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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 assess factors influencing 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, but 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), if they become invasive, can be drastic, with some invaders completely restructuring ecosystems, potentially leading to the loss of biodiversity and ecosystem services (Pejchar and Mooney 2009). This has led to a large body of literature focusing on the conditions of successful transport and naturalization 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 to the transport phase, the first major obstacle a NIS may encounter in a new environment is the abiotic environment. Abiotic factors have been shown to vastly influence species' evolution and the 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; Lejeusne 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 abiotic filter 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 on the apparent success (or failure) of NIS in various ecosystems. Being a major regulator of ecosystem functioning through regulation of prey populations and a driver of community structure, 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), the combined antagonistic interactions among 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 environments. 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). As such, NIS may perform better in introduced areas and gain competitive advantages over native species (Keane and Crawley 2002; Joshi and Vrieling 2005). Although the presence of generalist predators or host-switching specialists may partially undermine this assumption, the food selectivity of generalist predators, potentially favoring native species, may benefit NIS due to competitive release (Keane and Crawley 2002). NIS success can also be explained by the Novel Weapon Hypothesis (NWH; Callaway and Ridenour, 2004), implying specific adaptations of a NIS to its native habitat. In the absence of coevolution with NIS, native species may lack an evolutionary response to an NIS, leading to higher competitive performance of the invader (Ni et al. 2012). This NWH can involve direct competition and allelopathic interactions between NIS and natives, as extensively described for plant species (Callaway and Aschehoug 2000; Ni et al. 2012). However, such compounds may also be part of predator avoidance and thus indirectly result in competitive superiority (Hay et al. 1994; Cappuccino and Carpenter 2005).

While still debated, these hypotheses provide a complex interplay of ideas in the context of predation and acknowledge its importance. 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). Being predominantly tested in terrestrial habitats, few studies have been conducted on marine invasions often with inconsistent results with regard to these hypotheses (Chan and Briski 2017). Nonetheless, fouling communities and especially artificial harbor or marina communities are highly exposed to 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). This extensive exposure to NIS can lead to high economic and ecological costs, making it all the more important to better understand the factors behind NIS success in these ecosystems (Ojaveer et al. 2015). Despite apparent resemblances between plant and fouling communities, there is no consensus on whether their functioning is actually similar, limiting the conclusions that can be transposed from terrestrial studies to marine communities (Sutherland and Karlson 1977). Most notably, fouling communities in marinas experience strong anthropogenic disturbances, which have been the focus of numerous studies showing their effect on surrounding 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 (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 on NIS success in their range of introduction. Many studies have investigated biotic

resistance to invasions (Kimbro 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 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. The size-spectrum theory and the tendency of predatory fish to include small prey in their diet predicts higher predation pressure on small preys, i.e. recruits (Osman and Whitlatch 2004; Law et al. 2009). Recruits may also be more sensitive to environmental stressors (Saloni and Crowe 2015). We assumed that environmental conditions 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. We hypothesized that this resulting gradient of survival is less visible for the studied NIS species, because NIS tend to better resist disturbances due to their selection during introduction (Gröner et al. 2011). 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). This recreational marina is part of a larger marine urban zone with a commercial and military harbor leading to a highly anthropized environment. As a typical marina, it is made of many artificial substrata

that can be colonized by fouling communities, it is contaminated by elemental trace metals (such as copper and lead) and persistent organic pollutants (POPs), and it hosts many NIS (Kenworthy et al. 2018b). We focused on floating pontoons in our experimental setups because they are the most abundant substratum in the marina. This marina has significant variations in environmental conditions at small spatial scales (< 100 m), driving different fouling communities at the entrance, the middle and inner parts of the marina (Kenworthy et al. 2018b). In accordance with this previous study, 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 species in most marinas in Brittany (NW France), including the present one (Bouchemousse et al. 2016). This species is characterized by rapid growth, a high reproduction rate and a short life cycle, proprieties that otherwise make it highly invasive in many parts of the world (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 have convergent characteristics, being highly invasive in their respective areas of introduction, especially 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 (>15cm) 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 before *C. intestinalis*. 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. On a Petri dish, oocytes from two individuals (to maintain some variability between recruits) were distributed. Sperm was assigned to a sperm pool so as to avoid fertilizing oocytes from the same individual and mixed with filtered seawater. One pool of

