading to the loss of biodiversity and ecosystem services (Pejchar and Mooney 2009; Blackburn et al. 2011). These potential impacts have led to a large body of literature focusing on the conditions of successful transport and establishment in the new environment of these species, identifying several filters that constitute strong selective barriers that only few species successfully overcome (Williamson and Fitter 1996; Jarić and Cvijanovic 2012).

Upon survival of the transport phase, the first major obstacle a NIS may encounter in a new environment are the environmental conditions. Abiotic factors have been shown to vastly influence species' evolution and the local structure of ecosystems (Je et al. 2004; Benton 2009; Lewis et al. 2017). NIS seem to show higher resistance to abiotic stress and disturbance than their native counterparts (Gröner et al. 2011; Lejeune et al. 2014; Marie et al. 2017; Kenworthy et al. 2018a), which is attributed to two different processes. First, in some habitats, pre-adaptation of the considered NIS may attenuate the effect of abiotic filters of its new environment (Schlaepfer et al. 2010; MacDougall et al. 2018). Second, some NIS can undergo rapid evolution within several generations due to strong selective forces acting on them (Huey 2000; Hejda et al. 2009; Jarić and Cvijanovic 2012; Elst et al. 2016).

Biotic interactions, through a complex interplay between NIS and native species, may also condition successful introductions and invasions, and are the focus of numerous theories in invasion ecology. As a major regulator of ecosystem functioning, top-down regulation often occupies a central role in many of these theories (Hunter and Price 1992; Shurin et al. 2002; Heck and Valentine 2007; Riginos and Grace 2008). In the Biotic Resistance Hypothesis (Elton 1958), negative interactions between species, including competition and predation, may allow species-rich

communities to resist the establishment of invasive species through their more effective use of resources than species-poor communities. However, despite its popularity in the scientific community, the Biotic Resistance Hypothesis remains controversial (Jeschke et al. 2012). Another explanation for the success of NIS is provided by the Enemy Release Hypothesis (ERH; Keane and Crawley, 2002), which states that NIS may be released at least partially from their biotic regulators (predators, parasites and pathogens). NIS may thus perform better in introduced areas and gain competitive advantages over native species (Keane and Crawley 2002; Joshi and Vrieling 2005; Battini et al. 2021). As host-switching remains rare and as generalist predators expose a certain food selectivity potentially favoring native species, NIS may benefit from predators due to competitive release (Keane and Crawley 2002). These hypotheses provide a complex interplay of ideas in the context of predation and acknowledge the importance of it. Predation may be a key component in determining the success or failure of NIS (Keane and Crawley 2002; Cappuccino and Carpenter 2005; Joshi and Vrieling 2005; Skein et al. 2020; Battini et al. 2021). While these theories have predominantly been tested in terrestrial habitats, few studies have been conducted on marine invasions often with inconsistent results (Chan and Briski 2017).

Fouling communities and especially artificial harbor or marina communities hold many NIS due to the process of primary introduction via fouling on boat hulls and transport in ballast waters (Sylvester et al. 2011; Clarke Murray et al. 2012). The spread of NIS can lead to high economic and ecological costs, revealing the importance of better understanding the factors behind NIS success in these ecosystems (Ojaveer et al. 2015). Often related with human activities, permanent disturbances (press type disturbances) can have profound effects on communities and species (Nimmo et al. 2015). Fouling communities in marinas experience strong anthropogenic disturbances (Dafforn et al. 2015; Todd et al. 2019), which have been the focus of numerous studies showing their effect on local species. Artificial substrata are physically and chemically distinct from their natural counterparts and high concentrations of heavy metals and persistent organic pollutants (POPs) are present (Johnston et al. 2015; Saloni and Crowe 2015; Chase et al. 2016; Kinsella and Crowe 2016). The selection exerted by all these disturbances may

even result in differential selection and local adaptation at a very small spatial scale (ca. 50 m) with distinct communities adapted to local conditions (Kawecki and Ebert 2004; Colautti and Lau 2015; Kenworthy et al. 2018b). Despite an apparent focus on abiotic factors, studies have also assessed the applicability of the above-mentioned ecological hypotheses in marine environments. Among these theories, the main focus has laid on biotic resistance to invasions (Kimbrow et al. 2013). Biotic resistance due to interspecific competition seems to have a crucial importance on the success or failure of NIS (Fletcher et al. 2018; Gestoso et al. 2018), but interspecific competition may be modulated by predation (Oricchio et al. 2016a). Many studies have shown that predation strongly influences the population dynamics of several fouling species and contributes to the biotic resistance against NIS (Rogers et al. 2016; Rodemann and Brandl 2017; Yorisue et al. 2019). There is however no clear consensus on the importance of predation, with some studies indicating only minor effects masked by abiotic factors, and some others even indicating a facilitation of NIS due to predation on native competitors, probably due to generalist predators' preference for native prey (Astudillo et al. 2016; Gestoso et al. 2018; Kincaid and de Rivera 2020).

Studies however rarely include the complex interplay of biotic and abiotic factors, revealing the necessity for further research on how the combination of disturbance and generalist predation affects the successful establishment of NIS. In the present study, we aimed to assess these respective influences on the survival of young recruits of two ascidian species, the sea vase tunicate *Ciona intestinalis* (Linnaeus, 1767) and the Asian clubbed tunicate *Styela clava* Herdman, 1881, respectively native and non-indigenous in the North East Atlantic. Here we mainly focused on predatory fish, which were considered as a generalist predator model in this study. The recruit stage of tunicates is particularly vulnerable to predation due to its small size and lower structural defense. Recruits may also be more sensitive to environmental stressors (Saloni and Crowe 2015). Based on previous research we expected environmental conditions to vary in space, within the same marina, with levels of disturbance most likely organized in a gradient (Je et al. 2004; Kenworthy et al. 2018b). We hypothesized that this organization results in spatial variation in recruit survival, being lower in the inner part of the marina,

where pollution levels are likely maximal. NIS tend to better resist disturbances due to their selection during introduction (Gröner et al. 2011), which should result in better survival than native species in the marina. Predation intensity was predicted to vary in space and to negatively affect both species. However, we predicted that generalist predators prefer native prey, thus having a higher negative impact on the native species.

