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# Diversity and Functioning of Harbor Communities. Influence on Non Indigenous Species in a Climate Change Driven Context

Robin Gauff

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# Sorbonne Université

## Muséum national d'histoire naturelle

Ecole Doctorale 227 - Sciences de la nature et de l'Homme : évolution et écologie

Adaptation et Diversité en Milieu Marin (UMR 7144)

## Diversity and Functioning of Harbor Communities

Influence on Non-Indigenous Species in a Climate Change Driven  
Context

**Robin Gauff – These de Doctorat Ecologie Marine**

Conducted under the supervision of **Christophe Lejeusne** and **Dominique Davoult**

Presented publicly on 16<sup>th</sup> December 2021

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(Dog 2011)

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## Foreword

Dear reader, thank you for reading my thesis, which I hope you will find interesting and informative. This thesis combines different works that have been conducted during my PhD and have been submitted to various Journals (See title page of each chapter) or are currently being written. The here presented versions are preprint versions and have been edited in formatting and referencing to form a coherent experience with the rest of the manuscript. Additional illustrations were added to give more detail. If you use the PDF version of this thesis, you may use the links throughout the manuscript for quicker navigation. To return to the Table of contents, click on the page number.

## Résumé français

L'urbanisation des habitats côtiers a conduit à divers paradigmes écologiques s'interrogeant sur le fonctionnement de ces néo-écosystèmes. Tout en détruisant les habitats naturels, les infrastructures marines offrent de nouveaux substrats qui sont colonisés par un large éventail d'organismes (encrassement biologique). Cependant ces structures artificielles ne remplacent pas les écosystèmes naturels puisque : i) les filtres environnementaux associés et les processus écologiques clés peuvent y différer, ii) leur composition biotique diffère des habitats naturels, et iii) elles sont caractérisées par une grande diversité et abondance, voire une dominance, des espèces introduites. Les perturbations anthropiques se concentrent dans ces habitats relativement peu étudiés et caractérisés par de forts gradients environnementaux. Parmi les habitats urbains marins, les marinas ont une importance fondamentale en raison de leur prolifération au cours des dernières décennies et parce qu'elles représentent une exacerbation des effets anthropiques sur la biodiversité et les écosystèmes. En effet, l'artificialisation dans les marinas vise initialement à modifier l'hydrodynamisme, lequel par la réduction des échanges avec la mer ouverte change les caractéristiques de la colonne d'eau (par exemple la température, les nutriments) et peut favoriser la concentration de perturbations anthropiques comme les polluants. En conséquence, un gradient environnemental peut être présent avec des niveaux plus élevés de perturbation dans les parties les plus internes des marinas, ce qui pourrait agir comme filtre sélectif pour les organismes.

Le travail de thèse présenté dans ce manuscrit visait à comprendre la diversité et le fonctionnement des communautés sessiles dans les marinas en étudiant comment de tels gradients de perturbation (illustrés par la pollution par exemple) influencent la structure des communautés, et comment ils pourraient conduire à de l'adaptation locale. Une attention particulière a été portée aux espèces introduites car elles sont une composante majeure des communautés portuaires et représentent un

défi sociétal majeur. Pour ce faire, diverses expériences ont été menées dans des marinas (jusqu'à six) de deux régions différentes (la Méditerranée et l'Atlantique). La structure des communautés et des fonctions écologiques tels que la respiration communautaire, l'accumulation de polluants dans les tissus et le métabolome de certaines espèces clefs, ont été comparés entre l'entrée des marinas et leurs parties intérieures, plus perturbées. Des observations in situ ont été combinées à des approches expérimentales (transplantation réciproque). En se basant sur la littérature, nous avons testé l'hypothèse selon laquelle les espèces introduites seraient favorisées par les perturbations et seraient plus abondantes dans les parties les plus perturbées des marinas. Dans un premier temps, nous avons confirmé une corrélation entre la structure des communautés et les gradients environnementaux des marinas. Nous avons par la suite mis en évidence, par une approche expérimentale de transplantation réciproque, que les différences de structure des communautés sont causées par les niveaux locaux de pollution, auxquels est associée une adaptation locale à petite échelle spatiale (< 100m). Ceci a pu être confirmé dans une seconde étude élargissant le champ d'expérimentation à six marinas.

Cependant, nous avons également démontré que ces dynamiques peuvent être affectées par d'autres processus, tels que les interactions biotiques comme la prédation ou la compétition, et tels que les perturbations sporadiques comme les vagues de chaleur.

En prenant en compte ces résultats, on peut noter qu'au gradient décroissant de perturbation depuis le fond vers l'entrée des marinas est respectivement associé d'une part une sélection des espèces résistantes au stress et d'autre part de fortes interactions biotiques à l'entrée favorisant des espèces plus compétitives à croissance rapide.

Dans le contexte actuel de perturbation à grande échelle dû au changement climatique, nous avons également exploré comment un scénario de réchauffement de l'eau de mer (+3° C) peut avoir un impact sur les communautés de marinas et potentiellement favoriser les espèces introduites. Pour cela nous avons déployé des structures chauffantes in situ afin de voir l'effet de la hausse de température sur les communautés des marinas. Dans les deux marinas testées, l'effet du réchauffement des eaux avait un fort effet sur la communauté, mais son impact sur les espèces introduites était contraire provoquant leur hausse en Méditerranée et leur déclin en Atlantique.

Les résultats obtenus dans le cadre de cette thèse de doctorat visent à mieux comprendre comment les perturbations dans les écosystèmes urbains marins affectent la biodiversité et en particulier favorisent la prévalence des espèces introduites.

# State of the Art

## I Ecology, Community, Biotope and Ecosystems

### I.A What is an Ecosystem?

Ecology, which was first defined by E. Haeckel (1866), is the science of the interactions between organisms and their environment. All living organisms live in their environment in which they interact with other organisms and the abiotic environment. The term biocenosis first appeared in the works of K. A. Möbius (1877) who defined the biocenosis as a group of living beings linked by reciprocal interactions and whose individuals and species composition reflect the properties of the environment where they live. The term biocenosis is however less used in the present days and replaced by the synonym community. In 1905, F. Clements argued that communities can be considered as an organizational unit, as mature plant communities may behave like a superorganism evolving in time (Clements 1905, 1916). This holistic way of thinking has sparked strong debates in the scientific community, shifting into an integrative vision leading to the modern field of ecology, where abiotic environment and the community form one unity (ecosystem). The term ecosystem was first introduced by A.G. Tansley (1939) who considered that the environment and the communities living in it cannot be separated as they form a 'self-contained entity' (Tansley 1939). Other authors expanded on the concept by highlighting the importance of energy transfer, trophic interactions and food chains (Lindeman 1942, Odum 1953, 1957, Evans 1956). An ecosystem can be thus defined as "a unit comprising a community (or communities) of organisms and their physical and chemical environment, at any scale, desirably specified, in which there are continuous fluxes of matter and energy in an interactive open system." (Willis 1997).

### I.B Environmental context

As one of the two major components of an ecosystem, the environment is comprised of its physical (abiotic) and biological (biotic) properties. The abiotic environment may be composed by a set of geographical, geological, edaphic, climatic, physical and chemical properties that may affect living organisms and may have high impact on the communities living in the said environment (Odum 1953, Christopherson & Bryne 2009). Temperature and its variation is an important driver of communities and biodiversity as it profoundly affects the physiology of organisms (Waldock et al. 2018). Rainfall and water availability in general may also highly impact (mostly terrestrial) ecosystems (Webb et al. 2005, Yan et al. 2015). Climate may be one of the most important drivers on communities as a global

latitudinal biodiversity gradient exist from the poles to the equator (M.R. Willig et al. 2003, Hillebrand 2004). However geography and geology may also highly impact organisms as topography and bodies of water can isolate populations and drive evolution (Darwin 1859, MacArthur & Wilson 1967). In marine environments these rules equally apply as marine global biodiversity patterns are also affected by climate (M.R. Willig et al. 2003, Hillebrand 2004). Salinity, which is more affected by rivers and ice-melt rather than direct rain fall, may be an important driver of coastal communities (Zettler et al. 2007). Depth may also highly affect biodiversity patterns in oceans as, together with turbidity, it is an important driver of energy availability for organisms (Rex et al. 1993, Smith & Brown 2002, Danovaro et al. 2008, Costello & Chaudhary 2017). Virtually any physical or chemical property of the environment may affect the organisms living in it. Almost always these properties are organized as gradients from one level to another, allowing species to live along the different levels of the said gradient (Peres and Picard 1964; Willig et al. 2003; Zettler et al. 2007; Ryu et al. 2011; Costello and Chaudhary 2017). While physical, chemical, and geological processes may drive biodiversity patterns, species may in return also profoundly affect the environment. Some species (Ecosystem engineers) modify the abiotic environment in substantial ways, changing resource availability for other species or providing habitat for other species (Jones et al. 1994).

### **I.C Communities Functioning and Species Interactions**

A community is composed of two or more species which live in the same environment. In this environment each species occupies a certain ecological niche, a concept developed by Johnson in 1910 and elaborated on by Grinnell (1917) (Pocheville 2015). The niche was conceptualized as the space occupied by a species in its environment (Grinnell 1917). Trophic and non-trophic interactions with other species may be considered as a fundamental part of the niche (Elton 1927). In its most used definition, niche is a propriety of a considered species and is an 'n-dimensional hypervolume', corresponding to environmental conditions allowing a species to persist (Hutchinson 1957). Two species cannot occupy the same niche and overlapping niches may result in competitive exclusion (Gause 1934) or in niche partitioning, where each species occupies a different 'realized niche' (as opposed to its fundamental niche which depends only of interactions with the environment; Hutchinson 1957). Later definitions of the niche concept chose to focus on resource use rather than the environmental conditions (MacArthur & Levins 1967) but retain the idea of an occupied hypervolume. Biotic interactions within and between species play a major role in the niche concept as they can be a fundamental part of the niche itself (*e. g.*, predation of species A on species B makes B occupy a predator niche) or play a role on the occupation of the fundamental niche (*e. g.*, competition between A and B may result in niche partitioning, specialization and a smaller realized niche) (Elton



1927, Hutchinson 1957, MacArthur & Levins 1967). Several types of interactions exist between organisms that may more or less impact their niche (Tab. 1). Some of these interactions are considered as symbiotic if they are long lasting and intimate (Martin & Schwab 2012, Bronstein 2015). Previous definitions of symbiosis were based on the criterium of obligatory mutualistic relationships (*i. e.*, a reciprocally positive interaction; Bronstein 2015), which has however been replaced by the criterium of the strength of the interaction, as symbiosis may not always be mutualistic (Martin & Schwab 2012). As previously discussed, competition may strongly impact a species and the niche it occupies in an ecosystem (Gause 1934). This competition may be directly for a limited resource like space, food or water (Exploitative competition) (Gause 1934, Tilman 1982), which can lead to active interspecific aggression (Interference competition; Gordon 1988). Competition may however also be indirect and reside in the manner how species, which share a common predator or parasite, avoid its effect or increase its effect on the other species (Muller & Godfray 1997, Nouhuys & Hanski 2000). Since both species need to invest resources in this competition, it has a negative net effect on fitness for both (Gause 1934), which is the main reason for avoiding it by niche partitioning (Elton 1927, Hutchinson 1957, MacArthur & Levins 1967). Predation (herbivory included) is an interaction where one species feeds on the other one creating a unilateral positive-negative link. Conceptually, a predator is defined as a species which has a negative impact on another species by consuming all or part of it (Begon et al. 2005, Raffel et al. 2008). A parasite is a symbiont that has a negative impact on another species (host) by utilizing its resources (including feeding on it) and by using it as habitat (Raffel et al. 2008, Roberts & Janovy 2008). As symbiosis depends on the strength and intimacy of the interaction between two species, the definition of parasitism has changed over time and both interactions, predation and parasitism, somewhat overlap with each other as a spectrum of interactions exists between those two extremes (Raffel et al. 2008). Commensalism is an interaction where one species gains an advantage from the other without affecting this species (Odum 1953, Hellmann 2001), for example by hitchhiking a larger species for dispersal (Bartlow & Agosta 2021). Amensalism is the opposite, where a species has a negative effect on the other without being affected by its interaction (Odum 1953), humans trampling ants for example.

Tab. 1: Different interactions between species. All these interactions, except competition, may also constitute symbiosis if the link between both species is very intimate. The effect of one species on the other and in return are indicated.

	Species B			
Species A	Effect	Negative	Neutral	Positive
	Negative	Competition	Amensalism	Predation/Parasitism
	Neutral	Amensalism		Commensalism
	Positive	Predation/Parasitism	Commensalism	Mutualism

## II Evolution and Adaptation

### II.A Selection, Adaptation and Evolution

Early in the history of the study of nature, C. Linnaeus identified in his *Systema naturae*, that organisms could be classified according to morphological characteristics (Linnaeus 1758). It incorporates several levels at which organisms can be classified with the ultimate level being the species. Below an example of the classification of the Bryozoan *Bugula neritina* (Linnaeus, 1758), which plays an important role in the present manuscript (Tab. 2).

Tab. 2: Linnean classification illustrated by the example of *Bugula neritina*.

Kingdom ----->	Animalia
Phylum ----->	Bryozoa
Class ----->	Gymnolaemata
Order ----->	Cheilostomatida
Family ----->	Bugulidae
Genus ----->	<i>Bugula</i>
Species ----->	<i>Bugula neritina</i>

While C. Linnaeus recognized similarities between organisms and an apparent hierarchy, his classification was static in time and no evolution of organisms was envisioned. After G. Buffon (Buffon 1766) and G. Saint-Hillaire (Le Guyader 2000) laid the groundwork, J. Lamarck formulated the first fully fledged theory of evolution incorporating a driving process. Lamarck envisioned that species, given enough time could evolve according to their needs, depending on the use of certain of their characteristics (Lamarck 1809). It was however C. Darwin and A. Wallace who introduced a view of evolution predominantly based on natural selection (Darwin & Wallace 1858, Darwin 1859). Due to natural variations between individuals, natural selection causes a differential survival and reproduction among them, thus favoring or diminishing certain hereditary traits in the population (Darwin & Wallace 1858, Darwin 1859). These traits, if beneficial in the light of natural selection, may confer a higher fitness (the ability for survival and subsequent reproduction). By this process they can become fixed in the population as they consist in an adaptation for the considered environmental pressure (Darwin 1859, Williams 1966). On a macroevolutionary scale, selection can act in different ways (Fig. 1). For instance, it can stabilize traits through stabilizing selection (Fig. 1a Schmalhausen 1949, Waddington 1952). Directional selection appears to be the main cause of allopatric (species living at different places) speciation as it shifts traits to one extreme which causes the development of new phenotypes (Fig. 1b; Rieseberg et al. 2002). Disruptive selection occurs is the main cause for sympatric speciation (living at the same place) and niche partitioning (Fig. 1c; Maynard Smith 1966, MacArthur & Levins

1967). Selection can take many forms and be due to biotic or abiotic interactions between organisms. Intra specific competition and sexual selection may be major drivers of evolution as the most adapted individuals of a population will have a higher fitness than others (Darwin & Wallace 1858, Darwin 1859). Interspecific interactions may however also play an important role. Darwin (1859) conceived evolution as a struggle for survival which highlighted the biotic interactions between species. A biological arms race between species (Red Queen hypothesis) may be an important driver of evolution (Van Valen 1973, Benton 2009), especially on shorter time scales (Benton 2009). Opposed to this, the Court Jester hypothesis recognizes the importance of disturbance and environmental change on evolution as they seem to be the major actors on longer timescales (Barnosky 2001, Benton 2009). The abiotic environment may constitute selective filters that may constitute a physiological barrier requiring certain adaptations to survive (see I.B).

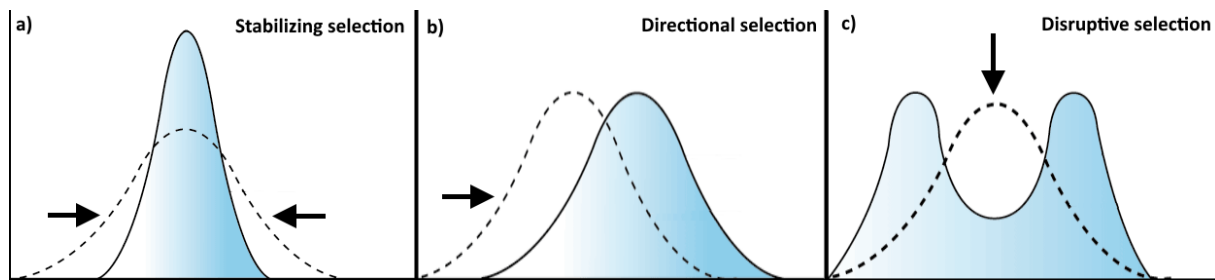


Fig. 1: Different types of selection. Selection pressures are indicated by arrows. The original population of a trait is shown with the dotted line, the new population after selection by the blue hued curve. a) Stabilizing selection where the selection acts on a trait to stabilize it. Extremes of this trait are counter selected. b) Directional selection where the selection pressure acts on only one extreme, evolving the trait in one direction. c) Disruptive selection where the average trait is counter selected and thus extremes favored. This can result in creating two species from a common ancestor. Modified from [principlesofevolution.blogspot.com](http://principlesofevolution.blogspot.com).

G. Mendel demonstrated that heredity was based on genetics, which had important implications on the conception of evolution (Mendel 1870). The importance of the phenotype, a species' appearance/expression which results from the interaction between its genes and its environment was now explained by its genotype, the hereditary information of its genes that are transmitted along generations. Selection acts on the individual phenotype and only indirectly on the genotype, it is however the hereditary genotype that is transmitted to generations and thus responsible for evolution. This has led to the modern understanding of evolution based on natural selection, genetic drift (stochasticity in allele frequency), gene flow (migration) and mutation (Darwin & Wallace 1858, Morjan & Rieseberg 2004, Masel 2011, Blanquart et al. 2013). While evolution is most conveniently illustrated by macroevolution (evolution on larger time scales) the exact same processes act on microevolution (evolution of adaptations and characteristics of a species on small spatial and temporal

scales) including local adaptation. At this level phenotypic plasticity (acclimatation) may also play a major role as it may affect the tolerances of an individual and can be in a certain extent heritable (Richards et al. 2010b, Moler et al. 2018).

## II.B Local Adaptation

A population is locally adapted if it adapts according to the local selective pressures and exposes a phenotype different from other populations of the same species (Williams 1966, Kawecki & Ebert 2004, Blanquart et al. 2013). If local adaptation is present, individuals of the given population have higher fitness (higher survival and/or higher reproduction and survival of descendants) at their original environment compared to individuals from other populations, and/or have higher fitness compared to individuals of this population elsewhere, which can be attributed to fitness trade-offs of the populations to accommodate for their home environment (Kawecki & Ebert 2004, Leimu & Fischer 2008). These two criteria are known as the 'foreign vs local' criterion and the 'home vs away' criterion respectively. The originally used 'home vs away' criterion for local adaptation requires that, in both directions, populations have a higher fitness at their home environment than in other environments (Kawecki & Ebert 2004). This criterion is however now used on a more subsidiary basis as it could be still satisfied in a theoretical case where one population outperforms the second on both locations (Fig. 2a; Kawecki and Ebert 2004; Leimu and Fischer 2008). The most important criterion for local adaptation is, thus, the 'foreign vs local' criterion which also integrates differences in the intrinsic quality of both locations (Fig. 2b; Kawecki and Ebert 2004). Here individuals' fitness is not compared with the fitness of individuals of this population in another location, but rather compared to individuals originating from another location in its own. If the fitness of a local individual is higher than for a foreigner to its habitat the criterion is satisfied (Fig. 2b; Kawecki and Ebert 2004). A combination of both criteria may however exist (which is the case in Fig. 2c). To test whether local adaptation is present (*i.e.*, if the fitness of a 'foreign' population is lower than the fitness of the 'local' population at a given location) it is required to transfer the 'foreign' population to the other location and reciprocally. Reciprocal transplant experiments are thus the standard experiment for detecting local adaptation (Kawecki & Ebert 2004, Angert & Schemske 2005, Leimu & Fischer 2008, Colautti & Lau 2015, Sork 2018, Martin et al. 2021).

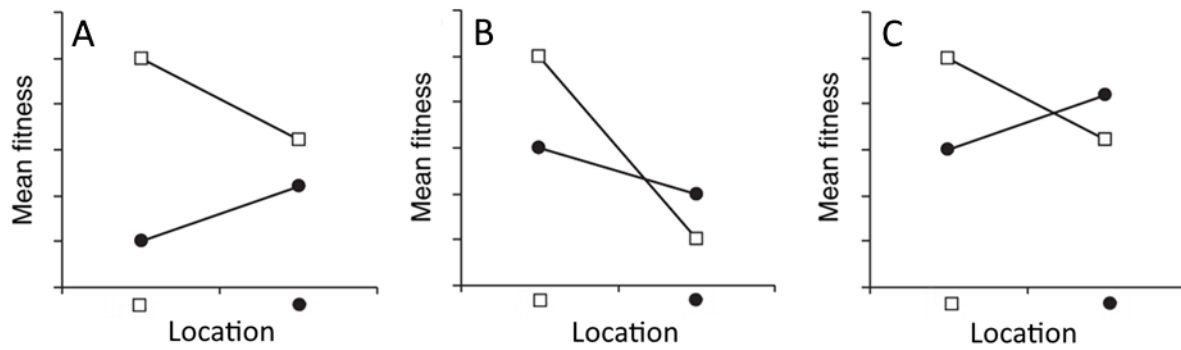


Fig. 2: Fitness of two theoretical populations (Circle and Square) in their own location or in another. The 'home vs away' criterion is satisfied in a) as populations have a higher fitness at their own location compared to another. a) however, exposes a theoretically impossible state as one population (square) outperforms the other (circle) in both locations and would competitively exclude it. Thus, the 'foreign vs local' criterion b) is the most important one for local adaptation. Here in both locations, the local population outperforms populations originating from the other location. In this situation, the circle location has a lower intrinsic quality and both populations have lower fitness at this location. However, the circle population has higher fitness compared to the square due to local adaptation. In c) both criteria are verified.

Local adaptation is fostered by strong divergent selective pressures (Williams 1966, Kawecki & Ebert 2004), but processes homogenizing individuals within a metapopulation may act against local adaptation (Kawecki & Ebert 2004). For instance, geneflow within a metapopulation or dispersion may restrain differentiation and thus local adaptation (Brown & Pavlovic 1992, Kisdi 2002, Kawecki & Ebert 2004, Savolainen et al. 2007). Spatial and temporal heterogeneity of the environment favors generalist genotypes and replaces local adaptation with phenotypic plasticity (Kawecki & Ebert 2004). Kawecki and Ebert (2004) argued that parental effects (when the phenotype of an organism is not only affected by its genotype and its environment, but also by the environment of its parents) may mimic local adaptation through plastic adaptive phenotypes. Yet, parental effects play an important role in adaptation (Mousseau & Fox 1998). Recently, hereditary adaptive plasticity through epigenetics is increasingly included in studies focusing on local adaptation and adaptation in general (Richards et al. 2010a, Platt et al. 2015, Moler et al. 2018, Sork 2018), since DNA-methylation has often been observed in association with environmental gradients (Dubin et al. 2015, Platt et al. 2015, Clark et al. 2018). Epigenetic variation may contribute to local adaptation if it affects adaptive traits (Richards et al. 2010b, Moler et al. 2018). As DNA-methylation might increase genetic mutation on the considered gene (Klironomos et al. 2013), locally adaptive phenotypes might even become genetically fixed. Epigenetics are challenging the current understanding of adaptation and evolutionary processes, but more research is required to clearly understand its contribution to them (Callinan & Feinberg 2006, Cushman 2014, Moler et al. 2018). For these reasons, in the present manuscript we do not limit local adaptation to genotypic variation, but also include hereditary adaptive plasticity.

## III Disturbance in Ecosystems

### III.A The Role of Disturbance in Ecosystems

Ecosystems and their associated communities have long been thought to evolve towards a stable, unchanging state (climax) through ecological succession (Cowels 1899, Clements 1916). This view has however been widely contested as, despite apparent stability at large scales, at local scales change and disturbance are commonplace (Connell & Slatyer 1977, Sousa 1984). Disturbance can be defined as a process or agent, changing biotic or abiotic conditions in comparison to a reference state, in a transient or permanent manner, thus causing a perturbation (the effect of a disturbance, a change in the considered ecological component) or stress (also an effect of a disturbance, but on a physiological or functional level; Rykiel 1985). Disturbances have three major components to consider: their intensity, duration and frequency, which all may impact the effect they have (Sutherland 1981, Rykiel 1985). Rykiel (1985) expanded on four categories of disturbance: 'Destruction' consisting in the destruction/removal of biomass, 'Discomposition' consisting in a change of selectivity advantaging/disadvantaging certain populations, 'Interference' consisting in the inhibition of exchange processes (biomass, energy, interactions), and ironically 'Suppression' consisting in the prevention of natural disturbance. However, a disturbance may fall within several of these categories. A disturbance is most often localized with an 'epicenter' and gradual decline in space towards the 'normal' state (Fig. 3). Small scale differences between environmental conditions may be seen as spatial disturbance, since they may locally favor certain groups or organisms (Rykiel 1985). This is however also true in time as the return to an initial state is gradual (Fig. 3). Yet, more disturbed patches need longer to recover than less disturbed ones.

Disturbances play a major role in ecosystems and constitute a fundamental part of their functioning and their diversity (Grime 1977, Connell 1978, Huston 1979). A disturbance may be so regular that organisms adapt to it or even integrate it as an essential part of their life-cycle (Pianka 1970, Sousa 1984, Montagnes et al. 2002, Rood et al. 2007, Pausas & Keeley 2014).

Disturbance may be profoundly anchored in evolutionary and reproductive strategies of a species (Pianka 1970, Montagnes et al. 2002, Pausas & Keeley 2014). Species might be categorized into two selective models depending on their parental investment and reproductive cycle (MacArthur & Wilson 1967, Pianka 1970, Grime 1977). *r*-selection species are species which thrive in unstable environments with high frequency of disturbance. Their characteristic trait is that almost all resources are channeled into the maximization of the number of descendants. They generally have short life cycles, are often opportunistic, and have low parental investment (MacArthur & Wilson 1967, Pianka 1970). This leads

to a high population growth, often allowing these species' populations to surpass an ecosystem's carrying capacity, ultimately leading to strong population fluctuations in boom-and-bust cycles (MacArthur & Wilson 1967, Pianka 1970, May 1975). *K*-selection species maximize their own survival and the survival of a small but 'costly' progeny. They are usually long-lived species with high parental investment in offspring production and parental care. They require higher ecosystem stability since the loss of descendants is 'costly' (MacArthur & Wilson 1967, Pianka 1970). In stable ecosystems, they have approximately constant populations around the ecosystem's carrying capacity (hence *K*) and tend to competitively exclude *r*-selection species (MacArthur & Wilson 1967, Pianka 1970, May 1975).

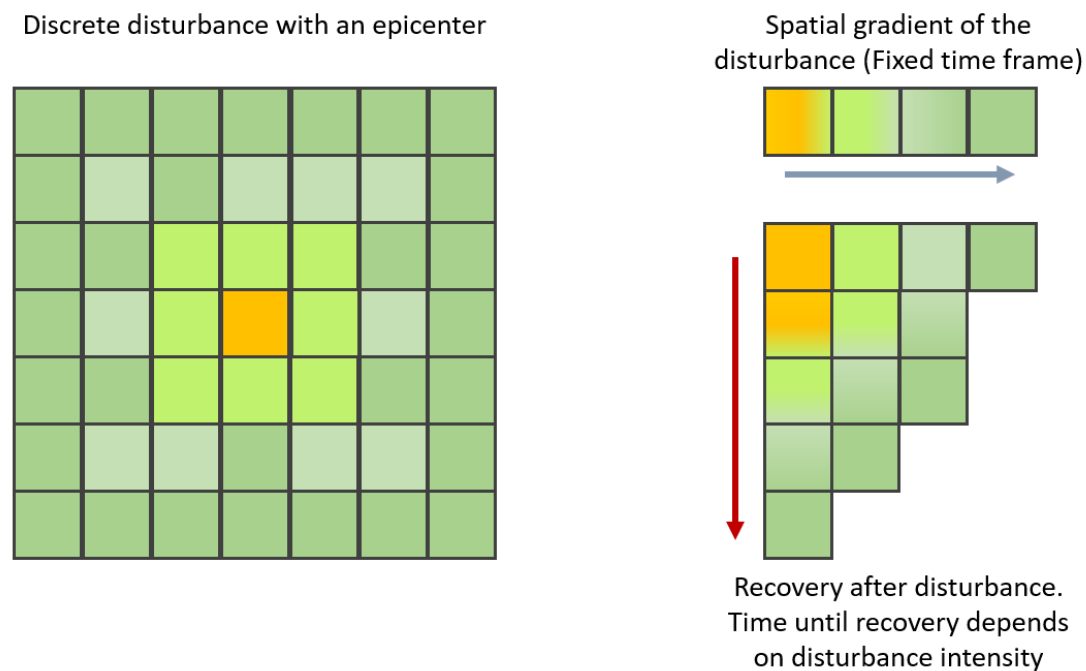


Fig. 3: Disturbance gradients in space and time. Left, disturbance acts on a local level with high intensity (Epicenter, yellow). A gradual decline of the intensity of the disturbance towards the other patches is visible (green tones). On the right side (top) the spatial gradient of disturbance intensity within a fixed timeframe (blue arrow) is shown. The intensity of the disturbance and thus its effect on the community diminishes further from the epicenter (left, yellow). Below (right side) the gradual recovery after disturbance is shown (red arrow). More disturbed zones (left) need more time to recover than less disturbed zones (right).

However perfectly stable ecosystems do not exist *in natura* and all species are situated on a spectrum between these two strategies, combining several of their characteristic traits (Pianka 1970, Grime 1977). Depending on the intensity and frequency of disturbances, different selection strategies can coexist in an ecosystem. Biodiversity is thus not maximized in ecosystems with low frequency and intensity of disturbance, where *K*-selection species competitively exclude *r*-selection species (Fig. 4a), nor in ecosystems with high frequency and intensity of disturbance (Fig. 4c), where *r*-selection species dominate, but in ecosystems with intermediate frequency and intensity of disturbance where they coexist (Fig. 4b; Intermediate Disturbance Hypothesis; Connell 1978; Sousa 1979, 1984). Disturbance

is thus an important driver of biodiversity patterns. While the  $r/K$ -selection model is mostly applied to animals, other classifications exist, most notably for plants (Grime 1977). The Grimes' (1977)  $C$ -,  $S$ - and  $R$ - selection model integrates three categories of selection, depending on if competitive ability in undisturbed condition is privileged (competitive,  $C$ -selection), stress (or disturbance) resistance is privileged (stress-tolerant,  $S$ -selection), or reproduction is maximized no matter the conditions (ruderal,  $R$ -selection). While  $C$ - and  $R$ -selection somewhat correspond to  $K$ - and  $r$ -selection, there are however slight differences between  $C$ - and  $K$ - as in plant communities competition often is realized by growth, while for animals this is not always the case (Grime 1977). The advantage of the  $C$ -  $S$ -  $R$ -selection model is that four secondary strategies exist mixing traits of the three, which corresponds to observations in plant communities ( $C$ - $S$ ,  $S$ - $R$ ,  $C$ - $R$ ,  $C$ - $S$ - $R$ ; Grime 1977).

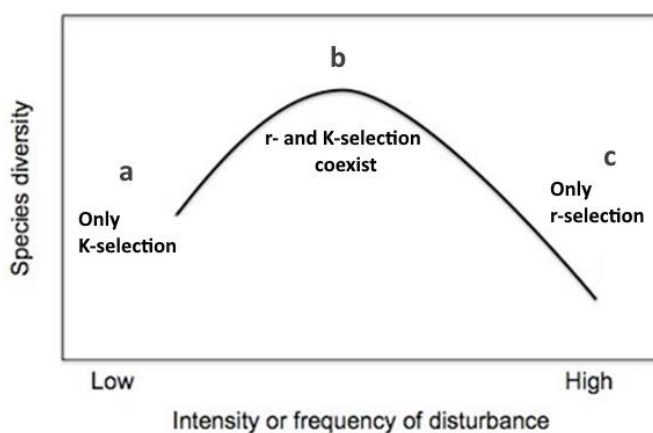


Fig. 4: Intermediate Disturbance Hypothesis (IDH; Connell 1978). Low or high intensity and/or frequency of disturbance results in low biodiversity (a and c), since only  $r$ -selection or  $K$ -selection species can exist. Intermediate disturbance results in higher diversity since both can coexist (b). Graphic modified from (Hughes 2010).

The interaction between disturbance and biodiversity is, however, not unidirectional. Biodiversity contributes to higher community stability and may impact the intensity and effect of disturbance (MacArthur 1955, Tilman 1996, Yachi & Loreau 1999). Originally explained by the insurance hypothesis (Tilman 1996, Yachi & Loreau 1999), this process is now attributed to the concept of functional redundancy (Lawton & Brown 1993, Rosenfeld 2002, Hubbell 2005, Biggs et al. 2020). Systems with high diversity, and thus higher number of potential functionally redundant species, have a higher probability of species occupying the functional niche of species lost during disturbance (Rosenfeld 2002). This increases ecosystem stability (Tilman 1996, Yachi & Loreau 1999, Biggs et al. 2020). Disturbance may more or less affect a community composition and state depending on its resistance and resilience, two important aspects of ecosystem stability (Fig. 5; Lewontin 1969, Holling 1973, Beisner et al. 2003, Lake 2013, Nimmo et al. 2015). Resistance is a community's ability to resist disturbance and change (Lake 2013, Nimmo et al. 2015). The disturbance is cushioned by the community and the ecosystem remains unchanged. Resilience describes a community's elasticity and its capacity to return to a stable state after being affected by disturbance (Holling 1973). Ecosystems and communities are conceptualized as dynamic entities as disturbance may provoke strong structural changes, affecting how communities are shaped and individual species interact with the environment



and with each other (Lewontin 1969, Beisner et al. 2003). Within the same ecological parameters, communities may shift/cycle between several alternative stable states when disturbed (Lewontin 1969, Beisner et al. 2003). The disturbance itself however is not the only parameter conditioning the community equilibrium. Its disturbance history may also play an important structuring role and conditions the evolution trajectory of communities (Hysteresis; Fig. 5; Beisner et al. 2003).

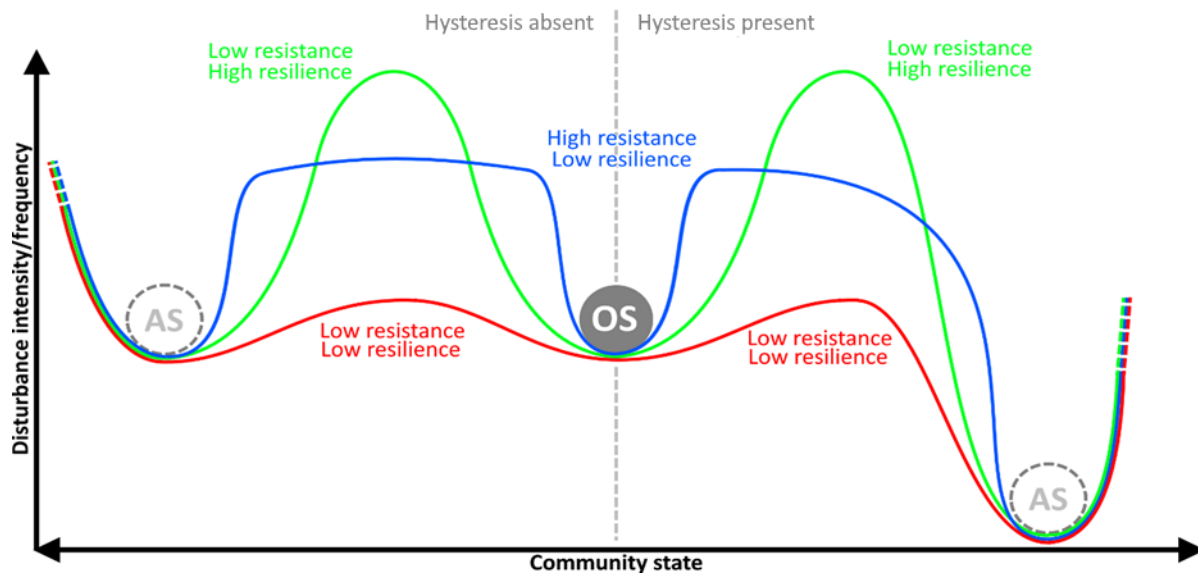


Fig. 5: Conceptualization of shifts between a community's alternate states (AS) in regard to community resistance, resilience and hysteresis. The current state, represented by a grey ball (OS) is moved by disturbance along the colored paths until it reaches a threshold and 'rolls' into its alternate state (Dashed balls, AS). Resistance is conceptualised as slope. Resilience is represented as high threshold. In a high resistance low resilience scenario (Blue line), disturbance acting on the ball will not significantly change the communities' state (balls' position) until it reaches the threshold and rolls into another alternative state. In a low resistance high resilience scenario (Green line), the ball can be moved away easily from its stable state but also rolls back easily; more disturbance is needed to move it to alternate states. If both are low, switches from the CS to AS require less disturbance (Red line). Hysteresis is represented by the difference in the intensity of disturbance needed by the community to move from an alternate state to the original state (CS). On the left side, hysteresis is absent. An equal amount of disturbance is needed to move back from the AS to the OS than disturbance was needed to move away from the OS to the AS (Symmetry). On the right side (Hysteresis present) more disturbance is needed to move back from the AS to the OS than was needed to move away from the OS. Inspired by Prof. Dr Dronkers (Vos 2021) and (Beisner et al. 2003).

While it is possible to conceptually define disturbance, it remains difficult to decide if a particular process or agent really is a disturbance since the reference state on which the disturbance may act is defined by the user of the definition (Rykiel 1985). Furthermore, scale (spatial and temporal) is highly important for quantifying the effect of a disturbance (Sutherland 1981, Sousa 1984, Rykiel 1985, Hamer & Hill 2000, White et al. 2000) and vastly conditions what is referenced as 'normal' as opposed to 'disturbed'. Spatial and temporal scale strongly impacts the perceived effect of disturbance (Fig. 6). This can be exemplified by the action of predators disturbing sediment; while this process causes disturbance on the small scale of the sediment micro fauna (Thistle 1981, VanBlaricom 1982, Hicks 1984, Sousa 1984), it is part of the normal patch dynamic on a larger ecosystemic scale (Thistle 1981).

Debates arise especially in regard to regular or even cyclic disturbances (Sousa 1984). Depending on the studied scale, cycles like day/night, seasons, tides etc. change the conditions for the studied communities enough so that certain organisms perceive stress, or are selected which could be regarded as disturbance (See def. 'Discomposition', Rykiel 1985). On another spectrum, hurricanes which are seen as major disturbance in Caribbean forests (Zimmerman et al. 1996), are frequent and normal on longer time-scales. Species may adapt to disturbance and integrate it to their life-cycle (Pianka 1970, Sousa 1984, Montagnes et al. 2002, Rood et al. 2007, Pausas & Keeley 2014). One might argue that at the considered event may not be a disturbance anymore (Allen & Starr 1982). But as any disturbance might affect evolutionary and reproductive strategies of a targeted species (Pianka 1970, Montagnes et al. 2002, Pausas & Keeley 2014), one could, adopt the view that disturbance itself becomes an integrative part of the normal ecosystem functioning, which implies that even very cyclical and regular disturbances may remain disturbances. This illustrates how the perception of disturbance may be arbitrary and how difficult it is to delimit and define disturbance by only considering it isolated.

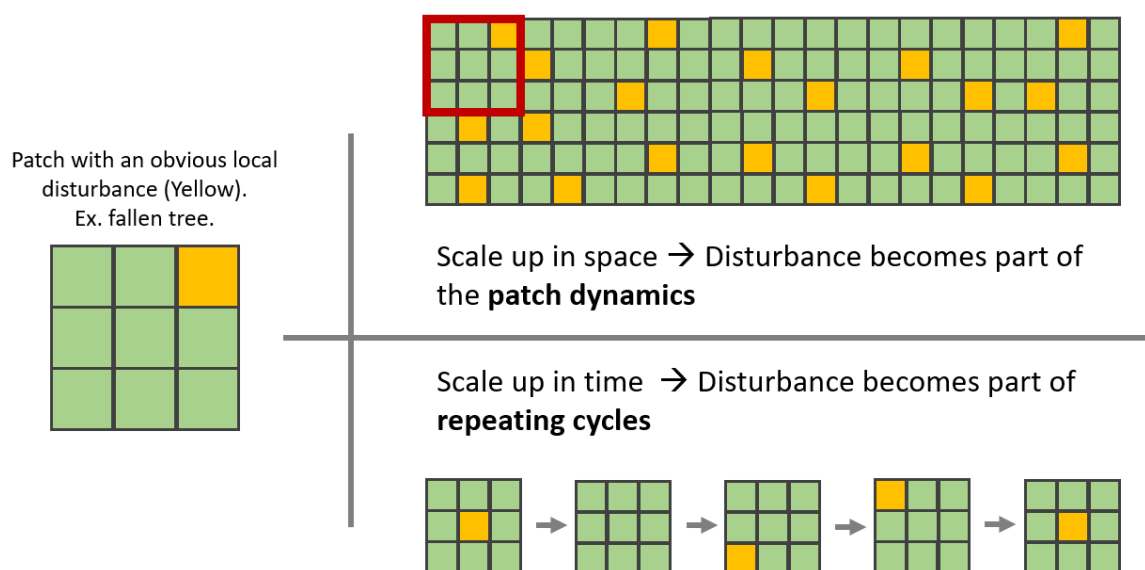


Fig. 6: The problem of scale for defining disturbances. On the left, a patch with an obvious local disturbance (yellow). We can imagine this disturbance as a fallen tree for example as it locally disturbs the ecosystem. If we scale up in space (top right) and look at the whole forest, the fallen tree is no longer a disturbance but becomes part of the normal patch dynamics of the forest. Equally, if we observe the patch for a long period of time, fallen trees are common and normal. This illustrates how important scale is to define the 'normal' state to which disturbance is opposed.

This problem illustrates the need for a classification of disturbances depending their impact rather than on the disturbance itself. Disturbances may for instance be categorized depending on their impact on community structure (Sutherland 1981, Boudouresque et al. 2009). By taking into account the resistance and resilience of communities it is possible to divide disturbances in three categories (Fig.