sperm cells was introduced into Petri dishes 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* ('Argenton') strains cultured at the Roscoff Culture Collection (RCC, http://roscoffculture-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 to avoid recruits overcrowding. Later, all dishes were individually fixed on 20 cm × 20 cm black PET panels to provide shade 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 crustacean 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 (~1 m depth; 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 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 shown by their position on the black dots. 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 at 50 days after deployment, especially considering that lab-grown recruits' mortality was high – and likely even 100%. 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. Natural *C. intestinalis* recruits were nevertheless used to compare their biomass between treatments. Given that the biomass of natural recruits is linked to their survival, this provides additional information on *C. intestinalis* survival between treatments. 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.

Feeding assay

In addition to the exclusion cages, predation intensity was directly measured with a feeding assay. It was developed to measure fish predation intensity in different environments and 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 and can only be detached as one piece. 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 x 3 locations x 3 dates = 225 baits were deployed. A snorkeler counted the remaining baits 1 h, 3 h, 6 h and 24 h after deployment to use survival analysis rather than comparisons of means, thus providing more detail on the bait consumption (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. During the 60 h of footage, no fish feeding on the bait were recorded.

Contaminant assessment

Three sediment samples (~ 0.4kg) were taken 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 (~ 0.4kg) were taken to quantify Metallic Trace Elements (MTE).

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. 1)

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 Tab. 1. PAHs were determined following a previously described method (Sarrazin et al. 2006; Ratier et al. 2018; Dron et al. 2019) that is also further detailed in the supplementary material. We focused on 16 PAH congeners defined by the US Environmental Protection Agency (USEPA) priority list (Tab. 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 Metallic Trace Elements (MTE) 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.

Statistical methods

Mean value and standard deviation were calculated for each contaminant. Differences between locations were identified via a Kruskal-Wallis test (R, CRAN, version 3.6.1). 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). 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). Since sediment samples (contaminants), feeding experiments (predation rate) 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 24h) as well as their interaction, on the survival of ascidian recruits. 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 modulization was repeated 10^5 times and p values and coefficients were saved in a separate table. The percentage of times $p < 0.05$ as well as the mean coefficient and mean hazard ratio were calculated for each model factor. We considered factors with above 25% of significative p-values as factors of interest and factors with above 50% as factors with a clear link to survival. To further investigate the effect of the interaction term between predation risk and caging on the recruits, linear models were established testing for an effect of predation intensity on the percentage of surviving recruits at the end of the experiment in both, caged and uncaged treatments.

Results

Recruit survival

The Kaplan-Meier survival curve of the recruits showed mortality for both species (Fig. 1). 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* (blue lines, Fig. 1) was consistently lower than for *S. clava* (red/orange lines, Fig. 1; $p < 0.001$). Location had a significant effect on the survival of caged *C. intestinalis*. Survival was the highest at the inner part, intermediate at the middle and the lowest at the entrance of the marina ($p < 0.001$). Conversely, location in the marina had no significant influence on survival of caged *S. clava*. 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 ($p < 0.01$). At the marina entrance, mortality was 100% for the caged and uncaged treatments for this species. No significant caging effect was observed in the log-rank test for *S. clava* at any location.

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. 2). The Wilcoxon tests revealed a significant caging effect for both species, but with opposite significant 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 2). Differences in biomass according to site were only identified for uncaged *C. intestinalis* between the inner and middle locations (Kruskal-Wallis multiple comparison; 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 (Tab. 1; CCME 1999; US

EPA 2005). Chrysene, phenanthrene, fluoranthene and fluorene largely exceeded values at which adverse effect on fauna are highly likely (Tab. 1). Total PCBs as well as two pesticides, lindane and pp'-DDD, also exceeded most guidelines used for comparison. Two PAHs, tPCB as well as seven pesticides had significant differences in concentration between locations (Kruskal-Wallis, $p < 0.05$; Tab. 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 barely 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 (Tab. 2; CCME 1999; US EPA 2005; Guerra-García et al. 2021).

Feeding assay

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 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 significatively different than either of the two other sites.

Statistical models

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 had a clear link with survival, caging and predation intensity had an influence on *C. intestinalis*. Caging reduced the mortality risk by 22% for *C. intestinalis* while it increased it by 33% for *S. clava*. While predation intensity explained only a small fraction of the survival of *C. intestinalis*, the interaction between predation intensity and caging also seemed to better explain its survival. Neither in the caged nor in the uncaged treatment, linear models showed a significant effect of predation intensity on the percentage of surviving recruits.