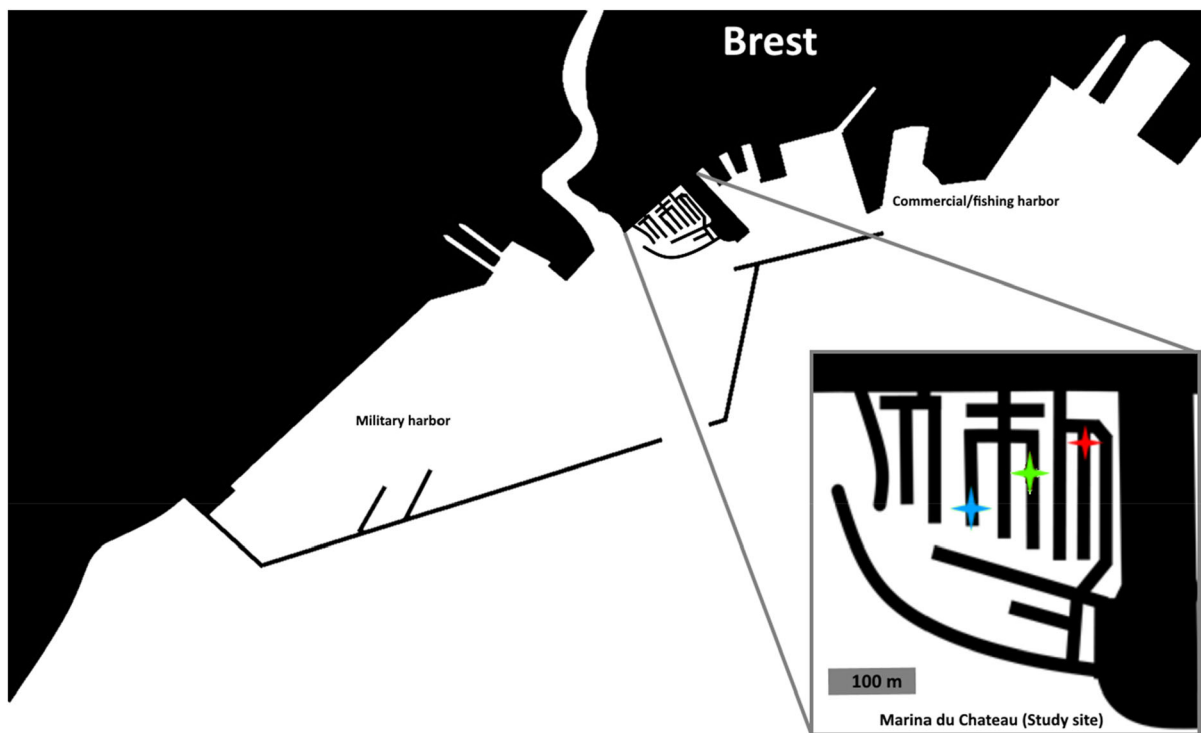
## Materials and methods

### Study site

The Marina du Château in Brest, France, was chosen as our study site (48° 22' 43.4" N; 4° 29' 22.1" W; Fig. 1). This recreational marina is part of a larger marine urban zone with a commercial and military harbor leading to a highly anthropized environment. In this marina numerous artificial substrata like jetties, pillars or pontoons are present, contamination by elemental trace metals (such as copper and lead) and persistent organic pollutants (POPs) is high, and NIS are abundant and diverse (Kenworthy et al. 2018b). We focused on the completely shaded environment of the floating pontoons in our experimental setups because they are the most abundant substratum in the marina. This marina has significant variations in environmental conditions among locations (spaced < 100 m), driving different fouling communities at the entrance, the middle and inner parts of the marina (Kenworthy et al. 2018b). In accordance with Kenworthy et al. (2018b), the same three locations (inner, middle and entrance) were selected which were spaced 80–90 m apart. These locations show higher disturbance (pollution) at the inner part of the marina and a lower disturbance at the entrance due to higher water exchanges with the outer environment.

### Study species

To assess the survival of ascidian recruits, we compared a native species and a NIS, being among the most abundant ascidian species in the whole marina. The native *Ciona intestinalis* (Linnaeus, 1767) is the dominant fouling animal in terms of abundance and biomass in most marinas in Brittany (NW France), including the present one



**Fig. 1** Map of the study region and site. Colored stars indicate the three locations within the marina. Blue: Entrance; Green: Middle; Red: Inner

(Bouchemousse et al. 2016). This species is characterized by rapid growth, a high reproduction rate and a short life cycle (Jackson 2008). The clubbed tunicate *Styela clava* (Herdman, 1881) was introduced from the NE Pacific into European waters, and was first recorded in Plymouth, UK in 1953. Since then, it has spread, constituting a potential pest on oyster and mussel farms (Carlisle 1954; Davis and Davis 2010). Both tunicate species are frequent in disturbed ecosystems such as harbors and marinas (Jackson 2008; Therriault and Herborg 2008; Davis and Davis 2010).

#### In situ assessment of recruit survival

Large adult individuals (> 15 cm) of both species were sampled in spring from the middle of the marina. Due to slower growth of *S. clava*, this species was sampled one month (April) before *C. intestinalis* (May). Both species were transferred into aquaria facilities with circulating sea water. After stabilization and fattening for two weeks to improve gamete production and quality, ca. 30 individuals of each

species were randomly selected for reproduction. Individuals of *S. clava* were separated into two pools to cross-fertilize and dissected by cutting the stipe at the base and opening the branchial sac, revealing the male and female reproductive apparatus. To avoid self-fertilization, oocytes and sperm were separately extracted with a thin pipette. Oocytes were rinsed and isolated from tissue debris on an 80 µm mesh filter with filtered (1.3 µm) seawater. Oocytes were distributed on Petri dishes, using two individuals per dish to maintain variability between recruits. Sperm was assigned to a sperm pool to avoid fertilizing oocytes from the same individual and mixed with filtered seawater. One portion of the sperm pool was introduced into each Petri dish containing the oocytes from different individuals. Similarly, *C. intestinalis* individuals were dissected to harvest gametes. Oocytes were collected by concentrating them in the narrow part of the oviduct and puncturing them with a glass pipette. Oocytes from different individuals were separated as described above for *S. clava*. Sperm was sampled similarly to the oocytes, by concentrating the mass in one side of the sacs and puncturing them

with a pipette. As described for *S. clava*, *C. intestinalis* individuals were separated into different genitor pools and mixed accordingly. For each species, 40 petri dishes with fertilized gametes were created. Cell divisions started at 1 h post fertilization, and larvae appeared after 24 h (seawater at constant 18 °C). Recruits of both species were fed with a mix of *Isochrysis affinis galbana* Tahiti (T-iso RCC 1349) and *Chaetoceros calcitrans* (so called 'Argenton' strain) strains cultured at the Roscoff Culture Collection (RCC, <https://roscoff-culture-collection.org/>). They were maintained for 15 days in the dark in a temperature-regulated environment (18 °C). After this period, the Petri dishes were transferred into temperature-regulated aquaria that were brought to match the varying outside water temperature of approx. 19 °C progressively over one week.