7; Type I, II and III; Sutherland 1981). In this framework, disturbances that are cushioned by the resistance of the community and that thus do not induce a notable change in community structure or function may be categorized as Type I disturbance (Fig. 7). This category can contain a vast number of processes which may be part of the normal functioning of the ecosystem. Seasonal cycles affect weather systems, which may lead to dry, hot, rainy or stormy weathers which cause sublethal and lethal stress to organisms and may shape communities (Lieberman et al. 1979, Power & Stewart 1987, Hanson & Weltzin 2000, Rood et al. 2007, Chang et al. 2018). Ice disturbance, which is commonplace in winter can have impacts on local species in terrestrial, aquatic and marine habitats (Robertson & Mann 1984, Conlan et al. 1998, Lafon 2004, Rood et al. 2007, Weber et al. 2013). Increased wave activity in the winter season constitutes a strong, regular disturbance for coastal communities (Lieberman et al. 1979, Blanchette 1996, Reed et al. 2011). On the other side summer drought and heat-waves equally impact many habitats (Hanson & Weltzin 2000, Humphries & Baldwin 2003, Garrabou et al. 2009, Verkaik et al. 2013, Stangler et al. 2016, Aoki et al. 2020, Strydom et al. 2020). On an even more regular basis, tides may cause a major disturbance for shore dwelling organisms. Tide caused emersion, salinity increase or heat increase causes major physiological stress for marine organisms (Montagnes et al. 2002, Contreras-Porcia et al. 2017) and coastal communities may be structured in levels depending on their emersion length and frequency (Peres & Picard 1964, Laborel 1986). The activity of other organisms may constitute a disturbance on smaller ones (Sousa 1984). Predation, herbivory and pathogen actions may vary in space and time, and constitute a disturbance on a local or populational scale by removing individuals (Hicks 1984, Ayres & Lombardero 2000, Wisdom et al. 2006). Likewise a common event like the death of a single individual may constitute a disturbance by selecting the very specific communities associated to carrion or rotting wood (Turner 1977, Kneidel 1984, Rayner & Boddy 1988, Zimmerman et al. 1996, Smith & Baco 2003, Bienhold et al. 2013). As all these disturbances fall within the tolerance of communities and their normal functioning due to their resistance, they may be categorized as Type I disturbances. Disturbances that induce a change in community structure, but where the community returns to its original state after disturbance may be categorized as Type II disturbance (Fig. 7). Weather fluctuations like drought, flooding, storms and tornadoes appear as obvious examples for high impact disturbances (Power & Stewart 1987, Zimmerman et al. 1996, Hanson & Weltzin 2000, Peterson 2000). Extraordinary heat or cold waves however also highly impact communities (Millward & Kraft 2004, Garrabou et al. 2009). This is particularly true for seasonal late summer droughts (Hanson & Weltzin 2000), flooding near rivers (Rood et al. 2007) and for wild-fires in fire-prone areas (Bond & van Wilgen 1996, Thonicke et al. 2001, Keeley & Pausas 2019). Most of the time however, communities return to their original stable state due to their resilience. High impact disturbances that induce large enough changes, that the community will evolve more or less permanently into an alternate state may be categorized as Type III

disturbances (Fig. 7). Geological activity may for instance induce high impact disturbances as volcanoes, earthquakes, tsunamis and landslides can virtually raze entire ecosystems (Zimmerman et al. 1996, Jaramillo et al. 2012, Seike et al. 2013, Crisafulli et al. 2015). Note that if successive Type II disturbances occur before the community returns to its original state due to resilience, they may stack and have the effect of a Type III disturbance (Sutherland 1981). While disturbance is commonplace in natural ecosystems, the most frequent disturbance type constitute Type II disturbance and ‘catastrophic’ Type III disturbances are relatively rare (Sutherland 1981). Disturbances due to anthropic activities, however, are very high intensity, long duration (if not permanent, so called ‘press’ disturbance), and high frequency (if not permanent) (Nimmo et al. 2015). The disturbances of the next chapter (Urbanization, Climate Change, Non-Indigenous species...) may thus all be categorized as Type III disturbances, as they all permanently change the communities in and around the affected habitat (Sutherland 1981, Nimmo et al. 2015).

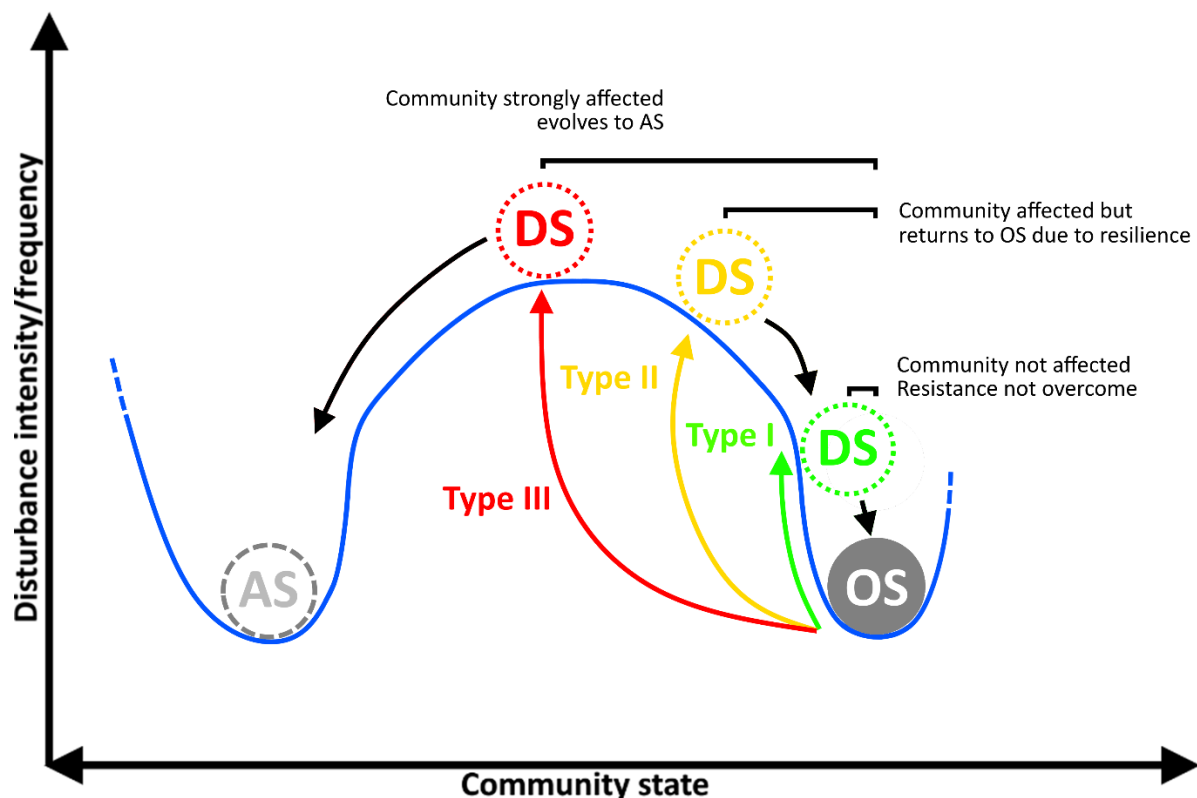


Fig. 7: Conceptualization of the three different types of disturbances explained by their effect on community state in regard to the resistance and resilience of the community. The current original stable state, represented by a grey ball (OS) is moved by disturbance along the colored path until it reaches a threshold and ‘rolls’ into its alternate stable state (Dashed ball, AS). High resistance is conceptualised as steep slope. Resilience is represented as high threshold. The community here has a high resistance and high resilience (case not represented in Fig. 4). The three different types of disturbances act on the OS which switches to an unstable disturbed state (Pointed balls, DS). The horizontal distance to OS symbolises the community change (i.e., the effect of the disturbance). The black arrow shows the direction of the evolution of the community after disturbance.

## III.B Anthropic Disturbances

### III.B.1 Habitat Loss, Landscape Change and Urbanization

The human population is growing. Projections estimate the human population to reach 9.3 billion in 2050, almost 70% of which will be living in urbanized areas (Bloom 2011). Previous growth has led to changes in the human use of landscapes and modification of natural spaces (Antrop 2004). One of the strongest impacts human activities may have on natural ecosystems is the direct modification of them. Habitat loss is the one of the most important contributor to the current mass extinction of fauna and flora (Fahrig 1997, Brooks et al. 2002, Powers & Jetz 2019). Habitat fragmentation further contributes to this process, as patches of habitat decrease in size and may be more separated (Fahrig 1997, 2003, Brooks et al. 2002, Bennett & Saunders 2011). Both processes tend to reduce available habitat, diminish population sizes and isolate populations from another. The reduced population sizes may contribute to inbreeding and mutational meltdown if certain populational thresholds are passed (Schaffer & Samson 1985, Rowe & Beebee 2003, Keyghobadi 2007, Griffen & Drake 2008) ultimately leading into extinction vortexes (Fagan & Holmes 2006, Blomqvist et al. 2010, Frankham et al. 2010). As the natural environment is patchy with somewhat isolated populations (MacArthur & Pianka 1966, MacArthur & Wilson 1967) this may evoke similarities to the human caused processes. However, for many species natural patchy landscapes consist in a mosaic of more or less suitable habitat as opposed to a mosaic between habitat and non-habitat in the context of human-caused fragmentation (Bennett & Saunders 2011). Part of the habitat loss and fragmentation can be directly attributed to urbanization (Antrop 2004, Lee et al. 2006). Urban land area has increased by 58 000 km<sup>2</sup> from 1970 to 2000, however it is projected to increase by 1 527 000 km<sup>2</sup> more by 2030 (Seto et al. 2012). This may particularly concern coastal areas as population density is three times higher in these regions (Small & Nicholls 2003). Increasing population will spark further population growth and displacement to coastal areas (Creel 2003, Mcgranahan et al. 2007) with the highest densities reached in areas with less than 10 m of elevation (Mcgranahan et al. 2007). Presently, nearly 50% of European and Asian coasts have been modified through artificial structures (Dafforn et al. 2015). This illustrates recent landscape changes and the necessity of increased territorial planning and management (Antrop 2004). Numerous works have been conducted to understand how organisms live in and interact with these new urban ecosystems (Rebele 1994, Breuste et al. 1998, Niemelä 1999). However, the marine realm has been less studied than their terrestrial counterpart (Bulleri 2006, Todd et al. 2019). As urbanization concentrates human populations and activities it may also cause and exacerbate other anthropic disturbances. Urbanization is associated with construction of artificial structures, but also increases

pollution and the presence of non-indigenous species (see III.B.4 which do not only interact with urbanization but among them as well).

### **III.B.2 Artificial Structures**

Artificial structures are human-made objects and infrastructures built to serve a certain purpose or that once served a purpose in the case of debris (Mineur et al. 2012). Coastal infrastructure plays a major role in urbanization as it is used to claim land from the sea and to protect against erosion (Davis et al. 2002, Charlier et al. 2005, Bulleri & Chapman 2010, Airoidi & Beck 2011, Lam & Todd 2013, Dafforn et al. 2015, Firth et al. 2016b). New trends in marine urbanization even turn away from the immediate coastline and artificial structures are built into the sea for aquaculture (Atalah et al. 2020), offshore wind energy and energy infrastructure (Ashley et al. 2014, Firth et al. 2016b, Taormina et al. 2018, 2020a), artificial islands like in Dubai (Burt 2014, Dafforn et al. 2015), and in a close future for recreational purposes with large floating structures (Dafforn et al. 2015, Bitterman 2018), or even floating cities like the Oceanix City project spearheaded by the former minister of tourism of French Polynesia and the architect Bjarke Ingels ([oceanixcity.com](http://oceanixcity.com)). These structures can be categorized in two large families: immobile and mobile. The most common are large immobile concrete structures like jetties, tetrapods and seawalls as they play a major role in marine urbanization and coastline protection (Bulleri & Chapman 2010, Airoidi & Beck 2011, Lam & Todd 2013, Dafforn et al. 2015, Firth et al. 2016b) as well as ships/boats as they transport goods and people (Mineur et al. 2012, Carrasco et al. 2017). But the number of different artificial structures is as high as the number of potential uses (examples in Fig. 8), ranging from shipwrecks to floating debris (Mineur et al. 2012), and listing them all is not the aim of the present manuscript.

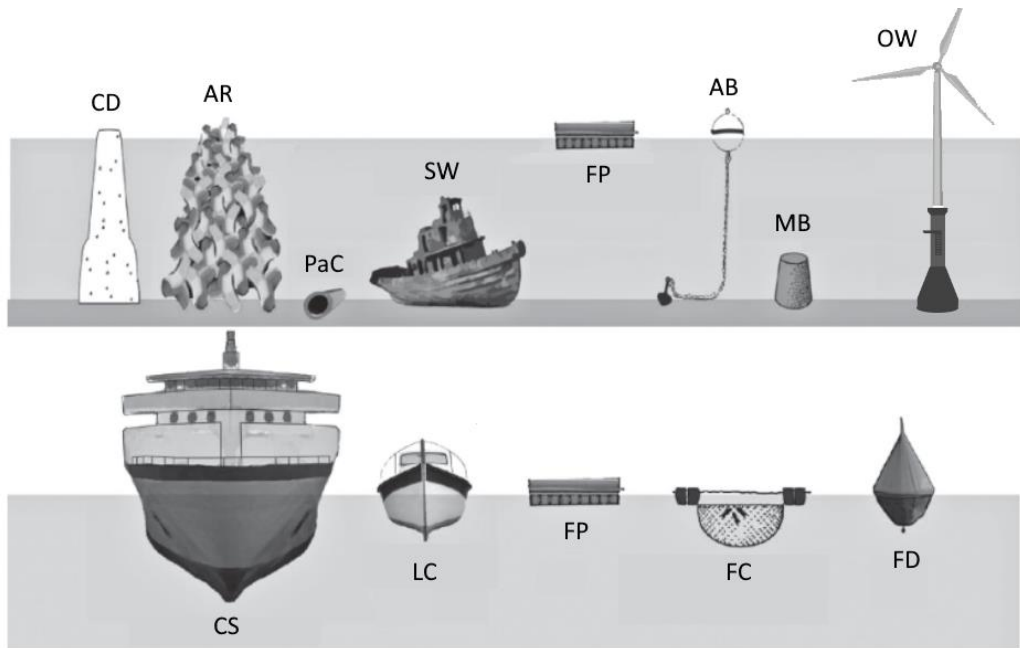


Fig. 8: Different examples of marine artificial structures. They are divided into: immobile (top) and mobile (bottom). Floating pontoons (FP) are in both. CD coastal defenses; AR artificial reefs; PaC pipes and cables; SW shipwrecks; FP floating pontoons; AB anchored buoys; MB mooring block; OW offshore windfarms; CS commercial ships; LC leisure crafts; FC fish cages (aquaculture); FD floating debris. Modified from (Mineur et al. 2012), windfarm from iberdrola.com.

Artificial structures may profoundly affect biodiversity. On one side, some artificial structures like breakwaters and jetties are intended to modify/reduce hydrodynamism resulting in altered connectivity and biodiversity (Floerl & Inglis 2003), increased temperature fluctuations (Menniti et al. 2020) and increased pollution (Lee & Arega 1999, Owen & Sandhu 2000, Tolun et al. 2001, Schiff et al. 2007, Mohammed et al. 2011, Aly et al. 2013). On the other side, artificial structures may serve as a habitat for some species. While green spaces constitute an important hotspot for biodiversity in urban areas (Lepczyk et al. 2017, Aronson et al. 2017), organisms living directly on artificial structures are rare in terrestrial urban habitats. However, in marine urban habitats, fouling communities heavily colonize artificial structures (Fig. 9). Fouling (or more specifically biofouling) is the unwanted accumulation of material or organisms on immersed surfaces (Dürr & Thomason 2010). It has always been a part of the human advent on the sea and was combatted on ships very early, as it consists in a nuisance for ships and reduces speed (WHOI US 1952). Artificial structures in marine habitats expose altered community composition with reduced biodiversity and function (Chapman 2003, Glasby et al. 2007, Mineur et al. 2012, Oricchio et al. 2016b, Chan & Briski 2017, Mayer-Pinto et al. 2018). This reduction of diversity may even be visible at the genetic level (Costantini et al. 2012). Artificial structures generally expose a high presence of opportunistic and introduced species (Glasby et al. 2007, Airoidi & Bulleri 2011, Mineur et al. 2012, Megina et al. 2016, Oricchio et al. 2016b, Ferrario et

al. 2017). Artificial substrates are often subject to other anthropic disturbances like pollution and introduced species (Piola & Johnston 2008, Mineur et al. 2012, Kenworthy et al. 2018b, Osborne & Poynton 2019, Todd et al. 2019), but also exacerbate them (Tolun et al. 2001, Floerl & Inglis 2003, Schiff et al. 2007, Mohammed et al. 2011, Aly et al. 2013, Menniti et al. 2020). They may also themselves be a source of disturbance like pollution leaching of ships and other artificial structures (WHO 1952; Voulvoulis et al. 1999; Briffa et al. 2020). It is thus difficult to disentangle their effect from other anthropic disturbances.



Fig. 9: Photos of fouling communities from the Marina du Chateau in Brest (NE Atlantic, France). Top left, an artificial substrate (ship propeller) entirely covered by fouling species (here mostly Non-Indigenous species). Top Right, different species of colonial ascidians (red and white), *Bugula neritina* (tuft) a blennie (fish, right) and a large barnacle (middle) on a floating pontoon. Bottom left, an encrusting ascidian (*Botrylloides* sp.) on a shaded pillar. Bottom right, a large Sabellidae below a floating pontoon with numerous *Ciona intestinalis* on its base.

### III.B.3 Pollutants and Contaminants

Pollution is a release of substances or energy, resulting from human activity, in the environment that may degrade the quality of the considered medium (Russell 1974). As such, pollution can be separated in two large categories: physical, and chemical pollution.

Pollution may take the form of energy. Thermal pollution for example is the release of heat produced by nuclear powerplants, thermal plants etc. in the water (Russell 1974). Thermal discharge may profoundly affect local communities and restructure ecosystems (Barnett 1971, Lardicci et al. 1999,



Arieli et al. 2011). However physical pollution may also be more subtle. Noise and light pollution have gained increasing attention in the recent years (Simmonds et al. 2014, Kunc et al. 2016, Bolton et al. 2017, Maggi et al. 2019, Di Franco et al. 2020, Giavi et al. 2020), as they increase without regard, certainly due to their unintuitive nature.

Chemical pollution however is more intuitive as chemical compounds may be toxic or harmful for organisms. As such Metallic Trace Elements (MTE, often also referred to as heavy metals) may be released into the environment by leaching of human made metallic objects (Briffa et al. 2020), but also with some pesticides (Briffa et al. 2020). Very early, toxic materials or coats like copper plating were used to reduce fouling on ship hulls (WHOI US 1952). Antifouling paints containing pesticides like tributylstannyl (TBT) were used until the late 80s which has now shifted to copper-based pesticides (Voulvoulis et al. 1999). While these paints have the merit to work in a certain extent, they leach toxic substances into the environment, consisting in a very impacting pollution (Bryan & Langston 1992, Voulvoulis et al. 1999, Schiff et al. 2007). Specifically Cu has been thoroughly studied as this metal is highly concentrated in urban areas and impact community composition and functioning (Schiff et al. 2007, Piola et al. 2009, Canning-Clode et al. 2011, Kinsella & Crowe 2016, Pardal et al. 2021), influences the recruitment and survival of many species (Johnston & Keough 2000, Bellas et al. 2001, 2004, Xie et al. 2005, Dafforn et al. 2008) and may favor introduced species (Piola & Johnston 2006a, Dafforn et al. 2008, Piola et al. 2009, Osborne & Poynton 2019). The effects of copper seem so pervasive that transgenerational adaptations may be induced (Piola & Johnston 2006b, Marshall 2008, McKenzie et al. 2011).

While MTE pollution is mostly considered as inorganic, some organic compounds may also consist in a significant pollution. Persistent Organic Pollutants (POPs) are a variety of mostly cyclic hydrocarbons with different chemical properties; however, they have all in common to be chemically very stable. Many of them have high acute and long-term toxicity and are bioaccumulated and biomagnified by organisms (Jensen 1972, Tarhanen et al. 1989, Kennish 2002). Some pesticides fall in the POP category. Pesticides strongly affect biodiversity as they are actively released in the environment and are designed to be toxic for some categories of organisms (Tang et al. 2021). Many POP pesticides are no longer in use but still have impacts on organisms due to their persistency. An example are the highly toxic Dichlorodiphenyltrichloroethane (DDT) and its derivative dichlorodiphenyldichloroethylene (DDE) which were most notably used for mosquito eradication in the mid-20<sup>th</sup> century and bioaccumulate and biomagnify in food chains (Jensen 1972, Kennish 2002, Wafo et al. 2006).

Another prime example of major POP category are Polychlorinated bisphenyls (PCBs). PCBs are among the most stable organic compounds in existence and were massively used for various applications

before they and their adverse effects were detected (Jensen 1972). PCBs were widely used as nonflammable oils with multiple industrial applications, in certain composite materials and in carbonless paper (Jensen 1972). PCBs are subject to strong bioaccumulation in the food chain and specially coplanar PCBs are extremely toxic (Jensen 1972, Tanabe 1988, Tarhanen et al. 1989). Their production was banned in most part of the world, but due to their stability they remain a major pollution problem (Jensen 1972, United Nations 2001, Webster et al. 2013).

Polycyclic Aromatic Hydrocarbons (PAH) are another category of POPs. They are a family of molecules generally associated to petroleum hydrocarbons as well as combustion of any kind and are released into the air and on burnt surfaces (Tobiszewski & Namie 2012, Cecinato et al. 2014). Through rain these molecules can be washed off into water where they expose high toxicity for organisms (Paul et al. 1998, Sarrazin et al. 2006, Tobiszewski & Namie 2012, Wang et al. 2017).

Another way to chemically pollute environments can be through nontoxic substances. The release of nitrates and phosphates into the environments can lead to the overfertilization of water bodies (eutrophication), resulting in algal proliferation and subsequent impacts (Schindler & The 2008, Chislock et al. 2013). These algal blooms can have large impacts leading to anoxic conditions after the die-off and decomposition of the created biomass (Chislock et al. 2013). Some algal blooms may however themselves be toxic or release toxic compounds (Harmful Algal Blooms, HAB; Chislock et al. 2013; Sanseverino et al. 2016). Worldwide HAB and green tides are on the rise as fertilized waters from farmland and animal farming are washed into water bodies (Schindler & The 2008, Ye et al. 2011, Smetacek & Zingone 2013, Sanseverino et al. 2016).

#### **III.B.4 Non-Indigenous Species (NIS)**

A Non-Indigenous Species (NIS; or also Introduced species, alien species, exotic species and sometimes non-native species) is a species present outside of its native range, due to the action of humans (Publications Office of the European Union 2012). Especially in recent history where interconnectivity among nations has drastically increased, accidental and voluntary species introductions have seen their numbers multiplied, which is predicted to even further increase with globalization (Levine & D'Antonio 2003, Seebens et al. 2016, Carrasco et al. 2017, Sardain et al. 2019). NIS are often categorized depending on the scale of their population and their impacts (Augsburger et al. 2000, Occhipinti-Ambrogi & Galil 2004, Lockwood et al. 2013, Ojaveer et al. 2015, Bradley et al. 2018, Spear et al. 2021). However, as NIS may cause impacts even with small populations, the most widely spread categorization integrates barriers a NIS needs to overcome to reach the next stage (Blackburn et al. 2011). The most important nomenclature used in this manuscript is detailed in Tab. 3.

Tab. 3: Definitions of terms used regarding non-indigenous species. The term population and species may be interchangeable depending on the considered scale.

Term	Synonyms	Definition	Source
Non-Indigenous species (NIS)	Alien, Introduced species	A species outside of its native range due to human activities (intentional or not)	(Publications Office of the European Union 2012)
Established	Naturalized	A NIS or population that reproduces in nature with a stable population	(Occhipinti-Ambrogi & Galil 2004, Blackburn et al. 2011, Bradley et al. 2018)
Sleeper species	Sleeper population	An established NIS or population that could become invasive but is hindered by abiotic factors (climate for ex.)	(Bradley et al. 2018, Spear et al. 2021)
Invasive species		A NIS that disperses, proliferates, and causes ecologic and/or economic impacts.	(Augspurger et al. 2000; Occhipinti-Ambrogi and Galil 2004; Blackburn et al. 2011; Publications Office of the European Union 2012; Lockwood et al. 2013; Bradley et al. 2018)

The ecological consequences of 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 & Mooney 2009, Johnston et al. 2015b, Walsh et al. 2016), causing economic impacts ranging from hundreds of millions to tens of billions of dollars for individual countries (Lovell et al. 2006, Olson 2006, Jardine & Sanchirico 2018). It is thus primordial to understand the factors conditioning the success of NIS in ecosystems (Ojaveer et al. 2015). This has prompted extensive research and reflection among ecologists, attempting to understand the ecological processes behind successful introductions and invasions. This has led to a large collection of literature focusing on the conditions of successful transport and successful naturalisation of these species in the new environment.

As each of the phases of the introduction of a species can constitute a selective filter for them, the tens rule was developed (Williamson 1993, Williamson & Fitter 1996). The concept behind this rule is that on every phase of introduction (transport, survival in the new environment, naturalisation, spread, invasion) only ca. 10% of the species reach the next phase (Williamson 1993, Williamson & Fitter 1996). While this rule should not be taken as an absolute, as it does not entirely reflect the reality of the introduction process (Lapointe et al. 2012, Jarić & Cvijanovic 2012), it is useful to conceptualize the pressures acting on NIS during their introduction. Upon surviving the transport phase, which may be difficult depending on the situational conditions, the environmental factors of the new environment may (or not) be profoundly different from the native one. Survival success of a NIS may be explained by preadaptation as these species might be already used to conditions similar to the ones of their new environment (Schlaepfer et al. 2010, MacDougall et al. 2018). However, they may also possess traits

that allow them to thrive in suboptimal habitats. NIS expose generally higher levels of adaptive phenotypic plasticity allowing them to cope with a wide range of environmental conditions (Davidson et al. 2011). Additionally, many NIS have *r*-selection traits (McMahon 2002, Davis 2005), which allows them to thrive in disturbed habitats. NIS seem to expose higher resistance towards abiotic stress and disturbance than their native counterparts (Lenz et al. 2011, Gröner et al. 2011, Lejeune et al. 2014, Marie et al. 2017, Kenworthy et al. 2018a). However both these assumptions might not always be true, as plant NIS tend to occupy similar *C-S-R*-selection strategies as natives (Dalle Fratte et al. 2019). As seen previously, biotic factors play a major role in the environment and may also affect NIS. A lot of antagonist interactions, dominated by interspecific competition and predation, may occur and the potential number of these interactions should be a function of the number of species in the environment, resulting in a resistance towards NIS (Elton 1958). Biotic resistance due to interspecific competition and predation seems to have a crucial importance on the success or failure of NIS (Garcia-Serrano et al. 2007, Fletcher et al. 2018, Gestoso et al. 2018). Concomitantly, areas where numerous NIS exist are often areas with altered communities and low biodiversity, often due to environmental influences (Chapman 2003, Glasby et al. 2007, Mineur et al. 2012, Oricchio et al. 2016b, Chan & Briski 2017, Mayer-Pinto et al. 2018), to which NIS are more resistant as seen previously. The biotic resistance hypothesis remains however controversial (Jeschke et al. 2012). Introduced species are often highly competitive and may exclude other species even if a certain biodiversity is present (Callaway & Ridenour 2004, Garcia-Serrano et al. 2007, Bøhn et al. 2008, Osborne & Poynton 2019). Different hypotheses have emerged to explain their competitive advantages. When being introduced into new areas, NIS leave their biotic regulators ('enemies') behind such as predators, parasites and pathogens (Enemy Release Hypothesis ERH; Keane and Crawley 2002). Without these regulators, NIS may perform better in introduced areas than in their native habitats and may gain competitive advantages towards native species (Torchin et al. 2001, Keane & Crawley 2002, Joshi & Vrieling 2005). The lack of enemies (or eventual constraining environmental conditions from their native area) may allow the reallocation of resources originally intended for survival in their native habitat, into production of biomass in their introduced area (Evolution of increased competitive ability EICA; Blossey and Notzold 1995). However, instead of not needing a certain set of adaptations in their new environment, they can also arrive with a specific adaptation, giving them a competitive edge (Novel Weapon Hypothesis). Novel weapons emerge in a NIS' native habitat, in absence of co-evolution between the NIS and the species native to its introduced habitat. Natives thus lack an evolutionary response to the considered NIS, leading to higher competitive performance of the invader (Ni et al. 2012). Novel weapons such as allelopathic chemical compounds can alter competition between natives and the NIS (Callaway & Aschehoug 2000, Jensen 2000, Ni et al. 2012). Although such compounds may also be part of predator avoidance and thus result in apparent competition (Hay et al. 1994, Cappuccino & Carpenter 2005). NIS seem to have

the competitive superiority in marine urban environments as they have higher resistance towards abiotic stress and disturbance (Davidson et al. 2011, Gröner et al. 2011, Lejeune et al. 2014, Marie et al. 2017, Kenworthy et al. 2018a) and are better competitors for space (Blossey & Notzold 1995, Callaway & Aschehoug 2000, Keane & Crawley 2002, Callaway & Ridenour 2004, Bøhn et al. 2008). These observations might be however due to survival bias (Wald Abraham 1949) as only hardy species survive the conditions of the transport and thus can become introduced (see also Tens Rule Williamson and Fitter 1996). However, NIS are also subject to rapid evolution, within several generations due to strong selective forces acting on them (Huey 2000, Hejda et al. 2009, Jarić & Cvijanovic 2012, Elst et al. 2016). On the other hand they have been shown to have very high phenotypic plasticity (Marshall 2008, Burgess & Marshall 2011, Davidson et al. 2011, Ardura et al. 2018). As discussed earlier, such phenotypic plasticity could in certain cases influence genotypic adaptation (Richards et al. 2010a b, Klironomos et al. 2013, Moler et al. 2018), which might give them a further adaptive advantage in their introduced range.

As stated in the Tens rule, not all NIS become invasive (Williamson 1993, Williamson & Fitter 1996). Certain NIS establish in stable populations, but do not proliferate causing ecologic and economic damages (Occhipinti-Ambrogi & Galil 2004, Bradley et al. 2018). As an example, marinas and harbors contain a disproportionately greater concentration of NIS than other areas (Mineur et al. 2008, Sylvester et al. 2011, Clarke Murray et al. 2012), and even support NIS exclusive to them (Ferrario et al. 2017). Although these NIS can slowly infiltrate native ecosystems (Simkanin et al. 2012), many seem thus to be constrained to highly anthropized environments. As dispersing significant distances from the location of introduction is a requirement for overcoming the dispersal barrier of introduction (Blackburn et al. 2011), and as transport by ships might not be considered autonomous dispersal, many of NIS in marinas are arguably not even invasive. These species and populations may however constitute sleeper species/populations as environmental conditions (including biotic conditions) do not (yet) allow their spread (Fig. 10; Bradley et al. 2018, Spear et al. 2021). This might change if environmental conditions change as for example in the light of climate change (Bradley et al. 2018, Spear et al. 2021).

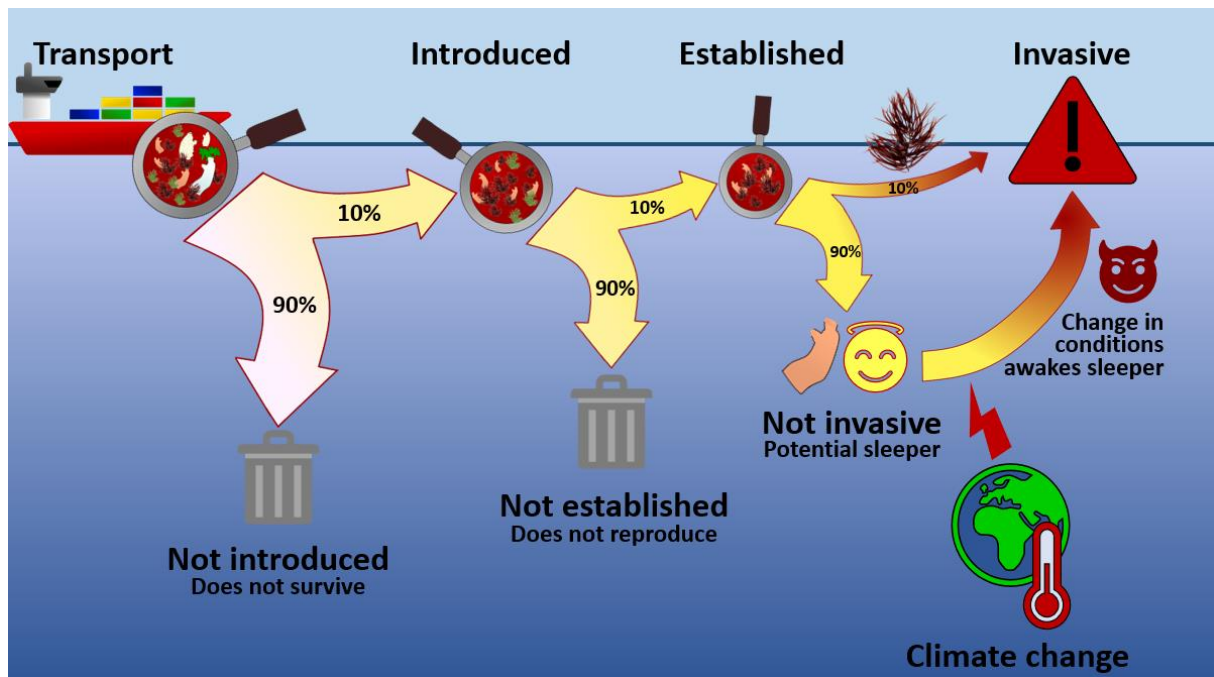


Fig. 10: The Tens rule and how established sleeper species may become invasive in the light of climate change. The tens rule (Williamson 1993, Williamson & Fitter 1996) was conceptually used to illustrate how species overcome different introduction steps. Most established species (90%) are not invasive but a certain unknown portion them could become invasive and cause damages if currently restricting conditions change due to climate change (Sleeper species; Bradley et al. 2018; Spear et al. 2021). Inspired by (Spear et al. 2021 Fig. 4)

### III.B.5 Climate Change

Earth's climate is regulated by astronomic, geologic but also to atmospheric regulators (Young 1991, Fluteau 2003). While sun activity may play a small role in climate (Young 1991, Lean 2010), it is mostly regulated by two processes, which are plate tectonics and atmospheric greenhouse gas concentrations (Young 1991, Zachos et al. 2008, Lacis et al. 2010, Beerling & Royer 2011). Plate tectonics are responsible for changes in sea levels, landmass position, mountain formation and regulate the albedo of the planet, which has substantially influenced past climates (Fluteau 2003). However atmospheric greenhouse gases and most importantly CO<sub>2</sub> are the main actor on climate on different timescales (Young 1991, Zachos et al. 2008, Lacis et al. 2010, Beerling & Royer 2011). Greenhouse gases - gases that absorb and emit infrared light (thermal radiation) thus trapping heat in the earth's atmosphere (IPCC 2021) - have a major importance in earth's climate as average planetary temperatures without them would be around -18°C rather than the current average of +15°C (Ma 1998, Ma & Tipping 1998). The quantity of these gases in the atmosphere can thus regulate the global average temperature (Young 1991, Zachos et al. 2008, Lacis et al. 2010, Beerling & Royer 2011, IPCC 2021). The most notable and abundant greenhouse gases in the atmosphere are water vapor (H<sub>2</sub>O), CO<sub>2</sub> and methane (CH<sub>4</sub>). Throughout the geologic eras, multiple greenhouse (warm temperature) and icehouse (cold

temperature) cycles have existed and changing climate has been (until now) a normal process of earth's history (Young 1991, Fluteau 2003, Zachos et al. 2008, Beerling & Royer 2011, IPCC 2021).

In recent history, human emissions, due to the combustion of fossil fuels and other human activities, have drastically increased atmospheric CO<sub>2</sub> levels (Malhi 2002, Lacis et al. 2010, Cook et al. 2016), which has led to human-driven global warming (Cook et al. 2016, Powell 2019, IPCC 2021). While having been controversial for many years due to political and economic implications, the general consensus is now that global surface temperatures do indeed rise and this, due to anthropogenic CO<sub>2</sub> emissions (Cook et al. 2016, Powell 2019, IPCC 2021). Current temperature rises are unexpected and too quick in the context of natural geological cycles and can be attributed to human activities beyond reasonable doubt (IPCC 2021). Climate change is now seen as a global emergency as it will cause large demographic displacements (Mcgranahan et al. 2007), impact global food security (Devereux & Edwards 2004) and human health (Harmer et al. 2020) but also because it will affect ecosystems and their services (Hanson & Weltzin 2000, Ayres & Lombardero 2000, Hughes et al. 2003, Lejeusne et al. 2010, Waldock et al. 2018, Zhang et al. 2020). This has prompted political engagements worldwide (Paris Agreements) to curb carbon emissions to levels that would limit global warming to less than +1.5°C (UN 2015; IPCC 2021). It is, however, unlikely that human efforts suffice to limit global heating to +1.5°C and a scenario with a global temperature increase of +3.2°C seems most likely (Raftery et al. 2017). Associated to atmospheric warming, global sea surface temperatures (and deeper temperatures) are projected to rise up to +3°C (IPCC 2019).

Climate change is a global disturbance with direct effects on ecosystems, but also acts synergistically with other disturbances. Global average temperatures have already surpassed +1°C compared to preindustrial times (IPCC 2021) and climate change strongly affects ecosystems with shifts in community composition, range changes in multiple species, and changes in trophic function of ecosystems (Hanson & Weltzin 2000, Ayres & Lombardero 2000, Hughes et al. 2003, Lejeusne et al. 2010, Waldock et al. 2018, Lonhart et al. 2019). It may substantially modify biological invasion patterns as new areas become available to invasive species (Zhang et al. 2020, Vilizzi et al. 2021), as it will favor introduced species with higher thermal resistance (Sorte et al. 2010b, Kelley 2014, Lord 2017a, Kenworthy et al. 2018a) and as some sleeper species that do not yet cause damages to ecosystems may become invasive (Bradley et al. 2018, Spear et al. 2021). Besides the long term heating, Climate change is projected to further increase the frequency of extreme weather events such as heat waves (Meehl et al. 2000, Holl 2009, Stott 2016) which can cause mass mortalities (Garrabou et al. 2009, Lejeusne et al. 2010, Sorte et al. 2010a, Pansch et al. 2018, Lonhart et al. 2019, Smale et al. 2019, Strydom et al. 2020) and further advantage certain NIS (Sorte et al. 2010b a, Kenworthy et al. 2018a, Castro et al. 2021). Warmer temperatures also have synergistic effects with other disturbances such

as pollution since they may impact the physiology, food uptake and metabolism of organisms (Sokolova & Lannig 2008, Alava et al. 2017, Cabral et al. 2019).



# Context and Objectives of the Present Work

## I Marinas – A Perfect Playground

Anthropic disturbances have a high impact on communities and may often act synergistically. Harbors/marinas are intrinsically linked with Urbanization of the coastal environment, as these are central hubs of human coastal activities. The number of marinas in the world is increasing drastically. In France for instance the number of marinas has increased from 180 in 2008 (FFPP 2011) to 473 in 2018 (OPP 2018), associated to an ever increasing demand for mooring places. These marinas are a major source of all previously listed anthropic disturbances for coastal ecosystems.

In marinas, artificial structures intended to modify/reduce hydrodynamism, like breakwaters and seawalls are vastly used, which may impact connectivity between communities (Floerl and Inglis 2003). Aside from the infrastructure associated to marinas, ships constitute a large substrate for marine fouling communities, however this substrate represents particular conditions due to efforts to combat these organisms through anti fouling paints (WHOI 1952; Minchin and Gollasch 2010; Sylvester et al. 2011). Artificial structures in marinas have, thus, specific associated communities with reduced biodiversity and function (Glasby 1999, Glasby et al. 2007, Oricchio et al. 2016b, Kenworthy et al. 2018b), but also with an increased proportion of opportunistic and introduced species (Glasby et al. 2007, Airoidi & Bulleri 2011, Megina et al. 2016, Oricchio et al. 2016b, Ferrario et al. 2017).

Due to the high abundance of ships in marinas and the use of anti-fouling coatings on their hulls, pollutants like Cu leach into the environment (Bryan & Langston 1992, Voulvoulis et al. 1999, Schiff et al. 2007), potentially constituting a major disturbance for the local fauna as they alter community composition and functioning (Schiff et al. 2007, Piola et al. 2009, Canning-Clode et al. 2011, Kinsella & Crowe 2016, Pardal et al. 2021). The reduction of water exchange with the open sea due to the artificial structures of marinas may favor concentration of these pollutants (Lee and Arega 1999; Owen and Sandhu 2000; Tolun et al. 2001; Schiff et al. 2007; Mohammed et al. 2011; Aly et al. 2013). This may be further fueled by the collection of runoff, sewage and industrial waters (Bryan & Langston 1992, Kennish 2002).

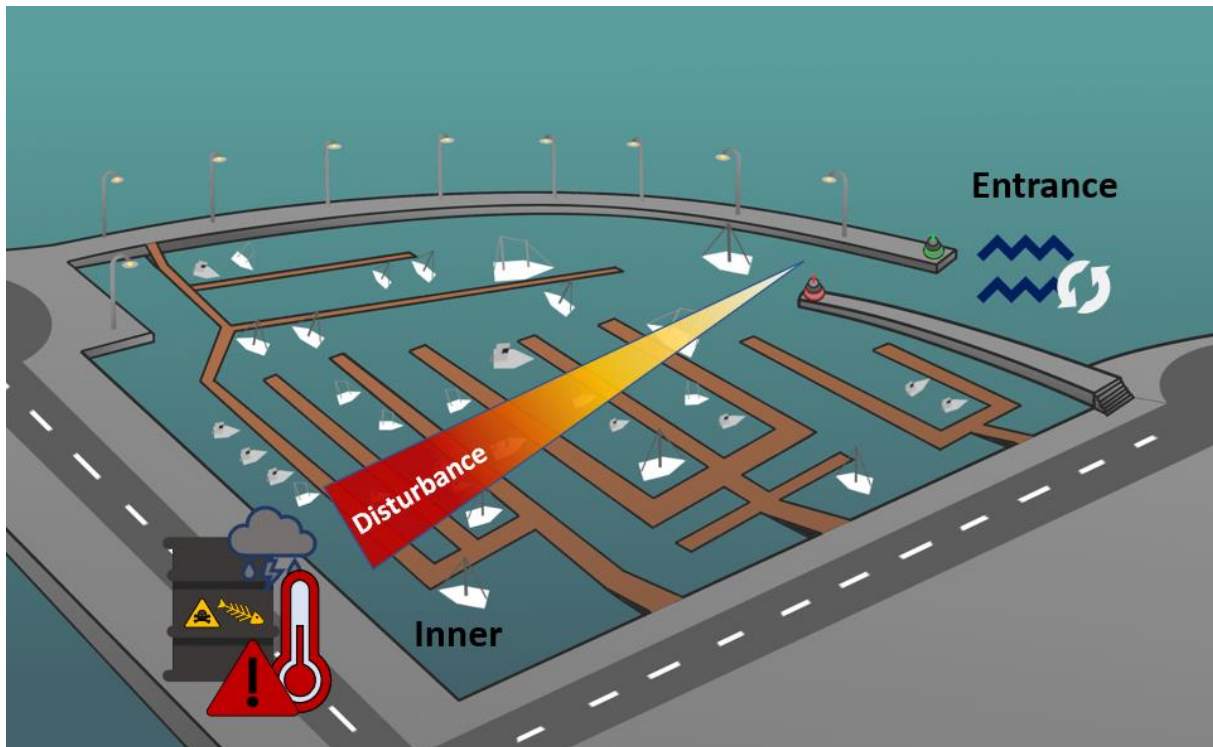
Due to the reduced water volume, low hydrodynamism and enclosed nature of marinas, fluctuations may also be favored in these environments, most notably temperature fluctuations (Menniti et al. 2020), but also salinity. As temperature and weather has been shown as determining for marina communities (Sorte et al. 2010b, Lord et al. 2015, Chang et al. 2018, Castro et al. 2021), this might exacerbate the effects extreme weather events and climate change.