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 inner-most part. Interestingly, the survival gradient was organized in the opposite direction of our prediction, based on a gradient of anthropogenic disturbance which should be maximal at the inner location of the marina. Metallic Trace Elements in sediments were distributed along this supposed gradient with maximal values in the innermost part of the marina, which is particularly visible for Cu, Pb and Zn. Our reasoning behind the hypothetic anthropogenic disturbance gradient was based on a sampling of those three MTE in 2016 (confirmed in the present), where copper, commonly used in anti-fouling painting on boats, has been shown to have significant effects on the community structure in this marina (Kenworthy et al. 2018b). In the present study, Benzo(g,h,i)perylene, Fluorene and total PCBs showed significant differences in concentrations between locations, but not along the supposed disturbance gradient. They had minimal values in the middle part 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 north American standards of sediment quality for Chrysene, Fluorene, Fluoranthene and Phenanthrene (PAH) as well as for total PCBs, with a 50% probability of toxicity on model amphipods if they were exposed to the sediment (CCME 1999; US EPA 2005). On the contrary MTE pollution, even if above some of these sediment quality guidelines, falls within quality categories described as good or moderate for Cu and Zn as proposed in (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 this species in the Cox models. This might indicate that another, not measured factor, varied between locations and influenced the survival of this species. 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 Whitlach 1995; Lavender et al. 2014; Leclerc et al. 2019). If these mesopredators are impacted by anthropogenic disturbance, 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. Unmeasured abiotic factors might also be at play. Factors like temperature, salinity and hydrodynamics, modulated by the artificial structures could impact the survival of the recruits (Walters and Wethey 1996; Kenworthy et al. 2018a). To decide between these variables, 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* suggests that this species is more adapted to the general marina environment and the factors impacting the survival of *C. intestinalis*. However, its abundance in marinas 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. This result is shown in the Log-Rank as well as in the Cox models with an associated 22% mortality risk decrease. 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. This predation intensity might be linked to the survival or *C. intestinalis* recruits in the Cox models since it slightly reduces their survival in 25% of iterations. The influence of the interaction of caging and predation intensity is due to the fact that predation could not apply on caged individuals. While not being able to demonstrate it, we propose that predation intensity acts negatively on the survival of *C. intestinalis* in uncaged treatments, while it is neutral for caged individuals. This interaction, however, further demonstrates that the increased survival in caged treatments may at least partly due to a protection against the predators partaking in the feeding experiment. As previously highlighted, the squid-bait feeding experiment targets almost exclusively fish (Duffy et al. 2015). However, we cannot exclude the possibility that native velvet swimming crabs *Necora puber* (Linnaeus, 1767) also partook in the feeding assay and the predation on *C. intestinalis* recruits. 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 did not record any direct attacks on baits, but camera surveillance observed the black seabream *Spondyliosoma cantharus* (Linnaeus, 1758) feeding on fouling communities close to the baits. This species is a plausible predator on ascidian recruits, as *Sparidae* 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* and its congener *Ciona robusta* (Hoshino & Tokioka, 1967) are vulnerable to predation in their introduced ranges, highlighting that predators may participate in biotic resistance against them in the areas that they attempt to invade (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 for the *S. clava*/*C. intestinalis* pair, thus making the Biotic Resistance Hypothesis unlikely in this study. *Styela clava,* especially adults are an unappealing food item for predators due to their tough tunic (Clarke and Thomas W. 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; Hay et al. 1994; 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*. One explanation 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. 3). Considering this competition it seems to

indicate that, in our study case, generalist predators 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 contribute to biotic resistance. In contrast to the many studies involving *Ciona* spp. as a non-indigenous model concluding that various predators exert biotic resistance (Dumont et al. 2011; Leclerc et al. 2019; Giachetti et al. 2020), we showed that in the native range of *C. intestinalis*, predation exerted by mobile generalist predators potentially leads to NIS facilitation. Thus, the issue of biotic resistance may depend more on the identity and characteristics of the considered native species, NIS and predator, rather than on the studied species 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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Declarations

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Conflicts 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.

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.

Compliance with Ethical Standards

We followed national and international ethical guidelines whenever applicable.