The number of *C. intestinalis* or *S. clava* individuals successfully attached on each Petri dish was counted and the position of the recruits was marked on the underside of each dish. We selected the 30 dishes with the highest abundance of recruits for each species. If the number of individuals exceeded 50 on a given Petri dish, we reduced it to this threshold by randomly removing individuals to avoid recruits overcrowding. Later, all dishes were individually fixed on 20 cm × 20 cm black PP panels to prevent from direct sunlight and were photographed (Olympus Tough TG5). For each location in the marina, 10 such panels were randomly selected for each species. Half of them were caged with plastic coated iron wire mesh with a 10 mm mesh size to exclude macro-predators, mostly fish (Dumont et al. 2011; Giachetti et al. 2019). Micro-predators and meso-predators such as small crustaceans and gastropods would still have access to caged treatments. The 20 randomly chosen panels (5 caged and 5 uncaged for each species) were suspended under the pontoon at each of the three locations (ca. 1 m depth; > 3 m away from the seafloor; 25 June 2019). After 20 and 28 days, cages and panels (but not the dishes) were cleaned to limit the effects of fouling on the recruits. Dishes were photographed to count the remaining individuals. Although fouling did occur on dishes, individuals stayed visible and could be identified on the dish and by their significantly larger size compared to additional natural recruits of the same species. The experiment ended 50 days after deployment. Dishes were recovered and individuals were counted. At this stage, heavy fouling on the

dishes, especially due to marina-recruited *C. intestinalis*, made it impossible to analyze photos for both in vitro recruited species. Thus, all *S. clava* individuals were manually counted and collected for drying (1 week at 60 °C) and weighing. All *S. clava* individuals present at the end of the experiment were lab-grown, as their positions were marked. While at 28 days after deployment of the recruits it was easy to discriminate lab-grown recruits of *C. intestinalis* from marina-recruited ones due the size difference, it was not possible anymore 50 days after deployment, especially considering that lab-grown recruits' mortality was high—likely even 100% since only few individuals were recorded at 28 days. We thus chose to consider the survival observations for this species after 28 days rather than at 50 days to avoid a potential underestimation of survival. Statistical analyses were conducted taking this into account. Nevertheless, the biomass of natural *C. intestinalis* recruits was compared between treatments, as these individuals are also influenced by environmental conditions and predation. The mass of potentially remaining lab-grown recruits can be neglected here due to their very low contribution to the total mass. For each dish, all recruits were collected and dried (1 week at 60 °C) for weighing.

#### Contaminant assessment

Three sediment samples (ca. 0.4 kg) were taken at ca. 3 to 7 m depth by divers at each of the locations for testing the concentration of Persistent Organic Pollutants (POP) including Polycyclic Aromatic Hydrocarbons (PAHs), Polychlorinated Biphenyls (PCBs) and the most frequent pesticides. Additionally, for each location, five sediment samples (ca. 0.4 kg) were taken to quantify Metallic Trace Elements (MTEs).

The analytical method for PCBs and pesticides is further detailed in our supplementary material and is fully described by Wafo et al. (2006). PCB determination focused on 33 individual congeners (Sup. 4) including target congeners proposed by the International Council for the Exploration of the Sea (ICES) as indicators of PCB contamination, complemented by congeners with high environmental prevalence (Webster et al. 2013). The list of the 16 quantified pesticides can be seen in Table 1. PAHs were determined following Sarrazin et al. (2006), Ratier et al. (2018) and Dron et al. (2019), which is detailed in the



supplementary material. We focused on 16 PAH congeners defined by the US Environmental Protection Agency (USEPA) priority list (Table 1, US EPA 2014). Each targeted PAH was identified based on the retention time and the mass spectrum from the chromatogram of standard solutions acquired in full scan mode. Quantification was then performed in the SIM mode for better selectivity.

For the quantification of MTEs the sediment samples were dissolved in a three-acid solution (HCL, HN03, HF) and were then analyzed using High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS; Jacquet et al. 2021). The spectrometer was calibrated via an external calibration method adding In as an internal standard.

#### Environmental variables

Temperature was monitored at each location with a HOBO® (Onset®) TidbiT v2 temperature logger during the whole duration of the experiment. Light intensity was monitored with a HOBO® (Onset®) Light-Temperature logger.

#### Feeding assay

In addition to the exclusion cages, predation intensity was directly measured with a feeding assay. This assay consists of 25 dried squid baits tied on fishing line, attached to fiberglass stakes on a 25 m long transect (Duffy et al. 2015). Squid, due to its firm consistence is particularly adapted to long feeding experiments since it does not detach without a strong attack from a larger predator (> 10 cm) and can only be detached as one piece. The experiment thus targets almost exclusively fish (Duffy et al. 2015). However, native velvet swimming crabs *Necora puber* (Linnaeus, 1767) could also feed on them. This assay has been used in various environments, including artificial habitats (Duffy et al. 2015; Rodemann and Brandl 2017). Here, a modified version of this assay was used and  $25 \times 3$  locations  $\times$  3 dates = 225 baits were deployed. A snorkeler counted the remaining baits 1 h, 3 h, 6 h and 24 h after deployment, allowing to use survival analysis rather than comparisons of means, thus providing detail on the bait consumption dynamics (Gauff et al. 2018). The bait was not deployed on the seafloor, because the height of the water column varied under each pontoon, but was suspended 1 m

below the pontoons, close to the ascidian recruits. The assay was performed 17, 24 and 31 days after the deployment of ascidian recruits at the inner, middle and entrance locations respectively, with one transect per location and date. Surveillance cameras (2 per transect, GoPro HD4) were installed.

#### Statistical methods

The survival data resulting from the feeding assay and collected from the ascidian recruits was analyzed using the ‘survival’ package (version 2.41–3, Therneau and Lumley 2017) in R (CRAN, version 3.6.1). The Kaplan–Meier curves for each treatment were established and compared using a log-rank pairwise comparisons with Bonferroni correction to avoid false positives due to multiple tests (Pyke and Thompson 1986; Bretz et al. 2011). The dry mass per *S. clava* individual as well as the log of the total dry mass of *C. intestinalis* were compared between caged and uncaged treatments with a Wilcoxon test, and individual differences of dry mass between locations were identified using a Kruskal–Wallis multiple comparison test with the ‘pgirmess’ package (version 1.6.2, Giraudoux et al., 2018). Mean value and standard deviation were calculated for each contaminant. Differences between locations were identified via a Kruskal–Wallis test. Differences in predation intensity between sites were assessed via a Log-Rank test.

Since sediment samples (contaminants), feeding experiments (predation intensity) and the survival rates of ascidian recruits could not be paired unambiguously, we chose to use R to randomly attribute samples according to the locations they are linked to. The generated table was submitted to a nested Cox regression from the ‘nested cohort’ package in R (version 1.3, Katki and Mark 2013). The two models, one for each species of ascidians, tested for the effect of all contaminants for which significative differences between locations were previously identified, the effect of caging and predation intensity (squid bait consumption after 24 h) as well as their interaction, on the survival of ascidian recruits. Predation intensity was here treated as an environmental variable. The interaction term here is required since predation cannot apply to caged individuals. Both models were nested within the Petri dishes which contained several ascidian recruits and location, as well as their interaction term. This process of random pairing and

modelization was repeated  $10^5$  times and  $p$  values and coefficients were saved in a separate table. For each model factor, we calculated the percentage of runs in which it resulted significant ( $p < 0.05$ ) as well as the mean hazard ratio (the risk increase/decrease caused by the factor). Factors significant in  $> 25\%$  of runs were considered as worth investigating (factor of interest) and those significant in  $> 50\%$  as factors with a clear link to survival.