Ships are the dominant vector for the introduction and dispersion of NIS in marine habitats, due to their ballast waters and fouling on hulls (Hewitt et al. 2009, Sylvester et al. 2011, Seebens et al. 2016). Many NIS seem to use artificial structures as privileged habitat (Dumont et al. 2011b, Mineur et al. 2012, Simkanin et al. 2012, Atalah et al. 2020), but NIS seem also to be favored by other disturbance such as pollution or temperature, potentially due to higher resistance and plasticity (Davidson et al. 2011, Gröner et al. 2011, Lejeusne et al. 2014, Marie et al. 2017, Kenworthy et al. 2018a). For instance, pollution disturbance due to Cu has been shown to often favor NIS (Piola & Johnston 2006a, Dafforn et al. 2008, Piola et al. 2009, Osborne & Poynton 2019). The numerous and intense disturbances in marinas may affect the communities in marinas, leading to reduced biodiversity (Dijkstra et al. 2007, Saloni & Crowe 2015, Kinsella & Crowe 2016), which might increase invasibility. For all these reasons marinas are highly subjected to NIS which may even lead to their dominance (Mineur et al. 2008, Sylvester et al. 2011, Clarke Murray et al. 2012). These habitats can contain a greater concentration of NIS than other urban areas like seawalls (away from marinas) or commercial harbors, and even support NIS exclusive to them (Marins et al. 2010, Ferrario et al. 2017).

For these reasons and for logistic reasons such as accessibility and ease of planning, marinas have become a privileged environment for studying ecological processes in marine urban habitats, as in the present work.

## **II Spatial Variability of Communities and Disturbance Gradients**

As water exchange with the open sea is reduced by artificial structures in and around marinas, disturbances like pollution and increased temperature variation are exacerbated (Lee & Arega 1999, Owen & Sandhu 2000, Tolun et al. 2001, Aly et al. 2013, Menniti et al. 2020). This is especially true in marinas due to their often semi-enclosed nature. As in these habitats, water exchange with the exterior operates at the entrance of the marina, the innermost parts may have even more stagnant water masses and may expose higher levels of disturbance compared to other parts (Schiff et al. 2007). As local disturbance sources are mostly inside marinas (Bryan & Langston 1992, Voulvoulis et al. 1999, Schiff et al. 2007) or apply on the marina as a whole (Menniti et al. 2020), this gradual decline of water exchange with the exterior may result in a disturbance gradient. Pollution in marinas and harbors for instance has often been observed to be organized as a gradient (Je et al. 2004, Ryu et al. 2011, Kenworthy et al. 2018b). Thus, with increasing intensity and frequency, it is possible to conceptualize a disturbance gradient in marinas, with maximal disturbance in the inner parts (Fig. 11). As disturbance is a major selective force and shaper of community structure (Grime 1977, Connell 1978, Huston 1979), it might constitute a selective filter for local fauna.



*Fig. 11: Illustration of the disturbance gradient in marinas. At the inner of a marina (left) reduced hydrodynamism favors the accumulation of disturbing agents like pollution, heat, or fresh water (even if the sources of these disturbances may be everywhere in the marina). At the entrance (right), water exchange with the open ocean occurs, which decreases disturbance. This results in a disturbance gradient inside the marina.*

If disturbance frequency and intensity are different between different parts of a marina, differential selective pressures should act on these parts. As a result, community composition should be different, depending on the individual tolerances of the present species to environmental filters and their selection strategies. Indeed, previous studies have observed local variation of fouling communities among different parts of harbors/marinas, which has sometimes been linked to pollution gradients (Je et al. 2004, Ryu et al. 2011, Kenworthy et al. 2018b). For instance, significant variation in fouling communities between pillars at the entrance and the inner part of three marinas has been linked with an environmental MTE gradient (Kenworthy et al. 2018b). This process was visible at very small spatial scales (< 100 m). The differential selective pressures between two parts of the marina, caused by different pollution levels, might even result in local adaptation if the pressure is strong enough and if dispersion remains low. This might for instance be the case for some introduced species as they are more abundant the inner parts of marinas (Kenworthy et al. 2018b) and have high adaptive potential (Piola & Johnston 2006b, Marshall 2008, Burgess & Marshall 2011, Davidson et al. 2011, Ardura et al. 2018).

### III Objectives of the Present Work

The present work aimed to understand the diversity and functioning of harbor communities by studying how disturbance gradients (illustrated by pollution for example) from the inner parts to the entrance of marinas shape community structure and how they might cause local adaptation at small spatial scale. A particular focus was given to NIS as they are a major part of these communities and constitute an important management challenge, especially in the context of climate change. To do so, various experiments were conducted in up to six different marinas from two regions (the Mediterranean and the Atlantic), by comparing community and/or ecological functions between the less disturbed entrance of a marina and its inner parts. Field observations were combined with *in situ* experimental approaches. We expected disturbance to be spatially heterogeneous within the studied marinas and that communities vary according to this disturbance. Based on the literature (See III.B.4) we assumed that NIS would be favored by disturbance, and that they would be more prevalent in the most disturbed parts of marinas (*i.e.*, the inner parts).

As a first effort, an in-depth study was conducted to complete the results of previous studies and particularly the one of Kenworthy et al. (2018b). Photographic data from three years of monitoring in the inner, middle and entrance part of one marina were analyzed to verify if spatial heterogeneity of communities is stable in time which would indicate a stable process determining community structures in marinas (Chapter I).

In the same marina, an experimental study tested for a causal link between the monitored disturbance gradient and the previously mentioned community structure (Chapter II). To expose this causal link between local environmental conditions and community structure, reciprocal transplant experiments were conducted. Classical tools of community analysis were combined with a physiological approach (community respiration and metabolomics) to investigate, at multiple organization levels, the response of the fauna to this environmental variation.

In the same marina, the effect of large, mobile predators, which might act independently from the disturbance gradient, was tested on the survival of two species of the fouling community which helped to disentangle the effects of predation and the disturbance gradient (Chapter III A). In certain situations, the effects of disturbance gradients might be masked by larger scale disturbances or biotic processes. In Chapter III B we expose results from an experiment in another marina, where a large scale extreme climatic event masked the effects of local spatial heterogeneity, homogenizing the community structure within the harbor.

As the effect of the disturbance gradient may depend on the disturbance intensity and as other processes have been shown to mask the effect of disturbance gradients, it was necessary to confirm the results observed in Chapter II. Thus, we replicated the reciprocal transplant experiment at a larger geographical scale in six marinas from two different regions (the Atlantic and the Mediterranean; Chapter IV). Marinas were chosen depending on the intensity of disturbance, with a strongly disturbed marina, a less disturbed marina and an intermediate one for each region. This allowed to replicate previous results and to generalize the observations.

Climate change is well underway and already profoundly impacts natural communities. We investigated if this global scale disturbance may impact marina communities. A prospective attempt was made by simulating *in situ* how a plausible global warming scenario would affect the community structure of two marinas especially considering the prevalence of NIS since they have previously been shown to be advantaged by disturbance (Chapter V).

As anthropic disturbances of all kinds are concentrated in marinas, the results obtained from this PhD dissertation can be used to discuss the place of NIS in marine urban ecosystems, how their prevalence is likely to evolve with climate change.

# **Chapter I      Description of the Spatial Variability of Fouling Communities in our study Marina**

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## Persistence of dominance of non-indigenous species in the inner part of a marina highlighted by multi-year photographic monitoring

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**Abstract.** As a result of urbanization, the coastal environment is being disturbed by various anthropogenic pressures. These are concentrated in harbor areas where the addition of artificial structures and the presence of pollutants seems to favor the settlement of non-indigenous species. Today, most of the studies working on these organisms are often carried-out in a single time window without integrating temporal variability. Our work consisted of analyzing multi-year photographic data of marina communities taken from three experiments held between 2016 and 2019 in the same marina. These photographs were taken from recruitment plates placed at the inner, middle and entrance locations of the marina, permitting us to discern the community differences and the distribution of non-indigenous taxa between these 3 locations. Over all the studied periods, the communities that grew at the entrance and the inner locations of the marina were always different. Non-indigenous taxa also appear to be more prevalent in the inner location of the marina. Our results suggest the presence of different environmental filters between the entrance and the inner location that could explain these observations. We suggest this could be due to a pollution gradient with high pollution at the inner location of the marina and to a competitive pressure exerted by the tunicate *Ciona intestinalis* at the marina entrance.

**Keywords:** Non-Indigenous species, *Ciona intestinalis*, Image analysis, Fouling communities, Marina

## Introduction

The urbanization of coastal environments has dramatically increased over the last few decades. Over 2,8 billion people, almost 40% of the world's population, live in coastal areas (< 100 km from the sea) and this figure is projected to increase to 3.2 billion before 2035 (Maul & Duedall, 2019). Presently, nearly 50% of European and Asian coasts have been modified with artificial structures (Dafforn et al., 2015). This growing urbanization is a major source of disturbance for coastal ecosystems (Lee et al., 2006; Burt, 2014), such as seagrass beds and rocky shores that support high biodiversity (Bowen & Valiela, 2001; Lee et al., 2006; Burt, 2014). Apart from destroying habitats, artificial structures also do not act as a substitute for natural substrata, but rather facilitate a differential recruitment of organisms, depending on the wide variety of materials they are composed of, *e.g.*, plastics, wood, metals, stones and concrete (Connell & Glasby 1999, Bulleri & Chapman 2010, Mineur et al. 2012). Furthermore, all these structures are also likely to transform the coastal hydrodynamics and larval dispersion (Burcharth & Lamberti 2005, Moschella et al. 2005). Coastal urbanization may, thus, impact the structure and diversity of communities along coastal environments (Inglis & Kross 2000, Deegan 2002, Rosa et al. 2003, Bulleri & Chapman 2010, Scherner et al. 2013).

Urbanization of the coastal environment is concentrated near harbor areas, which are a major source of pollutants for coastal ecosystems. Lubricating oils, exhaust gases and fuel spills are frequently released in the environment which cause various types of hydrocarbon pollution (Voudrias & Smith 1986). In addition, harbor areas often collect runoff, sewage and industrial waters, facilitating the input of large amounts of heavy metals and pesticides (Bryan & Langston 1992, Kennish 2002, Rivero et al. 2013). Compounds used to prevent biofouling on ship hulls also spread highly toxic copper and zinc molecules into the water (Voulvoulis et al. 1999, Karlsson & Eklund 2004, Lagerström et al. 2018). The distribution of these contaminants frequently appears structured with spatial heterogeneity. Indeed, the entrance of harbor areas are often less disturbed than the inner parts (Je et al. 2004, Ryu et al. 2011, Kenworthy et al. 2018b). This difference may be explained by the location of pollutant sources in the harbor and the hydrodynamics within resulting in higher water retention in the inner areas (Floerl & Inglis 2003, Schiff et al. 2007). Water retention in the innermost parts of the harbor might alter environmental parameters of natural (such as temperature) (Menniti et al. 2020) or anthropogenic origin (such as heavy metals) impacting organisms in harbor environments (Owen & Sandhu 2000, Schiff et al. 2007, Aly et al. 2013). The combined effects of these stressors may act in an additive manner or result in synergistic impacts (Sokolova & Lannig 2008, Saloni & Crowe 2015, Kinsella & Crowe 2016) and which may constitute a selective filter. Moreover, the loss of biodiversity induced by these disturbances is considered as one of the factors that may enhance the settling of non-indigenous species (NIS) in harbor areas (Elton 1958, Piola & Johnston 2008, Crooks et al. 2011).



NIS have mainly been dispersed in the marine environment through the development of maritime transport (Hewitt et al. 2009, Clarke Murray et al. 2012). As a result, they are found in high concentrations in harbor areas (Simkanin et al. 2012, Ferrario et al. 2017) where a number of species appear tolerant to disturbance (Lenz et al. 2011, Lejeusne et al. 2014, Marie et al. 2017). These include resistance to heating events (Kelley 2014, Kenworthy et al. 2018a) and heavy metals (Piola & Johnston 2006a b, Crooks et al. 2011). As these parameters can disturb the indigenous harbor biodiversity, NIS often appear more resistant than native organisms in these environments (Arenas et al. 2006, Piola & Johnston 2008). This tolerance, together with the presence of new artificial substrata, could then facilitate their settlement and prevalence in harbors (Glasby et al. 2007, Dafforn et al. 2012). NIS thus represent a fundamental issue for research and management of coastal ecosystems. It therefore appears essential to study the distribution of NIS populations in harbor environments and to understand the processes structuring these communities.

In this context, marinas represent a study environment that can contain a greater concentration of NIS than other areas, such as commercial harbors, and even support NIS exclusive to marinas (Marins et al. 2010, Ferrario et al. 2017). Although more studies are now focusing on the inventory of these organisms, they are often carried out for a single period without integrating the seasonal or interannual dynamics of the studied communities (Webb & Keough 2000, Ashton et al. 2006, Ferrario et al. 2017, Kenworthy et al. 2018b, Spagnolo et al. 2019). The study of these NIS then only occurs within a small-time window without knowing whether it is variable on a larger time scale. In the present study, we assess data from three community-scale experiments carried out in 2016, 2017 and 2019 in the Marina du Château (Brest, France) conducted independently but with similar overall methods. Most notably, each experiment collected photographic data of the evolution of communities recruited over a few months each year. Consequently, the photographs collected over these three years provide an opportunity to study the temporal variability of recruitment and evolution of the macrofauna in a marina context. The present work therefore aims to combine the photographic data from these three experiments to study the structure of fouling communities and the presence of NIS at the entrance, middle and inner parts of a temperate marina at a multi-year scale. We hypothesized that (1) the fouling communities are spatially heterogeneous among the marina's locations; (2) the inner location is characterized by a greater concentration of NIS; (3) and that the temporal variations of community structure in our study area are less important than spatial variations within the marina.

## Material and Methods

### Study site

The study site, the 'Marina du Château' is located in the Bay of Brest, France (48°22'44"N, 4°29'21.0"W). This marina is nested within a larger harbor complex with commercial and military activities. Three locations within the marina have been equipped with macrobenthic settlement plates in 2016, 2017 and 2019 (Fig. 12; Fig. 13).

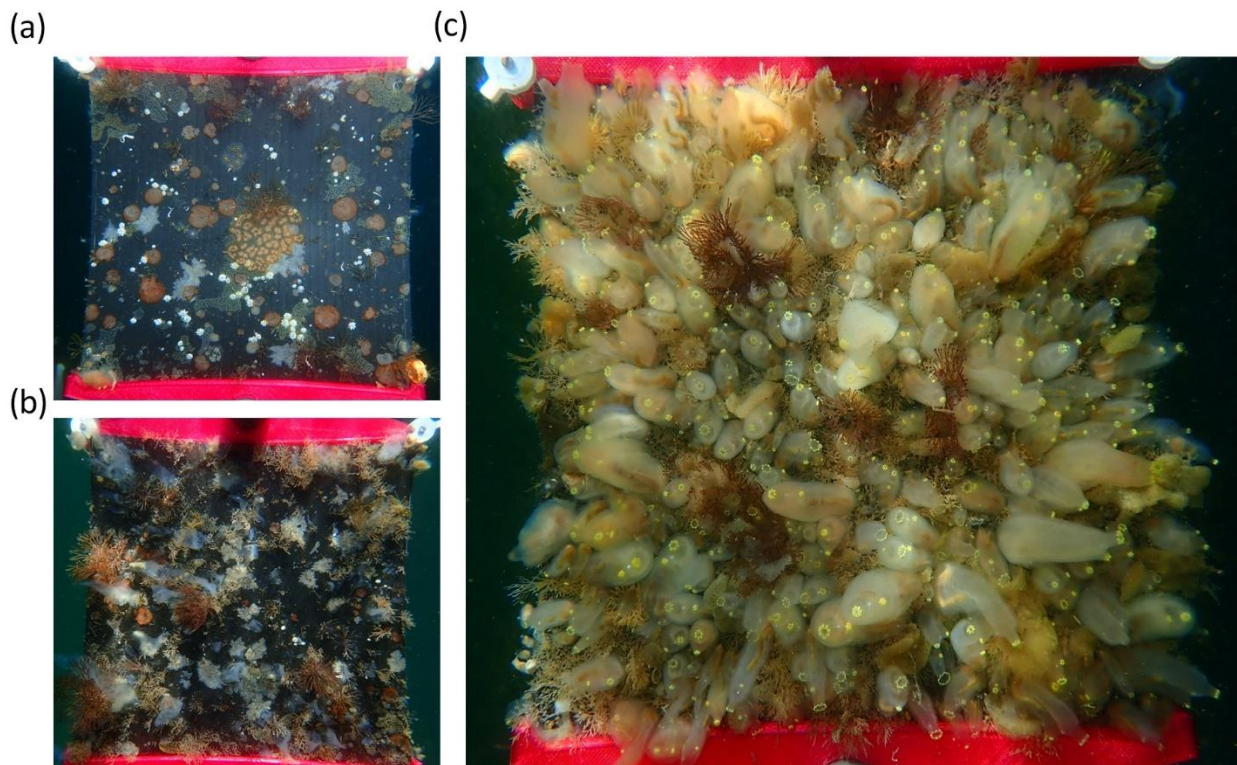


Fig. 12: Examples of photographic data of macrobenthic communities recruited on PET plates in June 2019; (a) inner, (b) middle and (c) marina entrance.

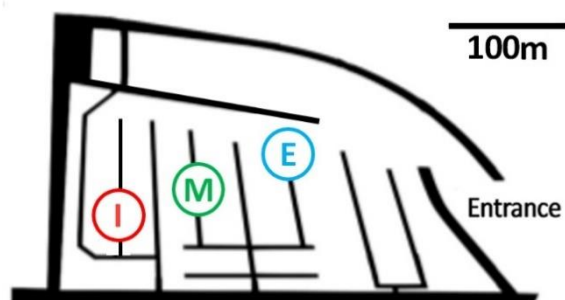


Fig. 13: Schematic diagram of the marina du Château in Brest and the different locations studied: (I) inner, (M) middle and (E) entrance.

## **Sampling Methods**

Photographic data were compiled from three different experiments, which all had the aim to characterize the spatial variability of the macrobenthic communities present at three different locations of the study area: the entrance, the middle and the inner locations of the marina (Fig. 12; Fig. 13). Settlement plates made of polyethylene or PE (20 cm x 20 cm), the same material composing the pontoons, were deployed at these three locations. In 2016, these plates were placed on the lower part of a triangular structure, whereas in 2017 and 2019 they were positioned on a grid frame. Panels were deployed in May for 5 months (October) in 2016. In 2017 and 2019 intermediate dates were added as panels were deployed in May 2017 and early April 2019, photographed in August 2017 and June 2019 and the photographed again in October 2017 and end of August 2019. The aim of the three experiments slightly varied between the years and some of the plates were sampled for other analyses. Consequently, the number of plates available per location and period is variable. In 2016, data was only collected for one period, 15 photos for the month of October (5 per location). In 2017, 48 photos for the month of August (16 per location) and then 15 in October (5 per location) were available, while for June and August 2019, 60 (20 per location) photos and 15 (5 per location) plates could be processed. Each experiment was therefore analyzed independently.

## **Photographic processing**

To avoid a border effect, a smaller quadrat (18 cm x 18 cm) has been digitally recreated on each photo. These quadrats were analyzed with the point intercept method in a random stratified design and with a total of 144 points randomly generated. This number above the threshold of 0.4 points per cm<sup>2</sup>, which allows to reliably assess the cover of species of more than 5% with a confidence interval above 95% (Taormina et al. 2020b). Most of these organisms were identified using morphological criteria. To further increase the quality of our photographic species identifications we crosschecked our data with previous identification work carried out in laboratory in 2019. The photo observer was able to train species identification with a taxonomic expert by working on additional recruitment plates. These observations were made on the very same recruitment plates as the ones used on our photographic identifications and permitted us to confirm the presence of certain taxa. We also used this data to briefly clarify if some biases of the image-analysis method, like the underestimated presence of certain taxa, could be a significant problem. A comparison of image-analysis and laboratory methods was thus carried out, revealing that communities are very similar. Therefore, the image-analysis method appears robust enough to study the overall structure of communities over the multi-year monitoring. Identified species (Tab. 4) were grouped within one of the three categories: indigenous species (IS),

non-indigenous species (NIS), and cryptogenic species (CS) (*i.e.*, species whose native or introduced status in the study area has not been clearly established).

Species	Authority	Status	Criteria
<b>Annelida</b>			
<i>Spirobranchus triqueter</i>	(Linnaeus, 1758)	Indigenous	
<b>Arthropoda</b>			
<i>Austrominius modestus</i>	(Darwin, 1854)	Non-Indigenous	
<b>Bryozoa</b>			
<i>Bugula neritina</i>	(Linnaeus, 1758)	Non-Indigenous	
<i>Bugulina flabellata</i>	(Thompson in Gray, 1848)	Indigenous	Obs
<i>Bugulina fulva</i>	(Ryland, 1960)	Cryptogenic	Obs
<i>Cryptosula pallasiana</i>	(Moll, 1803)	Non-Indigenous	
<i>Electra pilosa</i>	(Linnaeus, 1767)	Indigenous	
<i>Tricellaria inopinata</i>	d'Hondt & Occhipinti Ambrogi, 1985	Non-Indigenous	Obs
<i>Watersipora subatra</i>	(Ortmann, 1890)	Non-Indigenous	Obs
<b>Tunicata</b>			
<i>Asciidiella aspersa</i>	(Müller, 1776)	Indigenous	
<i>Asterocarpa humilis</i>	(Heller, 1878)	Non-Indigenous	
<i>Bortyllus schlosseri</i>	(Pallas, 1766)	Cryptogenic	
<i>Ciona intestinalis</i>	(Linnaeus, 1767)	Indigenous	Obs
<i>Clavelina lepadiformis</i>	(Müller, 1776)	Indigenous	
<i>Diplosoma listerianum</i>	(Milne-Edwards, 1841)	Cryptogenic	Obs
<i>Phallusia mammilata</i>	(Cuvier, 1815)	Indigenous	
<i>Styela clava</i>	Herdman, 1881	Non-Indigenous	
<b>Mollusca</b>			
<i>Anomia ephippium</i>	Linnaeus, 1758	Indigenous	

Tab. 4: Table of the different taxa and species identified via macro-morphological criteria as well as their ecological status within the marina. The abbreviation 'Obs' corresponds to an identification completed by the work carried out in the laboratory.

## Data analysis

Due to different methods used, each year was assessed separately. Community analyses were conducted using R (R core Team 2020, version 3.5.1) with the 'vegan' package (version 2.5-6; Oksanen et al. 2018). The cover of all species was used to construct several Bray-Curtis dissimilarity matrices which were graphically represented by non-metric multidimensional scaling (nMDS). For each year, the homogeneity of sample dispersions from the three study locations was tested via the 'betadisper' function from 'vegan'. While homogeneity was not systematically respected over all the studied periods, the PERMANOVA test has been shown to be robust to deviations from this homogeneity if the number of replicates is balanced between the different groups (Anderson et al., 2013), as it was in these experiments. When a PERMANOVA analysis ( $10^4$  permutations) showed significant differences between locations, a pairwise PERMANOVA ( $10^4$  permutations) was performed using the 'pairwiseAdonis' package (version 0.3; Martinez Arbizu 2019), applying a Benjamini-Hochberg adjustment method (Benjamini & Hochberg 1995). SIMPER tests were carried out for each period to characterize the taxa most contributing to the contrast between the communities of the different locations (Clarke 1993). Indicator species for each location and period were identified using the

multipattern analysis provided by the 'Multi Pattern Analysis' package (version 1.7.7; Cáceres et al. 2011). Histograms comparing the cover of indigenous, NIS, and cryptogenic species as well as unoccupied space were also created for each location and each period. The significant differences in percent cover between these different categories were identified using Kruskal-Wallis tests and Wilcoxon Mann-Whitney multiple comparison tests using the Benjamini-Hochberg adjustment method.

## Results

### Comparison of the overall structure of the recruited communities

Three distinct groups of macrobenthic communities (one for each location) can be discerned on the nMDS for October 2016, August 2017, October 2017, and June 2019 (Fig. 14), with PERMANOVA analysis indicating significant differences among locations for each time point (PERMANOVA, for all comparisons  $df=1$ ,  $p < 0.02$ ,  $R^2 > 0.17$ ; Tab. 5). In August 2019, no significant difference was found between the middle and inner communities, while both were significantly different from the entrance (PERMANOVA, Inner-Entrance and Middle- Entrance  $df=1$ ,  $p = 0.013$ ,  $R^2 > 0.49$ ; Inner-Middle  $df=1$ ,  $p = 0.182$ ,  $R^2 = 0.184$ ; Fig. 14, Tab. 5).

The same results were observed when analyzing the native taxa alone, with three distinct and significantly different groups of indigenous communities (Fig. 15) identified in October 2016, August 2017, October 2017, and June 2019 (PERMANOVA, for all comparisons  $df=1$ ,  $p < 0.01$ ,  $R^2 > 0.28$ ; Tab. 5). In August 2019, the inner and middle communities were not significantly different from each other, but they were both distinct from the communities at the entrance (PERMANOVA, Inner-Entrance  $df=1$ ,  $p = 0.02$ ,  $R^2 = 0.456$ ; Middle-Entrance  $df=1$ ,  $p = 0.036$ ,  $R^2 = 0.315$ ; Inner-Middle  $df=1$ ,  $p = 0.096$ ,  $R^2 = 0.257$ ; Fig. 15, Tab. 5).

Finally, three distinct groups of NIS communities (Fig. 16) were identified in August 2017 and June 2019 with significant differences among locations (PERMANOVA, for all comparisons  $df=1$ ,  $p < 0.006$ ,  $R^2 > 0.27$ ; Tab. 5). In October 2016 and 2017, the entrance and middle communities were not significantly different, but each were distinct from the inner community of the marina (PERMANOVA, for the two periods, Inner-Middle and Inner-Entrance  $df=1$ ,  $p < 0.035$ ,  $R^2 > 0.35$ ; Middle-Entrance  $df=1$ ,  $p > 0.05$ ,  $R^2 > 0.05$ ; Fig. 16, Tab. 5). However, in August 2019 no significant difference was found between the middle and inner communities but each of them was significantly different from the entrance (PERMANOVA, Inner-Entrance and Middle-Entrance  $df=1$ ,  $p = 0.012$ ,  $R^2 > 0.52$ ; Inner-Middle  $df=1$ ,  $p = 0.253$ ,  $R^2 = 0.173$ ; Fig. 16; Tab. 5).

Fig. 14: Non-metric Multidimensional scaling (nMDS) of the community data obtained from three different Château Marina locations in October 2016 (a), August 2017 (b), October 2017 (c), June 2019 (d) and August 2019 (e). Significant differences between the groups (Pairwise PERMANOVA,  $p < 0.05$ ) are represented by the letters A-B-C; details of these results are available in Tab. 5.

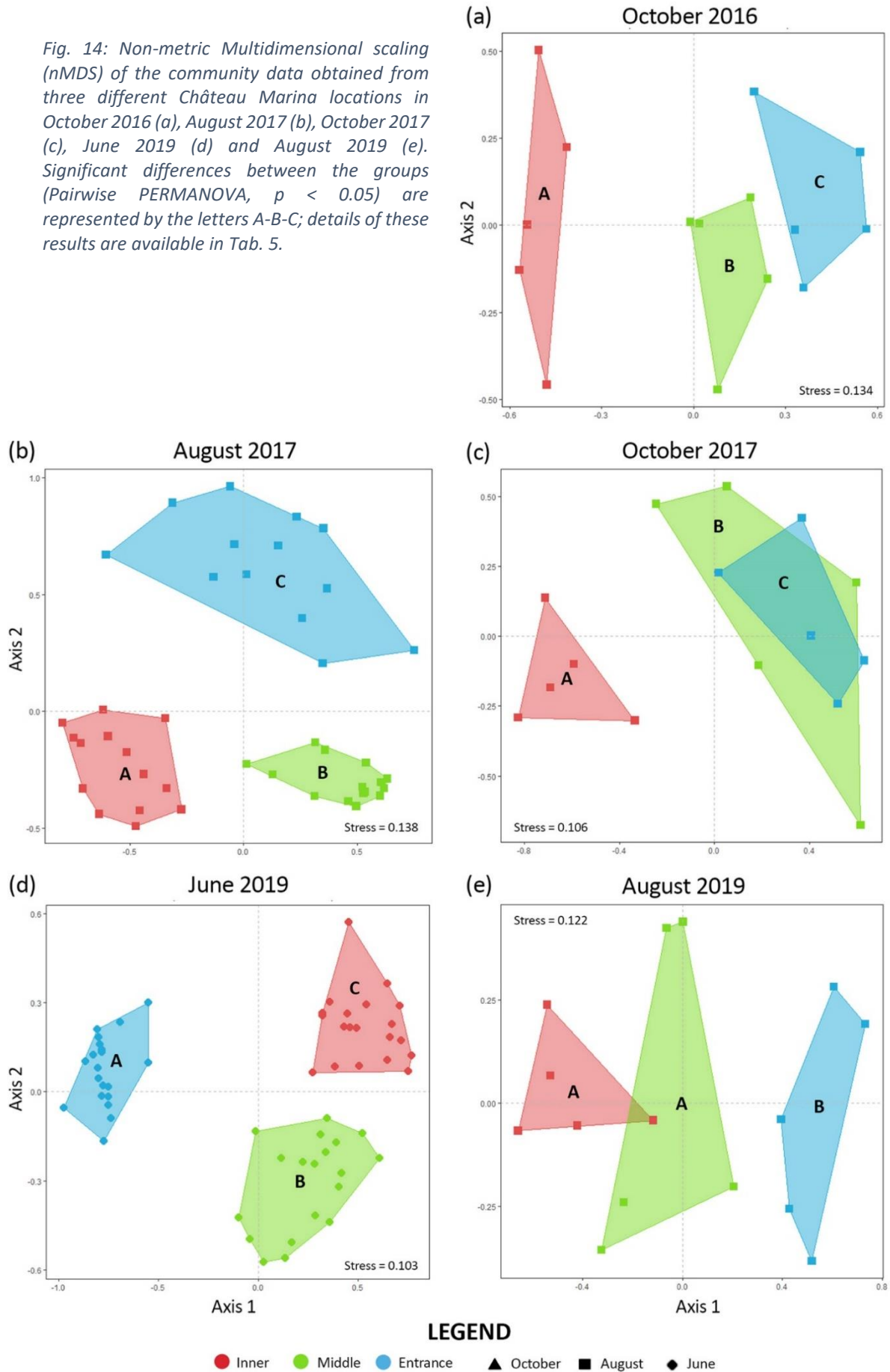


Fig. 15: Non-metric Multidimensional scaling (nMDS) of community data obtained from the indigenous taxa recruited on three different Château Marina locations in October 2016 (a), August 2017 (b), October 2017 (c), June 2019 (d) and August 2019 (e). Significant differences between the groups (Pairwise PERMANOVA,  $p < 0.05$ ) are represented by the letters A-B-C; details of these results are available in Tab. 5.

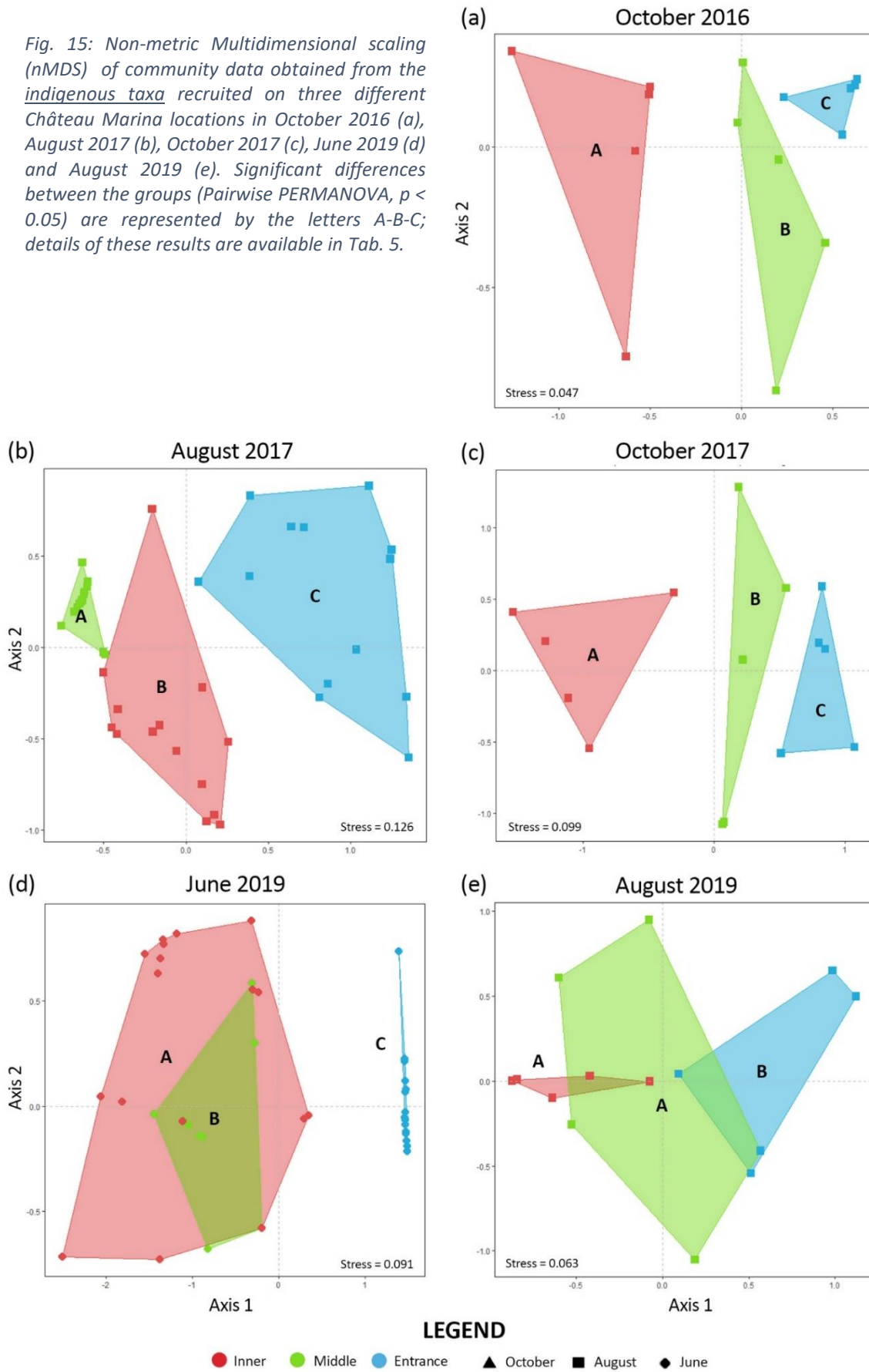
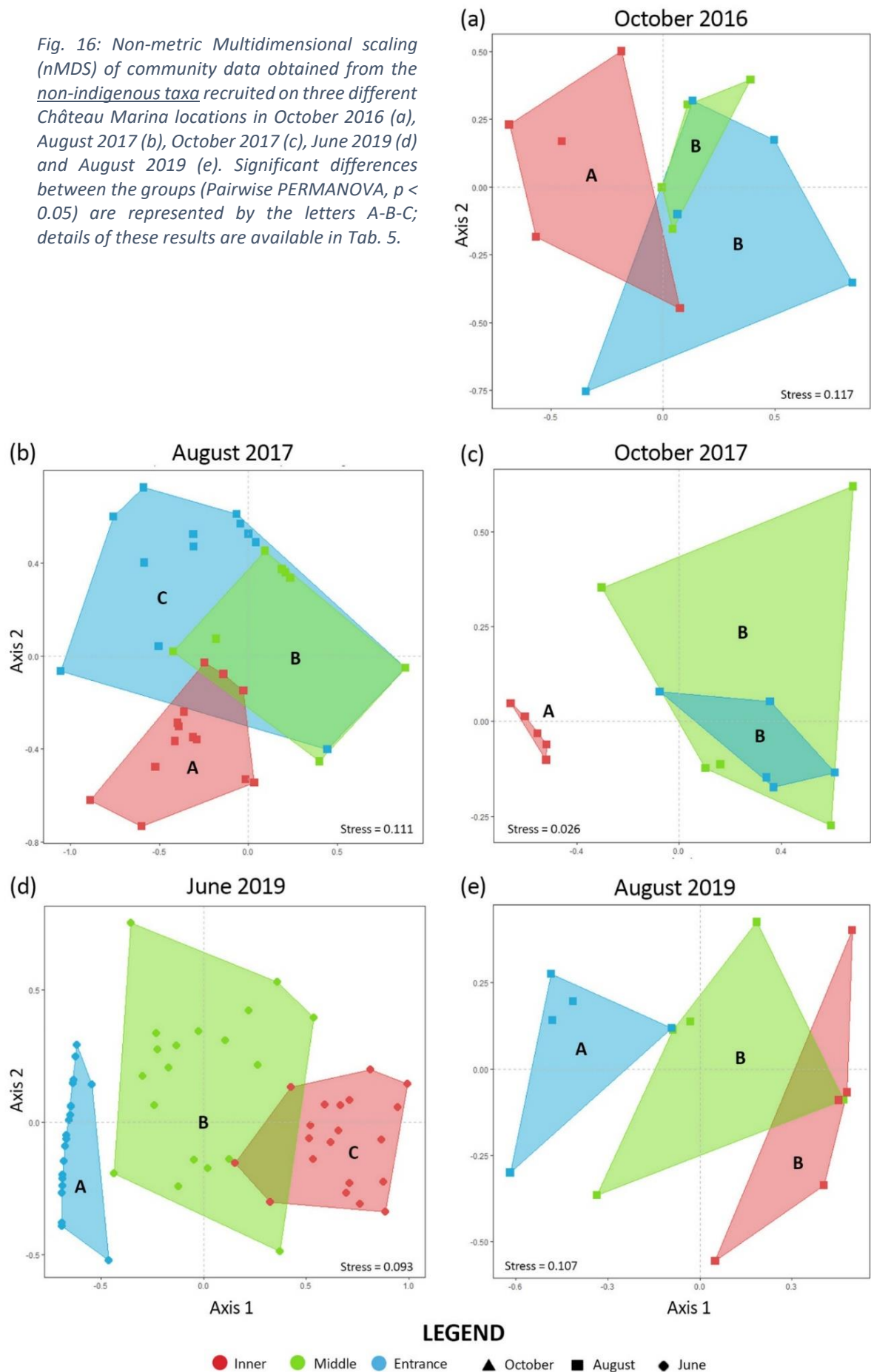


Fig. 16: Non-metric Multidimensional scaling (nMDS) of community data obtained from the non-indigenous taxa recruited on three different Château Marina locations in October 2016 (a), August 2017 (b), October 2017 (c), June 2019 (d) and August 2019 (e). Significant differences between the groups (Pairwise PERMANOVA,  $p < 0.05$ ) are represented by the letters A-B-C; details of these results are available in Tab. 5.





Tab. 5: Results of the different pairwise PERMANOVAs using the Benjamini & Hochberg correction showing significant differences between groups among locations for October 2016, August 2017, October 2017, June 2019 and August 2019.

Period	Community	Comparisons	df	R2	p.value	P.adjusted	
October 2016	Complete	Inner vs Middle	1	0.322	0.008	0.013	.
		Inner vs Entrance	1	0.819	0.006	0.013	.
		Middle vs Entrance	1	0.178	0.015	0.015	.
	Indigenous	Inner vs Middle	1	0.432	0.007	0.008	*
		Inner vs Entrance	1	0.562	0.008	0.008	*
		Middle vs Entrance	1	0.475	0.008	0.008	*
	Non- Indigenous	Inner vs Middle	1	0.352	0.023	0.034	.
		Inner vs Entrance	1	0.59	0.008	0.025	.
		Middle vs Entrance	1	0.291	0.057	0.057	.
August 2017	Complete	Inner vs Middle	1	0.801	6.25e-05	<0.001	***
		Inner vs Entrance	1	0.686	6.25e-05	<0.001	***
		Middle vs Entrance	1	0.653	6.25e-05	<0.001	***
	Indigenous	Inner vs Middle	1	0.613	6.25e-05	<0.001	***
		Inner vs Entrance	1	0.538	6.25e-05	<0.001	***
		Middle vs Entrance	1	0.688	6.25e-05	<0.001	***
	Non- Indigenous	Inner vs Middle	1	0.352	6.25e-05	<0.001	***
		Inner vs Entrance	1	0.59	6.25e-05	<0.001	***
		Middle vs Entrance	1	0.291	5.81e-03	5.81e-03	*
October 2017	Complete	Inner vs Middle	1	0.589	0.007	8.625e-03	*
		Inner vs Entrance	1	0.823	0.008	8.625e-03	*
		Middle vs Entrance	1	0.446	0.008	8.625e-03	*
	Indigenous	Inner vs Middle	1	0.576	0.008	9.06e-03	*
		Inner vs Entrance	1	0.815	0.009	9.06e-03	*
		Middle vs Entrance	1	0.534	0.008	9.06e-03	*
	Non- Indigenous	Inner vs Middle	1	0.568	0.007	0.011	.
		Inner vs Entrance	1	0.726	0.007	0.011	.
		Middle vs Entrance	1	0.057	0.749	0.749	.
June 2019	Complete	Inner vs Middle	1	0.539	1e-04	<0.001	***
		Inner vs Entrance	1	0.858	1e-04	<0.001	***
		Middle vs Entrance	1	0.778	1e-04	<0.001	***
	Indigenous	Inner vs Middle	1	0.281	1e-04	<0.001	***
		Inner vs Entrance	1	0.646	1e-04	<0.001	***
		Middle vs Entrance	1	0.862	1e-04	<0.001	***
	Non- Indigenous	Inner vs Middle	1	0.477	1e-04	<0.001	***
		Inner vs Entrance	1	0.730	1e-04	<0.001	***
		Middle vs Entrance	1	0.272	1e-04	<0.001	***
August 2019	Complete	Inner vs Middle	1	0.184	0.186	0.182	.
		Inner vs Entrance	1	0.612	0.008	0.013	.
		Middle vs Entrance	1	0.496	0.007	0.013	.
	Indigenous	Inner vs Middle	1	0.257	0.096	0.096	.
		Inner vs Entrance	1	0.456	0.006	0.020	.
		Middle vs Entrance	1	0.315	0.024	0.036	.
	Non- Indigenous	Inner vs Middle	1	0.173	0.253	0.253	.
		Inner vs Entrance	1	0.598	0.008	0.012	.
		Middle vs Entrance	1	0.521	0.008	0.012	.