Pollutant		Site mean ± SD			K-W	CCME		US EPA	
		Inner	Middle	Entry	test	ISQG	PEL	T20	T50
Polycyclic Aromatic Hydrocarbon (PAH) µg.kg⁻¹	Acenaphthene	39 ± 8	34 ± 2	34 ± 1		6,71	88,9	19	120
	Acenaphthylene	51 ± 17	66 ± 8	61 ± 22		5,87	128	14	140
	Anthracene	59 ± 8	52 ± 12	45 ± 14		46,9	245	34	290
	Benzo(a)pyrene	324 ± 69	425 ± 109	407 ± 43		88,8	763	69	520
	Benzo(a)anthracene	379 ± 23	343 ± 15	354 ± 2		74,8	693	61	470
	Benzo(g,h,i)perylene	407 ± 79	212 ± 51	283 ± 8	*			67	500
	Benzo(b)fluoranthene	700 ± 67	651 ± 141	880 ± 232				130	1110
	Benzo(k)fluoranthene	328 ± 72	413 ± 48	537 ± 78				70	540
	Chrysene	943 ± 185	1051 ± 61	1192 ± 173		108	846	82	650
	Dibenzo(a,h)anthracene	84 ± 19	70 ± 20	77 ± 19		6,22	135	19	110
	Fluorene	725 ± 19	567 ± 43	642 ± 56	*	21,2	144	19	110
	Fluoranthene	1532 ± 430	1049 ± 71	1829 ± 268		113	1494	120	1030
	Indeno(1,2,3-cd)pyrene	701 ± 106	713 ± 302	843 ± 313				68	490
	Naphthalene	232 ± 32	239 ± 37	344 ± 103		34,6	391	30	220
	Phenanthrene	1629 ± 363	1154 ± 66	1996 ± 239		86,7	544	68	460
	Pyrene	545 ± 8	510 ± 23	556 ± 17		153	1398	120	930
Polychlorinated Biphenyl (PCB) µg.kg⁻¹	tPCB	602,79 ± 5,93	506,03 ± 12,4	543,9 ± 2,54	*	21,5	189	35	370
Pesticides µg.kg⁻¹	aldrin	0,65 ± 0,25	0,85 ± 0,14	1,46 ± 0,09					
	Trans_chlordane	1,2 ± 0,06	0,15 ± 0,03	0,17 ± 0,06					
	cis_chlordane	0,06 ± 0,01	0,06 ± 0,01	0,07 ± 0,03					
	tChlordane	1,26 ± 0,05	0,21 ± 0,04	0,24 ± 0,09		2,26	4,79		
	diazinon	0,57 ± 0,11	0,83 ± 0,28	1,46 ± 0,12	*				
	dieldrin	1,11 ± 0,1	1,23 ± 0,04	2,19 ± 0,3	*	0,71	4,3	0,83	2,9
	pp'-DDD	10,65 ± 0,67	8,41 ± 1,09	2,53 ± 0,29	*	1,22	7,81	2,2	19
	pp'-DDE	17,62 ± 0,48	22,11 ± 0,32	15,55 ± 1,74	*	2,07	374	3,1	100
	pp'-DDT	0,62 ± 0,1	0,14 ± 0,03	0,1 ± 0,05	*	1,19	4,77	1,7	11
	endosulfan-1	3,09 ± 0,09	1,21 ± 0,07	2,33 ± 0,78	*				
	endosulfan- 2	14,11 ± 0,83	2,66 ± 0,47	13,18 ± 2,09					
	heptachlor	1,24 ± 0,2	0,72 ± 0,14	0,88 ± 0,3		0,6	2,74		
	heptachlor_epoxide_a	40,21 ± 4,65	21,44 ± 5,66	18,96 ± 1,66					
	heptachlor_epoxide_b	3,48 ± 0,29	2,76 ± 0,27	2,81 ± 0,05					
	isodrin	0,6 ± 0,16	0,21 ± 0,03	0,28 ± 0,07					
	lindane	2,03 ± 0,24	1,81 ± 0,32	1,94 ± 0,23		0,32	0,99		
	methoxychlor	7,43 ± 1,28	5,19 ± 0,21	2,52 ± 0,22	*				

Tab. 1: POP contaminants in the sediments of the three study locations. Mean values (µg/kg = ppb) with standard deviation. Differences between locations were tested via a Kruskal-Wallis test followed by a Kruskal-Wallis multiple comparison post-hoc. 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 Stated Environmental Protection Agency (US EPA 2005, Tab. 11; T20: probability of 20% of toxicity among samples; T50: probability of 50% of toxicity among samples). Gray text for concentrations: NS differences between locations (not integrated into COX models). Gray text for reference values: threshold not exceeded. Black text and gray background for reference values: threshold exceeded.