## Results

### Recruit survival

The Kaplan–Meier survival curve of the recruits showed mortality for both species (Fig. 2). Mortality for *S. clava* ranged from 15 to 30% within 50 days and from 60 to 100% for *C. intestinalis* within 28 days. A log-rank multiple comparison showed that survival for *C. intestinalis* (Fig. 2a) was consistently lower than for *S. clava* (Fig. 2b;  $\chi^2 > 255$ ,  $p < 0.001$ ). Location had a significant effect on the survival of caged *C. intestinalis*. Survival was the highest at the inner location, intermediate at the middle and the lowest at the entrance of the marina ( $\chi^2 > 32.5$ ,  $p < 0.001$ ). Caging had a significant influence on *C. intestinalis*, increasing the survival at the inner and the middle part of the marina compared with the uncaged treatment ( $\chi^2 > 13.4$ ,  $p < 0.01$ ). At the marina entrance, mortality was 100% for the caged and uncaged treatments

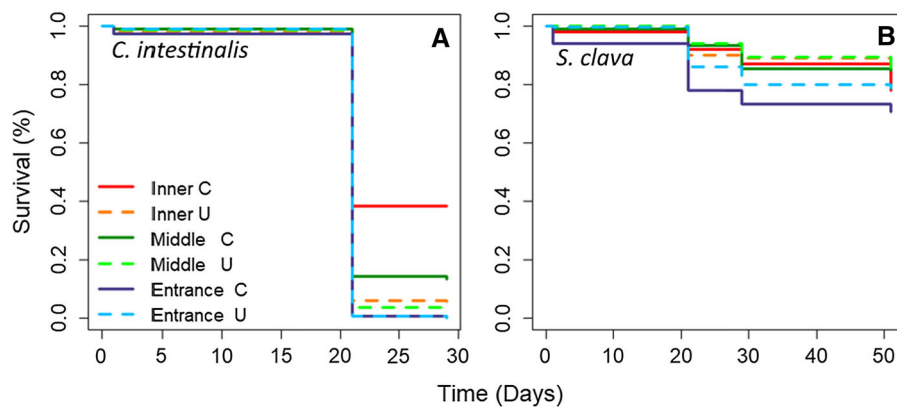
for this species. No significant effect of caging or location was observed in the log-rank test for *S. clava*.

### Dry mass

After 50 days, the total dry mass of natural *C. intestinalis* recruits and the dry mass per lab-grown *S. clava* individual varied between treatments (Fig. 3). The Wilcoxon tests revealed a significant caging effect for both species, but with opposite effects on species' biomass: higher biomass for *C. intestinalis* in caged treatments than in uncaged treatments and lower biomass for *S. clava* in caged treatments than in uncaged treatments ( $p < 0.05$  and  $p < 0.01$ , respectively; Fig. 3). Differences in biomass according to site were only identified for uncaged *C. intestinalis* between the inner and middle locations (Kruskal–Wallis multiple comparisons; adj.  $p < 0.05$ ).

### Contaminants

All tested PAHs concentrated in the sediments of the studied marina exceeded Canadian sediment quality guidelines and as well as concentrations at which 20% of sediments tested by the US Environmental Protection Agency would become toxic to model amphipods (Table 1; CCME 1999; US EPA 2005). Chrysene, phenanthrene, fluoranthene and fluorene largely exceeded values at which adverse effect on fauna are highly likely (Table 1). Total PCBs as well as two pesticides, lindane and pp'-DDD, also exceeded most guidelines used for comparison. Two PAHs, tPCB as



**Fig. 2** Kaplan–Meier survival curves for *Ciona intestinalis* (a) and *Styela clava* (b) for the inner, middle and entrance location of the marina. Dashed lines indicate uncaged

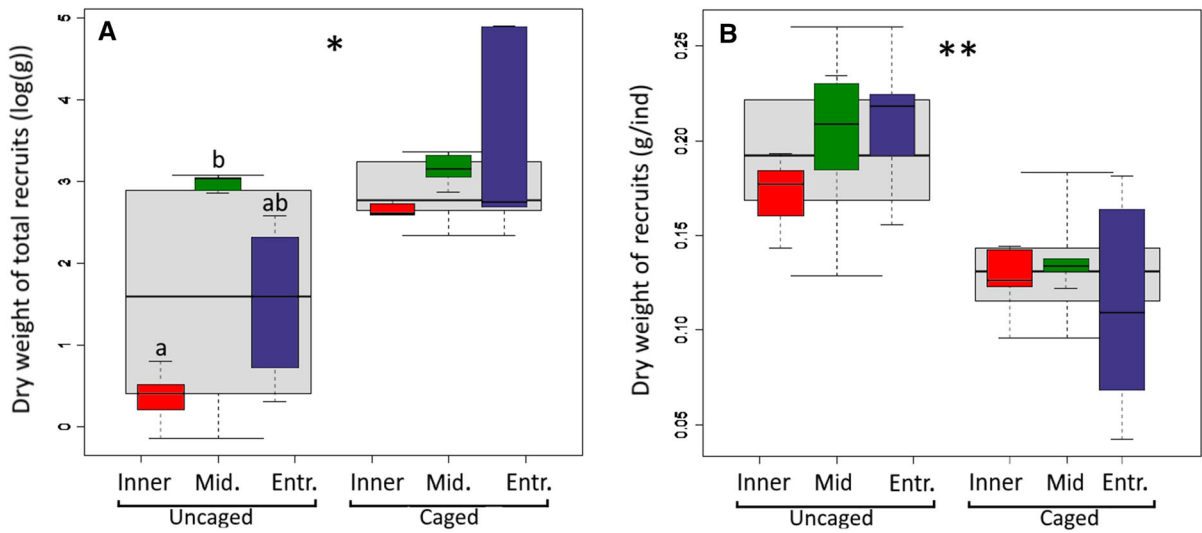
(U) treatments; solid lines indicate caged (c) treatments. *C. intestinalis* Entrance C and *C. intestinalis* Entrance U overlap. *S. clava* Inner U and *S. clava* Middle U overlap

**Table 1** Persistent Organic Pollutant (POP) contaminants in the sediments of the three study locations. Mean values ( $\mu\text{g}\cdot\text{kg}^{-1}$  = ppb) with standard deviation. Differences between locations were tested via a Kruskal–Wallis test followed by a Dunn Test. Reference values given by the Canadian Council of Ministers of the Environment (CCME 1999; ISQG: Interim