### Species cover

In October 2016, cover of native species was significantly lower in the inner location compared with the two other locations, which did not differ significantly (Wilcoxon's test, Inner-Entrance and Inner-Middle  $p = 0.019$ ; Entrance-Middle:  $p = 0.072$ ; Fig. 17). Conversely, NIS cover was significantly lower in the entrance and the two other locations (Wilcoxon's test, Inner-Entrance  $p = 0.019$ ; Inner-Middle  $p = 0.061$ ; Entrance-Middle  $p = 0.04$ ). The surfaces occupied by cryptogenic organisms and by unoccupied space were not significantly different between locations. The same proportion of native and NIS species was observed in the middle of the marina (Wilcoxon's test,  $p = 0.57$ ).

Cover of native species appeared to be significantly different among all locations in August 2017, with a higher cover in the middle of the marina (Wilcoxon test, for all comparisons  $p < 0.001$ ; Fig. 17). For NIS species, cover between locations did differ significantly with highest cover found at the inner location (Wilcoxon test, Inner-Entrance and Inner-Middle  $p < 0.001$ , Middle-Entrance  $p = 0.049$ ). The average proportion of unoccupied space was significantly different between locations with a higher presence at the entrance (Wilcoxon test, for all comparisons  $p < 0.001$ ). No significant difference was observed in the middle of the marina between the unoccupied space and NIS organisms on the one hand, and between cryptogenic species and the unoccupied space on the other hand.

Regarding October 2017, the native species cover appeared to be higher at the entrance, with significant differences compared to the middle and the inner location of marina, which did not differ significantly from each other (Wilcoxon test, Entrance-Inner  $p = 0.027$ ; Entrance-Middle  $p = 0.016$ , Middle-Inner  $p = 0.415$ ; Fig. 17). Conversely, NIS cover was greatest at the inner location, with significant differences with the entrance but not the middle (Wilcoxon test, Inner-Middle  $p = 0.068$ ; Entrance-Middle  $p = 0.556$ , Entrance-Inner  $p = 0.042$ ). The proportion of unoccupied space did differ significantly from entrance and inner to the middle, where its proportion was higher (Wilcoxon test, Inner-Entrance  $p = 0.916$ ; Middle-Entrance  $p = 0.016$ ; Inner-Middle  $p = 0.021$ ) and was not significantly different from the proportion of native species (Wilcoxon test, Native-Middle uncolonized space  $p = 0.161$ ).

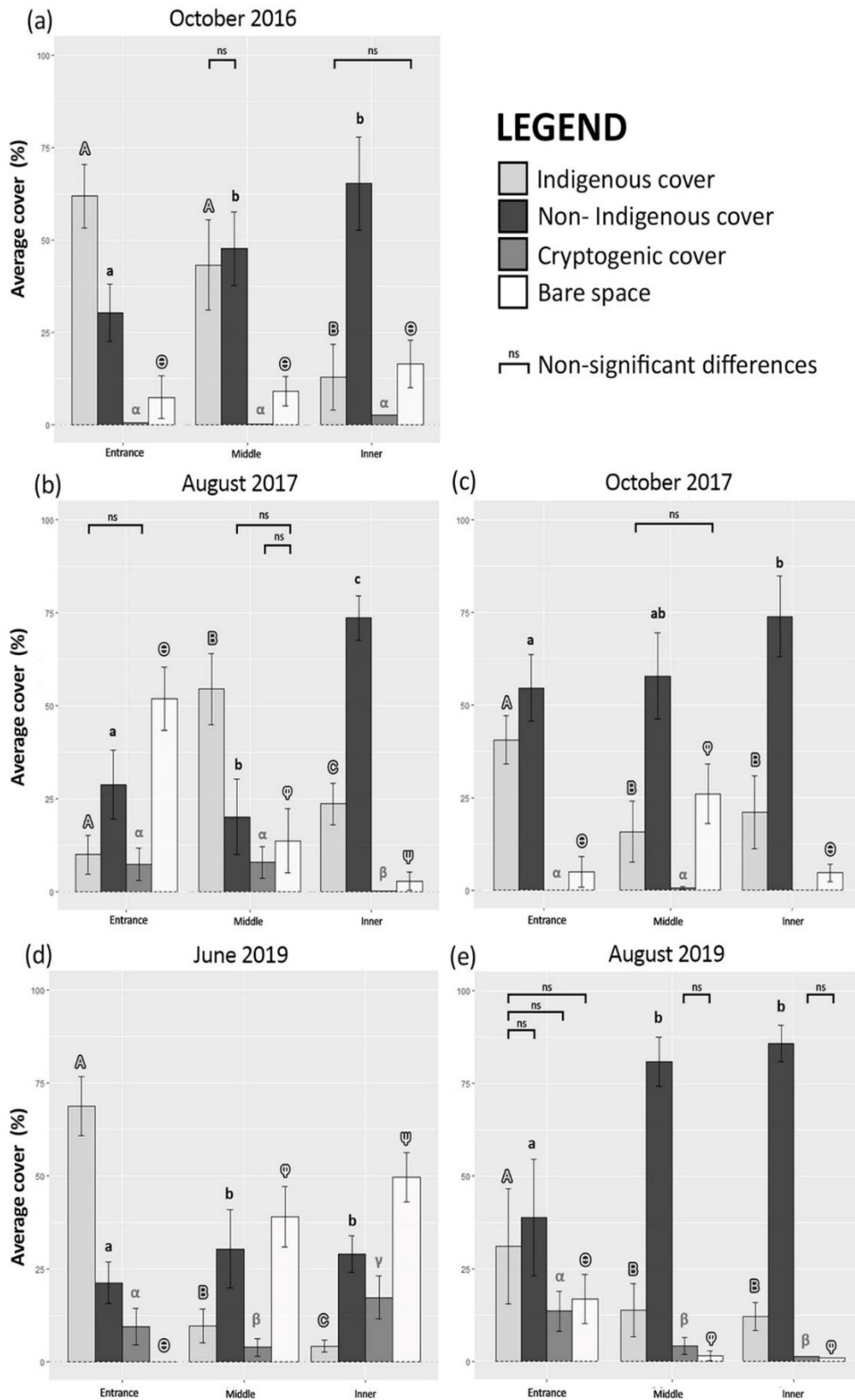


Fig. 17: Comparisons of average cover between different species categories at 3 locations within the marina du Château (Brest) in (a) October 2016, (b) August 2017, (c) October 2017, (d) June 2019 and (e) August 2019. The significance of the results within each category is indicated using symbols, and the significance of the results between categories within the same location is indicated using brackets for non-significant groups. An absence of a bracket indicates significant differences between intra-location groups.

In June 2019, cover of native species was significantly different among all locations, with a higher cover at the entrance (Wilcoxon test, Entrance-Inner and Entrance-Middle  $p < 0.0001$ ; Inner-Middle  $p = 0.0007$ ; Fig. 17), while NIS species cover was significantly lower at the entrance compared with inner or middle locations (Wilcoxon test, Entrance-Inner  $p = 0.011$ ; Entrance-Middle  $p = 0.0024$ ; Inner-Middle  $p = 0.704$ ). The cover of cryptogenic organisms showed significant differences between all location with a higher presence in the inner location of the marina (Wilcoxon test, Entrance-Inner  $p < 0.001$ ; Entrance-Middle and Middle-Inner  $p < 0.001$ ). The average proportion of unoccupied space was significantly different among all locations (Wilcoxon test,  $p < 0.001$ ).

Finally, cover of indigenous species in August 2019, was significantly lower at the entrance compared with the two other locations, which did not differ significantly from each other (Wilcoxon test, Entrance-Inner  $p = 0.017$ ; Entrance-Middle  $p = 0.114$ ; Middle-Inner  $p = 1.00$ ; Fig. 17). For NIS species, cover was higher in the inner location and middle of the marina and significantly different from the one at the entrance (Wilcoxon test, Entrance-Inner  $p = 0.017$ ; Entrance-Middle  $p = 0.017$ ; Middle-Inner  $p = 0.294$ ). The cover of cryptogenic organisms did differ significantly between inner or middle and the marina's entrance where it seems to be higher (Wilcoxon test, Entrance-Inner and Entrance-Middle  $p = 0.017$ ; Middle-Inner  $p = 0.074$ ). The same observation was present for the average proportion of unoccupied space (Wilcoxon test, Entrance-Inner and Entrance-Middle  $p = 0.017$ ; Middle-Inner  $p = 0.528$ ). Finally, the average proportion of indigenous and NIS species was not significantly different at the entrance of the marina (Wilcoxon test, Indigenous - NIS  $p = 0.463$ ).

### Identification of indicator species

In October 2016, the ascidian *Ciona intestinalis* (IS) (Linnaeus 1767) appeared as the taxon contributing most to differences between communities in each of the inter-location comparisons (39 to 52% of the SIMPER test; Fig. 18). It was also a characteristic species of the marina entrance (Multi Pattern Analysis; assoc. stat = 0.791,  $p < 0.001$ ). The annelid *Spirobranchus triqueter* (IS) (Linnaeus, 1758) and the bryozoans *Cryptosula pallasiana* (NIS) (Moll, 1803) and *Bugula neritina* (NIS) (Linnaeus, 1758) were indicator species of the inner location (Tab. 6). No indicator species were associated with the middle of the marina.

In August 2017, the bryozoan *Bugula neritina* (NIS) appeared as the highest contributive to differences communities in the Inner-Entrance and Inner-Middle comparisons (SIMPER test, 43.3% and 37.3% contribution respectively; Fig. 18). *Ciona intestinalis* (IS) was the second largest contributor (SIMPER test, Entrance-Inner 31.5% contribution, Middle-Inner 9.5% contribution) but was however the largest contributor for the Entrance-Middle comparison (SIMPER test, 53% contribution). *C. intestinalis* also appeared as a species characteristic of the marina entrance (Multi Pattern Analysis; assoc. stat = 0.888,

$p < 0.001$ ) while *B. neritina* appeared as an inner indicator (Multi Pattern Analysis; assoc. stat = 0.772,  $p < 0.001$ ). A set of ten other taxa was indicative of all three study locations (Tab. 6).

In October 2017, *Ciona intestinalis* (IS) appeared to be the taxon contributing the most to differences between communities for the Inner-Entrance and Middle-Entrance comparisons (SIMPER test, 36.5% and 51.7% contribution respectively). *Bugula neritina* (NIS) was the second largest contributor (SIMPER test, Inner-Entrance 20.4% contribution, Middle-Entrance 19.5%) but was the largest contributor for the Inner-Middle comparison (SIMPER test, 26.8% contribution). *C. intestinalis* also appeared as the only species characteristic of the marina entrance (Multi Pattern Analysis; assoc. stat = 0.89,  $p = 0.002$ ) while *B. neritina* was indicative of the inner (Multi Pattern Analysis; assoc. Stat = 0.645,  $p = 0.014$ ) as well as five other taxa (Tab. 6). However, no characteristic species was observed in the middle of the marina.

In June 2019, *Ciona intestinalis* (IS) appeared as the most significant contributor to differences between communities for the Inner-Entrance and Middle-Entrance comparisons (SIMPER test, 58% and 62.1% contribution respectively; Fig. 18). The bryozoan *Tricellaria inopinata* (NIS) d'Hondt & Occhipinti Ambrogi, 1985; accounted for most of the contrast in the Middle-Inner comparison (SIMPER test, 23.6% contribution). The results of this comparison also appeared to be more homogeneously distributed than those of the Middle-Entrance and Inner-Entrance comparisons, where *C. intestinalis* strongly dominated (Fig. 18). Moreover, this ascidian appeared again as a characteristic species of the marina entrance (Multi Pattern Analysis; assoc. Stat = 0.998,  $p = 0.001$ ). All the other eleven indicator species from the different locations are summarized in Tab. 6.

Finally, in August 2019, the bryozoan *Bugula neritina* (NIS) appeared as the most important contributor in community differentiation for all comparisons between the different locations (Fig. 18, SIMPER test, 30.2% contribution for the comparison Inner-Middle, 38% for Middle-Entrance and 28% for Inner-Entrance). The bryozoan *Watersipora subatra* (NIS) (Ortmann, 1890) was the second contributor for the Inner-Entrance and Inner-Middle comparisons (SIMPER test, 24.3% and 29.6% contribution respectively; Fig. 18). *Ciona intestinalis* (IS) also contributed to 18.5% of the contrast between the middle and entrance communities (Fig. 18). The bryozoans *Electra pilosa* (IS) (Linnaeus, 1767) and *Tricellaria inopinata* (NIS), as well as the colonial sea squirt *Diplosoma listerianum* (NIS) (Milne Edwards, 1841) appeared as characteristic species of the marina entrance (Tab. 6). The annelid *Spirobranchus triqueter* (IS) and the bryozoans *W. subatra* (NIS) and *C. pallasiana* (NIS) were indicators of the marina Inner. Finally, no indicator species were observed in the middle of the marina (Tab. 6).

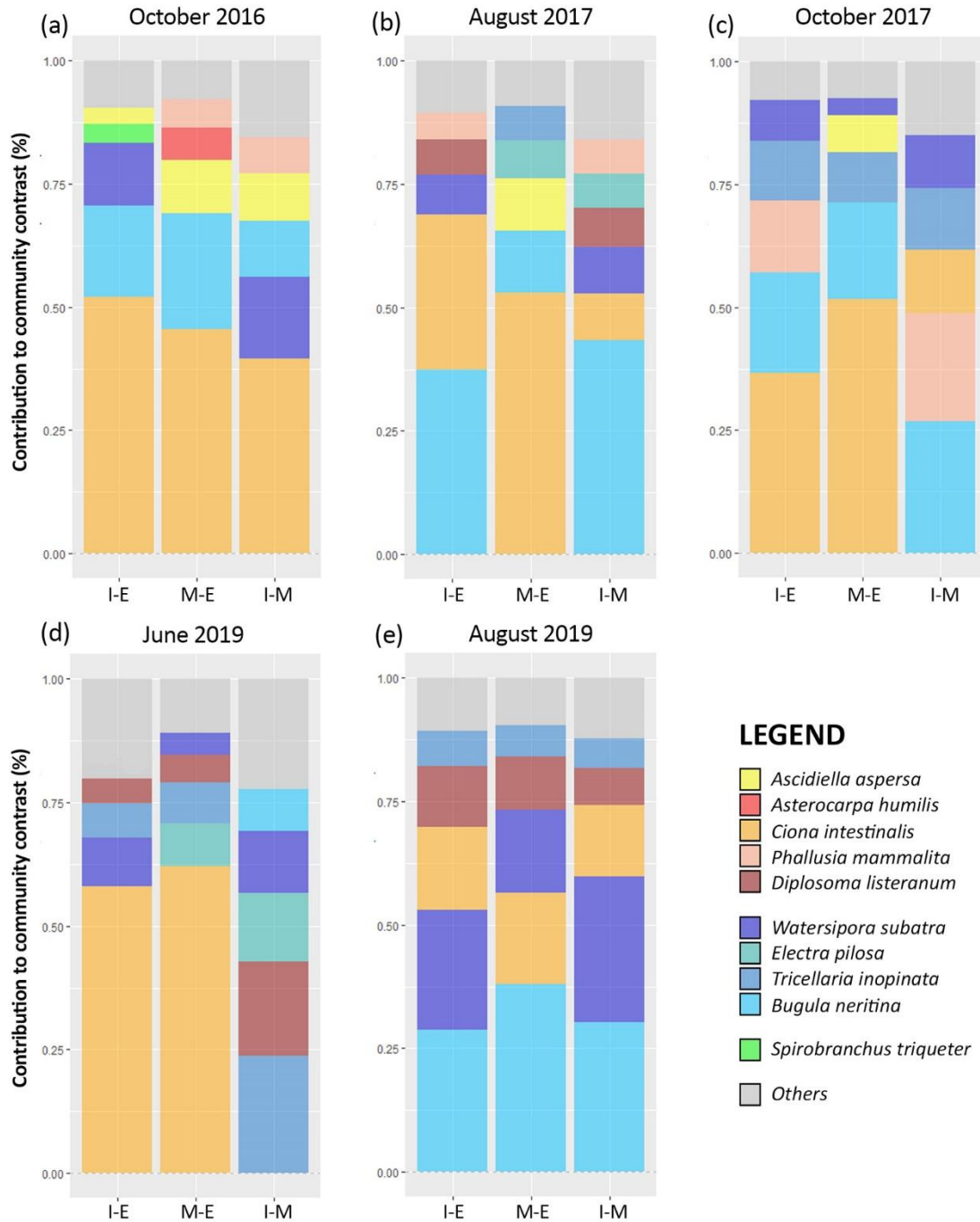


Fig. 18: SIMPER analysis of the contribution of the identified species to the community contrast between the different locations in October 2016 (a), August 2017 (b), October 2017 (c), June 2019 (d) and August 2019 (e). I-E comparison of the Inner-Entrance, M-E comparison of the Middle-Entrance and I-M comparison of the Inner-Middle.

Tab. 6: Indicator species associated to locations within the Chateau marina identified by multipattern analysis for October 2016, August 2017, October 2017, June 2019 and August 2019. Association statistic (stat.) of a species with a group and p value of the multipattern analysis as well as Non-indigenous species (NIS) are indicated.

Period	Locations	Indicator species	NIS	Stat.	p.value
October 2016	Entrance	<i>Ciona intestinalis</i>		0.791	<0.001 ***
	Inner	<i>Spirobranchus triqueter</i>		0.901	0.009 **
		<i>Cryptosula pallasiana</i>	NIS	0.894	0.017 *
		<i>Bugula neritina</i>	NIS	0.639	0.024 *
August 2017	Entrance	<i>Electra pilosa</i>		1.000	<0.001 ***
		<i>Tricellaria inopinata</i>	NIS	0.734	<0.001 ***
		<i>Bugulina flabellata</i>		0.568	0.002 **
		<i>Bortyllus schlosseri</i>		0.480	0.024 *
	Middle	<i>Ciona intestinalis</i>		0.888	<0.001 ***
		<i>Asciidiella aspersa</i>		0.761	0.002 **
		<i>Diplosoma listerantum</i>		0.723	<0.001 ***
	Inner	<i>Watersipora subatra</i>	NIS	0.941	<0.001 ***
		<i>Phallusia mammilata</i>		0.917	<0.001 ***
		<i>Asterocarpa humilis</i>	NIS	0.820	<0.001 ***
<i>Bugula neritina</i>		NIS	0.772	<0.001 ***	
<i>Spirobranchus triqueter</i>			0.628	0.010 **	
October 2017	Entrance	<i>Ciona intestinalis</i>		0.89	0.002 **
	Inner	<i>Phallusia mammilata</i>		0.966	0.002 **
		<i>Watersipora subatra</i>	NIS	0.869	0.002 **
		<i>Asterocarpa humilis</i>	NIS	0.839	0.010 **
		<i>Spirobranchus triqueter</i>		0.833	0.018 *
		<i>Bugula neritina</i>	NIS	0.645	0.014 *
June 2019	Entrance	<i>Ciona intestinalis</i>		0.998	<0.001 ***
		<i>Bugulina flabellata</i>		0.949	<0.001 ***
		<i>Bugulina fulva</i>		0.758	<0.001 ***
	Middle	<i>Electra pilosa</i>		0.901	<0.001 ***
		<i>Tricellaria inopinata</i>	NIS	0.783	<0.001 ***
	Inner	<i>Cryptosula pallasiana</i>	NIS	0.890	<0.001 ***
		<i>Austrominius modestus</i>	NIS	0.869	<0.001 ***
		<i>Watersipora subatra</i>	NIS	0.838	<0.001 ***
		<i>Bortyllus schlosseri</i>		0.837	<0.001 ***
		<i>Diplosoma listerantum</i>		0.735	<0.001 ***
<i>Asciidiella aspersa</i>		0.658	<0.001 ***		
August 2019	Entrance	<i>Electra pilosa</i>		0.926	0.004 **
		<i>Tricellaria inopinata</i>	NIS	0.849	0.006 **
		<i>Diplosoma listerantum</i>		0.848	0.002 **
	Inner	<i>Spirobranchus triqueter</i>		0.895	0.002 **
		<i>Cryptosula pallasiana</i>	NIS	0.817	0.035 *
		<i>Watersipora subatra</i>	NIS	0.702	0.010 **

## Discussion

The work presented in this paper aimed to characterize the macrobenthic communities settled under three pontoons of the Château marina (Brest), respectively at the inner location, middle and entrance of the marina. The main objective was to study over a multi-year period the heterogeneity of communities as well as the distribution of NIS among these locations. As assumed in our hypothesis, the results obtained illustrate a systematic difference between the communities settled at the entrance and at the inner part of the marina for all dates considered over three years. Such a distinction at this small spatial scale (< 100m between locations) is consistent with results previously obtained by Kenworthy et al. (2018b) in the same marina, although the habitats considered were different (pillars as opposed to pontoons). It thus appears that communities remain spatially discriminated over a multi-year scale and that the temporal variations of community structure are less important than spatial variations within the marina. While differences between the entrance and inner communities appear to be conserved, their differences with respect to the community settled in the middle of the marina appear much more variable. A distinction between communities of the three locations has sometimes been observed, but at other periods it has also been observed that no differentiation of the middle community with either the inner or the entrance communities of the marina is present.

Literature on spatial variability of communities within harbor areas shows rather divergent results. In some study areas, spatial variability is observed between communities at the entrance and inner locations of a harbor (Webb & Keough 2000, Ryu et al. 2011, Kenworthy et al. 2018b) but for others they appear relatively homogeneous (Lam & Todd 2013). The authors put forward different hypotheses to explain these results. Some highlight a presence of pollutant like copper or spatiotemporal variability in other abiotic parameters, but often without being able to formally demonstrate a causal link with the variation of community structures. These analyses are in fact complex due to the great diversity of biotic (*e.g.*, competition, predation) and abiotic (*e.g.*, hydrodynamics, pollution) factors that can participate in the structure of these communities (Floerl & Inglis 2003, Blum et al. 2007, Piola & Johnston 2008, Crooks et al. 2011, Kinsella & Crowe 2016). While the work presented here is not able to formally specify the causes of the differences observed at the study site, the details of our results suggest some possible explanations.

Firstly, as assumed, a systematic distinction between the communities at the inner location and at the entrance of the marina is accompanied by a difference in the distribution of indigenous and non-indigenous species. Over all the periods studied, the cover of NIS is always greater towards the inner location of the marina. Conversely, the cover of indigenous species is almost always maximal towards the entrance of the marina. Moreover, the nMDS, made respectively with indigenous and NIS data,



also highlighted distinct communities at the entrance and the inner locations of the marina throughout the studied period (Fig. 15, Fig. 16). These results suggest different environmental filters between the inner location and the entrance of the marina that impact the cover and the composition of each species category. A previous study conducted at the study site has shown the presence of a higher concentration of copper in the sediments in the inner location of the marina (Kenworthy et al. 2018b). It has already been observed that NIS are often more resistant than indigenous taxa to the presence of copper (Piola et al. 2009, Crooks et al. 2011). Therefore, that inverse distribution between indigenous and NIS species in our results could be related to the presence of this copper pollution gradient. In addition, the structure of marinas are known to often facilitate the formation of eddies that can lead to greater water retention in the inner parts of marina (Floerl & Inglis 2003). Consequently, this lower water mixing with the external environment may also result in a greater variability in temperature, pH or turbidity in the most enclosed areas (Rivero et al. 2013, Camp et al. 2017). Overall, this could induce heterogeneous environmental conditions within the marina and may induce the recruitment of distinct communities between the entrance and the inner locations, as observed in other marinas (Rivero et al. 2013).

Secondly, biotic factors may also play a role in the appearance of these differences. All our results highlighted the ascidian *Ciona intestinalis* as the species frequently explaining most of the contrast between communities among locations. Various works have already shown the importance of sea squirts in the structure and composition of fouling communities (Dijkstra et al. 2007, Lindeyer & Gittenberger 2011). In particular, *C. intestinalis* can lead to a decrease in species richness within the communities when present (Dijkstra et al. 2007, Blum et al. 2007). This may be explained by the highly competitive nature of *C. intestinalis* that tends to monopolize free space through rapid development/growth and massive arrival of larvae (Koechlin 1977, Paetzold et al. 2012). Available space appears as a key resource in the establishment of fouling communities (Osman 1977, Sutherland 1981). *C. intestinalis* thus may play an important structuring role in these environments. Consequently, its dominance at the entrance of our marina may explain the observed differences of biodiversity and community structure among locations. This heterogeneous distribution could be caused by various environmental factors such as predation (Schmidt & Warner 1986, Dumont et al. 2011; Chapter III A) and copper pollution which is known to negatively affect reproduction and larval development of *C. intestinalis* (Bellas et al. 2001, 2004) and has been observed at the inner part of the marina (Kenworthy et al. 2018b). The universally observed differences of community structure between the entrance and the inner of the marina could therefore be also explained by these parameters influencing the presence of *C. intestinalis*.

The work presented in this article has highlighted that spatial structure of the communities and some of its characteristics appear temporally preserved over all the studied periods. Fouling communities appear always significantly different between the entrance and the inner location of the marina, NIS cover is constantly higher toward the inner location of the marina and *C. intestinalis* seems to frequently play a major role in the communities' contrasts among locations. However, the temporal scale of our work has also revealed a large variability in our results, particularly about the presence of NIS. While the distribution of these taxa is always more prevalent in the inner of the marina, it is much more contrasted and visible at certain periods than at others. In June 2019, NIS did not exceed an average of 30% cover, but this cover sometimes appears much higher at other periods with more than 75% average cover in August 2019 in the middle and at the inner of the marina. Such results illustrate how important it is to quantify this temporal variability to conclude on the actual presence and distribution of these organisms throughout the year. Currently, most community-based studies conducted in marinas are attempting to increase the spatial scope of their results by integrating more and more different study sites (Webb & Keough 2000, Ashton et al. 2006, Ferrario et al. 2017, Spagnolo et al. 2019). In contrast, the number of studies integrating a temporal dimension is comparatively much smaller (Covazzi Harriague et al. 2007, Canning-Clode et al. 2013). Our results thus illustrate that the development of temporal monitoring of marina communities appears essential today in a context of management and assessment of the NIS populations of these ecosystems. To do so, the photographic analysis method appears as a tool of interest since it requires a lower logistical investment which could facilitate the implementation of future annual and interannual monitoring of several marina communities.

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## **Chapter II    Potential causes of the Spatial Variability of Fouling Communities in our study Marina**

Co-author approved version for submission to Science of the Total Environment (excl. Fig. 19)

# Pollution gradient leads to local adaptation and small-scale spatial variability of communities and functions in an urban marine environment

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**Abstract.** Urbanization of coastal habitats, of which harbors and marinas are the paragon, has led to various ecological paradigms about their functioning. Harbor infrastructures offer new hard substrata that are colonized by a wide variety of organisms (biofouling) including many introduced species. These structures also modify hydrodynamism and contaminant dispersal, leading to strong disturbance gradients within them. Differences in sessile community structure have previously been correlated to these gradients at small spatial scale (< 100 m). Local adaptation might be involved to explain such results, but as correlation is not causation, the present study aims to understand the causal link between the environmental gradients and community structure through a reciprocal transplant experiment among three sites of a marina (inner, middle, entrance). Our results highlighted strong small-scale spatial variations of contaminants (trace metals, PCB, pesticides, and PAH) in sediments and animal samples which have been causally linked to changes in community composition after transplant. But historical contingency and colonization succession also play an important role. Our results provided strong evidence for local adaptation since community structure, respiration, and pollutant uptake in *Bugula neritina*, as well as the metabolomes of *B. neritina* and *Ciona intestinalis* were impacted by the transplant with a disadvantage for individuals transplanted from the entrance to the inner location. The here observed results may thus indicate that the disturbance gradient in marinas might constitute a staple for selecting pollutant-resistant species and populations, causing local adaptation. This highlights the importance of conducting further studies into small scale local adaptation.

**Keywords:** local adaptation, fouling, pollution, metabolomics, respiration, marinas

## Introduction

With increasing anthropic pressure on ecosystems, understanding how communities and species respond and adapt to global change remains a major challenge. In natural habitats, the resilience of communities (*i.e.*, the elasticity of a community and its capacity to return to a stable state after disturbance) has been relatively well studied. However, resistance (*i.e.*, the capacity of communities to resist disturbance-induced changes) remains poorly studied although it is the major driver of community structure in urban habitats where disturbances do rarely relax and tend to even increase in pressure (Nimmo et al. 2015). As adaptation may be a major driver of resistance (Nimmo et al. 2015), understanding how eco-evolutionary feedbacks drive species adaptation in habitats subjected to anthropic disturbance seems crucial (Alberti 2015). Harbors and marinas constitute excellent study models to dive into these paradigms, as they combine multiple facets of anthropogenic disturbances (Bulleri 2006, Bulleri & Chapman 2010). Artificial structures are extremely numerous and diverse in marine urban areas where they constitute a major substratum for benthic communities (Mineur et al. 2012). Their properties are however different from natural substratum, resulting in different communities and diversity (Glasby et al. 2007, Oricchio et al. 2016b, Chan & Briski 2017). In harbors, some artificial structures like breakwaters and jetties are intended to modify/reduce hydrodynamism which may impact connectivity and local biodiversity (Floerl & Inglis 2003, Fauvelot et al. 2009). This reduction of water exchange with the open sea can also lead to an increased risk of eutrophication (Lee & Arega 1999), higher temperature fluctuations (Menniti et al. 2020), and can favor the accumulation of pollutants like Persistent Organic Pollutants (POPs) or Metallic Trace Elements (MTEs) (Owen & Sandhu 2000, Tolun et al. 2001, Schiff et al. 2007, Mohammed et al. 2011, Aly et al. 2013). These disturbances may thus affect the communities in harbors, leading to reduced biodiversity and increasing the presence of opportunistic species (Dijkstra et al. 2007, Saloni & Crowe 2015, Kinsella & Crowe 2016). The innermost parts of a marina may be the most affected by this phenomenon due to even lower water mixing (Schiff et al. 2007). Hence, as these disturbances may increase in intensity and frequency towards the inner parts of marinas, they might result in a disturbance gradient and constitute a selective filter for organisms, with gradient magnitude and orientation potentially modulated by harbor morphology (Floerl & Inglis 2003).

Harbors are a major area of species introduction as they are at the extremities of main introduction vectors through maritime traffic (Mineur et al. 2008, Sylvester et al. 2011, Ferrario et al. 2017). Introduced species, if they become invasive, may completely restructure ecosystems, leading to the loss of biodiversity and ecosystem services (Pejchar & Mooney 2009, Johnston et al. 2015b, Walsh et al. 2016). Together with the cost of mitigating invasive species, this loss of services can cause severe economic impacts, ranging from hundreds of millions to tens of billions dollars, depending on the

country (Lovell et al. 2006, Olson 2006, Jardine & Sanchirico 2018). Introduced species may be favored in disturbance regimes (Hobbs & Huenneke 1992, Altman & Whitlatch 2007, Bulleri et al. 2016) as they are often opportunistic and more resistant to disturbances. They have for instance been shown to be more resistant to extreme temperature events (Zerebecki & Sorte 2011, Kelley 2014, Marie et al. 2017, Kenworthy et al. 2018a) which are more common in harbors due to the reduced water volume and exchange with the open sea (Menniti et al. 2020). Copper, used as active component of many antifouling coatings of ships, demonstrated its detrimental effect on the native communities (Piola & Johnston 2008, Piola et al. 2009, Osborne & Poynton 2019), and with other pollutants has been shown to favor introduced species due to their higher resistance to them (Piola & Johnston 2006a, Dafforn et al. 2008, Osborne & Poynton 2019). The strong selective pressure exerted by copper can even lead to differential resistance among populations of the same introduced species (Piola & Johnston 2006b).

Previous studies have observed local variation of fouling (native and introduced) communities between different parts of harbors/marinas, which has been linked to pollution gradients (Je et al. 2004, Ryu et al. 2011, Kenworthy et al. 2018b). This may indicate that the selective pressure exerted by disturbance in marinas may operate at small spatial scales, potentially causing local adaptation. Such studies remain however putative regarding the causal link between disturbance and community composition as they show correlative results. As correlations between phenotypes and environmental conditions may be caused by phenotypic plasticity, manipulative experiments are required to expose the link between concurrent natural selection and observed variability (Kawecki & Ebert 2004, Thorpe et al. 2005). To expose the causal link between local adaptation and specific environmental factors, reciprocal transplant experiments should be conducted with one species or with entire communities (Kawecki & Ebert 2004, Angert & Schemske 2005, Chang & Marshall 2016, Sork 2018). It is then possible to reveal the presence of local adaptation, if fitness differences exist between 'locals' originating from a studied location and 'foreign' which were transplanted to this location ('locals vs foreign' criterion; Kawecki and Ebert 2004; Martin et al. 2021). Additionally, the 'home vs away' criterion can be satisfied if individuals at 'home' have higher fitness than their transplanted counterparts ('away'). This last criterion is however not a strict requirement to conclude on local adaptation (Kawecki & Ebert 2004). To occur, local adaptation requires a strong selective filter (Kawecki & Ebert 2004), such as anthropogenic disturbances. Using transplant experiments, some studies have been able to show local adaptation of various species, whether marine or terrestrial, to urban habitats, to pollution, and for an introduced species, to environmental conditions in its invaded areas (Roesijadi et al. 1984, Colautti & Lau 2015, Martin et al. 2021).

Kenworthy et al. (2018b) correlated a significant variation in contaminants, such as copper and zinc, and hydrocarbons in marina sediments with heterogeneity in fouling communities from pillars at small

spatial scale (< 100 m) which may indicate local adaptation. Therefore, a reciprocal transplant experiment in the very same marina was conducted to show if measured pollution levels may cause the observed differences in community composition. We chose to combine the classical tools of community analysis with a functional approach (community respiration, pollutant uptake and metabolomics) to investigate the response of the fauna to this environmental variation. There may be indeed a direct interaction between respiration or metabolite variations with the environment (Macel et al. 2010, Jones et al. 2013, Johnston et al. 2015a, Kenworthy et al. 2018a). As metabolomics constitutes a non-targeted analysis of molecules, it can be used to study non-model organisms in great detail (Macel et al. 2010, Jones et al. 2013). It has already been used to attempt to explain the success of certain introduced species in terrestrial and marine habitats (Nylund et al. 2011, Greff et al. 2017, Utermann et al. 2020, Skubel et al. 2020) and to observe how heavy metal pollution may impact different organisms (Booth et al. 2011, Kwon et al. 2012, Ji et al. 2015).

Our aim was to identify an anthropogenic disturbance gradient, illustrated by pollution levels and temperature differences, with higher values at the inner locations of the studied marina. We hypothesized that community structure would correlate with this gradient and that transplanted communities would resemble the communities of the location they were transplanted to, exposing a causal link between environmental conditions and community structure. Furthermore, we expected to verify the 'locals vs foreign' criterion of local adaptation on the respiration and metabolome of the studied models. Understanding if local variations of environmental conditions at small scales could lead to different communities and local adaptation may be crucial for understanding the processes of contemporary evolution of introduced species (Colautti & Lau 2015); and in consequence might be crucial for understanding the process of invasion itself.

## **Materials and methods**

### **Study site**

The Marina du Château (48°22'43.4"N; 4°29'22.1"W) in Brest, France was chosen as study site. This recreational marina, nested within the highly anthropized urban area of Brest, is integrated in a 250 ha harbor including a commercial (> 2.10<sup>6</sup> t.yr<sup>-1</sup> merchants, fishing and petroleum activities) and a military (2<sup>nd</sup> biggest French arsenal) part, which occupies the major part of the city coastline. The marina is characterized by numerous artificial substrata, colonized by fouling communities including several introduced species (Kenworthy et al. 2018b). We focused this study on floating pontoons as they are one of the most frequent substrata in the marina. In accordance with this previous study, the same three locations (inner, middle and entrance; spaced < 100 m) were studied.



### Reciprocal transplant experiment

At each of the 3 locations, 20 black polyethylene panels (Correx, 0.2 m x 0.2 m, 3 mm thick) were deployed in April 2019 for a reciprocal transplant experiment. The panels were sufficiently spaced to be considered as independent samples (Fig. 19). After two months in the field (60 days, June), panels were gathered and photographed. At each location, 5 panels were sampled for immediate species identification (Fig. 19). The remaining panels were randomly assigned to either be transplanted to one of the two other locations (10 panels, 5 per location), or stayed in place as control (5 panels per location; Fig. 19). Consequently, over a total of 15 panels present at each location, 10 were originating from the other two locations after transplant. Each panel could thus be sorted in further analysis according to a treatment depending on its origin (*i.e.*, where recruitment started before transplant) and its destination (*i.e.*, where it was transplanted to). Henceforth, we use the following code for treatment identification: 'I' Inner, 'M' Middle, 'E' Entrance; '>' indicates the direction of transplant (*e.g.*, I>E corresponds to panels from the inner location transplanted to the entrance E); C control (ex. IC is the inner control, *i.e.*, a treatment where panels stayed at the inner location during the whole experiment). After two additional months (70 days, September 2019) the whole set of panels was measured *in situ* for community respiration, then photographed, and fauna samples (individuals of key species) were immediately fixed in dry ice for contaminant and metabolomic assessment. The panels with the community were then transported in sea-water containers and stored in aquaria facilities (max. 1 week) for taxonomic determination on life communities.

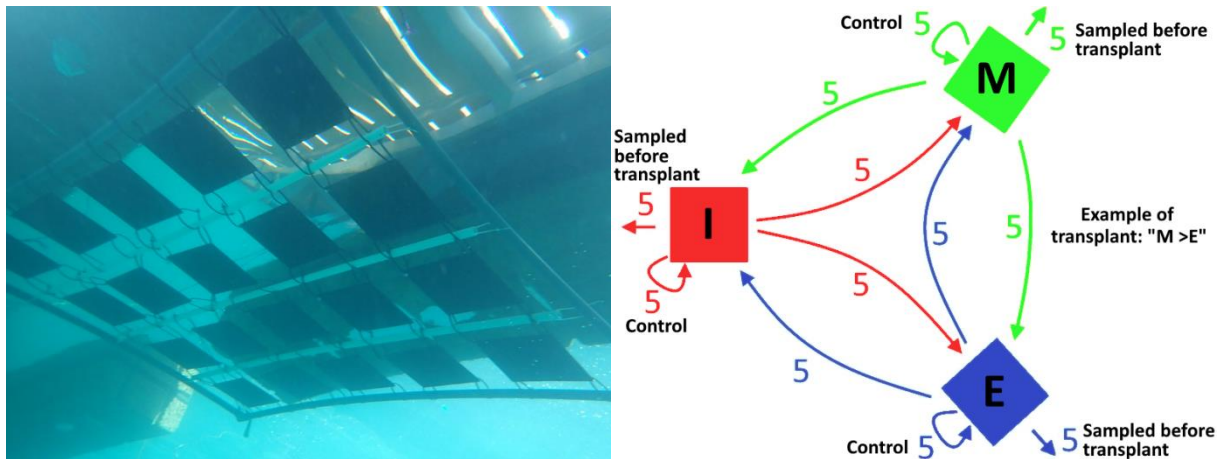


Fig. 19: Photo of the settlement plates *in situ* and design of the transplant experiment between the three locations of the marina. Treatments are labelled according to their origin followed (>) by their destination after transplant (I: inner, M: middle, E: entrance; C: controls *i.e.* identical Origin and Destination). For each treatment 5 panels were sampled for analysis before transplant.

## Environmental assessment

At each location, water temperature and light intensity were recorded with 1-hour interval all over the 4-month long experiment at 1 m depth (panels depth) using a HOBO® (Onset®) TidbiT v2 Water Temperature Data Logger and a HOBO® (Onset®) Pendant Temp/Light, 8K respectively. For temperature and light, the daily average was calculated. Hydrodynamism was measured with 5-min interval using a HOBO® (Onset®) Pendant G Data Logger until the battery went flat (73 days). Hydrodynamism was approximated by the daily total position change which was calculated as

$$\sum_{tn}^{t0} ([\vec{a}_x(t) - \vec{a}_x(t-1)] + [\vec{a}_y(t) - \vec{a}_y(t-1)] + [\vec{a}_z(t) - \vec{a}_z(t-1)])$$

where  $t$  is the measurement at a given time;  $n$  the number of measurements during a day,  $\vec{a}$  the acceleration in  $g$  ( $9.81 \text{ m}\cdot\text{s}^{-2}$ ) in the  $x$ ,  $y$ , or  $z$  axis. Two linear mixed effects (LME) model fit by restricted maximum likelihood (REML) were used (explaining temperature or hydrodynamism by location and using date as random factor) with t-test with Satterthwaite's method 'lme4' and 'lmeTest' package in R (Zeileis & Hothorn 2002, Bates et al. 2015). Light intensity data was not analyzed due to the data quality (See Sup. Fig. 1).

At the end of the experiment, at each location, surface sediments were sampled by divers for quantification of Metallic Trace Elements (MTEs, 5 replicates of 400 g each) and for Persistent Organic Pollutants (POPs, 3 replicates of 400 g each). Several individuals of the bryozoan *Bugula neritina* (Linnaeus, 1758), which was present at all locations all along the experiment, were sampled from each panel (5 replicates per treatment, sometimes pooled individuals) and frozen *in situ* in dry ice before storage in the lab at  $-80^\circ\text{C}$ . Due to the abundance of this species, the sampling of individuals was assumed to not influence the community structure.

MTEs samples were dissolved in a three-acid solution (HCl,  $\text{HNO}_3$ , HF). They were all analyzed using High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS) as in Jacquet et al. (2021). Indium was used as an external calibration standard. Concentrations were measured in sediments and animals (list of MTEs in **Erreur ! Source du renvoi introuvable.**)

The POPs analytical method for Polychlorinated biphenyls (PCBs) and pesticides in sediments has been described in Wafo et al. (2006). The protocol for *B. neritina* samples followed the same procedure. Our analysis focused on 33 individual PCB congeners (see Chapter III A Sup. Tab. 3) which include PCB contamination indicators targeted by the International Council for the Exploration of the Sea (ICES) and congeners with high environmental prevalence (Webster et al. 2013). A total of 17 pesticides including lindane, DDT, aldrin were measured (list in Tab. 8). Polycyclic Aromatic Hydrocarbons (PAH)

determination followed established protocols (Sarrazin et al. 2006; Ratier et al. 2018; Dron et al. 2019) and is also available in detail in the supplementary material of Chapter III A All 16 PAH congeners defined by the US Environmental Protection Agency (USEPA) priority list (US EPA 2014) were targeted (list in Tab. 9) and each was identified taking into account the retention time and the chromatogram mass spectrum in full scan mode.

For all contaminants, differences among locations were assessed using a Kruskal-Wallis test in R (version 4.0.3; R Core Team 2020) followed by a Dunn test. Reference values by the Canadian sediment quality guidelines (CCME 1999), by the US Environmental Protection Agency (US EPA 2005), and by the French national monitoring network of water and sediment qualities in maritime harbors (REPOM 2013) were added. For MTEs, an additional sediment quality category (SQC) by Guerra-García et al. (2021) was reported. We conducted a Principal Component Analysis (PCA; 45 variables) with auto-scaled variables using the R package 'FactoMineR' (version X; Lê et al. 2008) to observe the multivariate distribution of contaminants in *B. neritina* samples. In order to integrate all contaminants into one metrics, normalized contaminant concentrations in *B. neritina* were used:

$$C_{i(norm)} = \frac{(C_i - C_{min})}{(C_{max} - C_{min})}$$

where:  $C_{i(norm)}$  is the  $i^{th}$  normalized value;  $C_i$  is the  $i^{th}$  value;  $C_{min}$  and  $C_{max}$  are the minimum and maximum values of contaminant concentrations. The normalized data was plotted in a boxplot for each transplant and control treatment with 'ggplot2' in R (Wickham 2016). A two-level ANOVA was used to test for the effect of Origin and Destination on the normalized concentration of all contaminants in *B. neritina*, as well as for the effect of their interaction. A Tukey's honest significance test (Tukey HSD; Tukey 1949) was conducted to test for differences of the normalized concentration between treatments.