Pollutant		Site mean ± SD			K-W	CCME		US EPA		Quality category
		Inner	Middle	Entry	test	ISQG	PEL	T20	T50	
Metallic Trace Elements (MTE) mg.kg^{-1}	Al27(MR)	29207 ± 3825	26647 ± 9596	23125 ± 12905						
	As75(HR)	14 ± 3	11 ± 3	9 ± 5		7,24	41,6	7,4	20	Good
	Co59(MR)	5,78 ± 0,58	5,5 ± 1,15	4,72 ± 2,65						Good
	Cr52(MR)	57 ± 7	51 ± 11	44 ± 25		52,3	160	49	140	Good
	Cu63(MR)	84 ± 14	45 ± 10	33 ± 19	**	18,7	108	32	94	Moderate
	Fe56(MR)	13569 ± 1427	12639 ± 2980	10643 ± 6037						
	Mn55(MR)	150 ± 22	154 ± 29	134 ± 74						
	Ni60(MR)	18 ± 2	17 ± 4	14 ± 8				15	47	Good
	Pb208(LR)	85 ± 12	48 ± 14	46 ± 25	*	30,2	112	30	94	Moderate
	Ti47(MR)	2283 ± 291	2285 ± 506	1923 ± 1094						
	V51(MR)	90 ± 11	76 ± 19	65 ± 37						
	Zn66(MR)	236 ± 89	178 ± 77	111 ± 60	*	124	271	94	240	Good

Tab. 1: MTE contaminants in the sediments of the three study sites. Mean values (mg/kg = ppm) with standard deviation. Differences between sites were tested via a Kruskal-Wallis 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 Stated Environmental Protection Agency (US EPA 2005, Tab. 11; T20: probability of 20% of toxicity among samples; T50: probability of 50% of toxicity among samples). Quality category after Guerra-García et al. (2021) indicated. Gray text for concentrations: NS differences between sites (not integrated into COX models). Gray text for reference values: threshold not exceeded. Black text and gray background for reference values: threshold exceeded.