Sediment Quality Guideline; PEL: Probable Effect Level); and the United States Environmental Protection Agency (US EPA 2005, Table 11; T20: probability of 20% of toxicity among samples; T50: probability of 50% of toxicity among samples). Bold text and for reference values: threshold exceeded

	Site Mean $\pm$ SD			K-W test	CCME		US EPA	
	Inner	Middle	Entrance		ISQG	PEL	T20	T50
<b>Polycyclic Aromatic Hydrocarbon (PAH) <math>\mu\text{g}\cdot\text{kg}^{-1}</math></b>								
Acenaphthene	39 $\pm$ 8	34 $\pm$ 2	34 $\pm$ 1	ns	<b>6.71</b>	88.9	<b>19</b>	120
Acenaphthylene	51 $\pm$ 17	66 $\pm$ 8	61 $\pm$ 22	ns	<b>5.87</b>	128	<b>14</b>	140
Anthracene	59 $\pm$ 8	52 $\pm$ 12	45 $\pm$ 14	ns	<b>46.9</b>	245	<b>34</b>	290
Benzo[ <i>a</i> ]pyrene	324 $\pm$ 69	425 $\pm$ 109	407 $\pm$ 43	ns	<b>88.8</b>	763	<b>69</b>	520
Benzo[ <i>a</i> ]anthracene	379 $\pm$ 23	343 $\pm$ 15	354 $\pm$ 2	ns	<b>74.8</b>	693	<b>61</b>	470
Benzo[ <i>g,h,i</i> ]perylene	407 $\pm$ 79	212 $\pm$ 51	283 $\pm$ 8	*			<b>67</b>	500
Benzo[ <i>b</i> ]fluoranthene	700 $\pm$ 67	651 $\pm$ 141	880 $\pm$ 232	ns			<b>130</b>	1110
Benzo[ <i>k</i> ]fluoranthene	328 $\pm$ 72	413 $\pm$ 48	537 $\pm$ 78	ns			<b>70</b>	540
Chrysene	943 $\pm$ 185	1051 $\pm$ 61	1192 $\pm$ 173	ns	<b>108</b>	<b>846</b>	<b>82</b>	<b>650</b>
Dibenzo[ <i>a,h</i> ]anthracene	84 $\pm$ 19	70 $\pm$ 20	77 $\pm$ 19	ns	<b>6.22</b>	135	<b>19</b>	110
Fluorene	725 $\pm$ 19	567 $\pm$ 43	642 $\pm$ 56	*	<b>21.2</b>	<b>144</b>	<b>19</b>	<b>110</b>
Fluoranthene	1532 $\pm$ 430	1049 $\pm$ 71	1829 $\pm$ 268	ns	<b>113</b>	<b>1494</b>	<b>120</b>	<b>1030</b>
Indeno[1,2,3- <i>cd</i> ]pyrene	701 $\pm$ 106	713 $\pm$ 302	843 $\pm$ 313	ns			<b>68</b>	<b>490</b>
Naphthalene	232 $\pm$ 32	239 $\pm$ 37	344 $\pm$ 103	ns	<b>34.6</b>	391	<b>30</b>	<b>220</b>
Phenanthrene	1629 $\pm$ 363	1154 $\pm$ 66	1996 $\pm$ 239	ns	<b>86.7</b>	<b>544</b>	<b>68</b>	<b>460</b>
Pyrene	545 $\pm$ 8	510 $\pm$ 23	556 $\pm$ 17	ns	<b>153</b>	1398	<b>120</b>	930
<b>Polychlorinated Biphenyls (PCB) <math>\mu\text{g}\cdot\text{kg}^{-1}</math></b>								
tPCB	602.79 $\pm$ 5.93	506.03 $\pm$ 12.4	543.9 $\pm$ 2.54	*	<b>21.5</b>	<b>189</b>	<b>35</b>	<b>370</b>
<b>Pesticides <math>\mu\text{g}\cdot\text{kg}^{-1}</math></b>								
Aldrin	0.65 $\pm$ 0.25	0.85 $\pm$ 0.14	1.46 $\pm$ 0.09	ns				
Trans_chlordane	1.2 $\pm$ 0.06	0.15 $\pm$ 0.03	0.17 $\pm$ 0.06	ns				
Cis_chlordane	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01	0.07 $\pm$ 0.03	ns				
tChlordane	1.26 $\pm$ 0.05	0.21 $\pm$ 0.04	0.24 $\pm$ 0.09	ns	2.26	4.79		
Diazinon	0.57 $\pm$ 0.11	0.83 $\pm$ 0.28	1.46 $\pm$ 0.12	*				
Dieldrin	1.11 $\pm$ 0.1	1.23 $\pm$ 0.04	2.19 $\pm$ 0.3	*	<b>0.71</b>	4.3	<b>0.83</b>	2.9
pp'-DDD	10.65 $\pm$ 0.67	8.41 $\pm$ 1.09	2.53 $\pm$ 0.29	*	<b>1.22</b>	<b>7.81</b>	<b>2.2</b>	19
pp'-DDE	17.62 $\pm$ 0.48	22.11 $\pm$ 0.32	15.55 $\pm$ 1.74	*	<b>2.07</b>	374	<b>3.1</b>	100
pp'-DDT	0.62 $\pm$ 0.1	0.14 $\pm$ 0.03	0.1 $\pm$ 0.05	*	1.19	4.77	1.7	11
Endosulfan-1	3.09 $\pm$ 0.09	1.21 $\pm$ 0.07	2.33 $\pm$ 0.78	*				
Endosulfan-2	14.11 $\pm$ 0.83	2.66 $\pm$ 0.47	13.18 $\pm$ 2.09	ns				
Heptachlor	1.24 $\pm$ 0.2	0.72 $\pm$ 0.14	0.88 $\pm$ 0.3	ns	<b>0.6</b>	2.74		
Heptachlor_epoxide_a	40.21 $\pm$ 4.65	21.44 $\pm$ 5.66	18.96 $\pm$ 1.66	ns				
Heptachlor_epoxide_b	3.48 $\pm$ 0.29	2.76 $\pm$ 0.27	2.81 $\pm$ 0.05	ns				
Isodrin	0.6 $\pm$ 0.16	0.21 $\pm$ 0.03	0.28 $\pm$ 0.07	ns				
Lindane	2.03 $\pm$ 0.24	1.81 $\pm$ 0.32	1.94 $\pm$ 0.23	ns	<b>0.32</b>	<b>0.99</b>		
Methoxychlor	7.43 $\pm$ 1.28	5.19 $\pm$ 0.21	2.52 $\pm$ 0.22	*				