### **Community taxonomic assessment**

Photography analysis of panels was used to check for community homogeneity within each location before transplant. This allows to exclude experimental artifacts resulting from initial differences between control and transplanted panels as well as panels sampled before transplant. The outermost centimeter of the panels was excluded from our analysis to avoid an edge effect. All photos were analyzed by a single observer using a 144-point stratified random-point overlay. This number of points ( $> 0.4 \text{ points.cm}^{-2}$ ) provides a good tradeoff between efficiency and precision, providing a 0.95 confidence interval for species contributing at least to 5% cover (Taormina et al. 2020). Homogeneity of panels within each location before transplant, was checked using a PERMANOVA with the R package 'vegan' (version 2.4-6; Oksanen et al. 2018). Confirming this homogeneity allowed to entirely focus on

the analysis of panels sampled before transplant (5 per location) and at the end of the experiment (15 per location) for which higher data quality is expected due to higher taxonomic resolution.

At the two studied dates (before transplant and 2 months after transplant), the sampled panels were analyzed in the laboratory for taxonomic identification on living organisms. A 144-point grid was superimposed on the panels (stratified point design) and every individual of each species was counted at each point. This allowed to account for epibionts as well as the different strata of the community and to give more detail than a simple 2-D (photographic) analysis. Species were identified to the lowest possible taxonomic level (Hayward & Ryland 1979, 1985, 1995, 1998, Brunetti & Mastrototaro 2017a). All community data were explored with the R package 'vegan' (version 2.4-6; Oksanen et al. 2018). The multi-strata community matrix was transformed into a Bray-Curtis dissimilarity matrix. First, we analyzed the panels sampled before transplant. Upon confirming homogeneity of group dispersions with the 'betadisper' function of the 'vegan' R package (Oksanen et al. 2018), a PERMANOVA ( $10^4$  permutations) was conducted, followed by a pairwise PERMANOVA from the 'pairwisAodonis' R package (version 0.3; Martinez Arbizu 2019) including a Benjamini-Hochberg correction (Benjamini & Hochberg 1995). For all sampled panels, the mean species richness, Shannon diversity index and Pielou's evenness were calculated and the mean cover of the three most abundant species was assessed for each treatment. A PERMANOVA ( $10^4$  permutations) testing for the effect of origin and destination of panels as well as their interaction was conducted for panels at the end of the experiment. For the factor 'Origin' as well as the interaction of Origin and Destination (*i.e.*, Treatment), homogeneity of group dispersions was compromised. However PERMANOVA shows high robustness in balanced designs such as here (Anderson & Walsh 2013). A pairwise PERMANOVA ( $10^4$  permutations) including a Benjamini-Hochberg correction was conducted to test for differences of communities between treatments. The results were visualized using a Principal Coordinates Analysis (PcoA). The 'envfit' function of 'vegan' was used to overlay vectors, which were significantly correlated to the ordination (with  $p < 0.05$  and  $R^2 > 0.2$ ; Oksanen et al. 2018). To identify the indicator species in our experiment, we used a multipattern analysis from the 'indicspecies' R package (Cáceres et al. 2011). Parameters were set to  $10^4$  permutations while testing for individual treatments, allowing combinations of treatments within the same origin or within the same destination (ex.: origin inner = IC + I>M + I>E).

### **Respiration measures**

Community respiration was measured *in situ* for all panels at the end of the experiment. Panels were detached from the pontoons and individually hooked in 23.3 L transparent, hermetically sealed methacrylate chambers. A magnetic stirrer was used to ensure water circulation within the chamber.

Chambers were placed under the pontoons for similar light conditions as during the experiment. Three empty control chambers (blank) were incubated at each location to control for potential photosynthesis or respiration caused by microbial activity within the water. Dissolved oxygen concentration was measured extracting 200 mL of water from a valve on the chamber using a syringe and with an oxygen probe with temperature sensor (Hach-Lange LDO101). Oxygen concentration was measured right after sealing off the chamber and at the end of incubation (between 14 and 23 min). The measured  $\Delta O_2$  (in  $\text{mg O}_2 \cdot \text{L}^{-1}$ ) was then rated according to the volume of the chamber, the incubation time, and the ash free dry mass (AFDM) to obtain the respiration in  $\text{mg O}_2 \cdot \text{h}^{-1} \cdot \text{g}_{\text{AFDM}}^{-1}$ . The AFDM was determined after the community analyses in the laboratory were finished. The whole community was dried for one week at  $60^\circ\text{C}$  for each panel. After a first mass measure, it was burned at  $520^\circ\text{C}$  for 6 h to allow to calculate the AFDM. For respiration, two extreme values were excluded from analysis. Respiration measurements were compared between treatments *via* an ANOVA followed by a Tuckey HSD test.

### Metabolome assessment

Three species occurring at the three locations in the harbor were sampled for metabolomic analyses: two large colonies of the bryozoan *Bugula neritina* per panel and one individual per panel of the two solitary ascidians *Ciona intestinalis* (Linnaeus, 1767) and *Ascidella aspersa* (Müller, 1776). For *B. neritina* and *C. intestinalis*, only four samples could be constituted for the E>I. Additionally, the IC and M>I treatment are missing entirely for *C. intestinalis* due to the absence of individuals for these conditions. After sampling on the field, samples were immediately frozen in dry ice and then stored at  $-80^\circ\text{C}$ . Samples were analyzed according to different mass spectrometry approaches. The detailed extraction, injection protocols and MS modalities (LCMS+, LCMS+2, LCMS-, LCMS-2; GCMS) can be consulted in the supplementary material. For each species, all features from the LCMS+, the LCMS- as well as the GCMS were combined into one matrix and treated with 'Metaboanalyst' (Xia et al. 2009). The imported matrix (with less than 5000 features) was log transformed and auto scaled. We conducted a Partial Least Squares Discriminant Analysis (PLS-DA) with class order assuming a gradient from the inner to the entrance location. In parallel a PPLS-DA driven permutation Model Validation Analysis (MVA) as well as a pairwise MVA test using the 'RVAideMemoire' package in R (version 0.9-79; Hervé 2021) were performed to test for statistical differences between treatments ( $10^4$  permutations, 6 components). After revealing significant differences between treatments for *B. neritina*, the 100 first Variable Important in Projection (VIP) scores were selected and tentatively annotated with the help of the  $\text{MS}^2$  data. The annotated VIP were gathered in a table including an expression heatmap ranging from the highest to the lowest ion intensity in  $\text{MS}^1$  for each treatment. Mean intensity was used when a feature was detected as a VIP in  $\text{MS}^{1+}$  and  $\text{MS}^{1-}$ . Annotated

metabolites were sorted following their heatmap similarity in the dendrogram, while allowing compromise for keeping chemical families together for clarity. Molecules within a family were sorted by numbers of carbons, oxygens, and unsaturations. For *C. intestinalis* a more streamlined approach was used for simplification as some treatments were missing. Using a molecular network generated on Global Natural Products Social Molecular Networking (GNPS; Wang et al. 2016) and visualized on 'cytoscape' (version 3.7.2), we localized clusters in MS2+ and MS2- corresponding to glycerophospholipids and only highlighted VIP within these clusters.

## Results

### Environmental factor characterization

Significantly higher hydrodynamism indicating higher water exchanges with external mass waters was noted at the entrance location (LME;  $t = 3.61$ ;  $p < 0.001$ ; Sup. Fig. 1 **Erreur ! Source du renvoi introuvable.**). Temperature did also significantly vary between locations (LME;  $t = 5.33$ ;  $p < 0.05$ ). However, these differences ( $< 0.01^{\circ}\text{C}$ ) are well below temperatures that are expected to have an impact on fauna (Brunetti et al. 1980, Hitoshi & Kazutsugu 1984, Qiu & Qian 1998, Sorte et al. 2011, Lord 2017a).

Almost all MTEs were distributed along a decreasing concentration gradient from the inner location to the entrance, although differences were not supported by statistics. Only Cu, Pb and Zn showed significantly different concentrations between locations (Kruskal-Wallis tests;  $\chi^2 > 8.07$ ;  $p < 0.05$ ), with Cu and Zn significantly higher at the inner location (Dunn Test;  $Z > 2.83$ ;  $p.\text{adj} < 0.05$ ). When applicable, most MTE concentrations are barely above the Canadian sediment quality guideline (CCME) and correspond to moderate to good Sediment Quality Categories for Cu and Pb according to Guerra-García et al. (2021; Tab. 7). For Cu this constitutes an increase compared to 2011 and 2012 as concentrations did originally not exceed the REPOM category N1 (REPOM 2013), while they did now.

Seven pesticides, two PAHs, as well as total PCB (tPCB), also showed significant differences in concentration in sediments between locations (Kruskal-Wallis test;  $\chi^2 > 6.47$ ;  $p < 0.05$ ; Tab. 8; Tab. 9). All tested PAHs exceed CCME guidelines and US-EPA concentrations for which 20% of sediments may be toxic to model amphipods (Tab. 9; CCME 1999; US EPA 2005). REPOM classification for PAHs was constant between 2012 and 2019 (REPOM 2013), and a slight improvement of the contamination in Benzo(a)pyrene decreasing below N1 can be noted. The PAHs chrysene, fluoranthene, fluorene and phenanthrene largely exceeded values at which adverse effects on fauna may be encountered (**Erreur ! Source du renvoi introuvable.**; CCME 1999; US EPA 2005). Total PCBs (tPCB) and two pesticides (Lindane and pp'-DDD) were also more concentrated than recommended (Tab. 8; Tab. 9; CCME 1999).

Tab. 7: Mean values (mg.kg<sup>-1</sup> = ppm) with standard deviation of the Metallic Trace Element (MTE) concentrations in the sediments of the three locations (n = 5) sampled at the end of the experiment and in the bryozoan *Bugula neritina* (control individuals; n = 5). Bold font and stars indicate significant differences between locations (Kruskal-Wallis test; \*: p < 0.05; \*\*: p < 0.01). Sediment quality guidelines provided by the Canadian Council of Ministers of the Environment (CCME 1999; ISQG: Interim Sediment Quality Guideline; PEL: Probable Effect Level); the United States Environmental Protection Agency (US EPA 2005, Tab. 11; T20: probability of 20% of toxicity among samples; T50: probability of 50% of toxicity among samples); the Réseau de surveillance des ports maritimes (REPOM 2013, Tab. 4; N1 level of contamination requiring further assessment; N2 level of contamination with probable negative impact); and Sediment Quality category (SQC) by Guerra-García et al. (2021), are indicated. Bold font for reference values: threshold exceeded at least at one location.

Metallic Trace Elements (mg.kg <sup>-1</sup> )		Location (mean ± SD)			K-W test	CCME		US EPA		REPOM		SQC
		Inner	Middle	Entrance		ISQG	PEL	T20	T50	N1	N2	
<sup>27</sup> Al	Sediment	29207 ± 3825	26647 ± 9596	23125 ± 12905	ns							
	<i>B. neritina</i>	416 ± 142	246 ± 85	123 ± 36	**							
<sup>75</sup> As	Sediment	14.4 ± 3.2	11.3 ± 2.9	8.77 ± 5.1	ns	<b>7.24</b>	41.6	<b>7.4</b>	20	25	50	Good
	<i>B. neritina</i>	3.46 ± 0.9	3.51 ± 0.8	2.56 ± 0.1	ns							
<sup>59</sup> Co	Sediment	5.78 ± 0.6	5.5 ± 1.2	4.72 ± 2.7	ns							Good
	<i>B. neritina</i>	0.2 ± 0.1	0.18 ± 0.1	0.07 ± 0.03	*							
<sup>52</sup> Cr	Sediment	57.4 ± 7.3	50.7 ± 11	43.6 ± 25	ns	<b>52.3</b>	160	<b>49</b>	140	90	180	Good
	<i>B. neritina</i>	0.96 ± 0.1	0.64 ± 0.2	0.29 ± 0.1	**							
<sup>63</sup> Cu	Sediment	83.9 ± 14	45 ± 10	32.8 ± 19	**	<b>18.7</b>	108	<b>32</b>	94	<b>45</b>	90	<b>Moderate</b>
	<i>B. neritina</i>	3.08 ± 3.5	1.1 ± 1.2	4.61 ± 5.7	ns							
<sup>56</sup> Fe	Sediment	13569 ± 1427	12639 ± 2980	10643 ± 6037	ns							
	<i>B. neritina</i>	521 ± 105	408 ± 138	172 ± 56	*							
<sup>55</sup> Mn	Sediment	150 ± 22	154 ± 29	134 ± 74	ns							
	<i>B. neritina</i>	22.6 ± 5.1	20.2 ± 10	8.1 ± 3.3	*							
<sup>60</sup> Ni	Sediment	18.1 ± 1.8	16.6 ± 3.8	14.3 ± 8.2	ns			<b>15</b>	47	37	74	Good
	<i>B. neritina</i>	0.72 ± 0.2	0.54 ± 0.2	0.27 ± 0.1	**							
<sup>208</sup> Pb	Sediment	85 ± 12	48.2 ± 14	45.8 ± 25	*	<b>30.2</b>	112	<b>30</b>	94	100	200	<b>Moderate</b>
	<i>B. neritina</i>	5.3 ± 2.3	2.71 ± 0.5	1.56 ± 0.4	**							
<sup>47</sup> Ti	Sediment	2283 ± 291	2285 ± 506	1923 ± 1094	ns							
	<i>B. neritina</i>	6.15 ± 4	3.44 ± 1.5	1.53 ± 0.7	*							
<sup>51</sup> V	Sediment	89.8 ± 11	75.6 ± 19	64.6 ± 37	ns							
	<i>B. neritina</i>	2.84 ± 1.4	2.53 ± 0.7	1.23 ± 0.3	*							
<sup>66</sup> Zn	Sediment	236 ± 89	178 ± 77	111 ± 60	*	<b>124</b>	271	<b>94</b>	240	276	552	Good
	<i>B. neritina</i>	58.5 ± 16	53.5 ± 13	31.9 ± 4.2	*							

Tab. 8: Mean values ( $\mu\text{g.kg}^{-1} = \text{ppb}$ ) with standard deviation of pesticide concentrations in the sediments of the three locations ( $n = 3$ ) and in the bryozoan *Bugula neritina* control individuals ( $n = 5$ ). Stars indicate significant differences between locations (Kruskal-Wallis test; \*:  $p < 0.05$ ). Sediment quality guidelines provided 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, Tab. 11; T20: probability of 20% of toxicity among samples; T50: probability of 50% of toxicity among samples) are indicated. Bold font for reference values: threshold exceeded at least at one location.

Pesticides ( $\mu\text{g.kg}^{-1}$ )		Location mean $\pm$ SD			K-W test	CCME		US EPA	
		Inner	Middle	Entrance		ISQG	PEL	T20	T50
Aldrin	Sediment	0.65 $\pm$ 0.2	0.81 $\pm$ 0.1	0.14 $\pm$ 0.1	ns				
	<i>B. neritina</i>	1.31 $\pm$ 0.1	0.89 $\pm$ 0.1	1.76 $\pm$ 0.3	*				
Cis_chlordane	Sediment	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01	0.01 $\pm$ 0.03	ns				
	<i>B. neritina</i>	1.07 $\pm$ 0.3	2.03 $\pm$ 0.3	1.43 $\pm$ 0.3	ns				
Trans_chlordane	Sediment	1.2 $\pm$ 0.1	0.12 $\pm$ 0.03	0.03 $\pm$ 0.1	ns				
	<i>B. neritina</i>	6.15 $\pm$ 0.8	5.68 $\pm$ 0.7	7.56 $\pm$ 0.9	ns				
tChlordane	Sediment	1.26 $\pm$ 0.1	0.18 $\pm$ 0.04	0.04 $\pm$ 0.1	ns	2.26	4.79		
	<i>B. neritina</i>	7.22 $\pm$ 1.2	7.71 $\pm$ 0.9	8.99 $\pm$ 0.9	ns				
Diazinon	Sediment	0.57 $\pm$ 0.1	0.74 $\pm$ 0.3	0.28 $\pm$ 0.1	*				
	<i>B. neritina</i>	5.05 $\pm$ 0.3	3.76 $\pm$ 0.5	6.16 $\pm$ 0.2	*				
Dieldrin	Sediment	1.11 $\pm$ 0.1	1.25 $\pm$ 0.04	0.04 $\pm$ 0.3	*	<b>0.71</b>	4.3	<b>0.83</b>	2.9
	<i>B. neritina</i>	1.1 $\pm$ 0.2	4.31 $\pm$ 0.2	2.54 $\pm$ 0.7	*				
Endosulfan- 2	Sediment	14.1 $\pm$ 0.8	2.87 $\pm$ 0.5	13.2 $\pm$ 2.1	ns				
	<i>B. neritina</i>	6.43 $\pm$ 0.8	5.53 $\pm$ 0.8	2.02 $\pm$ 0.3	ns				
Endosulfan-1	Sediment	3.09 $\pm$ 0.1	1.22 $\pm$ 0.1	0.07 $\pm$ 0.8	*				
	<i>B. neritina</i>	0.17 $\pm$ 0.04	0.8 $\pm$ 0.6	0.51 $\pm$ 0	ns				
Heptachlor	Sediment	1.24 $\pm$ 0.2	0.74 $\pm$ 0.1	0.14 $\pm$ 0.3	ns	<b>0.6</b>	2.74		
	<i>B. neritina</i>	0.22 $\pm$ 0.04	2.48 $\pm$ 0.3	2.6 $\pm$ 0.3	ns				
Heptachlor_epoxide_a	Sediment	40.2 $\pm$ 4.7	21.4 $\pm$ 5.7	19 $\pm$ 1.7	ns				
	<i>B. neritina</i>	1.54 $\pm$ 0.1	0.31 $\pm$ 0.1	0.57 $\pm$ 0.1	*				
Heptachlor_epoxide_b	Sediment	3.48 $\pm$ 0.3	2.97 $\pm$ 0.3	0.27 $\pm$ 0.05	ns				
	<i>B. neritina</i>	3.28 $\pm$ 0.1	1.49 $\pm$ 0.1	1.22 $\pm$ 0.1	*				
Isodrin	Sediment	0.6 $\pm$ 0.2	0.23 $\pm$ 0.03	0.03 $\pm$ 0.1	ns				
	<i>B. neritina</i>	0.74 $\pm$ 0.1	1.59 $\pm$ 0.5	0.69 $\pm$ 0.1	ns				
Lindane	Sediment	2.03 $\pm$ 0.2	1.97 $\pm$ 0.3	0.32 $\pm$ 0.2	ns	<b>0.32</b>	<b>0.99</b>		
	<i>B. neritina</i>	1.86 $\pm$ 0.4	2.08 $\pm$ 0.2	1.52 $\pm$ 0.4	ns				
Methoxychlor	Sediment	7.43 $\pm$ 1.3	5.16 $\pm$ 0.2	0.21 $\pm$ 0.2	*				
	<i>B. neritina</i>	2.97 $\pm$ 0.3	1.04 $\pm$ 0.2	4.09 $\pm$ 0.2	*				
pp'-DDD	Sediment	10.7 $\pm$ 0.7	9.58 $\pm$ 1.1	1.09 $\pm$ 0.3	*	<b>1.22</b>	<b>7.81</b>	<b>2.2</b>	19
	<i>B. neritina</i>	2.47 $\pm$ 0.1	3.09 $\pm$ 0.7	4.68 $\pm$ 0.5	*				
pp'-DDE	Sediment	17.6 $\pm$ 0.5	22.1 $\pm$ 0.3	15.5 $\pm$ 1.7	*	<b>2.07</b>	374	<b>3.1</b>	100
	<i>B. neritina</i>	8.88 $\pm$ 2.2	2.25 $\pm$ 0.6	4.39 $\pm$ 0.8	*				
pp'-DDT	Sediment	0.62 $\pm$ 0.1	0.1 $\pm$ 0.03	0.03 $\pm$ 0.1	*	1.19	4.77	1.7	11
	<i>B. neritina</i>	0.29 $\pm$ 0.04	0.23 $\pm$ 0.01	0.18 $\pm$ 0.05	ns				



Tab. 9: Mean values ( $\mu\text{g.kg}^{-1} = \text{ppb}$ ) with standard deviation of Polycyclic Aromatic Hydrocarbons (PAH) and Total Polychlorinated Biphenyl (tPCB) concentrations in the sediments ( $n = 3$ ) and in the bryozoan *Bugula neritina* control individuals ( $n = 5$ ) of the three locations. Stars indicate significant differences between locations (Kruskal-Wallis test; \*:  $p < 0.05$ ). Sediment quality guidelines provided by the Canadian Council of Ministers of the Environment (CCME 1999; ISQG: Interim Sediment Quality Guideline; PEL: Probable Effect Level); the United States Environmental Protection Agency (US EPA 2005, Tab. 11; T20: probability of 20% of toxicity among samples; T50: probability of 50% of toxicity among samples); and the Réseau de surveillance des ports maritimes (REPOM 2013, Tab. 5 and 6; N1 level of contamination requiring further assessment; N2 level of contamination with probable negative impact) are indicated. Bold font for reference values: threshold exceeded at least at one location.

PAH ( $\mu\text{g.kg}^{-1}$ )		Location mean $\pm$ SD			K-W test	CCME		US EPA		REPOM	
		Inner	Middle	Entrance		ISQG	PEL	T20	T50	N1	N2
Acenaphthene	Sediment	39 $\pm$ 8.5	34.1 $\pm$ 2	33.6 $\pm$ 1	ns	<b>6.71</b>	88.9	<b>19</b>	120	<b>15</b>	260
	<i>B. neritina</i>	1.97 $\pm$ 1.2	2.09 $\pm$ 0.1	0.88 $\pm$ 0.1	ns						
Acenaphthylene	Sediment	50.6 $\pm$ 17	65.7 $\pm$ 8.3	60.6 $\pm$ 22	ns	<b>5.87</b>	128	<b>14</b>	140	<b>40</b>	340
	<i>B. neritina</i>	2.81 $\pm$ 0.7	2.26 $\pm$ 0.1	1.21 $\pm$ 0.2	ns						
Anthracene	Sediment	58.6 $\pm$ 7.9	52.1 $\pm$ 12	44.6 $\pm$ 14	ns	<b>46.9</b>	245	<b>34</b>	290	85	590
	<i>B. neritina</i>	5.3 $\pm$ 0.2	5 $\pm$ 0.1	1.87 $\pm$ 0.1	ns						
Benzo[a]anthracene	Sediment	379 $\pm$ 23	343 $\pm$ 15	354 $\pm$ 1.7	ns	<b>74.8</b>	693	<b>61</b>	470	<b>260</b>	930
	<i>B. neritina</i>	8.43 $\pm$ 0.5	2.46 $\pm$ 0.1	6.1 $\pm$ 0.8	ns						
Benzo[a]pyrene	Sediment	324 $\pm$ 69	425 $\pm$ 109	407 $\pm$ 43	ns	<b>88.8</b>	763	<b>69</b>	520	430	1015
	<i>B. neritina</i>	9.93 $\pm$ 0.7	8.9 $\pm$ 0.3	7.4 $\pm$ 0.8	ns						
Benzo[b]fluoranthene	Sediment	700 $\pm$ 67	651 $\pm$ 141	880 $\pm$ 232	ns			<b>130</b>	1110	<b>400</b>	900
	<i>B. neritina</i>	23.5 $\pm$ 2.3	26.8 $\pm$ 3.8	21.4 $\pm$ 1.2	ns						
Benzo[g,h,i]perylene	Sediment	407 $\pm$ 79	212 $\pm$ 51	283 $\pm$ 7.9	*			<b>67</b>	500	1700	5650
	<i>B. neritina</i>	10 $\pm$ 0.3	7.95 $\pm$ 0.3	10 $\pm$ 0.1	ns						
Benzo[k]fluoranthene	Sediment	328 $\pm$ 72	413 $\pm$ 48	537 $\pm$ 78	ns			<b>70</b>	540	<b>200</b>	<b>400</b>
	<i>B. neritina</i>	26.5 $\pm$ 4.1	13.4 $\pm$ 0.2	13.1 $\pm$ 0.2	ns						
Chrysene	Sediment	943 $\pm$ 185	1051 $\pm$ 61	1192 $\pm$ 173	ns	<b>108</b>	<b>846</b>	<b>82</b>	<b>650</b>	<b>380</b>	1590
	<i>B. neritina</i>	21.5 $\pm$ 1.3	24.8 $\pm$ 2	15.7 $\pm$ 1	ns						
Dibenzo[a,h]anthracene	Sediment	83.6 $\pm$ 19	69.9 $\pm$ 20	76.9 $\pm$ 19	ns	<b>6.22</b>	135	<b>19</b>	110	<b>60</b>	160
	<i>B. neritina</i>	4.73 $\pm$ 0.5	3.94 $\pm$ 0.1	2.85 $\pm$ 0.3	ns						

Table Continued on next page

Tab. 9 continued											
Fluoranthene	Sediment	1532 ± 430	1049 ± 71	1829 ± 268	ns	<b>113</b>	<b>1494</b>	<b>120</b>	<b>1030</b>	<b>600</b>	2850
	<i>B. neritina</i>	43.4 ± 1.6	33.9 ± 2.6	15.5 ± 0.1	ns						
Fluorene	Sediment	725 ± 19	567 ± 43	642 ± 56	*	<b>21.2</b>	<b>144</b>	<b>19</b>	<b>110</b>	<b>20</b>	<b>280</b>
	<i>B. neritina</i>	3.42 ± 0.1	2.58 ± 0.2	1.28 ± 0.1	ns						
Indeno[1,2,3-cd]pyrene	Sediment	701 ± 106	713 ± 302	843 ± 313	ns			<b>68</b>	<b>490</b>	1700	5650
	<i>B. neritina</i>	14 ± 1.4	11.6 ± 0.4	12.5 ± 0.9	ns						
Naphthalene	Sediment	232 ± 32	239 ± 37	344 ± 103	ns	<b>34.6</b>	391	<b>30</b>	<b>220</b>	<b>160</b>	1130
	<i>B. neritina</i>	17 ± 2.7	12.5 ± 0.3	7.23 ± 0.3	ns						
Phenanthrene	Sediment	1629 ± 363	1154 ± 66	1996 ± 239	ns	<b>86.7</b>	<b>544</b>	<b>68</b>	<b>460</b>	<b>240</b>	<b>870</b>
	<i>B. neritina</i>	12.6 ± 0.5	44.9 ± 3.9	26.3 ± 3	ns						
Pyrene	Sediment	545 ± 7.8	510 ± 23	556 ± 17	ns	<b>153</b>	1398	<b>120</b>	930	<b>500</b>	1500
	<i>B. neritina</i>	10.8 ± 0.7	9.58 ± 0.6	5.66 ± 0.6	ns						

PCB ( $\mu\text{g}\cdot\text{kg}^{-1}$ )		Location mean ± SD			K-W test	CCME		US EPA		REPOM	
		Inner	Middle	Entrance		ISQG	PEL	T20	T50	N1	N2
tPCB	Sediment	603 ± 5,9	506 ± 12	544 ± 2,5	*	<b>21.5</b>	<b>189</b>	<b>35</b>	<b>370</b>	<b>500</b>	1000
	<i>B. neritina</i>	360 ± 13	216 ± 10	155 ± 7.1	*						

For control individuals of *B. neritina* sampled at the end of the experiment, all MTEs excepted Cu and As showed significant differences between the three locations, with systematically higher concentrations from individuals sampled at the inner location (Kruskal-Wallis test;  $\chi^2 > 6.76$ ;  $p < 0.05$ ; **Erreur ! Source du renvoi introuvable.**). tPCB and eight pesticides did vary with distinct distribution profiles (Kruskal-Wallis test;  $\chi^2 > 7.2$ ;  $p < 0.05$ ; **Erreur ! Source du renvoi introuvable.**; Tab. 9). None of the PAHs significantly varied in *B. neritina* (Tab. 9).

The PCA biplot revealed differences of contaminant compositions of *B. neritina* between the treatments (Fig. 20). The PC1 axis (30.2%) represents general pollution levels with MTE, PCBs, Pesticides (group 2, see Fig. 20 legend) and HAP being correlated to the axis, and Phenantrene and pesticides (group 1, see Fig. 20 legend) being anticorrelated. Individuals transplanted from other locations to the inner, most notably E>I, were correlated to PC1 indicating high levels of contaminants in their tissues. Individuals which had the entrance as their destination were anticorrelated with MTE, PCBs, pesticides (group 2) and HAP and correlated with pesticides (group 1) and Phenantrene. On the PC2 (21%) MTE, PCBs and Pesticides distinguish from HAP pollutants forming two large vector groups. Most individuals were however in the center of both PC, indicating intermediate levels for all contaminant groups.

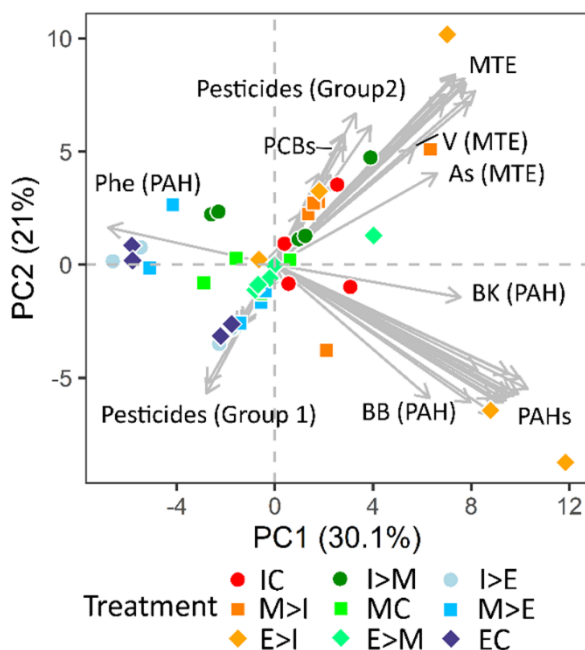


Fig. 20: Principal Component Analysis (PCA) biplot of contaminant concentrations in the bryozoan *Bugula neritina* according to their treatment. Treatments are labelled according to their origin followed (>) by their destination after transplant (I: inner, M: middle, E: entrance; C: controls i.e. identical Origin and Destination) and indicated in the biplot according to their destination (red = inner; green = middle; blue = entrance) and origin (circle = inner, square = middle, diamond = entrance). MTE: all MTEs except As and V; PAH: all PAHs except Phe (Phenanthrene), BK (Benzo[k]fluoranthene) and BB (Benzo[b]fluoranthene); Pesticides (Group 1): heptachlor, methoxychlor, pp.DDT, Endosulfan 1, Cis\_chlordan, Trans\_Chlordan; Pesticides (Group 2): heptachlor\_epoxide\_b, pp.DDE, Diazinon, heptachlor\_epoxide\_a, lindane, dieldrin.

The normalized concentrations of all combined contaminants varied significantly between treatments for *B. neritina* with a significant effect of 'origin' (two-way ANOVA;  $p < 0.001$ ;  $F = 7.23$ ), 'destination' (two-way ANOVA;  $p < 0.001$ ;  $F = 77.86$ ) and their interaction (i.e. treatment; two-way ANOVA;  $p < 0.001$ ;  $F = 9.70$ ). The Tuckey HSD test revealed that contaminant concentrations for the inner control IC had significantly higher values than the two other controls ( $p < 0.01$ ; Fig. 21). Higher contaminant

concentrations in *B. neritina* samples transplanted to the inner location were systematically observed, compared to samples present at the entrance location at the end of the experiment, regardless of their origin ( $p < 0.001$ ; Fig. 21). It was only possible to observe an effect of origin for samples transplanted to the inner location: E>I individuals had significantly higher normalized contaminant concentrations than M>I and IC ( $p < 0.01$ ; Fig. 21).

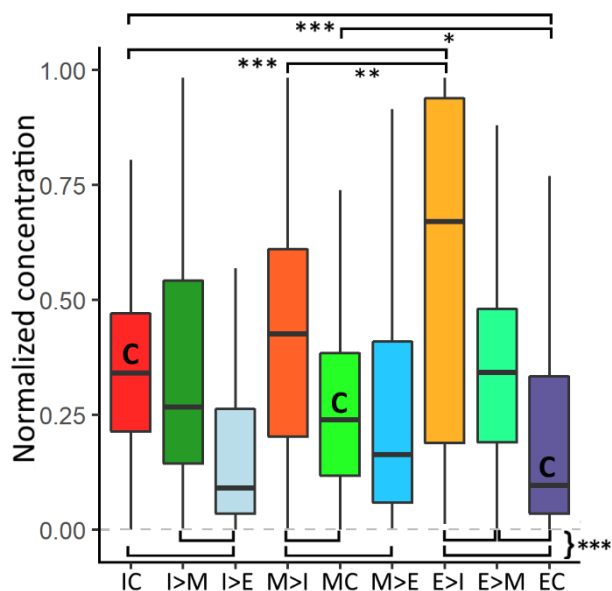


Fig. 21: Boxplot of normalized concentrations of all contaminants in the bryozoan *Bugula neritina* according to their treatment. Treatments are labelled according to their origin followed (>) by their destination 2 months after transplant (I: inner, M: middle, E: entrance; C: controls i.e. identical Origin and Destination) and indicated according to their destination (red = inner; green = middle; blue = entrance). Significant pairwise differences (Tukey's honest significance test of a 2-way Anova) are indicated by black brackets. \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ ; no bracket: non-significant. All bottom brackets are \*\*\*. ( $n = 5$ )

## Community

The photography-based community analysis did not show any difference among panels within each location before transplant (PERMANOVA;  $p > 0.57$ ;  $R^2 = 0.001$ ), thus excluding experimental artifacts due to panel selection. Panels sampled before transplant were significantly different according to their origin: IC vs MC (pairwise PERMANOVA;  $p = 0.009$ ;  $R^2 = 0.55$ ), MC vs EC (pairwise PERMANOVA;  $p = 0.009$ ;  $R^2 = 0.87$ ), IC vs EC (pairwise PERMANOVA;  $p = 0.009$ ;  $R^2 = 0.89$ ). The PCoA (Fig. 22a) clearly discriminated EC from IC and MC along the first PCoA axis (76.6% of variability) while the second PCoA axis discriminated the two latter (13.2% of variability). The cover of several species varied between treatments before transplant. *C. intestinalis*, *A. aspersa* and *B. neritina* had the highest cover at the entrance location, while *W. subatra* had a lower cover at this location and higher at the inner location (Dunn Test;  $p < 0.05$ ; Sup. Fig. 2). Bare space was the highest at the inner location compared to the entrance (Dunn Test;  $p = 0.002$ ). The total cover of introduced species was higher at the entrance location than at the inner location (Dunn Test;  $p = 0.012$ ).

Two months after transplant, the effect of 'origin' and 'destination' of panels as well as their interaction was tested and showed a significant effect of all three (PERMANOVA;  $p < 0.001$ ;  $R^2 > 0.22$ ). The PCoA explained a total of 79.3% of the variability (Fig. 22b), with the first axis (56.6%) associated with the destination effect (inner locations on the right side). Most species correlated positively along this axis

( $p < 0.05$ ;  $R^2 > 0.2$ ) are encrusting species including NIS like *Cryptosulla pallasiana* (Moll, 1803) and *Watersipora subatra*. The second axis (22.7% of variability) reflects an origin effect (all panels originating from the entrance are on the top side). E>M treatment were very distinct from all other treatments in relation with *C. intestinalis* and *Diplosoma listerianum* (Milne Edwards, 1841). Subsequent pairwise PERMANOVA for panels after the 2-months transplant revealed significant differences between almost all treatments ( $p < 0.05$ ;  $R^2 > 0.23$ ). Mean species richness (S), Shannon index (H') and Pielou's evenness (J') varied among sites (Sup. Tab. 1). The most abundant species (cover > 30%) were *Watersipora subatra* (Ortmann, 1890), *B. neritina* and *C. intestinalis*. Their cover was highly variable depending on the treatment (Sup. Tab. 1 and Sup. Fig. 2). Diversity indices for each treatment varied between treatments, ranging from 14.6 to 18.4 for mean species richness, from 2.0 to 2.9 for Shannon index and from 0.52 to 0.70 for Pielou's evenness. The only exception were E>M panels with very low diversity (S = 11; H' = 0.6; J' = 0.19; Sup. Tab. 1). *W. subatra* had a higher cover at the inner location compared to the entrance (Dunn Test;  $p = 0.01$ ). Further details on the cover of each can be seen in Sup. Fig. 2

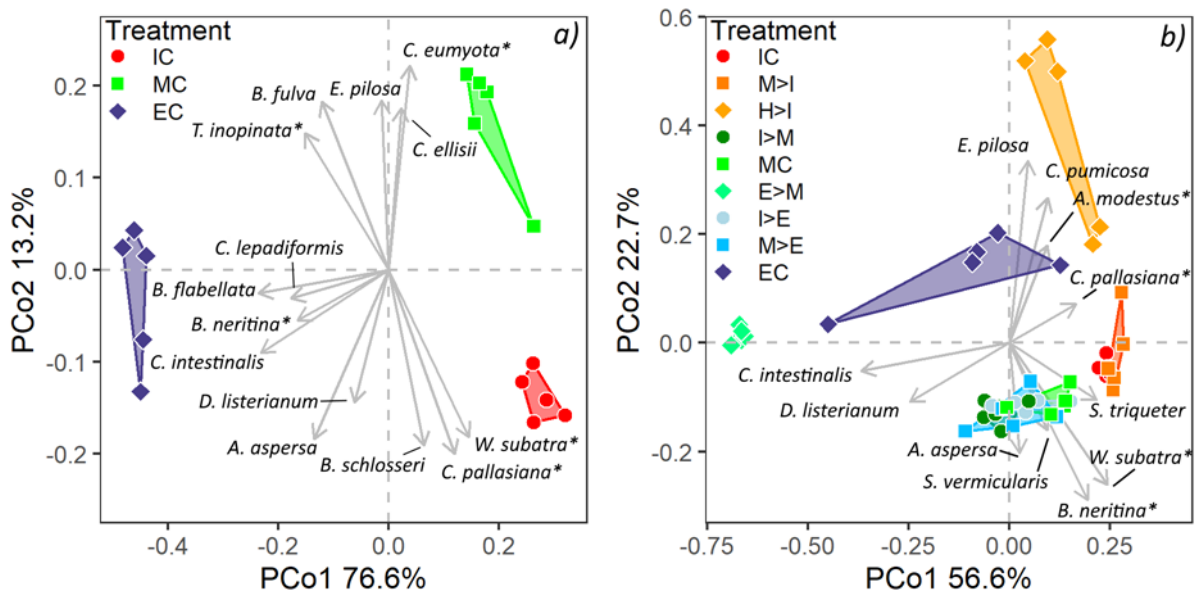


Fig. 22: Principal Coordinate Analysis (PCoA) biplot of the community structure before transplant (a) and 2 months after transplant (b). Treatments are labelled according to their origin followed (>) by their destination after transplant (I: inner, M: middle, E: entrance; C: controls i.e. identical Origin and Destination) and indicated in the biplot according to their destination (red = inner; green = middle; blue = entrance) and origin (circle = inner, square = middle, diamond = entrance). Vector overlay indicates species with a positive correlation ( $p < 0.05$ ;  $R^2 > 0.2$ ) with groups. Non-Indigenous species are indicated by an asterisk. Species list: *Asciidiella aspersa*, *Austrominius modestus\**, *Botryllus schlosseri*, *Bugula neritina\**, *Bugulina flabellata*, *Bugulina fulva*, *Ciona intestinalis*, *Cellepora pumicosa*, *Clavelina lepadiformis*, *Corella eumyota\**, *Cradoscrupocellaria ellisii*, *Cryptosulla pallasiana\**, *Diplosoma listerianum*, *Electra pilosa*, *Tricellaria inopinata\**

The multipattern analysis identified several species associated to the three locations before transplant as well as multiple species according to specific origins (origin effect) or to specific destinations

(destination effect; Tab. 10). Over both periods, between 40% and 45% of these indicator species were NIS. While NIS were associated to every location before transplant, no NIS was associated to panels having the entrance as origin or destination after transplant.

Tab. 10: Indicator species associated to treatments identified by multipattern analysis before and 2-months after transplant. Association statistic (stat.) of a species with a group and p value of the multipattern analysis as well as Non-indigenous species (NIS) are indicated. Groups are labelled according to their origin or destination, composed by the three treatments within the respective origin or destination (ex: Origin inner = Inner Control, Inner>Middle and Inner>Entrance; where > represents the direction of transplant).

Group	Indicator Species	Authority	NIS	Stat.	p value	
<b>Before transplant</b>						
Inner	<i>Cryptosula pallasiana</i>	(Moll, 1803)	NIS	0.87	< 0.001	***
	<i>Botryllus schlosseri</i>	(Pallas, 1766)		0.78	< 0.001	***
	<i>Watersipora subatra</i>	(Ortmann, 1890)	NIS	0.74	< 0.001	***
	<i>Austrominius modestus</i>	(Darwin, 1854)	NIS	0.72	0.041	*
Middle	<i>Corella eumyota</i>	Traustedt, 1882	NIS	1.00	0.001	***
	<i>Electra pilosa</i>	(Linnaeus, 1767)		0.74	0.047	*
Entrance	<i>Ciona intestinalis</i>	(Linnaeus, 1767)		1.00	0.001	**
	<i>Bugulina flabellata</i>	(Thompson in Gray, 1848)		0.98	0.001	**
	<i>Tricellaria inopinata</i>	d'Hondt & Occhipinti Ambrogi, 1985	NIS	0.87	0.01	**
	<i>Clavelina lepadiformis</i>	(Müller, 1776)		0.83	0.03	*
	<i>Asciidiella aspersa</i>	(Müller, 1776)		0.81	0.003	**
	<i>Bugulina fulva</i>	(Ryland, 1960)		0.80	0.014	*
	<i>Bugula neritina</i>	(Linnaeus, 1758)	NIS	0.65	0.005	**
<b>After transplant</b>						
Origin Inner	<i>Asterocarpa humilis</i>	(Heller, 1878)	NIS	0.91	< 0.001	***
	<i>Asciidiella aspersa</i>	(Müller, 1776)		0.79	< 0.001	***
	<i>Serpula vermicularis</i>	Linnaeus, 1767		0.72	0.002	**
	<i>Styela clava</i>	Herdman, 1881	NIS	0.71	0.026	*
	<i>Mytilus edulis</i>	Linnaeus, 1758		0.63	0.023	*
Origin Middle	<i>Watersipora subatra</i>	(Ortmann, 1890)	NIS	0.71	< 0.001	***
	<i>Bugula neritina</i>	(Linnaeus, 1758)	NIS	0.66	0.008	**
Origin Entrance	<i>Electra pilosa</i>	(Linnaeus, 1767)		0.82	< 0.001	***
Destination inner	<i>Spirobranchus triqueter</i>	(Linnaeus, 1758)		0.81	< 0.001	***
	<i>Cryptosula pallasiana</i>	(Moll, 1803)	NIS	0.73	0.005	**
	<i>Cellepora pumicosa</i>	(Pallas, 1766)		0.71	0.009	**
	<i>Austrominius modestus</i>	(Darwin, 1854)	NIS	0.69	0.015	*
Destination Middle	<i>Ciona intestinalis</i>	(Linnaeus, 1767)		0.86	< 0.001	***
	<i>Diplosoma listerianum</i>	(Milne Edwards, 1841)		0.68	0.026	*
Entrance Control	<i>Laomedea flexuosa</i>	Alder, 1857		0.63	0.038	*

## Community respiration

Community respiration varied considerably among treatments with panels having the inner location as destination (including IC) showing 3 to 5 times higher respiration rates than any other treatments (Fig. 23). Respiration was significantly different between IC ( $17.66 \text{ mg O}_2 \cdot \text{h}^{-1} \cdot \text{gAFDM}^{-1}$ ) and the two other controls (MC:  $3.39 \text{ mg O}_2 \cdot \text{h}^{-1} \cdot \text{gAFDM}^{-1}$ ; EC:  $4.83 \text{ mg O}_2 \cdot \text{h}^{-1} \cdot \text{gAFDM}^{-1}$ ; Tuckey HSD;  $p < 0.001$ ). M>I and E>I had each higher respiration rates than treatments in other destinations (Tuckey HSD;  $p < 0.001$ ). However, E>I respiration was even higher than the IC (E>I:  $21.40 \text{ mg O}_2 \cdot \text{h}^{-1} \cdot \text{gAFDM}^{-1}$ ; Tuckey HSD;  $p = 0.033$ ).