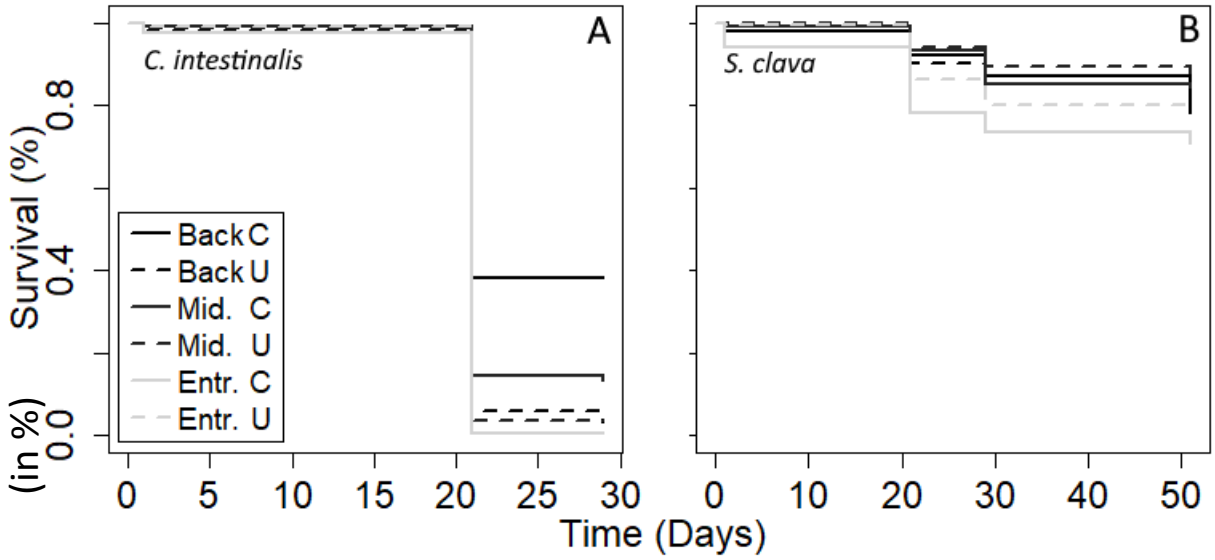

Fig. 1: Kaplan-Meier survival curves for *Ciona intestinalis* (A) and *Styela clava* (B) recruits. Inner = Inner marina location; Mid. = middle location; Entr. = entrance location of the marina. Dashed lines indicate uncaged (U) treatments; solid lines indicate caged (C) treatments. *Ciona* Entrance C and *Ciona* Entrance U overlap. *Styela* Inner U and *Styela* Mid U overlap.

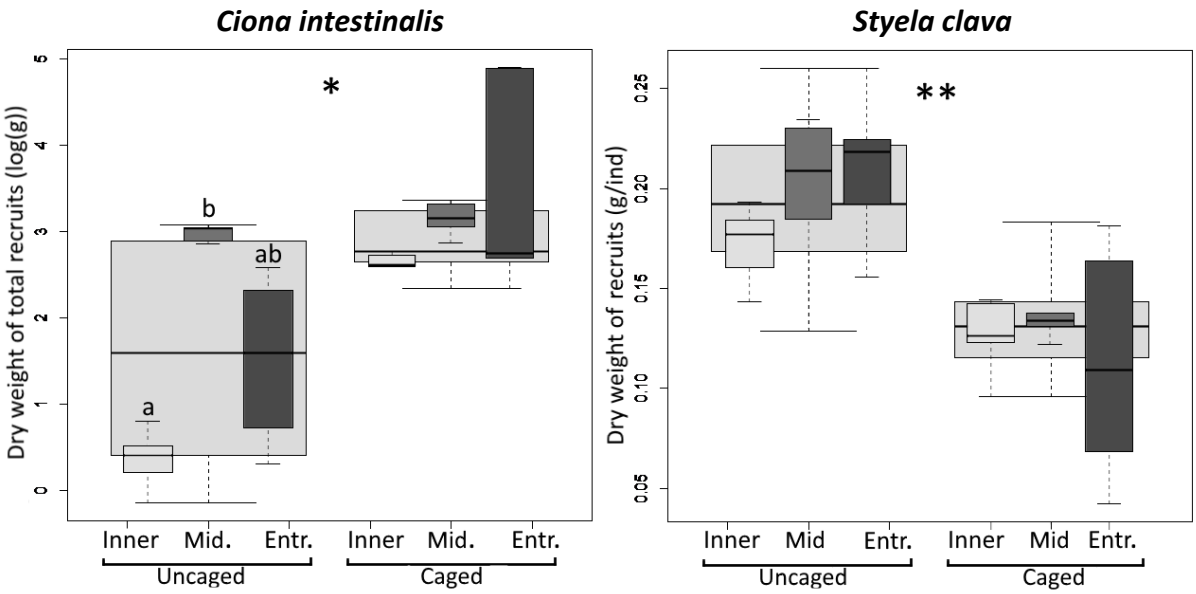

Fig. 2: Log of total dry mass of naturally recruited *Ciona intestinalis* and dry mass per lab grown *Styela clava* individual. Significant differences between caged and uncaged treatments: * $p < 0.05$ and ** $p < 0.01$ (Wilcoxon test). Significant differences within the uncaged *C. intestinalis* (a-b) $p < 0.05$ (Kruskal-Wallis multiple comparison).