**Fig. 3** Logarithm of total dry mass of naturally recruited *Ciona intestinalis* (a) and dry mass per lab grown *Styela clava* individual (b) at 50 days. Significant differences between caged

and uncaged treatments: \*  $p < 0.05$  and \*\*  $p < 0.01$  (Wilcoxon test). Significant differences within the uncaged *C. intestinalis* (a-b)  $p < 0.05$  (Dunn test)

**Table 2** Metallic Trace Element (MTE) contaminants in the sediments of the three study locations. Mean values (mg/kg = ppm) with standard deviation. Differences between locations were tested via a Kruskal–Wallis test followed by a Dunn Test. Reference values given by the Canadian Council of Ministers of the Environment (CCME 1999; ISQG: Interim Sediment Quality Guideline; PEL: Probable Effect Level); and

the United States Environmental Protection Agency (US EPA 2005, Table 11; T20: probability of 20% of toxicity among samples; T50: probability of 50% of toxicity among samples). Sediment quality category after Guerra-García et al. (2021) indicated. Bold text and for reference values: threshold exceeded

Metallic trace elements (MTE) mg.kg <sup>-1</sup>	Site mean ± SD			K-W test	CCME		US EPA		Guerra-García et al. 2021
	Inner	Middle	Entrance		ISQG	PEL	T20	T50	
Al27(MR)	29207 ± 3825	26647 ± 9596	23125 ± 12905	ns					
As75(HR)	14 ± 3	11 ± 3	9 ± 5	ns	<b>7.24</b>	41.6	<b>7.4</b>	20	Good
Co59(MR)	5.78 ± 0.58	5.5 ± 1.15	4.72 ± 2.65	ns					Good
Cr52(MR)	57 ± 7	51 ± 11	44 ± 25	ns	<b>52.3</b>	160	<b>49</b>	140	Good
Cu63(MR)	84 ± 14	45 ± 10	33 ± 19	**	<b>18.7</b>	108	<b>32</b>	94	<b>Moderate</b>
Fe56(MR)	13569 ± 1427	12639 ± 2980	10643 ± 6037	ns					
Mn55(MR)	150 ± 22	154 ± 29	134 ± 74	ns					
Ni60(MR)	18 ± 2	17 ± 4	14 ± 8	ns			<b>15</b>	47	Good
Pb208(LR)	85 ± 12	48 ± 14	46 ± 25	*	<b>30.2</b>	112	<b>30</b>	94	<b>Moderate</b>
Ti47(MR)	2283 ± 291	2285 ± 506	1923 ± 1094	ns					
V51(MR)	90 ± 11	76 ± 19	65 ± 37	ns					
Zn66(MR)	236 ± 89	178 ± 77	111 ± 60	*	<b>124</b>	271	<b>94</b>	240	Good

well as seven pesticides had significant differences in concentration between locations (Kruskal–Wallis,  $p < 0.05$ ; Table 1).

Almost all MTE were distributed as a gradient from the inner location (max.) to the entrance location (min.). Cu, Pb and Zn showed significant differences

between locations (Kruskal–Wallis,  $p < 0.05$ ). Cu and Zn concentrations in sediment were significantly higher at the inner location compared to the entrance location (Kruskal–Wallis multiple comparisons, adj.  $p < 0.05$ ). Most of the MTE concentrations were slightly above the Canadian sediment quality guideline and above the concentration of 20% probability of toxicity, falling within a sediment quality category of good or moderate for Cu and Pb (Table 2; CCME 1999; US EPA 2005; Guerra-García et al. 2021).

#### Environmental variables

Temperature variation between locations was lower than logger precision ( $< 0.2$  °C). Mean light intensity ranged from 7.6 and 8.5 Lux at the entrance and middle respectively to 45.4 Lux at the inner location.

#### Feeding assay

During the 60 h of video footage, no fish or other predators feeding on the bait were recorded but the black seabream *Spondyliosoma cantharus* (Linnaeus, 1758) was observed feeding on fouling communities close to the baits. A total of 42 out of 225 baits (18.67%) were consumed in the experiment. Predation intensity was relatively low at all locations, but varied in space, ranging from 10 and 16% at inner and entrance location respectively to 30% (middle location) consumption after 24 h. Using survival analysis on the baits, a log-rank comparison showed a significant difference ( $p < 0.01$ ) between the middle and the inner location. The entrance location had an intermediate predation intensity not significantly different to either of the two other sites.

#### Effect of contaminants and predation intensity on recruit survival

The results of the  $10^5$  iterations of Cox models after random pairing of samples from the same location showed that while none of the tested contaminants influenced ascidian recruit survival; caging and predation intensity had an influence on *C. intestinalis* (Table 3). Caging reduced the mortality risk by 22% for *C. intestinalis* ( $p < 0.05$  in 100% of iterations) while it increased it by 33% for *S. clava* ( $p < 0.05$  in 86% of iterations). While predation intensity explained only a small fraction of the survival of *C.*

*intestinalis* ( $p < 0.05$  in 25% of iterations but risk increase of 0%), the interaction between predation intensity and caging seemed to have a stronger association with its survival ( $p < 0.05$  in 52% of iterations, risk increase 1%).

#### Discussion

Our experiments yielded insights on the survival dynamics of the two species during their juvenile phase. They showed that the native *C. intestinalis* was strongly affected by environmental heterogeneity and predation (significant differences according to location variability and caged/uncaged treatments, respectively), but that the alien *S. clava* was less affected by either factor.

The survival of *C. intestinalis* varied significantly among locations within the marina, following an increasing gradient from the entrance to the innermost part. Interestingly, the survival gradient was organized in the opposite direction of our prediction. We based this prediction on a sampling of three MTE (Cu, Pb and Zn) in 2016, which revealed a pollution gradient with maximal values in the innermost location of the marina, structuring the community along this gradient (Kenworthy et al. 2018b). In the present study, we confirm significant gradient of Cu, Pb and Zn among locations. Additionally, Benzo[*g,h,i*]perylene, Fluorene and total PCBs showed significant differences in concentrations among locations, but not as gradient. They had minimal values in the middle location of the marina. Additionally, seven pesticides had significant differences between locations with varying distribution profiles. It is notable that the studied marina sediment largely exceeds adverse effect levels indicated by Canadian (PEL) and USA (50% probability of toxicity) sediment quality guidelines for Chrysene, Fluorene, Fluoranthene and Phenanthrene (PAH) as well as for total PCBs (CCME 1999; US EPA 2005). On the contrary MTE pollution, even if it is above some of these sediment quality guidelines, falls within quality categories described as good or moderate (Guerra-García et al. 2021). Despite the significant effect of location variability on *C. intestinalis* as shown by the Log-Rank test, none of the contaminants varying significantly between locations could explain the survival profile of any of both tested species in the Cox models. It may be that the sediment