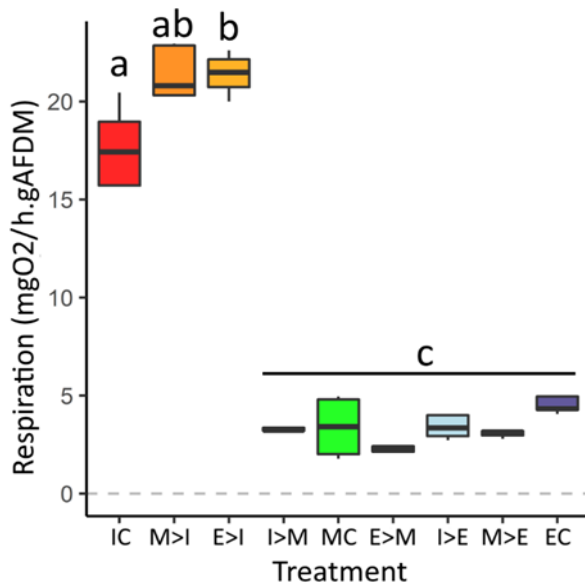


Fig. 23: Boxplot of the community respiration ( $\text{mg O}_2 \cdot \text{h}^{-1} \cdot \text{gAFDM}^{-1}$ ) for all treatments. Treatments are labelled according to their origin followed (>) by their destination after transplant (I: inner, M: middle, E: entrance; C: controls i.e. identical Origin and Destination) and indicated according to their destination (red = inner; green = middle; blue = entrance). Significant differences (Tuckey HSD;  $p < 0.05$ ) indicated by letter groups ( $n = 5$ ).

## Metabolome

The merged data matrix groups 597 features (LCMS+: 303, LCMS-: 282 and GCMS: 12) after metabolome analysis for *B. neritina*, 522 features (LCMS+: 181, LCMS-: 344 and GCMS: 7) for *C. intestinalis* and 428 features (LCMS+: 165, LCMS-: 171 and GCMS: 92) for *A. aspersa*. The PLS-DA of the detected metabolome (LCMS+, LCMS- and GCMS) of the three studied species was established (Fig. 24; Sup. Fig. 3). For *B. neritina* and *C. intestinalis* the treatments are well separated on the Component 1 axis, grouped by destination with the inner origin at the left side and the entrance origin on the right for each destination group. Component 2 reflects the variability within each treatment. The PPLS-DA driven permutation Model Validation Analysis (MVA) revealed significant differences among locations after transplant in the metabolomes of *B. neritina* (CER = 0.53;  $p < 0.001$ ; Fig. 24a) and of *C. intestinalis* (CER = 0.44;  $p < 0.001$ ; Fig. 24c), but not of *A. aspersa* (CER = 0.82;  $p = 0.095$ ; Sup. Fig. 3). The pairwise MVA test showed a strong effect on the metabolome of *B. neritina* with differences according to the treatments (Fig. 24b). The IC metabolome was significantly different from the two other controls (MVA;  $p < 0.05$ ). Several times, transplant resulted in significantly different metabolomes compared to

origin controls (destination effect) but also to destination controls (origin effect). For *C. intestinalis*, it was not possible to recover samples from the IC and M>I panels due to low biomass. Only the metabolome of E>I panels was significantly different from all other treatments, except from M>I and MC ( $p < 0.047$ ).

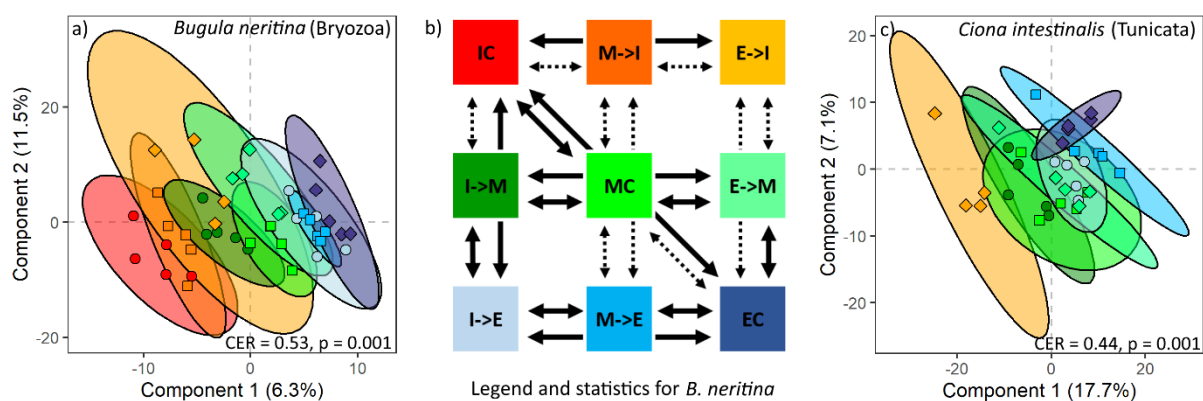


Fig. 24: a) Partial Least Squares Discriminant Analysis (PLS-DA) of the metabolomes of *Bugula neritina* with b) its associated Statistical metrics for the pairwise comparisons and c) PLS-DA of the metabolome of *Ciona intestinalis*. Ellipses indicate confidence intervals of 0.95 for each treatment. Treatments are labelled according to their origin followed (>) by their destination after transplant (I: inner, M: middle, E: entrance; C: controls i.e. identical Origin and Destination) and indicated in the PLS-DA according to their destination (red = inner; green = middle; blue = entrance) and origin (circle = inner, square = middle, diamond = entrance). Full line arrow: pairwise MVA with  $p < 0.05$ . Dashed line arrow: non-significant. For both species one sample is missing in E>I while the treatments IC and M>I are completely absent for *C. intestinalis*. CER = Classification Error Rate with  $p$ -value after permanova test (permutations 104).

For *B. neritina* the 100 highest Variable of Importance in Projection (VIP) scores linked to the PLS-DA were selected to putatively identify the metabolites linked to metabolome differentiation according to treatments. Almost two third (59 %) of the VIPs were either sulfated or phosphated compounds. Using MS<sup>2</sup> spectra to annotate metabolites, 46 of the 100 VIPs could be connected to a specific chemical family (Tab. 11), including 16 glycerophosphoethanolamines (35%) and 13 sulfoethanolamines (28%). While sulfoethanolamine VIPs had varying expression profiles, associated to the inner or the entrance destinations, glycerophosphoethanolamine VIPs were more prevalent in the MC and treatments including the entrance as a destination. Among other families, we were also able to annotate some oxylipins, amines and purines. For *C. intestinalis* only some glycerophospholipids were highlighted due to the absence of IC (Sup. Tab. 2). However, their pattern was similar to glycerophosphoethanolamines in *B. neritina*.



## Chapter II

Tab. 11: Variables of Importance in Projection (VIP) scores associated to the PLS-DA of *Bugula neritina* metabolomes. 46 of 100 VIP were annotated to at least family level. Heatmap expression profiles (Prof.) were sorted while keeping chemical families together. Molecules within a family are sorted with increasing number of carbons, number of oxygens, and number of unsaturations. The two most prominent molecular families are in bold. Phosphated molecules are hued in green, sulphated molecules in yellow, oxylipins in grey and purines in blue. Treatments are labelled in the heatmap according to their origin (top) followed by their destination (below) after transplant (I: inner, M: middle, E: entrance; C: controls i.e. identical Origin and Destination). Heatmap legend above heatmap.

Prof.	Chemical family	Molecular formula	Putative molecular structure	Heatmap																			
				Low			High			Low			High										
				I	M	E	I	M	E	I	M	E	I	M	E								
Inner Destination	Amine	C <sub>8</sub> H <sub>11</sub> N	Phenylethylamine																				
		C <sub>10</sub> H <sub>12</sub> N <sub>2</sub>	Tryptamine																				
	Carnitine	C <sub>12</sub> H <sub>23</sub> NO <sub>4</sub>	Valerylcarnitine																				
	<b>Glycerophosphoethanolamine</b>	<b>C<sub>23</sub>H<sub>46</sub>NO<sub>6</sub>P</b>																					
	Glycerophosphocholine	C <sub>28</sub> H <sub>48</sub> NO <sub>7</sub> P	PC(20:5)																				
	Fatty Alcohol Sulfate	C <sub>10</sub> H <sub>20</sub> O <sub>4</sub> S	Decenol, hydrogen sulfate																				
Middle Destination	<b>Sulfoethanolamine</b>	<b>C<sub>10</sub>H<sub>21</sub>NO<sub>4</sub>S</b>	<b>SE(8:1)</b>																				
		<b>C<sub>12</sub>H<sub>34</sub>N<sub>2</sub>O<sub>5</sub>S</b>																					
		<b>C<sub>14</sub>H<sub>36</sub>N<sub>2</sub>O<sub>5</sub>S</b>																					
		<b>C<sub>22</sub>H<sub>41</sub>NO<sub>6</sub>S</b>	<b>SE(17:3)</b>																				
	Brominated imidazole	C <sub>3</sub> H <sub>1</sub> Br <sub>3</sub> N <sub>2</sub>	1H-Imidazole, 2,4,5-tribromo-																				
	Purine	C <sub>11</sub> H <sub>14</sub> N <sub>6</sub> O <sub>4</sub>	Adenosine, amino-																				
Middle Control and Entrance Destination	<b>Glycerophosphoethanolamine</b>	<b>C<sub>21</sub>H<sub>44</sub>NO<sub>6</sub>P</b>	<b>LysoPE(P-16:0)</b>																				
	<b>Sulfoethanolamine</b>	<b>C<sub>12</sub>H<sub>21</sub>NO<sub>4</sub>S</b>																					
	Sulfo serine ester	C <sub>16</sub> H <sub>33</sub> NO <sub>5</sub> S	Serine, docosyl ester, hydrogen sulfate (ester)																				
	Sulfated acid	C <sub>8</sub> H <sub>8</sub> O <sub>7</sub> S	Sulf. benzoic/benzenacetic acid																				
	Oxylipins	C <sub>18</sub> H <sub>32</sub> O <sub>7</sub> C <sub>20</sub> H <sub>34</sub> O <sub>8</sub> C <sub>20</sub> H <sub>34</sub> O <sub>8</sub>																					
	Fatty acyl glycerol	C <sub>21</sub> H <sub>34</sub> O <sub>4</sub>	1-Oxo-octadecatetraenyl-glycerol																				
Entrance Destination	<b>Glycerophosphoethanolamine</b>	<b>C<sub>22</sub>H<sub>44</sub>NO<sub>7</sub>P</b>	<b>LysoPE(17:1)</b>																				
		<b>C<sub>23</sub>H<sub>46</sub>NO<sub>7</sub>P</b>	<b>LysoPE(18:1)</b>																				
		<b>C<sub>23</sub>H<sub>42</sub>NO<sub>10</sub>P</b>	<b>LysoPE(18:3/3-OH)</b>																				
		<b>C<sub>25</sub>H<sub>48</sub>NO<sub>7</sub>P</b>	<b>LysoPE(20:2)</b>																				
		<b>C<sub>25</sub>H<sub>48</sub>NO<sub>7</sub>P</b>	<b>LysoPE(20:2)</b>																				
		<b>C<sub>25</sub>H<sub>44</sub>NO<sub>7</sub>P</b>	<b>LysoPE(20:4)</b>																				
		<b>C<sub>27</sub>H<sub>44</sub>NO<sub>7</sub>P</b>	<b>LysoPE(22:6)</b>																				
		<b>C<sub>27</sub>H<sub>50</sub>NO<sub>7</sub>P</b>	<b>LysoPE(22:3)</b>																				
		<b>C<sub>27</sub>H<sub>48</sub>NO<sub>7</sub>P</b>	<b>LysoPE(22:4)</b>																				
		<b>C<sub>27</sub>H<sub>46</sub>NO<sub>7</sub>P</b>	<b>LysoPE(22:5)</b>																				
		<b>C<sub>27</sub>H<sub>46</sub>NO<sub>7</sub>P</b>	<b>LysoPE(22:5)</b>																				
		<b>C<sub>27</sub>H<sub>44</sub>NO<sub>7</sub>P</b>	<b>LysoPE(22:6)</b>																				
		<b>C<sub>28</sub>H<sub>47</sub>NO<sub>9</sub>P</b>	<b>C20:2-diGly-PE</b>																				
		Glycerophosphocholine	C <sub>23</sub> H <sub>42</sub> NO <sub>7</sub> P	PC(15:3)																			
	<b>Sulfoethanolamine</b>	<b>C<sub>16</sub>H<sub>35</sub>NO<sub>5</sub>S</b>																					
Entrance Destination	<b>Sulfoethanolamine</b>	<b>C<sub>10</sub>H<sub>19</sub>NO<sub>5</sub>S</b>	<b>SE(8:1)</b>																				
		<b>C<sub>11</sub>H<sub>23</sub>NO<sub>5</sub>S</b>	<b>SE(9:0)</b>																				
		<b>C<sub>12</sub>H<sub>25</sub>NO<sub>5</sub>S</b>	<b>SE(10:0)</b>																				
		<b>C<sub>13</sub>H<sub>25</sub>NO<sub>5</sub>S</b>	<b>SE(11:1)</b>																				
		<b>C<sub>14</sub>H<sub>23</sub>NO<sub>4</sub>S</b>	<b>SE(12:4)</b>																				
		<b>C<sub>19</sub>H<sub>29</sub>NO<sub>7</sub>S</b>																					
		<b>C<sub>20</sub>H<sub>41</sub>NO<sub>4</sub>S</b>																					
	Purine	C <sub>10</sub> H <sub>13</sub> N <sub>5</sub> O <sub>8</sub> S	Guanosine, sulfate																				
		C <sub>12</sub> H <sub>17</sub> N <sub>5</sub> O <sub>8</sub> S	Guanosine, N-dimethyl-, sulfate																				
	<b>Glycerophosphoethanolamine</b>	<b>C<sub>21</sub>H<sub>44</sub>NO<sub>7</sub>P</b>	<b>LysoPE(16:0)</b>																				
Oxylipins	C <sub>18</sub> H <sub>37</sub> ClO <sub>10</sub>																						

## Discussion

Previous studies have observed local variations of fouling communities between different parts of harbors/marinas, which have been linked to pollution gradients (Je et al. 2004, Ryu et al. 2011, Kenworthy et al. 2018b). The associated mechanistic process might be linked to a selective pressure exerted by such disturbances inducing the observed heterogeneity and local adaptation. To expose the causal link between local adaptation and environmental factors, reciprocal transplant experiments represent one of the best way though their use remains scarce in marine ecology due to technical difficulties to run such experiments (Kawecki & Ebert 2004, Angert & Schemske 2005, Chang & Marshall 2016, Sork 2018). Based on previous results in the very same marina few years before (Kenworthy et al. 2018b), we hypothesized a disturbance (here POPs and MTEs) gradient from the entrance to the inner of the marina and assumed that community structure would correlate with this gradient as previously observed. We also hypothesized that transplanted communities would be driven according to their new environmental conditions, thus revealing a causal link between the environment and community structure. We also hypothesized that such filter was acting at different biological organization levels, including community structure, community respiration, pollutant accumulation, and species metabolomes, indicating local adaptation.

### Contamination in sediments and animals

While no official common European guidelines for contamination in marine sediments do exist, we could compare our results with two North American and two European guidelines. The harbor sediments largely exceeded the various thresholds for some PAHs (chrysene, fluorene, fluoranthene and phenanthrene) as well as for total PCBs (33 congeners). This may constitute an important stress for local species, as the encountered concentrations would exceed a 50% probability of toxicity on model amphipods exposed to the sediment (US EPA 2005). MTEs pollution presents higher global values than recommended by CCME and US EPA guidelines, especially in the inner parts (**Erreur ! Source du renvoi introuvable.**). According to the Sediment Quality Category (SQC) established from southern Spanish harbors (Guerra-García et al. 2021), MTEs appear at good or moderate (Zn and Cu) levels. This is in agreement with the French national monitoring network REPOM, for which the studied marina was sampled in 2011 and 2012 (and had levels below the lowest concentration category N1 for most contaminants; REPOM 2013). However, our present values and those recorded in 2016 by Kenworthy et al. (2018b), indicate increasing Cu concentrations, exceeding the REPOM N1 category at various locations.

Among the different locations studied within the marina, contaminants did, as expected, vary between the three locations spaced by less than 100 m. We observed significant differences in

benzo[*g,h,i*]perylene, fluorene and total PCBs concentrations among locations, with maximal values at the inner location, but not organized as a gradient. MTEs in sediments had maximal concentrations in the innermost part of the marina and diminished towards the entrance. This trend was particularly marked for Cu, Pb and Zn, two of which have been previously reported in 2016 (Kenworthy et al. 2018b). Such lower contaminant concentrations at the entrance might be linked to the higher hydrodynamism and higher depth that we noted at this location (Sup. Fig. 1), as stronger water mixing might dilute pollutants (Schiff et al. 2007).

Contaminant concentrations in the filter feeder *Bugula neritina* approximately followed the same trends as in sediments with, however, lower concentrations in general but high heterogeneity between samples. An exception lays in pesticides which were equivalently concentrated or even higher (aldrin, diazinon and chlordanes) in *B. neritina* samples than in the sediments. As previously reported for various benthic species (Reynoldson 1987), the filter-feeding *B. neritina* might have bioaccumulated these contaminants following sediment resuspensions due to meteorological or hydrodynamic events. Interestingly, Cu presented low concentrations and no significant differences between locations in *B. neritina* samples while it had a strong spatial variability and a higher pollution levels than most MTEs in sediments (moderate, according to Guerra-García et al. 2021). Due to its high toxicity, it is a major driver of harbor fouling communities and of the prevalence of non-indigenous species (Dafforn et al. 2008, Canning-Clode et al. 2011, Kenworthy et al. 2018b, Osborne & Poynton 2019). *Bugula neritina* is particularly resistant to Cu, even compared to other introduced bryozoans like *Watersipora* spp. commonly present in harbors (Piola & Johnston 2006a). Detoxification mechanisms may thus explain why despite high Cu concentrations in sediments, *B. neritina* had overall low concentrations.

### Community analyses

Previous studies have shown that pollution gradients may structure communities by inducing local differences among them (Je et al. 2004, Ryu et al. 2011, Kenworthy et al. 2018b). In the same marina as the present study, Kenworthy *et al.* (2018b) could correlate the high but varying levels of some sediments contaminants (Cu, Zn, and hydrocarbons) at small spatial scales (< 100 m) with heterogeneity in fouling communities of vertical pillars. While increasing the contaminant number, the present study showed similar results with significant differences maintained throughout the whole duration of the experiment among communities from control panels (Fig. 22). Such similar spatial patterns maintained over time might suggest local adaptation. However, only an experimental approach such as reciprocal transplant may establish a causal relationship between the environmental conditions and biological functions (Osman & Whitlatch 2004, Cifuentes et al. 2010, Chang & Marshall 2016).

It was possible to observe a strong effect of the transplant on community structures. The global PERMANOVA revealed a strong effect of origin, destination, and their interaction (Treatment). The destination effect directly results from the transplant experiment. Pollution and specifically Cu pollution has been thoroughly shown to highly influence fouling communities (Piola & Johnston 2008, Canning-Clode et al. 2011, Kinsella & Crowe 2016). Here we confirmed differences in community composition between locations as previously observed (Je et al. 2004, Ryu et al. 2011, Kenworthy et al. 2018b), but we also demonstrated a causal relationship between location and community composition through the transplant experiment. This might indicate that the selective pressure exerted by pollution in the inner part of the harbor is a major driver of community composition.

On the other side, population dynamics of *C. intestinalis* might explain the effect of panel origin. Before transplant, *C. intestinalis* was completely covering panels from the entrance (Sup. Fig. 2 and Sup. Fig. 4), while at the end of the experiment, EC and E>I panels had a comparatively lower cover. Conversely E>M panels were completely covered by very large individuals that were most likely the same individuals noted before transplant (Sup. Fig. 4, middle). Indeed, this species has a rather short life cycle in the present harbor, recruiting twice a year (spring and late summer) and living 2-4 months before reproducing and dying (Bouchemousse et al. 2016b). Moreover lethal and sublethal effects were reported for some environmental factors such as heavy metal pollution (Bellas et al. 2001, 2004). Considering the contaminant levels recorded in the present harbor, the entrance may have been the most favorable location for this species' high recruitment in the early phases of our experiment, then for completing its reproduction cycle and subsequent mass mortality (hence its decline at the end of the experiment). At the inner location, lethal effects of contaminants may explain the low presence of *C. intestinalis* in inner controls and the sharp decline in E>I treatments. Individuals on E>M panels however, may have encountered sublethal effects of pollution delaying their reproductive cycle and explaining the high cover by large individuals at the end of the experiment. This illustrates the origin effect observed in the PERMANOVA. Niche preemption as priority effect may highly influence the historical contingency of a community (Fukami 2015). Previous transplant studies on fouling communities have already demonstrated the importance of primary colonization on the subsequent community composition in harbors (Cifuentes et al. 2010, Chang & Marshall 2016). Stochastic colonization may also have a high structuring role (Chase & Myers 2011). *Ciona intestinalis* is a highly competitive species with a tendency to monopolize substratum and reduce diversity (Blum et al. 2007). Considering all this, the monopolization of space by *C. intestinalis* at the entrance before transplant may explain why communities originating from this location strongly differentiate from all others even if this species dies off if transplanted to the inner location.

The indicator species analysis revealed that species may be associated to certain origins or to certain destinations, which reflects the coexistence of those effects as observed at the community level. The indicator species associated to specific destinations revealed the causal effect between environment and community structure. *Cryptosula pallasiana* and *Austrominius modestus* seem both to be species associated with the inner location, as they were indicator species of this location before transplant and are indicator species of the inner destination (Tab. 10). In general, the inner part of the marina seems to be more frequently correlated or associated with encrusting species and NIS (see Fig. 22 and Tab. 10). The indicator species associated to specific origins reveal that historical contingency plays an important role in the community structure and species present on panels. Interestingly, none of the indicator species for any location before transplant is an indicator species for the same origin after transplant (Tab. 10). Some of the indicator species associated to origin effects (ex. *Mytilus edulis* or *Asterocarpa humilis*) were not detected at all before transplant. Two very notable indicator species, *B. neritina* and *W. subatra* (both introduced bryozoans), were associated to the middle origin. These were also the panels where their percent cover was often the highest compared to panels from the same destination and the panels for which introduced species cover was the highest (Sup. Fig. 1). This might indicate that once these species gain a foothold on the substratum, they persist even if environmental conditions change, which may pose problems for their management in fluctuating environments.

#### **Local adaptation is revealed at various organization levels and functions**

We investigated several biological functions to see if the ‘local vs foreign’ criterion (fitness differences among transplanted and destination control individuals) for local adaptation was respected (Kawecki & Ebert 2004). For this, we chose to focus on metabolic metrics to discuss the potential fitness of organisms.

Increased stress due to disturbance may engage energetically costly regulatory mechanisms like osmoregulation or detoxification (Roast et al. 1999, Pook et al. 2009, Lenz et al. 2011), which may in turn increase respiration (Kenworthy et al. 2018b). Here we did observe higher respiration rates in IC, M>I and E>I communities, which could indicate that the inner location may constitute a high stress environment for them. Interestingly, community respiration from the E>I treatment is even higher than the IC respiration, which would indicate an even higher stress level for these communities. This would be consistent with a ‘foreign’ disadvantage of these communities compared to IC (locals) and be a potential indicator that at least some species might be locally adapted to the environmental conditions. Respiration was 3-5 times higher than what has been reported in the literature (Bakke & Skjoldal 1979, Lenz et al. 2011, Kenworthy et al. 2018a, Oh et al. 2020). Bacterial respiration might thus

contribute to the elevated values at the inner location as it may account for between 30% and 60% of community respiration (Smith 1973, Martínez-García et al. 2013).

Contamination in animal tissues may be an important measure to be accounted for, as it may impact fitness through reproduction and survival (Piola & Johnston 2006a b, Ruiz et al. 2011). The contaminant composition of *B. neritina* (see Fig. 20 and **Erreur ! Source du renvoi introuvable.**, Tab. 8, Tab. 9) is mainly driven by MTE and PAH. Consequently, the EC (control at the least contaminated location) had a significantly lower normalized concentration than the two other controls (Fig. 21), but the transplant had a strong effect on *B. neritina* normalized contaminant concentrations: whatever their origin, individuals transplanted towards the entrance showed the lowest concentrations, while individuals transplanted to the inner location had systematically higher concentrations (Fig. 21). Interestingly, it is also possible to observe a differential accumulation of contaminants among individuals transplanted to the inner (most contaminated) location, with E>I individuals having a significantly higher concentration than IC and M>I individuals (Fig. 21). This might be an indication for local adaptation at very small scale (< 100 m) for this species, where individuals from less contaminated locations (entrance) may have a lower detoxification activity (or higher contaminant uptake) compared to their counterpart in contaminated locations (inner). There are many molecular and cellular mechanisms used for detoxification which can be explained by genetic adaptation and differences in expression regulation of detoxification pathways (Rainbow et al. 1990, Janssens et al. 2009). This species has been shown to have differential resistance to Cu among populations (Piola & Johnston 2006b). Maternal effects seem to have a particularly strong effect on *B. neritina* facing spatial variability of environmental factors (Marshall 2008, Burgess & Marshall 2011). Local adaptation through epigenetics and maternal effects might explain the differential accumulation/detoxification of this species in our case and need to be further explored.

Environmental conditions and pollution levels may be an important driver of the metabolome of a species (García-Sevillano et al. 2015, Xue et al. 2019). The metabolomes of *B. neritina* and *C. intestinalis* were significantly influenced by location and transplant. For *B. neritina*, it was possible to observe differences in the metabolome among locations (IC distinct from other controls). Furthermore, a clear pattern linked to the transplant could be observed (distinct metabolomes among panels exchanged between the inner and entrance locations with their respective controls). For *C. intestinalis*, a clear difference between metabolomes of EC and E>I individuals has been identified, but due to the absence of IC metabolomes (not enough individuals for sampling), no clear patterns could be highlighted for this species. Destination effects on the metabolome of *C. intestinalis* and *B. neritina* showed that both species were able to modify their metabolism to accommodate for new environmental conditions. Changes in the metabolome might be a direct response to variable contamination levels among

locations. For *B. neritina*, this destination effect however coincides with an origin effect since the E>I metabolome was not only distinct from EC, but also from IC. This effect is even more visible for metabolomes originating from the middle, which were different from MC and from both their destination controls (EC and IC). These results are in accordance with the normalized pollutant concentration in *B. neritina*, whose entrance individuals transplanted to the inner location seem to present a lower detoxification activity (or higher pollutant uptake) compared to the inner control. Since these activities are dependent on metabolic pathways (Rainbow et al. 1990, Janssens et al. 2009), they should also manifest in the metabolome and at least partly influence it. Indeed, such a tendency may be also reported from the expression profiles of some of *B. neritina*'s metabolites (Tab. 11). Glycerophospho-ethanolamines (GlyPE), more specifically Lyso-phospho-ethanolamines (Lyso-PE), have a very distinct expression pattern with high expression in MC, M>E and EC. Expressions of this chemical family varied according to their transplant destination ( $I>E > I>M > IC$  and  $EC > E>M > E>I$ ). Interestingly, an origin effect is also visible for Lyso-PE, since the concentration of  $E>I < IC < EC$ . This would further indicate that local adaptation of this species may be present at this small spatial scale. Data from literature suggest a potential link between GlyPE expression and reproductive activity. They have been shown to vary with the reproductive cycle of the sponge *Oscarella tuberculata* (Schmidt, 1868), being highly expressed during embryogenesis and early development (Ivanisevic et al. 2011). Moreover, lyso-phospholipids also play a role in mammalian reproduction (Birgbauer & Chun 2006). Phospholipids in general appear to be impacted by various types of pollutions in vertebrates and invertebrates (Al-Salhi et al. 2012, García-Sevillano et al. 2015, Salihovic et al. 2015, Wang et al. 2017). Lyso-PE levels have an almost exact opposite distribution than pollution levels in *B. neritina* indicating a negative effect of pollution on Lyso-PE levels. It is possible to assume that the inner, more polluted location is metabolically at higher cost for *B. neritina* due to higher energy cost of detoxification, leaving less resources to allocate to reproduction.

Although it is difficult to make clear statements upon the metabolome of *C. intestinalis*, the analysis of its metabolome allowed to identify several VIP as glycerophosphocholines (GlyPC) and GlyPE. They had a similar distribution pattern to *B. neritina*'s ones. However, GlyPC and GlyPE levels in *C. intestinalis* had a slightly lower expression at the EC compared to  $I>E$ ,  $M>E$  and compared to MC (Sup. Tab. 2). Their expression profile would be compatible with the low survival of *C. intestinalis* when transplanted from the entrance to the inner location and the delay in reproductive activity due to less favorable conditions when transplanted to the middle (Sup. Fig. 2, Sup. Fig. 4). This suggests that glycerophospholipids may be excellent markers of environmental stress linked to pollution across multiple animal classes, since they seem to be linked to a tradeoff between reproduction and



detoxification. Subsequent studies could use targeted metabolomics to assess levels of these molecules in several study species.

Here we show strong indications for local adaptation, since the 'local vs foreign' criterion is satisfied for the community respiration, contaminant accumulation and the metabolome of *B. neritina*. Our results do however not satisfy the 'home vs away' (fitness differences among transplanted and origin control individuals) criterion since I>E individuals had lower pollutant concentrations and higher Lyso-PE levels than IC. This is due to differences in the intrinsic habitat quality, which is why the 'foreign vs local' criterion is the foremost one to satisfy to conclude on local adaptation (Kawecki & Ebert 2004). Strong selective forces are primordial in generating local adaptation and contemporary evolution for introduced species (Kawecki & Ebert 2004, Colautti & Lau 2015). Pollutants constitute a strong selective filter that can act on at the community level (Je et al. 2004, Canning-Clode et al. 2011, Ryu et al. 2011), but also on the species which might develop hereditary resistances (Piola & Johnston 2006b, Marshall 2008, McKenzie et al. 2011). While for the E>I treatment the 'foreign' disadvantage is striking, it is much more subtle for the I>E treatment. However, if we use the detoxification activity as example, transplant from the inner to the entrance should lead to a fitness disadvantage compared to EC 'locals'. Detoxification depends on costly metabolic pathways since these require trade-offs between metabolism and other activities (Handy et al. 1999, Pook et al. 2009). High detoxification activity in less polluted environments could thus impair fitness and cause a competitive disadvantage (wasted energy) even if we were not able to measure it. This may explain why glycerophospholipids, which we presume as fitness markers, are lower in the I>E treatment compared to EC for *B. neritina*, which would satisfy the 'foreign vs local' criterion in both directions. Many introduced species seem to be exclusive to anthropic structures and do not 'escape' to natural habitats (Glasby 1999, Marins et al. 2010, Simkanin et al. 2012), which could at least partially be explained by such disadvantage.

### **Concluding remarks**

The present results highlight strong small-scale spatial variation of contaminants in sediments and animal samples. The community composition did not only vary in space in accordance to pollution as it has already previously been observed (Kenworthy et al. 2018b), but it was causally linked to environmental conditions as shown by the changes in community composition after transplant. However, this destination effect is concomitantly associated with an effect of origin location indicating that historical contingency and colonization succession may still play an equally crucial role. Our results provide strong evidence for local adaptation at a very small spatial scale (< 100 m) since community structure, community respiration, pollutant content of *B. neritina* and the metabolome of two species were impacted. We expose a clear disadvantage for the 'foreign' condition for individuals transplanted

from the entrance to the inner, more polluted, location in community respiration, pollutant accumulation and metabolome. The here observed results may thus indicate that the disturbance gradient in marinas might constitute a staple for selecting pollutant-resistant species and populations and cause local adaptation, which may explain why many NIS remain exclusive to anthropic structures and marinas. However, in natural and urbanized habitats surrounding marinas, pollution may occur in pulses due to wash off and water mixing events. Such pulse-pollution has already been shown to have drastic impacts on settled communities (Johnston & Keough 2000, 2002, Johnston et al. 2002). This highlights the importance of conducting further studies into small scale local adaptation, using reciprocal transplant experiments to determine if the selective pressure in marinas might play an important role in restricting many introduced species to marinas and similarly polluted environments; or if it confers an advantage in areas with variable pollution levels.

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## Supplementary Material

# Pollution gradient leads to local adaptation and small-scale spatial variability of communities and functions in an urban marine environment

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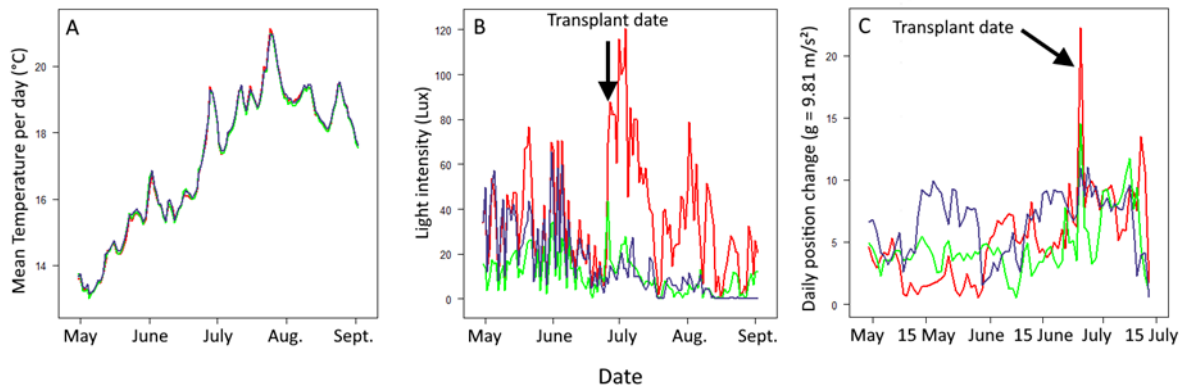
3 Aix Marseille Univ, Université Toulon, CNRS, IRD, MIO UM 110, Mediterranean Institute of Oceanography, Marseille, France

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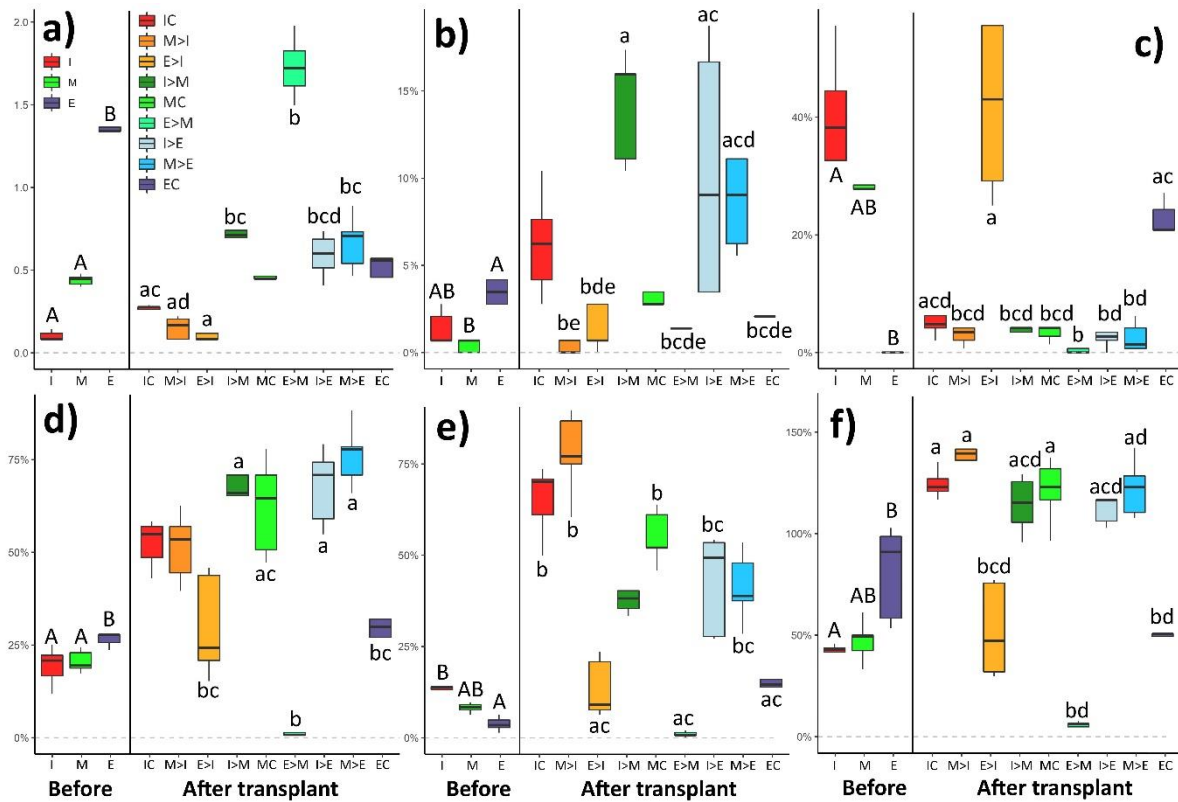
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**Abstract.** Urbanization of coastal habitats, of which harbors and marinas are the paragon, has led to various ecological paradigms about their functioning. Harbor infrastructures offer new hard substrata that are colonized by a wide variety of organisms (biofouling) including many introduced species. These structures also modify hydrodynamism and contaminant dispersal, leading to strong disturbance gradients within them. Differences in sessile community structure have previously been correlated to these gradients at small spatial scale (< 100 m). Local adaptation might be involved to explain such results, but as correlation is not causation, the present study aims to understand the causal link between the environmental gradients and community structure through a reciprocal transplant experiment among three sites of a marina (inner, middle, entrance). Our results highlighted strong small-scale spatial variations of contaminants (trace metals, PCB, pesticides, and PAH) in sediments and animal samples which have been causally linked to changes in community composition after transplant. But historical contingency and colonization succession also play an important role. Our results provided strong evidence for local adaptation since community structure, respiration, and pollutant uptake in *Bugula neritina*, as well as the metabolomes of *B. neritina* and *Ciona intestinalis* were impacted by the transplant with a disadvantage for individuals transplanted from the entrance to the inner location. The here observed results may thus indicate that the disturbance gradient in marinas might constitute a staple for selecting pollutant-resistant species and populations, causing local adaptation. This highlights the importance of conducting further studies into small scale local adaptation.

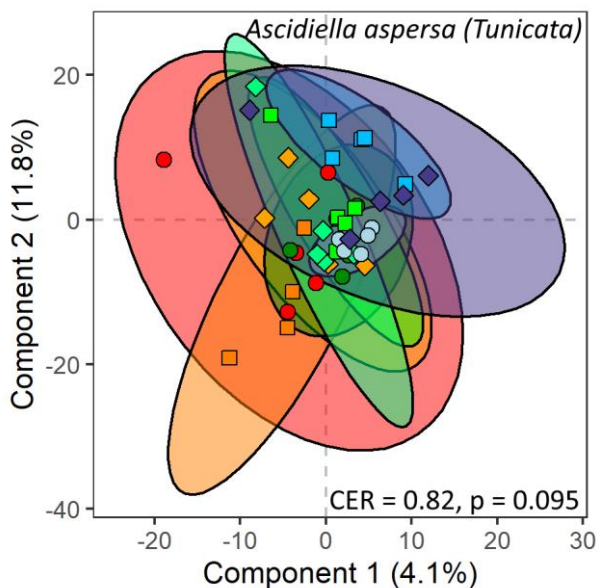
**Keywords:** local adaptation, fouling, pollution, metabolomics, respiration, marinas



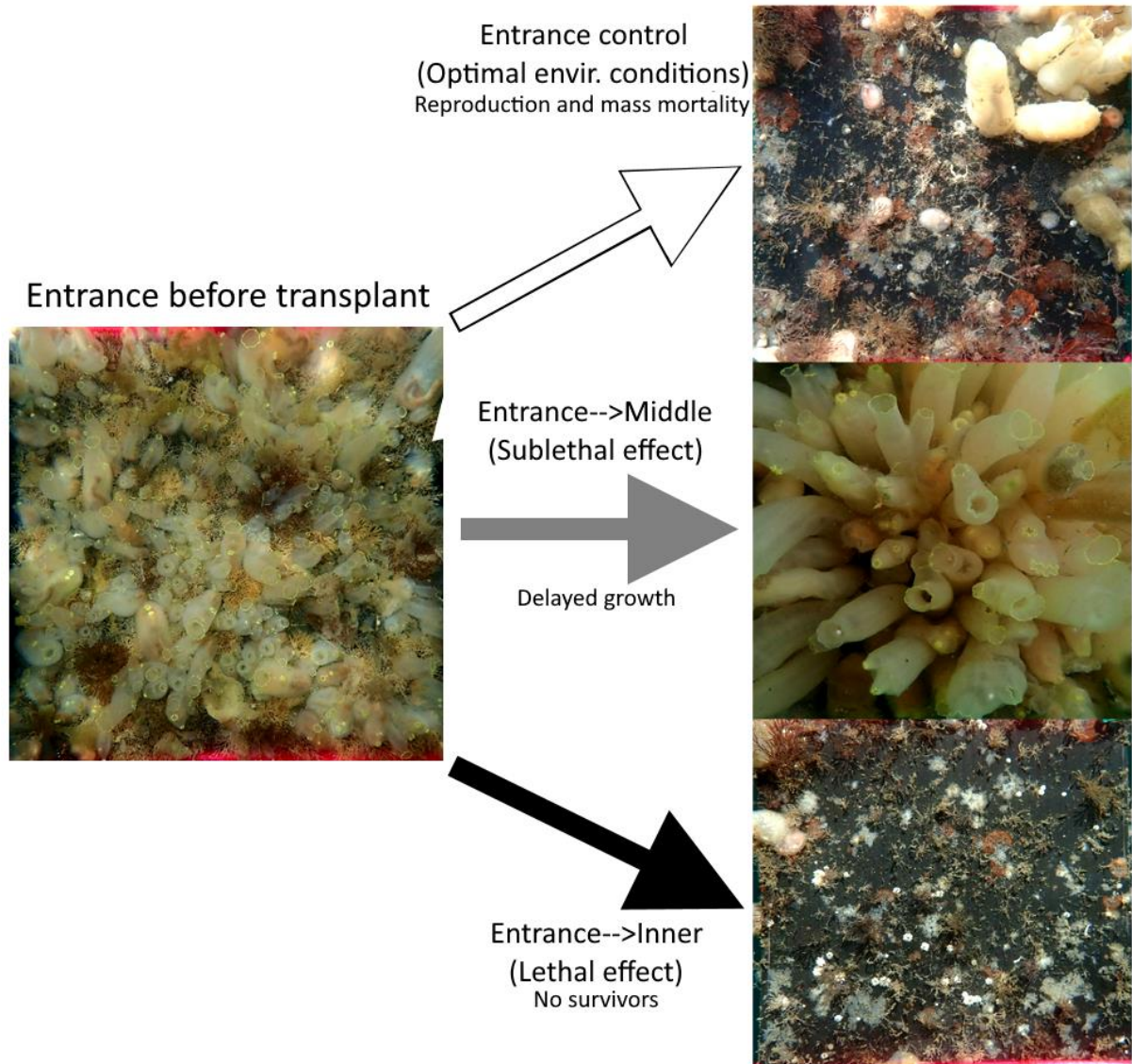
Sup. Fig. 1: Temperature (A), light intensity (B) and hydrodynamism (C) at the three locations. Red: Inner; Green: Middle; Blue: Entrance. For Temperature, the curves overlap although significant differences between pontoons were detected (LME,  $t = 5.33$ ;  $p < 0.05$ ). On a biological perspective, the differences were however negligible ( $< 0.1^{\circ}\text{C}$ ). We chose to discard light measures as the position of the sensor and the fouling on the sensor highly influenced the results. For instance, a high light intensity on the inner sensor just after the transplant was observed when structures were handled and the sensors were cleaned, indicating a strong manipulation bias. Daily position change in  $g$  ( $9.81 \text{ m}\cdot\text{s}^{-2}$ ) calculated from the difference between tridimensional vectors at  $T_n$  and  $T_{n-1}$ , revealed a significant higher hydrodynamism at the entrance compared to the inner location (LME;  $t = 3.61$ ;  $p < 0.001$ ).