Factor		Species	Mean coefficient	Mean risk increase (Mean HR-1)	% of p value below 0.05
Predation	Caging (uncaged compared to caged)	*Ciona intestinalis*	0,20	22%	100%
		Styela Clava	-0,40	-33%	86%
	Predation intensity	*Ciona intestinalis*	0,004	0%	25%
		Styela Clava	-0,01	-1%	6%
	Predation intensity : Caging	*Ciona intestinalis*	-0,01	-1%	52%
		Styela Clava	0,02	2%	10%
PAHs	Benzo(g,h,i)perylene	*Ciona intestinalis*	0,00	0%	6%
		Styela Clava	0,00	0%	5%
	Fluorene	*Ciona intestinalis*	0,00	0%	6%
		Styela Clava	0,00	0%	5%
PCB	tPCB	*Ciona intestinalis*	0,00	0%	9%
		Styela Clava	0,00	0%	6%
Pesticides	diazinon	*Ciona intestinalis*	0,02	2%	7%
		Styela Clava	0,06	14%	6%
	dieldrin	*Ciona intestinalis*	0,02	2%	8%
		Styela Clava	0,07	13%	6%
	pp'-DDD	*Ciona intestinalis*	-0,01	-1%	14%
		Styela Clava	-0,04	-4%	8%
	pp'-DDE	*Ciona intestinalis*	0,00	0%	6%
		Styela Clava	-0,01	-1%	6%
	pp'-DDT	*Ciona intestinalis*	-0,08	-6%	8%
		Styela Clava	-0,04	50%	5%
	endosulfan-1	*Ciona intestinalis*	0,00	0%	5%
		Styela Clava	0,01	2%	5%
	methoxychlor	*Ciona intestinalis*	-0,01	-1%	7%
		Styela Clava	-0,02	-1%	6%
MTE	Cu63(MR)	*Ciona intestinalis*	0,00	0%	6%
		Styela Clava	0,00	0%	5%
	Pb208(LR)	*Ciona intestinalis*	0,00	0%	5%
		Styela Clava	0,00	0%	5%
	Zn66(MR)	*Ciona intestinalis*	0,00	0%	6%
		Styela Clava	0,00	0%	5%

Tab. 3: Mean Coefficient, mean risk increase (Mean Hazard Ratio -1) and percentage of significant p-values after 10^5 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 font: factor of interest; Gray background: link between survival and factor.

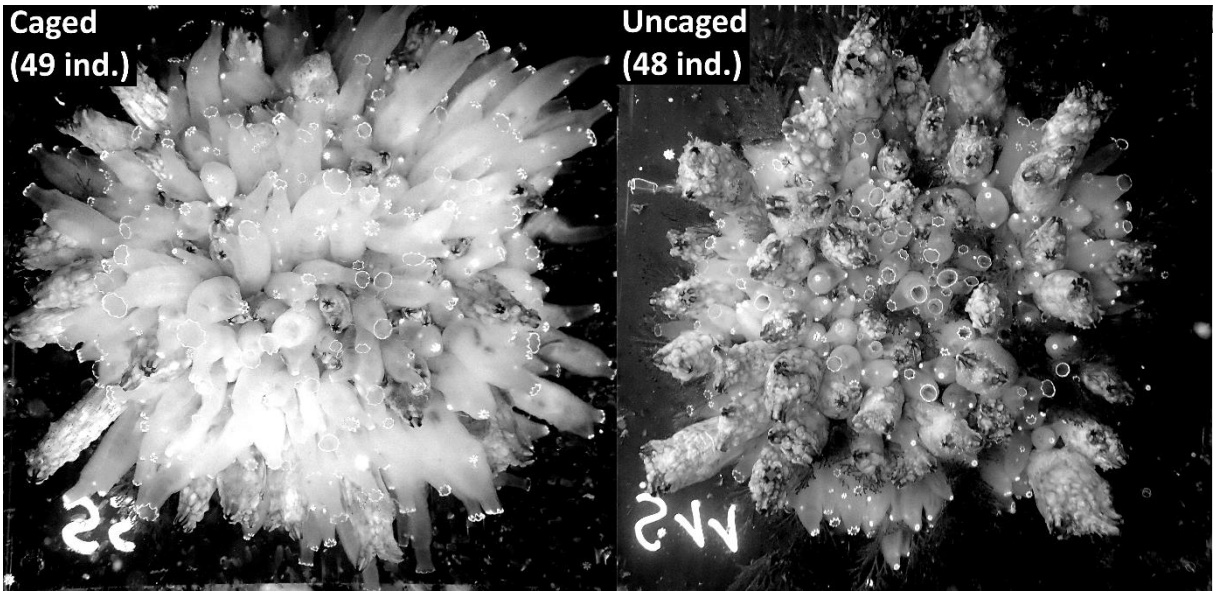

Fig. 3: Caged and uncaged Petri dish of *Styela clava* recruits after 50 days in the field. Panels originating from the inner location of the marina. Natural recruits of *C. intestinalis* are more visible (larger, more abundant) in the caged treatment. Individual number refers to final *S. clava*.

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