**Table 3** Statistics associated to multiple iterations of random sample pairing followed by a Cox regression. Mean Coefficient, mean risk increase (Mean Hazard Ratio - 1) and percentage of significant *p* values after 10<sup>5</sup> iterations of random sample linking followed by a Cox model (nested within Petri dish, site and their interaction) for each species, integrating all factors indicated in the first column. Black and bold font: factor of interest

Factor	Species	Mean coefficient	Mean risk increase (mean HR-1) (%)	% of <i>p</i> values below 0.05
<b>Predation</b>				
Caging (uncaged compared to caged)	<i>Ciona intestinalis</i>	<b>0.20</b>	<b>22</b>	<b>100</b>
	<i>Styela Clava</i>	<b>- 0.40</b>	<b>- 33</b>	<b>86</b>
Predation intensity	<i>Ciona intestinalis</i>	<b>0.004</b>	<b>0</b>	<b>25</b>
	<i>Styela Clava</i>	- 0.01	- 1	6
Predation intensity: Caging	<i>Ciona intestinalis</i>	<b>- 0.01</b>	<b>- 1</b>	<b>52</b>
	<i>Styela Clava</i>	0.02	2	10
<b>PAHs</b>				
Benzo(g,h,i)perylene	<i>Ciona intestinalis</i>	0.00	0	6
	<i>Styela Clava</i>	0.00	0	5
Fluorene	<i>Ciona intestinalis</i>	0.00	0	6
	<i>Styela Clava</i>	0.00	0	5
<b>PCB</b>				
tPCB	<i>Ciona intestinalis</i>	0.00	0	9
	<i>Styela Clava</i>	0.00	0	6
<b>Pesticides</b>				
diazinon	<i>Ciona intestinalis</i>	0.02	2	7
	<i>Styela Clava</i>	0.06	14	6
dieldrin	<i>Ciona intestinalis</i>	0.02	2	8
	<i>Styela Clava</i>	0.07	13	6
pp'-DDD	<i>Ciona intestinalis</i>	- 0.01	- 1	14
	<i>Styela Clava</i>	- 0.04	- 4	8
pp'-DDE	<i>Ciona intestinalis</i>	0.00	0	6
	<i>Styela Clava</i>	- 0.01	- 1	6
pp'-DDT	<i>Ciona intestinalis</i>	- 0.08	- 6	8
	<i>Styela Clava</i>	- 0.04	50	5
endosulfan-1	<i>Ciona intestinalis</i>	0.00	0	5
	<i>Styela Clava</i>	0.01	2	5
methoxychlor	<i>Ciona intestinalis</i>	- 0.01	- 1	7
	<i>Styela Clava</i>	- 0.02	- 1	6
<b>MTE</b>				
Cu63(MR)	<i>Ciona intestinalis</i>	0.00	0	6
	<i>Styela Clava</i>	0.00	0	5
Pb208(LR)	<i>Ciona intestinalis</i>	0.00	0	5
	<i>Styela Clava</i>	0.00	0	5
Zn66(MR)	<i>Ciona intestinalis</i>	0.00	0	6
	<i>Styela Clava</i>	0.00	0	5

contaminant concentrations, which integrate long term tendencies, might not have reflected the very variable water contaminant concentration at the time of our experiment. This also might indicate that another, not measured confounding factor, varied between locations and influenced the survival of this species. Factors like salinity, physical disturbance and hydrodynamics, modulated by the artificial structures could impact the survival of the recruits (Walters and Wethey 1996; Clark and Johnston 2009; Saloni and Crowe 2015; Kenworthy et al. 2018a; Ferrario et al. 2020). Additionally, mesopredators or micropredators like nudibranchs and caprellids, which have been shown to have strong influence on benthic communities, would still have access to the inside of the cages and could thus affect recruit survival independently of caging (Osman and Whitlatch 1995; Lavender et al. 2014; Leclerc et al. 2019). If these mesopredators are impacted by contaminants, their abundance could vary in space and be maximal at the entrance of the marina, which would explain the observed survival pattern of *C. intestinalis* recruits. To disentangle the effect of mesopredators and abiotic factors, future experiments should integrate a way of identifying the effect of smaller predators on survival and integrate more environmental factors (Lavender et al. 2014). The systematically higher survival of *S. clava* compared to *C. intestinalis* suggests that this species is more resistant to the environmental variables impacting the survival of *C. intestinalis*. However, its abundance in marinas of the studied region is much lower than that of *C. intestinalis*, indicating this survival advantage may only occur at the recruit stage or indicate that *C. intestinalis* dominates due to its high fecundity and growth rate and not survival (Jackson 2008). The higher survival of *S. clava* in its recruit stage supports the idea that NIS are more resistant to abiotic stress and is consistent with studies indicating that NIS in marina environments show higher abundance in anti-fouling polluted environments (Dafforn et al. 2008; Piola and Johnston 2008; Piola et al. 2009).

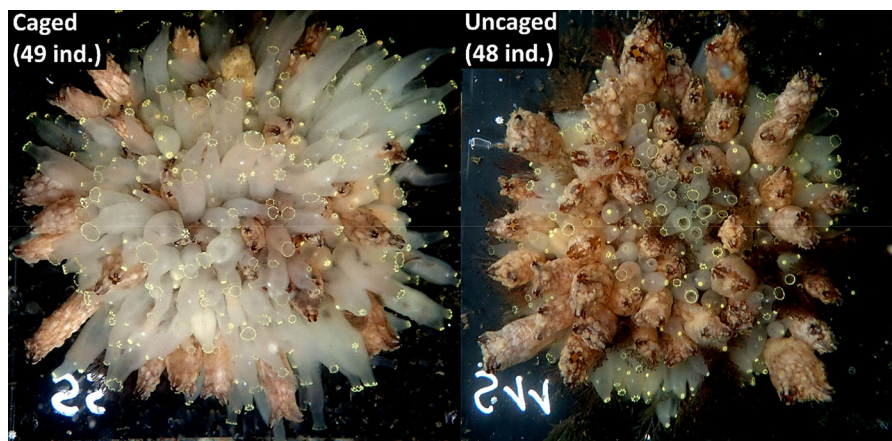
In addition to the location, caging also seemed to strongly affect the survival of *C. intestinalis* recruits, their survival increased when they were caged. At the entrance location, this effect was however not observable in the Log-Rank test due to the high mortality in both caged and uncaged treatments. This probably also leads to an underestimation of the risk decrease in the Cox model. Predation intensity estimated by the