Sup. Fig. 2: Boxplot of the cover of several important species before transplant (June) and 2 months after transplant (September). a) percent-cover of *Ciona intestinalis* (squares-root-transformed data) b) percent cover of *Asciidiella aspersa* c) percent cover of empty space without organisms, d) percent cover of *Bugula neritina* e) percent cover of *Watersipora subatra* and f) percent cover of introduced species. Cover can exceed 100% if species overlap (See methods). Treatments are labelled according to their origin followed (>) by their destination upon transplant (I: inner, M: middle, E: entrance; C: controls i.e. identical Origin and Destination). Statistic groups (Dunn Test) indicated by letter groups. Treatments before transplant were compared among them (Capital letters). Treatments 2 months after transplant were compared among them (lower case letters). Boxes without letters are NS with all other.

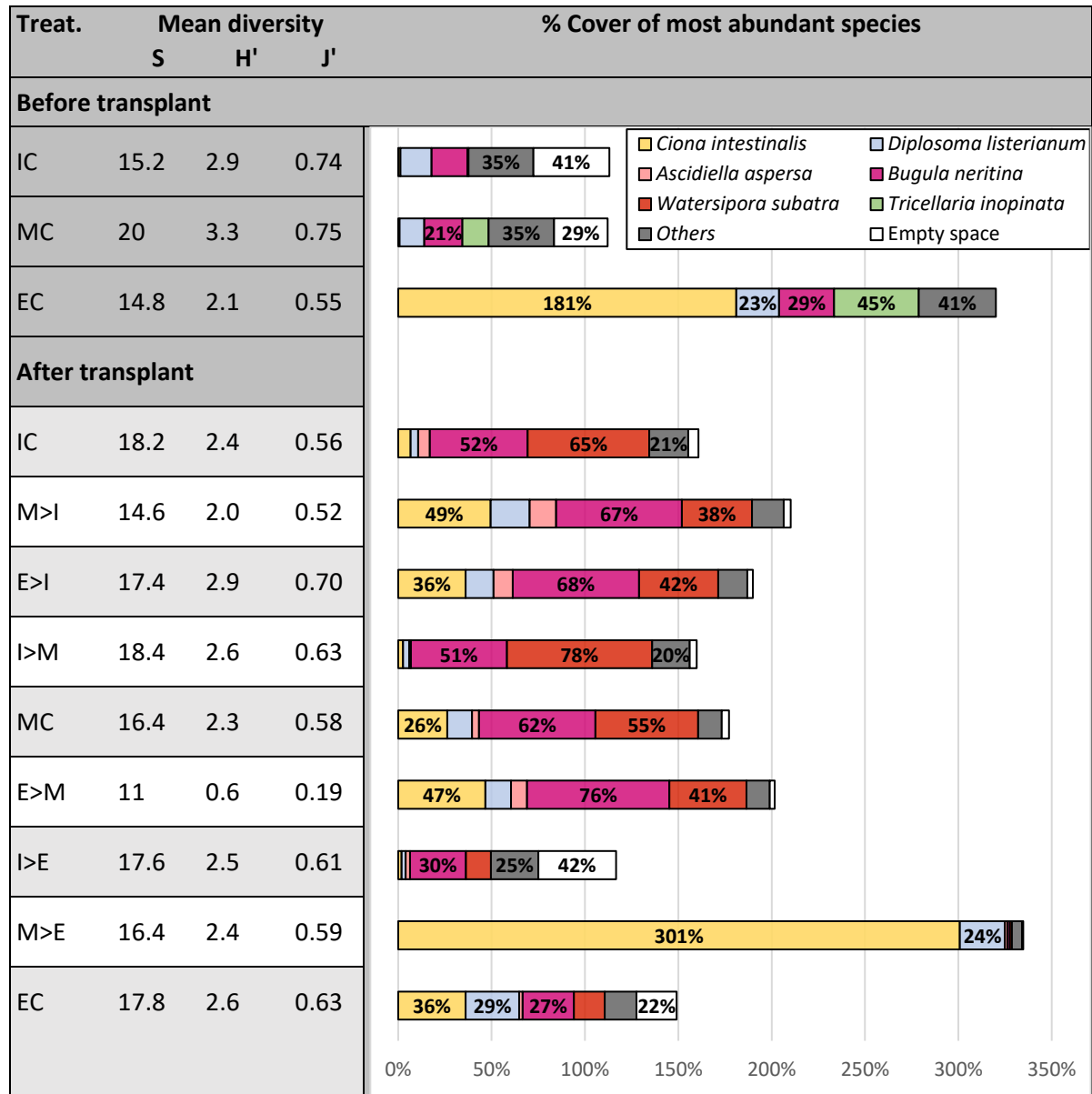


Sup. Fig. 3: Partial Least Squares Discriminant Analysis (PLS-DA) of the metabolome of *Asciidiella aspersa*. Ellipses indicate confidence intervals of 0.95 of each treatment (MVA test;  $p = 0.095$ , CER (Classification Error Rate) = 0.82). Treatments are labelled according to their origin followed (>) by their destination after transplant (I: inner, M: middle, E: entrance; C: controls i.e. identical Origin and Destination) and indicated in the biplot according to their destination (red = inner; green = middle; blue = entrance) and origin (circle = inner, square = middle, diamond = entrance).



Sup. Fig. 4: Photos of the panels from the entrance before transplant (single left) and after transplant: control (top right), to the middle (middle right) and to the inner location (bottom right). Hypotheses concerning the cover of *Ciona intestinalis* are indicated.

Sup. Tab. 1: Mean diversity indices (Species richness: *S*; Shannon index: *H'*; Pielou's evenness: *J'*) and percent cover of the most abundant species for each treatment before transplant and 2 months after transplant. Treatments are labelled according to their origin followed (>) by their destination upon transplant (I: inner, M: middle, E: entrance; C: controls i.e. identical Origin and Destination). Cover can exceed 100% if species overlap (See methods).



Sup. Tab. 2: Variable Importance in Projection (VIP) scores associated to the PLS-DA of *Ciona intestinalis*. Molecules within a family are sorted with increasing number of carbons, number of oxygens, and number of unsaturations. Treatments are labelled in the heatmap according to their origin (top) followed by their destination (below) 2 months after transplant (I: inner, M: middle, E: entrance; C: controls i.e. identical Origin and Destination). Heatmap legend on top of heatmap.

Chemical family	Molecular formula	Putative Molecular structure	Heatmap								
			Low			High			High		
			I	M	E	I	M	E	I	M	E
			C	I	I	M	C	M	E	E	C
Glycerophosphocholine derivatives	$C_{26}H_{44}NO_8P$	LysoPC(19:4-O)									
	$C_{29}H_{52}NO_9P$	LysoPC(22:2-O <sub>2</sub> )									
	$C_{31}H_{52}NO_9P$	LysoPC(24:4-O <sub>2</sub> )									
Glycerophosphocholines	$C_{28}H_{48}NO_7P$	LysoPC(20:5)									
	$C_{30}H_{50}NO_7P$	LysoPC(22:6)									
Glycerophosphoethanolamines	$C_{23}H_{46}NO_7P$	LysoPE(18:1)									
	$C_{23}H_{44}NO_7P$	LysoPE(18:2)									
	$C_{25}H_{44}NO_7P$	LysoPE(20:4)									
	$C_{25}H_{42}NO_7P$	LysoPE(20:5)									
	$C_{27}H_{44}NO_7P$	LysoPE(22:6)									

### Metabolome extraction and mass spectrometry methods

Three species occurring at all three locations in the harbor were sampled for metabolomic analyses on every settlement plate: two sufficiently large colonies of the bryozoan *Bugula neritina* and one individual of the two solitary ascidians *Ciona intestinalis* and *Ascidella aspersa* were sampled. They were immediately frozen in dry ice in the marina, and then stored at  $-80^{\circ}C$  at the laboratory. The samples were then all freeze-dried and mechanically ground to dust. The metabolome of the three species was extracted out of 40 mg of dry powder with 3 times 1 mL MeOH/DCM ( $CH_2Cl_2$ ; 1:1) and ultrasonication during 2 min at room temperature. The supernatants were filtered with PTFE syringe filters (13 mm, 0.22  $\mu m$ , Restek®) and transferred to vials containing silica powder (40 mg, C-18 Polygoprep® 60-50, Macherey-Nagel®). The resulting volume of 3 mL of extract was dried, first under the hood (for DCM evaporation) and secondly under vacuum (SpeedVac®, SPD111V, Thermo Scientific® Savant). The dried extract fixed on silica was fractionated by SPE on C-18 cartridges (Strata® C18-E 55  $\mu m$ , 70 Å, 500 mg / 6 mL, Phenomenex®). The cartridges were flushed two times with 2 x 5 mL MeOH and 2 x 5 mL DCM, intensively dried under vacuum and conditioned with 2 x 5 mL H<sub>2</sub>O. The extracts were transferred onto the cartridges, rinsed with 3 x 5 mL H<sub>2</sub>O to remove salts and eluted with 5 mL MeOH and 5 mL DCM. The MeOH fractions were dried under vacuum, while the DCM fractions were dried under the hood, and stored in a freezer. Samples were resuspended in 500  $\mu L$  MeOH for the polar fractions and in 500  $\mu L$  DCM for non-polar fractions before analysis in LCMS and GCMS, respectively. For each species and fractions, a pool sample was created by mixing 30  $\mu L$  of each sample.



Three blank samples, that underwent the entirety of the extraction and fractioning process, were made for each species and fraction.

DCM fractions were analyzed with a gas chromatograph (7890B GC System - 7693 autosampler, Agilent Technologies®) coupled to a mass selective detector (5977A MSD, Agilent Technologies®). A volume of 1 µL was injected in splitless mode at 250 °C. Metabolites were separated on a HP-5MS 5% Phenyl-Methyl Siloxane column (30 m × 0.25 mm, 0.25 µm, Agilent Technologies®) using helium as carrier gas. The temperature was fixed at 40°C for 5 min and progressively raised from 40 °C to 300 °C (10 °C.min<sup>-1</sup>). The fixed flow rate was set to 1 mL.min<sup>-1</sup> all along the run. A solution of C8-C20 and C21-C40 alkanes (Fluka Analytical) was injected for the determination of compound retention indexes.

GC-MS Agilent data was exported into CDF format using MSD Chemstation (F.01.001903, Agilent Technologies®) and processed in R using the 'eRah' package (version 1.1.0; Domingo-Almenara et al. 2016) following these different steps : preprocessing, peak deconvolution [min. peak width = 5, min. peak height = 500, noise threshold = 100, avoid processing  $m/z = c$  (73:75,147:149,207:208)], peak alignment (min. spectra correlation = 0.90, max. time distance = 20,  $m/z$  range = 40:500) and missing compound recovery (compound detected in > 2 samples). CGMS compound annotation was performed by comparison of mass spectra with those of the NIST 2014 database and Kovàts' index (van Den Dool & Dec. Kratz 1963).

The injections in LCMS were performed with an UHPLC instrument (Dionex Ultimate 3000 equipped with RS Pump, autosampler, thermostated column and UV diode array, Thermo Scientific®) coupled to a Quadrupole Time of Flight spectrometer (QqToF) equipped with an ESI source (Impact II, Bruker Daltonics®). UHPLC separation was conducted on an Acclaim™ RSLC 120 C18 column (150 mm x 2.1 mm, 2.2 µm, ThermoScientific®) at 40 °C with an elution rate of 0.5 mL.min<sup>-1</sup>. We used water (LC/MS grade, Carlo Erba®) with 0.1% formic acid (A) and acetonitrile (LC/MS grade, Carlo Erba®) with 0.1% formic acid (B) as chromatographic solvents. The chromatography cycled through 1) a mix of 5:95 of A:B during 2 min, 2) a linear increase up to 100% B during 8 min followed by 100% B during 4 min, 3) then a return to initial conditions (5:95, A:B) for 3 min for a total runtime of 17 min per sample. An injection of 2 µL in MS1+ and MS2+ (positive mode) and of 0.2 µL in MS1- and MS2- (negative mode) was performed for *B. neritina*. For the other species, the injection volume was of 1 µL in MS1+ and MS2+ and of 0.2 µL in MS1- and MS2-. MS parameters were set as follows: nebulizer gas, N<sub>2</sub> at 3.5 bars; dry gas, N<sub>2</sub> at 12 L.min<sup>-1</sup>, capillary temperature at 200°C and voltage at 3500 V. A full scan from 50 to 1200 mass units at 2 Hz was performed for both modes. The spectrometer was calibrated with a formate:acetate solution in basic media (Bruker) and before each sample for mass calibration. The Pooled sample of each species was injected at regular intervals (every 9 samples) to

correct for possible mass spectrometer drift during the sequence. Blank samples were injected to filter for ions linked to the solvent and the extraction process.

LCMS profiles were automatically recalibrated using internal calibration in 'Bruker Compass DataAnalysis' (version 4.3) assuring a  $m/z$  precision of 2-5 ppm on the mass range. The generated netCDF data were processed with the R package 'XCMS' (version 3.12; Smith et al. 2006). The first step consisted in peak picking for the detection of different features, followed by a retention time correction (obiwarp method), a grouping of features in a fixed time frame (5 s) and peak filling to integrate portions where peaks were initially absent. The report and data matrix were then generated in Microsoft Excel format. Ions were normalized in Microsoft Excel following an established protocol (Van Der Kloet et al. 2009). The normalized matrix was subjected to three consecutive filtering in-house steps on R. These filtering methods consist in filtering ions originating from the blanks based on signal/noise ratio ( $S/N = 10$ ), filtering ions for which intensities were highly variable in the pooled sample ( $> 0.2$ ), filtering ions that were auto-correlated ( $> 0.8$ ) to discard redundancy.



## Chapter III Disruption of the Spatial Variability of Fouling Communities in Marinas Trough Biotic and Abiotic Factors

Previously, we were able to observe on different levels of biological function (community structure, community respiration, pollutant uptake of a model species, metabolomic analyses) how a pollution gradient affected communities and individuals. A reciprocal transplant affected biological functions, exposing the causal link. However, the effects the disturbance gradient on community structure and on local adaptation might be masked/erased larger scale stochastic processes acting similarly on all locations. This is especially true for local adaptation as stochastic processes select phenotypes independent from the original selection pressures (Kawecki & Ebert 2004). These stochastic processes may originate from the abiotic but also the biotic environment of the considered species/community.

Biotic interactions like predation exerted by mobile predators might also mask the effect of local adaptation to the disturbance gradient, since this process might be locally stochastic and act on a larger scale than the gradient. Predation may additionally constitute an independent selection pressure. Predation, may be a major driver of community composition in fouling communities (Osman & Whitlatch 1995, Osman & Whitlatch 2004, Oricchio et al. 2016b a). In Chapter III A we present a study which aimed to disentangle the effect of the disturbance gradient and the effect of predation on the juveniles of two species of the marina, one native, another one introduced.

In Chapter III B, we present a study which originally had the same protocol as in Chapter II but was conducted in a Mediterranean marina. A reciprocal transplant experiment was used to assess the local variation of community structure in regard of spatially variable pollution. While we were able to identify differences in community structure before transplant, overall similar communities were identified after transplant, along with a strong decrease in biodiversity and abundance of numerous species. We link these observations with a larger scale extreme climatic event masking the effects of spatial variability and homogenizing the community structure within the marina. For simplicity, we chose to exclude the reciprocal transplant experiment in this publication and rather focused on the control panels of the two periods (before transplant and after transplant), which allowed to synthesize the most important observations for readers unaccustomed to the principles of the transplant experiment.



## **Chapter III A Effects of Large Mobile Predators (Biotic)**

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## Alien vs. Predator – Influence of the environment and predation on the survival of native and alien ascidian recruits

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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

## 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 & 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 & 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 & Fitter 1996, Jarić & 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 & 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 & Price 1992, Shurin et al. 2002, Heck & Valentine 2007, Riginos & 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 & Crawley 2002, Joshi & 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 & 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 & 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 & 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 & Crawley 2002, Cappuccino & Carpenter 2005, Joshi & 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 & 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 & 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 & Crowe 2015, Chase et al. 2016, Kinsella & 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 & Ebert 2004, Colautti & 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 (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 several fouling species and contributes to the biotic resistance against NIS (Rogers et al. 2016, Rodemann & 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 & 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 & Whitlatch 2004, Law et al. 2009). Recruits may also be more sensitive to environmental stressors (Saloni & 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. 2016a). This species is characterized by rapid growth, a high reproduction rate and a short life cycle, properties 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 & 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 & Herborg 2008, Davis & 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. 2011a, 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 & Brandl 2017). Here, a modified version of this assay was used and 25 x 3 locations x 3 dates = 225 baits were deployed (Fig. 25). 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. Tab. 3) including target congeners proposed by the International Council for the Exploration of the Sea (ICES)

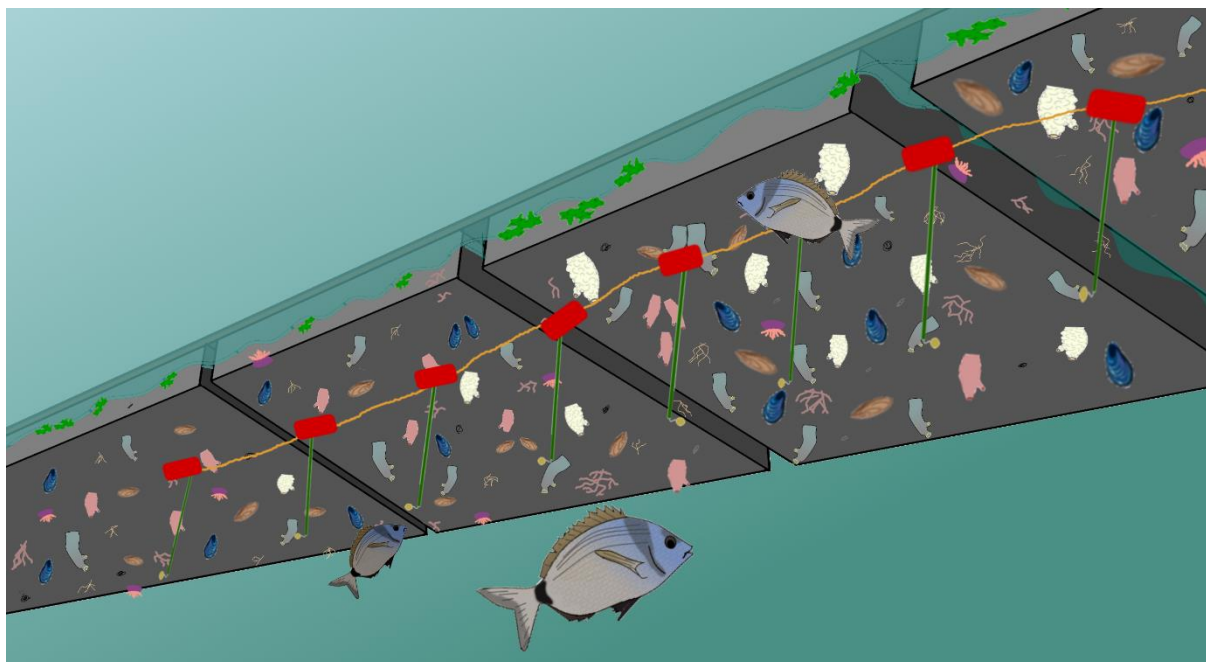


Fig. 25: Illustration of the modified feeding experiment. Baits were deployed for 24h and counted 1, 3, 6 and 24h after deployment.

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. 12. 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. 12, 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 the element 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 & 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 Dunn 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 significant 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 significant 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. 26). 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. 26) was consistently lower than for *S. clava* (red/orange lines, Fig. 26;  $p < 0.001$ ). 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.

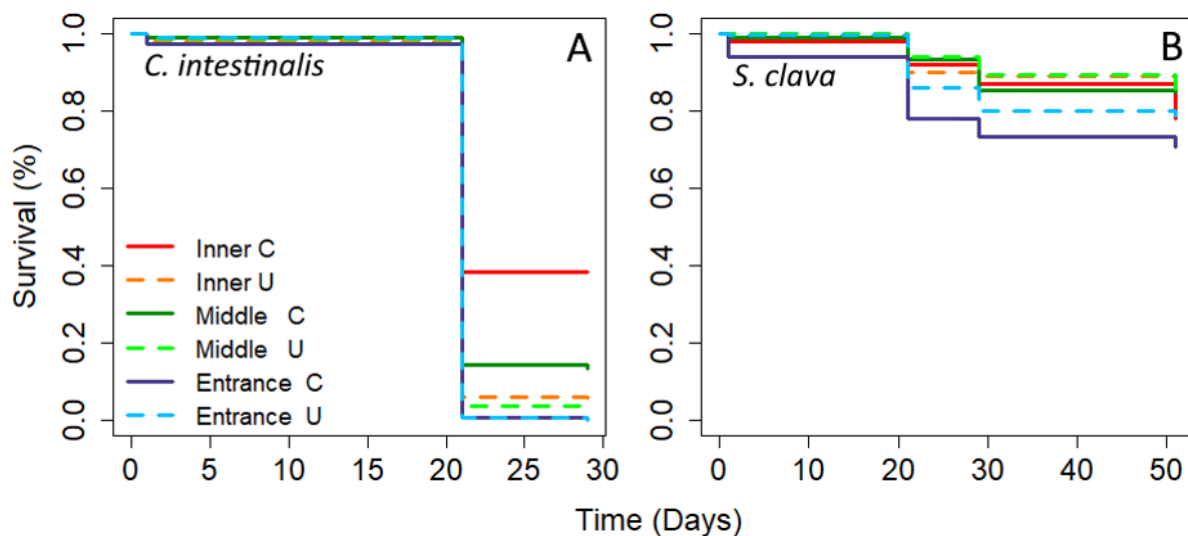


Fig. 26: Kaplan-Meier survival curves for *Ciona intestinalis* (A) and *Styela Clava* (B) recruits for the Inner, middle and entrance location of the marina. Dashed lines indicate uncaged (U) treatments; solid lines indicate caged (C) treatments. *Ciona* Entrance C and *Ciona* Entrance U overlap. *Styela* Inner U and *Styela* Mid U overlap.

### 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. 27). 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. 27). Differences in



biomass according to site were only identified for uncaged *C. intestinalis* between the inner and middle locations (Dunn Test; adj.  $p < 0.05$ ).

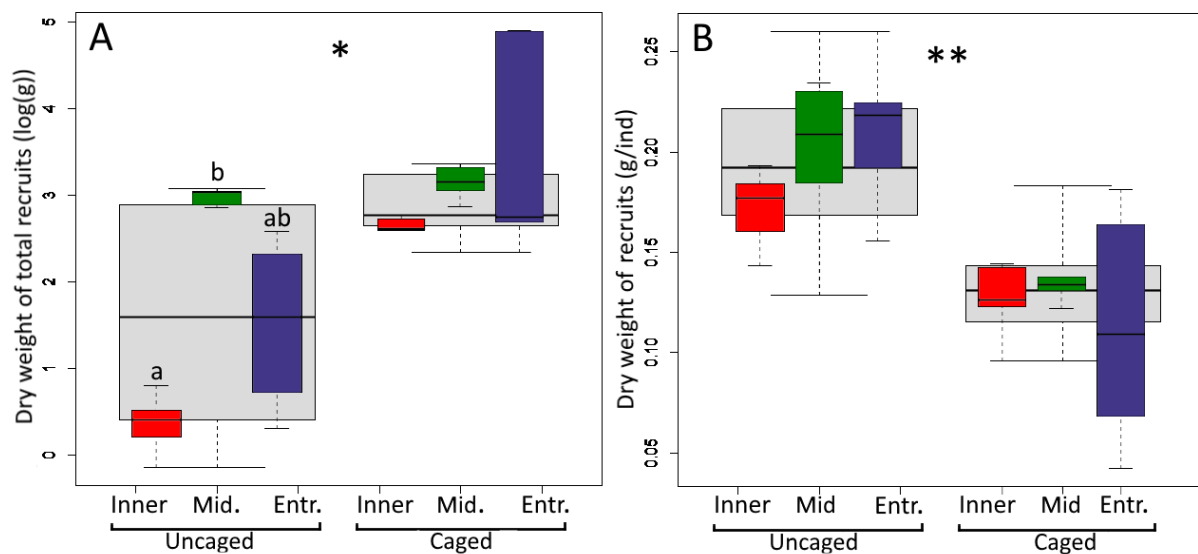


Fig. 27: Log of total dry mass of naturally recruited *Ciona intestinalis* (A) and dry mass per lab grown *Styela clava* individual (B). Significant differences between caged and uncaged treatments: \*  $p < 0.05$  and \*\*  $p < 0.01$  (Wilcoxon test). Significant differences within the uncaged *C. intestinalis* (a-b)  $p < 0.05$  (Dunn test).

## 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. 12; CCME 1999; US EPA 2005). Chrysene, phenanthrene, fluoranthene and fluorene largely exceeded values at which adverse effect on fauna are highly likely (Tab. 12). 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. 12).

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 (Dunn Test, 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. 13; 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 significantly 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* (Tab. 14). 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.

Tab. 12: Persistent Organic Pollutant (POP) contaminants in the sediments of the three study locations. Mean values ( $\mu\text{g}/\text{kg} = \text{ppb}$ ) 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, Tab. 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.

Polycyclic Aromatic Hydrocarbon (PAH) $\mu\text{g}\cdot\text{kg}^{-1}$	Site mean $\pm$ SD			K-W test	CCME		US EPA	
	Inner	Middle	Entrance		ISQG	PEL	T20	T50
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[a]pyrene	324 $\pm$ 69	425 $\pm$ 109	407 $\pm$ 43	ns	<b>88,8</b>	763	<b>69</b>	520
Benzo[a]anthracene	379 $\pm$ 23	343 $\pm$ 15	354 $\pm$ 2	ns	<b>74,8</b>	693	<b>61</b>	470
Benzo[g,h,i]perylene	407 $\pm$ 79	212 $\pm$ 51	283 $\pm$ 8	*			<b>67</b>	500
Benzo[b]fluoranthene	700 $\pm$ 67	651 $\pm$ 141	880 $\pm$ 232	ns			<b>130</b>	1110
Benzo[k]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[a,h]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-cd]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 Biphenyl (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	*				

Tab. 13: 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, Tab. 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. Gray text for concentrations: NS differences between sites (not integrated into COX models). Bold text for reference values: threshold exceeded.

Pollutant		Site mean ± SD			K-W test	CCME		US EPA		Guerra-García et al. 2021 Quality category
		Inner	Middle	Entrance		ISQG	PEL	T20	T50	
Metallic Trace Elements (MTE) mg.kg <sup>-1</sup>	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

Tab. 14: 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 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 p value below 0.05
Predation	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 : Caging	<i>Ciona intestinalis</i>	<b>0,004</b>	<b>0%</b>	<b>25%</b>
		<i>Styela Clava</i>	-0,01	-1%	6%
		<i>Ciona intestinalis</i>	<b>-0,01</b>	<b>-1%</b>	<b>52%</b>
	<i>Styela Clava</i>	0,02	2%	10%	
PAHs	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%
PCB	tPCB	<i>Ciona intestinalis</i>	0,00	0%	9%
		<i>Styela Clava</i>	0,00	0%	6%
Pesticides	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%
MTE	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%

## 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 & Whitlatch 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 & Wetthey 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 & 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 of *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 & 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. 2011a, 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 & 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 & Pawlik 2002, Koplovitz & 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 & Ryland 1994, Koplovitz & 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 & 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. 28). 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.

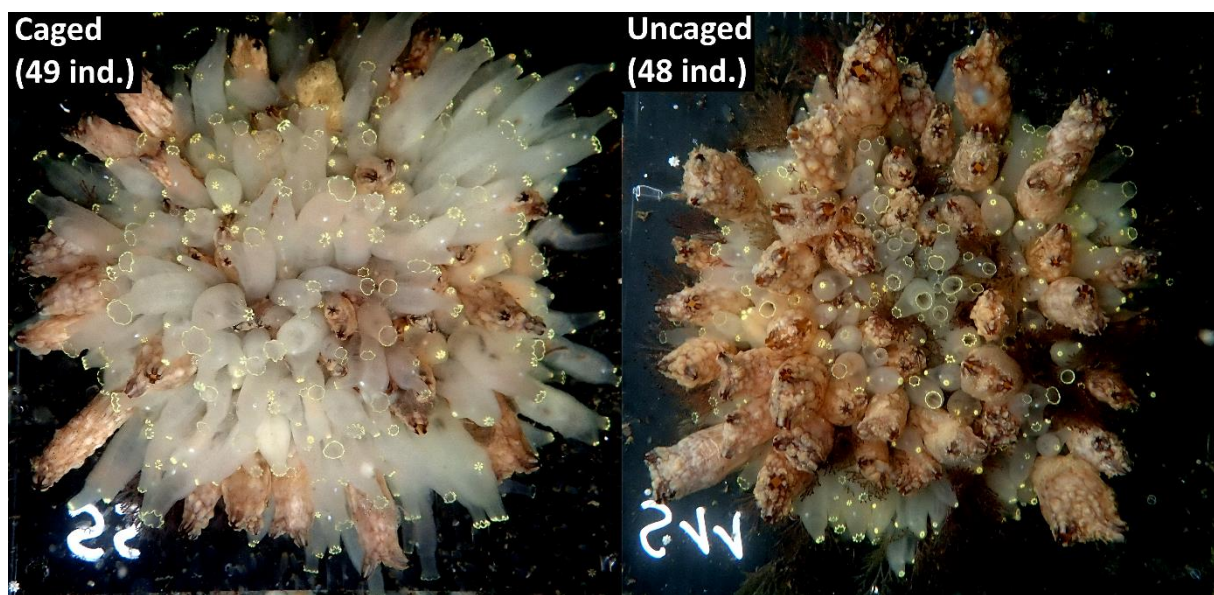


Fig. 28: Caged and uncaged Petri dish of *Styela clava* recruits after 50 days in the field. Both panels originating from the inner location of the marina. Natural recruits of *C. intestinalis* are more visible (larger, more abundant) in the caged treatment. Individual number refers to final *S. clava*.

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 & Crawley 2002, Kincaid & 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. 2011a, 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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## Supplementary Material

### Alien vs. Predator – Influence of the environment and predation on the survival of native and alien ascidian recruits

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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

### Detailed POP contaminant assessment method

Here we would like to further detail the process of POP analyses conducted on the sediment samples taken for our study. The PCB determination focused on 33 individual congeners (IUPAC Nos: 17, 18, 28, 31, 33, 44, 49, 52, 70, 74, 82, 87, 95, 99, 101, 105, 110, 118, 128, 132, 138, 149, 151, 153, 156, 170, 177, 180, 183, 187, 191, 199, 201; Sup. Tab. 3) in each sample. This list contains the seven target congeners (PCBs 28, 52, 101, 118, 138, 153, 180) 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). Care was taken to include representatives of major PCB chlorination classes in the environment (tri- to Octo-chlorinated) to have a wide congener range for the spatial comparison of congener profiles. Besides PCBs, the 16 selected pesticides were pp'-DDT, pp'-DDD, pp'-DDE, isodrin, heptachlor, heptachlor-epoxides ( $\alpha$ - and  $\beta$ -isomers), dieldrin, aldrin, lindane, cis and trans-chlordane, endosulfan ( $\alpha$ - and  $\beta$ -isomers), methoxychlor and diazinon (Tab. 1). The 16 PAH congeners defined by the US Environmental Protection Agency (USEPA) priority list, naphthalene (Nap), acenaphthylene (Ace), acenaphthene (Ac), fluorene (Flu), phenanthrene (Phe), anthracene (An), fluoranthene (Fluo), pyrene (Pyr), benzo[a]anthracene (Bean), chrysene (Chr), benzo[b]fluoranthene (BB), benzo[k]fluoranthene (BK), benzo[a]pyrene (BaPy), benzo[g,h,i]perylene (Bepe), dibenzo[ah]anthracene (DiBe), and indeno[1,2,3-cd]pyrene (InPy), were focused on (Sup. Tab. 3; US EPA 2014).

The analytical method for PCBs and pesticides is fully described by Wafo et al. (2006). Freeze-dried samples (~1 g) were hexane-extracted in Whatman cellulose thimbles (22-80 mm, No. 350211, Schleicher & Schuell) during 16 h. The extracts were reduced by evaporation to a small volume (2mL), and then subjected to clean-up, with concentrated sulfuric acid by the method described by Murphy (1972), followed by desulfuration with TBA-Sulfite Reagent (Jensen et al. 1977). Each extract was then submitted to fractionation by liquid chromatography on a silica–alumina column (Wells et al. 1985). The fraction (I) contains PCBs and the pesticides: Aldrin, pp'-DDE, Diazinon and Heptachlor (50%), while fractions II and III contained Heptachlor (50%) and pp'-DDT; ( $\gamma$ -HCH), pp'-DDD, Dieldrin and Heptachlor epoxide. Evidence of pp'-DDT and metabolites (pp'-DDD, pp'-DDE) was confirmed by dehydrochlorination with alcoholic potassium hydroxide in selected samples. The PCBs were analyzed by gas chromatography (GC) with an Agilent Technologies 7890B coupled with Mass Spectrometers (SMD 5977A). The column was an Rxi-XLB RESTEK (60 m length x 0.25 mm i.d. x 0.25 $\mu$ m film thickness). The carrier gas was helium, held at a constant flow rate of 1 mL min<sup>-1</sup>. The column initial 60°C; hold time 0.1; PRGM1 temp. 160°C rate 10°C min<sup>-1</sup>, hold time 0.00 min, total time 10.1; PRGM2 temp. 280°C rate 2°C min<sup>-1</sup>, hold time 10 min, total time 80.1; Samples were injected in the splitless mode at 250°C and the injector was purged with helium after 0.1 min. The transfer line was held at 280°C.

Variation in the MSD response was corrected by daily calibration with a mixture prepared from standard solutions of the 33 analyzed PCB congeners and Pesticides (AccuStandard Inc., New Haven, CT, USA). Each targeted compound was identified based on the retention time and the mass spectrum from chromatogram of standard solutions acquired in full scan mode. Quantification was then performed in the MS in SIM mode for better selectivity. The same column was used to analyze Pesticides compounds (program: from 60 to 160°C at 10°C min<sup>-1</sup> and from 160 to 280°C at 2°C min<sup>-1</sup>). Total run time: 72 min. Injector and transfer line temperature were 250 and 280°C respectively. The carrier gas was helium. A standard mixture containing all pesticides in this study was used for calibration.

The variation in the response of the MS detector was corrected by a daily calibration with a standard solution of PCBs congeners, and for pesticides. The sample response was matched to that of the standard solution by dilution or concentration of the sample. The detection limits (DL, in ng g<sup>-1</sup> dry mass) are 0.01 ng g<sup>-1</sup> for PCB congeners, 0.2 ng·g<sup>-1</sup> for dieldrin and  $\alpha$ -endosulfan, for aldrin, diazinon,  $\beta$ -endosulfan, endrin, heptachlor,  $\gamma$ -HCH, pp'-DDT, pp'-DDE and pp'-DDD, the DL is 0.1 ng·g<sup>-1</sup> and 0.01 ng·g<sup>-1</sup> for heptachlor epoxide ( $\alpha$ - and  $\beta$ -isomers) and chlordane.

In all cases, for each batch, a blank and a certified material (RTC), was systematically introduced to validate the obtained results. For quality insurance and quality control, 7 IUPAC congeners (28, 52, 101, 118, 138, 153 and 180) were analyzed as well as all the pesticides detailed previously. The results obtained for the reference materials were used to plot control charts and to decide upon acceptance or rejection of the data produced for each sample batch. Rejected batches were reanalyzed. Five replicated RTC were conducted on the same day, in order to determine the average recovery for each PCBs' congener and each pesticide: results for the PCBs' congeners varied from 89 to 96 % and from 85 and 101 % for pesticides. Recoveries for the seven ICES congeners and pesticides agreed with the global results.

PAHs were determined following previously described method (Sarrazin et al. 2006, Ratier et al. 2018, Dron et al. 2019). Samples (~0.5 g) were extracted with 20 mL acetone in an ultrasonic bath (JP Selecta). An aliquot of the supernatant was filtered through a glass microfiber filter (pore size 2.7  $\mu$ m, Grade GF/D, Whatman). Then, 15 mL of ultrapure water were added, and the solution was passed through a 1-g C18 cartridge (6 mL volume, Bond Eluted, Agilent). The PAH congeners were eluted with 3  $\times$  1 mL acetone followed by 2  $\times$  1 mL methanol.

The final extracts were analyzed by GC (Agilent Technology 6890 N, equipped with a splitless injector coupled to mass spectrometry (MS), by a gas chromatograph (GC) equipped with a deactivated fused silica guard column (Agilent Technologies, 5 m, 0.25 mm i.d.) and a fused-silica capillary Phenomenex

ZB-50 (30 m length, 0.25 mm i.d., 0.25 mm film thickness) and coupled with a Mass Spectrometer (MSD). The carrier gas was helium, held at a constant flow rate of 1 mL min<sup>-1</sup>. Samples were injected in the splitless mode and the injector was purged with helium after 1 min. The transfer line and the injector were held at 280 °C. The oven temperature was programmed as follows: 100°C during 1min. PRGM1 from 100 to 240°C at 10°C min<sup>-1</sup>, PRGM2 from 240 to 280°C at 1.5 °C min<sup>-1</sup>; hold time 15 min.

Each targeted PAH was identified based on the retention time and the mass spectrum from chromatogram of standard solutions acquired in full scan mode. Quantification was then performed in the SIM mode for better selectivity. Variation in the MSD response was corrected by daily calibration with a mixture prepared from standard solutions of the 16 analyzed PAH compounds (AccuStandard Inc., New Haven, CT, USA). Each targeted compound was identified based on the retention time and the mass spectrum from chromatogram of standard solutions acquired in full scan mode. Quantification was then performed in the MS in SIM mode for better selectivity. The analytical precision determined by replicate injections of PAH standards indicated maximum error of ±10% and average standard deviation of ±5%. Field blanks, laboratory blanks, matrix spikes and duplicates were included, and PAH external standards added. The validation results were carried out by analyzing the certified material IAEA-451 (Gafrarium tumidum homogenate) within the real sample sequences. In each batch, a blank and a certified material, were systematically introduced in order to validate the results obtained. For quality insurance and quality control, each compound was analyzed as detailed previously. The results obtained for the reference materials were used to plot control charts and to decide upon acceptance or rejection of the data produced for each sample batch. Rejected batches were reanalyzed. Results are expressed in ng.g<sup>-1</sup> or µg kg<sup>-1</sup> of dry weight.

Sup. Tab. 3: Full list of contaminant concentrations for each site. Differences tested by Kruskal-Wallis test.