feeding experiment varied in space with maximal predation pressure in the middle of the marina and a minimal pressure at the inner part of the marina. When treated as an explanatory variable, the spatially heterogeneous predation intensity may be linked to the survival of *C. intestinalis* recruits in the Cox models since it slightly reduces their survival in 25% of iterations. The interaction of caging and predation intensity further reveals a potential link between the increased survival in caged treatments and the impact of predators partaking in the feeding experiment. As previously highlighted, the squid-bait feeding experiment targets mostly fish with a probable contribution of swimming crabs. Everything considered, we suggest that a significant part of predation on ascidians may thus be exerted by highly mobile generalist predators. The surveillance cameras installed around the feeding assay observed the black seabream *Spondyliosoma cantharus* (Linnaeus, 1758) feeding on fouling communities. This species is a plausible predator on ascidian recruits, as multiple *Sparidae* species can significantly influence marina communities, and also feed on squid bait (Oricchio et al. 2016b; Rodemann and Brandl 2017). Studies frequently demonstrated that *C. intestinalis* (see Astudillo et al. 2016) and its congener *Ciona robusta* (Hoshino & Tokioka, 1967) are vulnerable to predation in their respective introduced ranges, highlighting that predators may participate in biotic resistance against them (Dumont et al. 2011; Astudillo et al. 2016; Leclerc et al. 2019; Giachetti et al. 2020). Our results are consistent with observations of the predation on *Ciona* spp., although in the present case, *C. intestinalis* was studied in its native range. The most abundant solitary non-indigenous ascidian in the Brest marina, *S. clava*, was not affected by predation intensity and had a higher mortality risk if caged. Considering the invasive status of *S. clava*, biotic resistance – as exerted by predators – does not seem to occur in the context of the present study. *Styela clava*, especially adults are an unappealing food item for predators due to their tough tunic (Clarke and Thomas 2007). Moreover, its congener *Styela plicata* (Lesueur, 1823) has been shown to be unpalatable to fish due the accumulation of chemically deterrent secondary metabolites in their gonads (Pisut and Pawlik 2002; Koplovitz and McClintock 2011). We suggest that this may also be the case for *S. clava* as adults or recruits. On the other hand, *C. intestinalis* has no chemical deterrents with

regard to palatability and is readily consumed by crabs and fishes (Teo and Ryland 1994; Koplovitz and McClintock 2011). Our results thus may provide support for the Novel Weapons Hypothesis (NWH), as in the absence of co-evolution of the native species, predator avoidance strategies of a NIS can indirectly result in competitive superiority (Hay et al. 1994; Callaway and Ridenour 2004; Cappuccino and Carpenter 2005). The non-indigenous *S. clava* may possess a strong predator avoidance system, whereas the native *C. intestinalis* may be comparatively heavily affected by predation.

The total dry mass of naturally recruited *C. intestinalis* was significantly lower in uncaged treatments than in caged ones. This difference in dry mass was either due to lower abundance or smaller individual size since predation would cause mortality before recruits could grow in uncaged treatments. In both cases, this difference can be attributed to higher mortality due to predation. Interestingly, no natural *S. clava* recruits were observed in this study. Having strong differences in reproductive season according to locality, this species seems to reproduce and recruit in late summer/autumn, explaining the absence of natural recruits (Clarke and Thomas 2007). For *S. clava*, the individual dry mass was significantly higher in uncaged treatments than in caged treatments, suggesting a negative effect of caging on *S. clava*. This result is further supported by the Cox model indicating a 33% increased risk of mortality when caged. This may appear intriguing because predators cannot directly have a positive influence on *S. clava*. A cage control

treatment could have helped to understand if these results were linked to the presence of the cage itself or due to the influence of predation (Giachetti et al. 2019). Here we were however not able to integrate a cage control treatment as we were not able to produce enough dishes with a sufficient number of recruits of both species in the laboratory for three treatments per location. It might be, that the cage acted as a refuge for meso- and micro-predators, which could feed on ascidians, here especially *S. clava*, as devoid of predation by macro-predators (Lavender et al. 2014). However, such an effect of mesopredation would uniquely affect survival and not the mean individual mass as it has been affected in our experiment. Furthermore, a previous study conducted in the same marina has shown no effect of caging (protection from large predators) on small mobile fauna (mesopredators) assemblage and abundance (Leclerc and Viard 2018). It thus seems unlikely that the cages acted as refuge for mesopredators and that they may be responsible for the negative effect of caging on *S. clava*. Another mechanism seems more likely to explain the observed results. When observing photos of the *S. clava* dishes, it is striking that uncaged *S. clava* were prominent, while they were smothered by naturally recruited *C. intestinalis* in caged treatments. We thus think that an indirect positive interaction might be induced by the predation on natural *C. intestinalis* recruits in uncaged *S. clava* panels, leading to a decrease in the spatial and trophic competition exerted on *S. clava* by *C. intestinalis* (Fig. 4). Considering this potential competition our study case



**Fig. 4** Caged and uncaged Petri dish of *Styela clava* recruits after 50 days in the field. Both panels originating from the inner location of the marina. Natural recruits of *Ciona intestinalis* are

more visible (larger, more abundant) in the caged treatment. Individual number refers to final *S. clava* at 50 days



seems to indicate that generalist predators might facilitate the NIS *S. clava* through competitive release, a specific mechanism of the Enemy Release Hypothesis (ERH; Keane and Crawley 2002). This hypothesis could be confirmed by conducting a similar experiment to ours, but mixing both species' recruits on the dishes from the beginning.

In the present study, we showed that the survival of the native *C. intestinalis* was highly influenced by both location and predation, itself variable in space. To date, the very few studies that have focused on the very small-scale spatial variability of communities in marina environments have not considered the possibility of spatially varying predation. For the studied ascidians, we observed a preference of predators for the native prey similar to Cuthbert et al. (2018) and Kincaid and de Rivera (2020). The present results support the idea that generalist predation may play a crucial role in the success of NIS due to facilitation through competitive release (Keane and Crawley 2002; Kincaid and de Rivera 2020). The results also provide a new rationale for how generalist predators may or may not contribute to biotic resistance. Many studies involving *Ciona* spp. in its introduced range, conclude that various predators exert biotic resistance against this species (Dumont et al. 2011; Leclerc et al. 2019; Giachetti et al. 2020). Here, we showed that in the native range of *C. intestinalis*, predation exerted by mobile generalist predators potentially leads to the facilitation of a NIS. Thus, the question if biotic resistance against NIS is exerted by predators may depend more on the identity and characteristics of the considered species (native, NIS and predator), rather than on their status (NIS vs native; Skein et al. 2020). Predictions on whether a NIS encounters resistance or facilitation by local predators in its introduced range could thus be formulated by looking at how various types of predation affect it in its native environment.

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**Data accessibility** All data tables generated or analyzed during this study are included in this published article and its supplementary material files. Photos analyzed shall be shared upon request (corresponding author).

**Code availability** Code for the program 'R' can be requested upon the corresponding author.

#### Declarations

**Conflict of interest** All authors declare that they have no potential conflicts of interest with any organization or entity with financial or non-financial interest in the subject discussed in this manuscript.

**Ethical standards** We followed national and international ethical guidelines whenever applicable.

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