Pollutant	Site mean ± SD			K-W test	
	Inner	Middle	Entrance		
Polycyclic Aromatic Hydrocarbon (PAH) µg.kg <sup>-1</sup>	Acenaphthene	39 ± 8	34 ± 2	34 ± 1	
	Acenaphthylene	51 ± 17	66 ± 8	61 ± 22	
	Anthracene	59 ± 8	52 ± 12	45 ± 14	
	Benzo[ <i>a</i> ]pyrene	324 ± 69	425 ± 109	407 ± 43	
	Benzo[ <i>a</i> ]anthracene	379 ± 23	343 ± 15	354 ± 2	
	Benzo[ <i>g,h,i</i> ]perylene	407 ± 79	212 ± 51	283 ± 8	*
	Benzo[ <i>b</i> ]fluoranthene	700 ± 67	651 ± 141	880 ± 232	
	Benzo[ <i>k</i> ]fluoranthene	328 ± 72	413 ± 48	537 ± 78	
	Chrysene	943 ± 185	1051 ± 61	1192 ± 173	
	Dibenzo[ <i>a,h</i> ]anthracene	84 ± 19	70 ± 20	77 ± 19	
Fluorene	725 ± 19	567 ± 43	642 ± 56	*	
Continued on next page					

		Sup. Tab. 3 continued			
Polycyclic Aromatic Hydrocarbon (PAH) $\mu\text{g}\cdot\text{kg}^{-1}$	Fluoranthene	1532 ± 430	1049 ± 71	1829 ± 268	
	Indeno[1,2,3- <i>cd</i> ]pyrene	701 ± 106	713 ± 302	843 ± 313	
	Naphthalene	232 ± 32	239 ± 37	344 ± 103	
	Phenanthrene	1629 ± 363	1154 ± 66	1996 ± 239	
	Pyrene	545 ± 8	510 ± 23	556 ± 17	
Polychlorinated Biphenyl (PCB) $\mu\text{g}\cdot\text{kg}^{-1}$	cb17	2,77 ± 0,1	1,49 ± 0,25	2 ± 0,24	*
	cb18	18,35 ± 2,14	8,02 ± 0,41	10,59 ± 1,28	*
	cb28	20,15 ± 4,08	11,05 ± 0,66	12,17 ± 1,37	
	cb31	5,54 ± 0,88	4,12 ± 0,14	4,56 ± 0,05	*
	cb33	14,05 ± 0,55	11,99 ± 0,76	12,87 ± 0,6	*
	cb44	13,45 ± 0,29	10,09 ± 0,44	13,49 ± 1,16	
	cb49	12,69 ± 1,05	8,53 ± 0,73	10,56 ± 0,37	*
	cb52	20,17 ± 1,58	15,94 ± 0,64	18,38 ± 0,4	*
	cb70	27,3 ± 2,12	23,86 ± 1,08	27,17 ± 1,02	
	cb74	27,7 ± 1,4	22,83 ± 0,99	26,36 ± 0,32	*
	cb82	1,83 ± 0,08	1,54 ± 0,08	1,81 ± 0,03	
	cb87	22,22 ± 1,15	17,71 ± 0,95	20,16 ± 0,69	*
	cb95	7,12 ± 0,21	7,4 ± 0,66	6,9 ± 0,45	
	cb99	31,4 ± 0,07	25,16 ± 0,39	29,3 ± 1,02	*
	cb101	29,03 ± 0,93	24,6 ± 0,93	27,95 ± 0,73	*
	cb105	1,96 ± 0,07	1,39 ± 0,04	1,75 ± 0,1	*
	cb110	40,69 ± 0,18	42,57 ± 1,24	35,68 ± 1,45	*
	cb118	12,79 ± 0,24	11,9 ± 0,37	10,55 ± 0,28	*
	cb128	17,94 ± 0,89	15,09 ± 0,43	20,82 ± 0,48	*
	cb132	5,96 ± 0,46	5,52 ± 0,29	6,14 ± 0,08	
	cb138	31,27 ± 0,83	23,7 ± 0,92	28,68 ± 0,98	*
	cb149	25,83 ± 0,34	22,79 ± 2,03	23,06 ± 0,95	
	cb151	15,86 ± 0,81	14,68 ± 0,46	14,05 ± 0,51	
	cb153	27,83 ± 1,97	24,74 ± 1,6	27,84 ± 0,6	
	cb156	19,36 ± 2,1	17,25 ± 2,57	17,8 ± 1,17	
	cb170	20,57 ± 1,27	15,84 ± 0,99	17,35 ± 1,08	*
	cb177	15,34 ± 0,62	14,28 ± 1,16	19,67 ± 1,11	
cb180	23,33 ± 0,64	21,01 ± 0,79	21,93 ± 0,22	*	
cb183	14,89 ± 0,69	14,66 ± 0,53	13,56 ± 0,35		
cb187	19,63 ± 0,26	18,72 ± 1,22	17,62 ± 0,4		
cb191	25,07 ± 1,52	20,72 ± 1,06	21,4 ± 0,25		
cb199	4,97 ± 0,33	7,33 ± 3,12	4,98 ± 0,58		
cb201	25,73 ± 2,94	19,53 ± 1,92	16,76 ± 2	*	
tPCB	602,79 ± 5,93	506,03 ± 12,4	543,9 ± 2,54	*	
Pesticides $\mu\text{g}\cdot\text{kg}^{-1}$	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	
	diazinon	0,57 ± 0,11	0,83 ± 0,28	1,46 ± 0,12	*

Continued on next page

		Sup. Tab. 3 continued			
Pesticides $\mu\text{g}\cdot\text{kg}^{-1}$	dieldrin	1,11 ± 0,1	1,23 ± 0,04	2,19 ± 0,3	*
	pp'-DDD	10,65 ± 0,67	8,41 ± 1,09	2,53 ± 0,29	*
	pp'-DDE	17,62 ± 0,48	22,11 ± 0,32	15,55 ± 1,74	*
	pp'-DDT	0,62 ± 0,1	0,14 ± 0,03	0,1 ± 0,05	*
	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	
	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	
	methoxychlor	7,43 ± 1,28	5,19 ± 0,21	2,52 ± 0,22	*
	Metallic Trace Elements (MTE) $\text{mg}\cdot\text{kg}^{-1}$	Al27(MR)	29207 ± 3825	26647 ± 9596	23125 ± 12905
As75(HR)		14 ± 3	11 ± 3	9 ± 5	
Co59(MR)		5,78 ± 0,58	5,5 ± 1,15	4,72 ± 2,65	
Cr52(MR)		57 ± 7	51 ± 11	44 ± 25	
Cu63(MR)		84 ± 14	45 ± 10	33 ± 19	**
Fe56(MR)		13569 ± 1427	12639 ± 2980	10643 ± 6037	
Mn55(MR)		150 ± 22	154 ± 29	134 ± 74	
Ni60(MR)		18 ± 2	17 ± 4	14 ± 8	
Pb208(LR)		85 ± 12	48 ± 14	46 ± 25	*
Ti47(MR)		2283 ± 291	2285 ± 506	1923 ± 1094	
V51(MR)		90 ± 11	76 ± 19	65 ± 37	
Zn66(MR)	236 ± 89	178 ± 77	111 ± 60	*	





## **Chapter III B Effects of Extreme Climatic Events (Abiotic)**

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## Dead Heat - Heat waves can contribute to environmental homogenization with few winners in marine urban communities

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**Abstract.** Urbanization of coastal habitats, often exemplified by harbors and marinas, has led to various ecological paradigms, questioning the functioning of these new ecosystems. In the present study we investigated, in a large Mediterranean harbor, whether spatial variation of pollution is present and if this variability drives the structure of the sessile community. We hypothesized locations to have significantly different communities, based on the assumption on the occurrence of environmental gradients of pollution that would constitute selective filters. Three distinct community types were identified in June, coinciding with spatially variable contaminants in sediments. We observed an unexpected shift of the community between June and August associated to a sharp decrease in biodiversity and a decline of most species, masking the effects of local variation and thus leading to the homogenization of the biodiversity within the harbor. This shift may be caused by three successive heatwaves (the longest lasting 13 days at over 25°C, and 28°C at the thermal peak) which might have caused a die-off in the harbor communities, regardless of location. In the dead-heat race of post disturbance recruitment and proliferation, the few winners may thus profit from small advantages, allowing them to shape the community's evolution and permitting introduced species to gain a foothold.

**Keywords:** fouling communities, harbor, Marina, local variation, pollution, climate, introduced species

## Introduction

Urbanization of coastal habitats has led to various ecological paradigms, questioning the functioning of these new ecosystems (Bulleri 2006, Bulleri & Chapman 2010). At the forefront, harbours and marinas have been used as models, representative of marine urbanisation. A particular focus has been accorded to the role of introduced species, established and abundant in these environments (Mineur et al. 2012). The number of species introductions rose exponentially since the beginning of marine trade traffic, a trend which will continue in the coming decades (Levine & D'Antonio 2003, Seebens et al. 2016, Carrasco et al. 2017, Sardain et al. 2019). Non Indigenous Species (NIS), if they become invasive, can have high ecological impacts, completely restructuring the ecosystems which can lead to the loss of biodiversity and ecosystem services (Pejchar & Mooney 2009, Johnston et al. 2015b, Walsh et al. 2016). This loss of services and the investment to combat these species can also cause severe economic impacts, ranging from hundreds of millions to tens of billions dollars for individual countries (Lovell et al. 2006, Olson 2006, Jardine & Sanchirico 2018).

Artificial structures constitute a new substratum to be colonized by sessile fauna, but diverging from natural habitats with distinct communities and altered diversity (Glasby et al. 2007, Mineur et al. 2012, Oricchio et al. 2016b, Chan & Briski 2017). Diversity at all scales is generally regarded as a contributor to biotic resistance against introduced species (Elton 1958, Arenas et al. 2006, Megina et al. 2016, Gestoso et al. 2018). Associated with the proximity to the primary vector of species introductions (ships' hull and ballast waters) it makes these substrates highly susceptible to marine introduced species (Glasby et al. 2007, Megina et al. 2016, Oricchio et al. 2016b, Ferrario et al. 2017). High ambient pollution in Persistent Organic Pollutants (POPs), Polycyclic Aromatic Hydrocarbons (PAHs) and Elemental Trace Metals (ETMs) constitutes strong selective filters which profoundly affect local communities, potentially favouring introduced species and leading to strong environmental patchiness (Je et al. 2004, Oricchio et al. 2016b, Kenworthy et al. 2018b). Copper, which is used as active component in many antifouling coatings of ships, has been shown to be a major selective agent in harbor environments and seems to favor introduced species, due to their higher tolerance (Piola et al. 2009, Canning-Clode et al. 2011, Osborne & Poynton 2019). This selective pressure can even lead to differential resistance among populations of the same introduced species (Piola & Johnston 2006b). Local adaptation as a response to such selective pressure, can however be impaired by larger scale disturbances exerting identical pressure on the studied environments, favoring originally genotypes not linked to the original pressure which can homogenize the local populations (Kawecki & Ebert 2004, Colautti & Lau 2015). As such disturbance, large scale climatic variation and extreme climatic events can affect local communities (Sorte et al. 2010a, Astudillo et al. 2016, Chang et al. 2018). Heat waves have particularly been shown to have severe consequences on marine communities and their

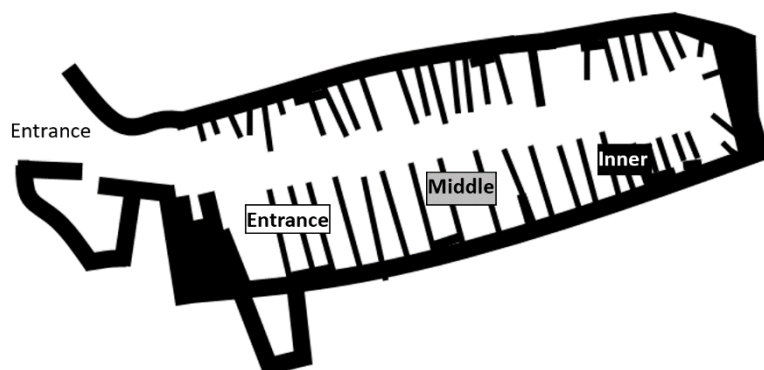
ecosystem services (Garrabou et al. 2009, Pansch et al. 2018, Lonhart et al. 2019, Smale et al. 2019). In many cases, introduced species seem to expose higher thermic resistance than native species (Sorte et al. 2010a, Kelley 2014, Lejeusne et al. 2014, Kenworthy et al. 2018a). This resistance may be explained by rapid molecular responses to stressors (Zerebecki & Sorte 2011, Marie et al. 2017). It is yet however unknown how extreme weather events act on local adaptation and community structure in harbors where communities include a significant higher proportion of introduced species compared to other marine habitats.

Previous studies showed that, in some harbors, pollution may be organized as gradient with maximal pollution in the inner parts of the harbor and less strong pollution at the entrance. Communities are organized along these gradients and display thus high intra-harbor variability, that can be greater than inter-harbor variability when comparing the same functional areas (Je et al. 2004, Kenworthy et al. 2018b). In the present study we investigated, in a large Mediterranean harbor, how spatial variation of pollutants drives the structure of the sessile community. Due to the almost absence of tide-induced hydrodynamism and the higher frequency of larger scale climatic events like heat waves, the processes acting upon such a Mediterranean harbor might be profoundly different from the ones previously reported in cold-temperate harbors (*e.g.*, Je et al. 2004; Kenworthy et al. 2018b).

## Material and Methods

### Study site

We focused on the Vieux Port de Marseille, a recreational marina with approximately 3200 boat mooring and zones dedicated to careening activities, artisanal fisher boats and short distance ferry transports. The high number of boats and the multiplicity of maritime activities renders this site a key example of human disturbance in anthropized habitats. Within this harbor three locations were chosen to show intra-harbor variability of the environment and the associated community. The locations were oriented on a hypothetic gradient of anthropic disturbance from the entrance to the inner part of the harbor (Fig. 29; Je et al. 2004; Kenworthy et al. 2018b).



*Fig. 29: Site of the Vieux Port de Marseille. The three study locations are indicated.*

### **Experimental design**

At each location, 10 panels (0.2 x 0.2 m) were installed at 1 m depth in April 2019. Panels were sufficiently spaced (> 0.4 m) to be considered independent for recruitment. After two months (65 days, June 2019), allowing sessile communities to settle, all panels were photographed and 5 panels per location were randomly sampled for laboratory analysis. After two additional months (70 days more, August) the remaining 5 panels per location were sampled for laboratory analysis.

The photos of the panels were used to verify whether there was homogeneity of the community settled in all panels within each location or if large stochasticity was present. They were analyzed by excluding the outermost centimeter of the panels to avoid an edge effect. All photos were analyzed by one observer using a 144 point stratified random-point overlay ( $\sim 0.4$  points  $\text{cm}^{-2}$ ) allowing an optimal resolution for species contributing to at least 5% cover (Taormina et al. 2020b).

For both dates, the sampled panels were additionally analyzed in the laboratory. A 144-point grid was superposed on the panels (stratified point design) and at each point all species were counted. If one species superposed another, both were noted to account for epibionts as well as the different strata of the community. Species were identified to the lowest taxonomic level possible (Riedl 1983, Zabala & Maluquer 1988, Hayward & Ryland 1995, Brunetti & Mastrototaro 2017a).

### **Contaminants and Temperature**

Per location, three surface sediment samples of approximately 0.4 kg were taken for Persistent Organic Pollutant analysis, including PolyChlorinated Biphenyls (PCBs), Polycyclic Aromatic Hydrocarbons (PAHs) and the most frequent pesticides (see Tab. 15). Five more were sampled for quantifying Metallic Trace Elements (MTEs). All were sampled by a diver underneath the settlement plates (depth ranged from 2-7 m). Samples were frozen and lyophilized before analysis.

The PCBs and pesticides assessment method has been described in detail in the supplementary material of Chapter III A and in Wafo et al. (2006). We quantified 33 individual PCB congeners which include molecules targeted by the International Council for the Exploration of the Sea (ICES). The targeted pesticides can be seen in Tab. 15 PAH quantification of all 16 PAH targeted by the US Environmental Protection Agency (USEPA) priority list (Tab. 15, US EPA 2014), was conducted following Sarrazin et al. (2006), Ratier et al. (2018) and Dron et al. (2019) and is described in detail in the supplementary material of Chapter III A.

Samples for Metallic Trace Elements (MTE) quantification were dissolved in a three-acid solution (HCL, HN03, HF). Analysis was conducted with a High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS; Jacquet et al. 2021).

Water temperature at 1 m depth was logged at each location with HOBO® (Onset®) TidbiT v2 Water Temperature Data Logger which was attached to close to the settlement plates. We used a 15-minute measure interval for the whole duration of the experiment (135 days).

### **Statistical analyses**

Contaminant data were compiled in a table with the individual contaminant name, its family, and its mean concentration for all three locations along with standard deviation. We used a Kruskal-Wallis test (R version 4.0.3; R Core Team 2020) to test for significant differences between sites. Reference values given by the Canadian sediment quality guidelines and by the US Environmental Protection Agency are indicated for all contaminants (CCME 1999, US EPA 2005). For MTEs an additional sediment quality category by Guerra-García et al. 2021 is indicated.

All community data were analyzed in the R package 'vegan' (version 2.4-6; Oksanen et al. 2018). To assess whether panels sampled in June and panels left until August were comparable, the photos of all panels in June were compared at each location. The community matrix containing the species cover for all panels was submitted to a PERMANOVA, to assess for possible differences that would compromise this comparability, if for instance the panels sampled in June would be significantly different from the panels staying until August at the same location.

After confirming the comparability of panels from the same location with the photo analysis, it was possible to analyze, all together, panels sampled in June and panels sampled in August and treated at the laboratory with a higher taxonomic resolution. The community matrix containing the multi strata cover of all present species for each panel was transformed into a Bray-Curtis dissimilarity matrix used in a nMDS comparing panels from the 3 locations in June and August. Multivariate homogeneity of groups was verified via the 'betadisper' function of the R package 'vegan' (Oksanen et al. 2018). Afterwards, a pairwise PERMANOVA from the 'pairwiseAdonis' R package (version 0.3; Martinez Arbizu 2019) including a Benjamini-Hochberg correction was conducted to spatially and temporally compare individual locations (Benjamini & Hochberg 1995). For each panel, species richness, the Shannon-Weaver and Simpson indices as well as the Pielou evenness were calculated. They were compared among sites at each date using a Dunn test from the 'FSA' R package (version 0.8.32; Ogle et al. 2021) accounting for the Benjamini-Hochberg procedure and they were compared between dates using a Kruskal-Wallis test. SIMPER analysis was conducted to determine the species' contribution to the observed temporal difference (Clarke 1993). The percent covers of the five highest contributing species in the SIMPER analysis were compared between June and August with a Kruskal-Wallis test.

## Results

### Contaminants

All contaminants, for which reference values were present, largely exceeded their Canadian sediment quality guidelines as well as concentrations at which 20% and 50% of sediments tested by the US Environmental Protection Agency would become toxic to model amphipods (Tab. 15; CCME 1999; US EPA 2005). PAHs in the sediments of the Vieux Port de Marseille were highly concentrated. Most notably, Benzo[*a*]pyrene, Benzo[*k*]fluoranthene and Phenanthrene varied significantly between locations (Kruskal-Wallis test,  $p < 0.05$ ) and had their highest concentrations in the middle of the harbor (lowest at the entrance), where their concentrations were exceeding three to four times the concentration at which a 50% probability of toxicity is reached (Tab. 15, US EPA 2005). A similar situation was observed for total PCBs which exceeded these thresholds by orders of magnitude and significantly varied in space (K-W,  $p < 0.05$ ). Seven pesticides varied significantly in space with distinct distribution profiles (Tab. 15; K-W,  $p < 0.05$ ).

Most tested MTEs had significant higher concentrations at the inner location compared to the entrance location (K-W,  $p < 0.05$ ), however concentrations in the middle location were close to the inner concentrations. MTE concentrations were all above the Canadian sediment quality guideline and above the concentration of 20% probability of toxicity and fell within a sediment quality category of moderate for As and Cr and bad for Cu and Pb and Zn (Tab. 16; CCME 1999; US EPA 2005; Guerra-García et al. 2021). The Cu concentration in sediments even exceeded a 80% probability of toxicity at all locations (US EPA 2005).



Tab. 15: Contaminants with significant differences between locations (Kruskal-Wallis test,  $p < 0.05$ ) indicated by \*. 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, Tab. 11; T20: probability of 20% of toxicity among samples; T50: probability of 50% of toxicity among samples). Bold font for reference values: threshold exceeded.

Pollutant	Site mean $\pm$ SD			K-W test	CCME		US EPA	
	Inner	Middle	Entrace		ISQG	PEL	T20	T50
<b>Polycyclic Aromatic Hydrocarbon (PAH) <math>\mu\text{g.kg}^{-1}</math></b>								
Acenaphthene	192 $\pm$ 64.3	230 $\pm$ 33.5	70 $\pm$ 21.4	ns	<b>6.71</b>	<b>88.9</b>	<b>19</b>	<b>120</b>
Acenaphthylene	96.8 $\pm$ 14.4	84.3 $\pm$ 13.2	37.1 $\pm$ 18.4	ns	<b>5.87</b>	128	<b>14</b>	140
Anthracene	660 $\pm$ 75.7	677 $\pm$ 21.3	389 $\pm$ 21.4	ns	<b>46.9</b>	<b>245</b>	<b>34</b>	<b>290</b>
Benzo[a]pyrene	1185 $\pm$ 170	1687 $\pm$ 230	590 $\pm$ 106	*	<b>88.8</b>	<b>763</b>	<b>69</b>	<b>520</b>
Benzo[a]anthracene	1210 $\pm$ 115	1571 $\pm$ 221	838 $\pm$ 127	ns	<b>74.8</b>	<b>693</b>	<b>61</b>	<b>470</b>
Benzo[g,h,i]perylene	665 $\pm$ 35.3	509 $\pm$ 160	369 $\pm$ 48.8	ns			<b>67</b>	<b>500</b>
Benzo[b]fluoranthene	1184 $\pm$ 180	1184 $\pm$ 120	702 $\pm$ 4.6	*			<b>130</b>	<b>1110</b>
Benzo[k]fluoranthene	1523 $\pm$ 275	1971 $\pm$ 242	953 $\pm$ 106	*			<b>70</b>	<b>540</b>
Chrysene	103 $\pm$ 16	174 $\pm$ 77.1	62.9 $\pm$ 14.6	*	<b>108</b>	846	<b>82</b>	650
Dibenzo[a,h]anthracene	1652 $\pm$ 272	2112 $\pm$ 313	1112 $\pm$ 98.6	ns	<b>6.22</b>	<b>135</b>	<b>19</b>	<b>110</b>
Fluorene	580 $\pm$ 31.5	1099 $\pm$ 716	290 $\pm$ 30.3	ns	<b>21.2</b>	<b>144</b>	<b>19</b>	<b>110</b>
Fluoranthene	486 $\pm$ 117	575 $\pm$ 128	207 $\pm$ 23.2	*	<b>113</b>	1494	<b>120</b>	1030
Indeno[1,2,3-c,d]pyrene	570 $\pm$ 20.6	581 $\pm$ 142	494 $\pm$ 80.1	*			<b>68</b>	<b>490</b>
Naphthalene	57.4 $\pm$ 15.3	78 $\pm$ 13	41.2 $\pm$ 12.4	ns	<b>34.6</b>	391	<b>30</b>	220
Phenanthrene	1233 $\pm$ 169	1691 $\pm$ 293	621 $\pm$ 42.9	*	<b>86.7</b>	<b>544</b>	<b>68</b>	<b>460</b>
Pyrene	767 $\pm$ 46.3	962 $\pm$ 144	389 $\pm$ 65.2	ns	<b>153</b>	1398	<b>120</b>	<b>930</b>

Polychlorinated Biphenyl (tPCB) $\mu\text{g.kg}^{-1}$	1252 $\pm$ 6.27	1395 $\pm$ 15.9	455 $\pm$ 7.61	*	<b>21.5</b>	<b>189</b>	<b>35</b>	<b>370</b>
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<b>Pesticides <math>\mu\text{g.kg}^{-1}</math></b>								
aldrin	2.74 $\pm$ 0.22	2.53 $\pm$ 0.14	5.32 $\pm$ 0.36	ns				
Trans_chlordane	1.57 $\pm$ 0.28	1.18 $\pm$ 0.05	2.42 $\pm$ 0.03	*				
cis_chlordane	0.73 $\pm$ 0.22	0.11 $\pm$ 0.04	0.38 $\pm$ 0.21	ns				
tChlordane	2.3 $\pm$ 0.2	1.29 $\pm$ 0.08	2.81 $\pm$ 0.19	*	<b>2.26</b>	4.79		
diazinon	4.3 $\pm$ 0.38	2.92 $\pm$ 0.65	2.09 $\pm$ 0.55	ns				
dieldrin	7.11 $\pm$ 0.09	11.3 $\pm$ 1.44	2.4 $\pm$ 0.26	*	<b>0.71</b>	<b>4.3</b>	<b>0.83</b>	<b>2.9</b>
pp'-DDD	5.33 $\pm$ 0.55	12.3 $\pm$ 1.59	13.1 $\pm$ 1.46	ns	<b>1.22</b>	<b>7.81</b>	<b>2.2</b>	19
pp'-DDE	8.58 $\pm$ 1.39	15 $\pm$ 1.61	17.8 $\pm$ 1.96	*	<b>2.07</b>	374	<b>3.1</b>	100
pp'-DDT	0.66 $\pm$ 0.19	1.82 $\pm$ 0.1	2.55 $\pm$ 0.16	*	<b>1.19</b>	4.77	<b>1.7</b>	11
endosulfan-1	1.83 $\pm$ 0.44	3.74 $\pm$ 0.53	2.26 $\pm$ 0.1	ns				
endosulfan- 2	21.2 $\pm$ 0.94	13.8 $\pm$ 1.26	2.57 $\pm$ 0.17	*				
heptachlor	6.09 $\pm$ 0.54	6.02 $\pm$ 0.43	3.89 $\pm$ 0.28	ns	<b>0.6</b>	<b>2.74</b>		
heptachlor_epoxide_a	23.9 $\pm$ 0.95	15.3 $\pm$ 2.67	5.8 $\pm$ 0.57	*				
heptachlor_epoxide_b	1.6 $\pm$ 0.4	0.84 $\pm$ 0.14	0.47 $\pm$ 0.11	*				
isodrin	5.68 $\pm$ 0.38	5.52 $\pm$ 0.43	8.45 $\pm$ 0.14	ns				
lindane	3.89 $\pm$ 0.34	3.83 $\pm$ 0.28	2.07 $\pm$ 0.4	ns	<b>0.32</b>	<b>0.99</b>		
methoxychlor	5.95 $\pm$ 0.48	15.7 $\pm$ 0.83	16 $\pm$ 1.03	ns				

Tab. 16: Concentrations of Metallic Trace Element (MTE) contaminants in the sediments of the three study locations. Mean values ( $\text{mg.kg}^{-1} = \text{ppm}$ ) with standard deviation. Contaminants with significant differences between locations (Kruskal-Wallis test) indicated by \*  $p < 0.05$  or \*\*  $p < 0.01$ . 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, 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. Bold font for reference values: threshold exceeded.

Metallic Trace Elements (MTE) $\text{mg.kg}^{-1}$	Site mean $\pm$ SD			K-W test	CCME		US EPA		Quality category <sup>1</sup>
	Inner	Middle	Entrace		ISQG	PEL	T20	T50	
Al27(MR)	12927 $\pm$ 3151	8400 $\pm$ 3829	5501 $\pm$ 1881	**					
As75(HR)	29.2 $\pm$ 5.95	31.5 $\pm$ 3.62	18.6 $\pm$ 2.78	*	<b>7.24</b>	<b>41.6</b>	<b>7.4</b>	<b>20</b>	Moderate
Co59(MR)	4.55 $\pm$ 1.65	4.82 $\pm$ 2.13	2.07 $\pm$ 0.2	**					Good
Cr52(MR)	68.6 $\pm$ 14.6	66.4 $\pm$ 9.42	27.8 $\pm$ 4.54	**	<b>52.3</b>	160	<b>49</b>	140	Moderate
Cu63(MR)	611 $\pm$ 111	545 $\pm$ 73.4	279 $\pm$ 103	*	<b>18.7</b>	<b>108</b>	<b>32</b>	<b>94</b>	Bad
Fe56(MR)	12881 $\pm$ 3158	12867 $\pm$ 1357	6859 $\pm$ 2341	*					
Mn55(MR)	164 $\pm$ 33.3	158.2 $\pm$ 13.2	126 $\pm$ 9.19	**					
Ni60(MR)	17.7 $\pm$ 2.76	17.3 $\pm$ 2.36	7.89 $\pm$ 1.49	**			<b>15</b>	47	Good
Pb208(LR)	299 $\pm$ 63.7	262 $\pm$ 26.2	136 $\pm$ 27.5	**	<b>30.2</b>	<b>112</b>	<b>30</b>	<b>94</b>	Bad
Ti47(MR)	1373 $\pm$ 301	1414 $\pm$ 251	723 $\pm$ 129	**					
V51(MR)	68.5 $\pm$ 16.7	70.8 $\pm$ 5.28	28.7 $\pm$ 1.65	**					
Zn66(MR)	665 $\pm$ 151	531 $\pm$ 85.8	198 $\pm$ 18.5	*	<b>124</b>	<b>271</b>	<b>94</b>	<b>240</b>	Bad

## Community

Since we focus on fouling communities with small individuals, we wanted to have the most accurate level possible for species identification and cover assessment. We thus opted for a destructive sampling of communities by sampling five panels for each location in June and five others in August. This however leaves the risk of initial differences between panels of the same location in June subsequently causing differences between panels sampled in June and panels sampled in August. To exclude this risk, we used the photographic analysis of all panels in June to compare the panels that were sampled at this date to the ones that were destined to stay until August. This analysis showed no difference between these panels for any location, showing that panels sampled in August are indeed representative of the evolution of panels sampled in June, allowing us to directly compare them.

The analysis of community data derived from the laboratory analysis of panels in June and August showed a significant variability both in space and time (Fig. 30). A PERMANOVA pairwise comparison showed a systematic difference for each location between June and August ( $p$  adj.  $< 0.05$ ,  $R^2 > 0.75$ ). All sites differed significantly from each other in June ( $p$  adj.  $< 0.05$ ;  $R^2 > 0.54$ ), while they were only significantly different between the inner and the middle site in August ( $p$  adj. = 0.02,  $R^2 = 0.44$ ).

Total percent cover of the community significantly increased from June to August ( $p = 0.002$ , Fig. 31). Significant differences of total cover between locations could only be identified in June between the middle and the entrance site ( $p$  adj. = 0.04). While a total of 28 species were identified during this study, the maximal species richness per panel was 12 species. Diversity indices ranged from 1.09 to 2.58 for the Shannon index, 0.37 to 0.80 for the Simpson index, and Pielou's evenness ranged between 0.39 and 0.85. Significantly higher values of indices were detected for the Shannon, Simpson and Pielou's evenness in June (Dunn test,  $p < 0.001$ , Fig. 32), but not for species richness. For each date, no significant differences of diversity indices were found among locations (Dunn test).

The species most contributing to differences between June and August were identified via the SIMPER analysis. The polychaete annelids Serpulinae (Rioja, 1923), the serpulid worm *Simplaria pseudomilitaris* (Thiriot-Quievreux, 1965), the bryozoan *Bugula neritina* (Linnaeus, 1758) and the colonial ascidians *Botryllus schlosseri* (Pallas, 1766) and *Diplosoma listerianum* (Milne Edwards, 1841) accounted to over 85% of the contrast between June and August (Fig. 33). These species are also the five most abundant species that have a mean cover of more than 5% at both dates. Most of these species suffered a significant reduction of their percent cover between June and August ( $p < 0.01$ , Fig. 34) except the sub-family of the Serpulinae which significantly increased ( $p < 0.001$ , Fig. 34).

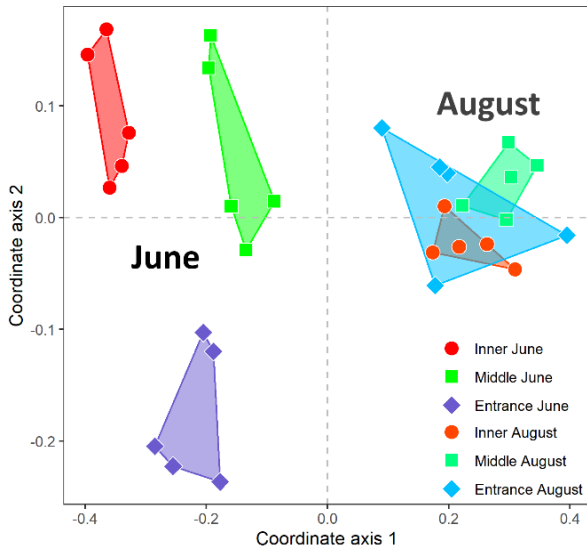


Fig. 30: Nonmetric Multidimensional Scaling (nMDS) of analyzed communities. Communities from a location are systematically different between June and August (PERMANOVA,  $p$  adj.  $< 0.05$ ,  $R^2 > 0.75$ ). Communities differ between locations in June (PERMANOVA,  $p$  adj.  $< 0.05$ ,  $R^2 > 0.54$ ). Inner and Middle differ in August (PERMANOVA  $p$  adj.  $= 0.02$ ,  $R^2 = 0.44$ )

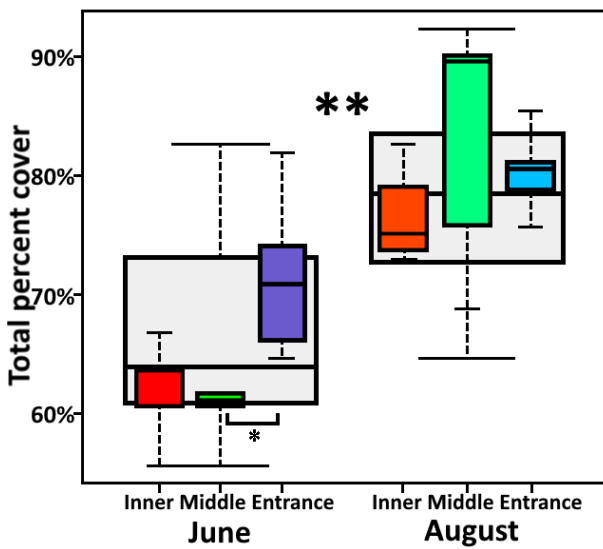


Fig. 31: Spatial and temporal variation of the total percent cover. Significant differences between dates are indicated with big stars (Kruskal-Wallis test). Significant differences between locations are indicated by brackets (Dunn test). \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

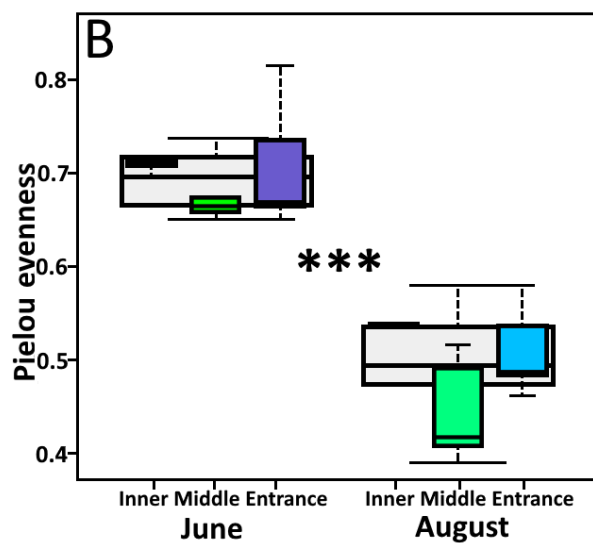
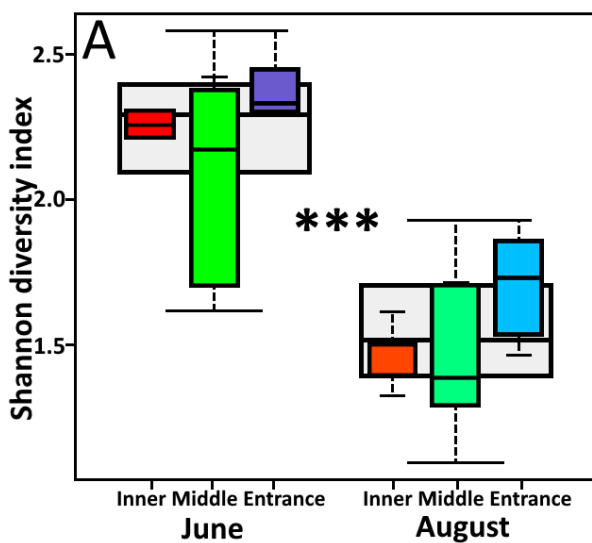


Fig. 32: Spatial and temporal variation of the Shannon-Weiner diversity index (A) and Pielou evenness (B). Significant differences between dates are indicated with big stars (Kruskal-Wallis test). No significant differences between locations within dates. \*\*\*  $p < 0.001$

Temperature

The temperature curve of all three sites reveals a similar trend among locations. Between June and August three major heatwaves can be noted with temperatures exceeding 25°C during 13, 7 and 3 days, respectively, and a maximum temperature of 28°C (Fig. 35).

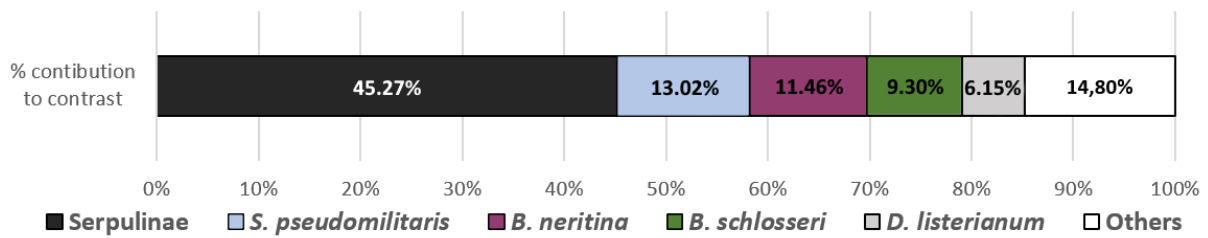


Fig. 33: SIMPER analysis showing the species that contribute the most to the temporal contrast among communities between June and August (percentage, SIMPER analysis).

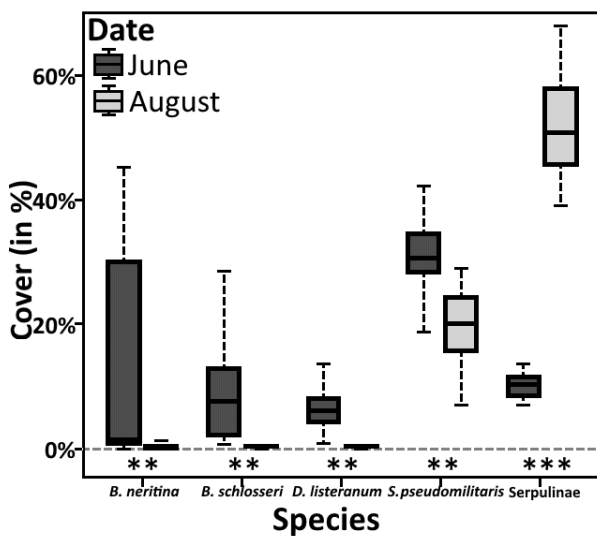


Fig. 34: Comparison of the percent cover of the five most abundant species (which are also the species, most contributing to the contrasts between June and August) between June (dark grey) and August (light grey). Significant differences (Kruskal-Wallis test) between dates for each species are indicated by stars: \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

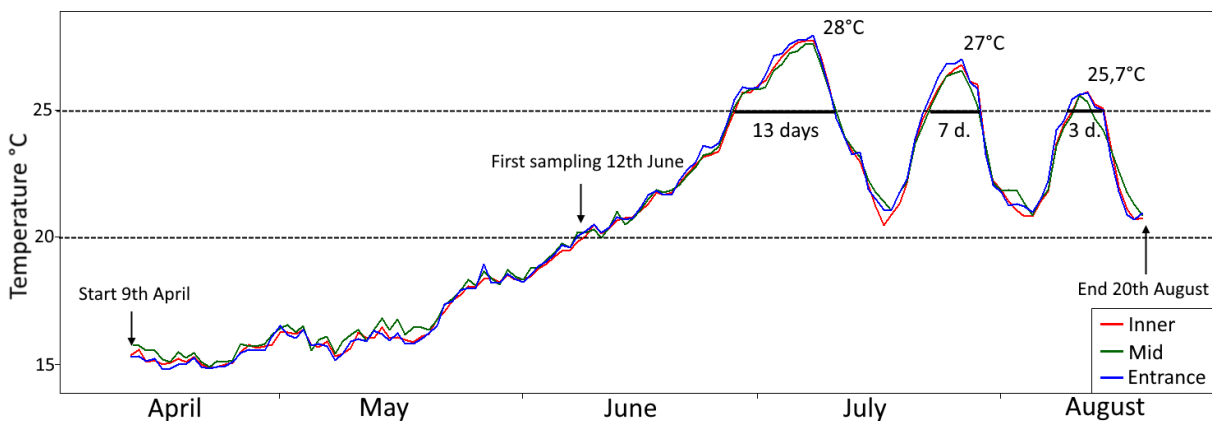


Fig. 35: Temperature curve for each location. Start and sampling dates as well as notable values are indicated.

## Discussion

The present study aimed to describe how the sessile sciaphilic communities of the Vieux Port de Marseille are composed and how they vary in space and in time. We hypothesized different locations in the harbor have significantly different communities, in relation with the occurrence of potential environmental gradients constituting selective filters resulting in local variation as reported from the literature (Je et al. 2004, Kenworthy et al. 2018b). In accordance with these studies, most contaminant concentrations were minimal at the entrance of the harbor, where more exchange with the outer water masses occur. Conversely, we did not observe a clear pollution gradient, as concentrations in the middle and the inner parts of the harbor were similar which might be explained by local hydrodynamic conditions. The community analysis of sampled panels showed a significant difference between all three locations of the harbor in June, with three distinct community types which did not entirely correspond to the patterns observed with the pollution.

Community heterogeneity among locations observed in June was in contrast strongly reduced in August with overall similar communities. In addition to this loss of spatial variability between locations in August, it was possible to observe a strong temporal shift of the communities between June and August. Such evolution of communities with time is nothing unexpected and even normal due to ecological succession (Raja 1959, Gardner & Wear 2006, Loke et al. 2016). This phenomenon is particularly expected for settlement plates as used in the present case (Marraffini et al. 2017). The here observed change in community structure might however not solely be due to temporal succession of species. Such conclusions might arise by focusing on the significant decrease of all diversity indices and the significant decrease of the cover of almost all major species between June and August. Indeed, *Simplaria pseudomilitaris*, *Bugula neritina*, *Botryllus schlosseri* and *Diplosoma listerianum* had their cover significantly reduced. Conversely, calcareous worms of the Serpulinae sub-family are the only group increasing their cover between both periods. However, we were not able to identify most Serpulinae species on the settlement plates, due to their small size making dissection impossible. Some species of this sub-family could however be identified: *Salmacina incrustans* Claparède, 1870, *Hydroides elegans* (Haswell, 1883), followed by *Serpula vermicularis* Linnaeus, 1767 were the most abundant identified Serpulinae while we noted the anecdotal presence of *Hydroides norvegica* Gunnerus, 1768 and *Serpula concharum* Langerhans, 1880.

The observed temporal decrease of the cover of most species (including the dominant ascidians and bryozoan) can be either caused by biotic interactions among the organisms of the fouling community or by an abiotic factor causing mass mortality for these species. Focusing on biotic interactions, the present case might reflect competitive exclusion due to the strong increase of the cover of Serpulinae

(Hardin 1960, Mayfield & Levine 2010). However, while the total species cover increased between June and August, it reached 65%-90% in August with Serpulinae covering 39-68%, which would still leave enough space for other species to grow and thus limit competition for space. Furthermore, Serpulinae are mostly prone species forming calcareous tubes which should vastly limit spatial competition they could exert towards *B. neritina* an erect bryozoan. *B. neritina* could also simply overgrow the calcareous tubes. Equally, encrusting colonial ascidians *B. schlosseri* and *D. listerianum* are unlikely to be competitively excluded by Serpulinae. Both frequently grow on other species meaning they could overgrow the tube worms due to their high competitive ability (Grosberg 1981). They potentially have larvotoxicity towards several fouling species and are avoided by other species' larvae (Teo & Ryland 1995, Dijkstra et al. 2007). The Spirorbinae serpulid *Simplaria pseudomilitaris* is the only major species that occupies a very similar ecological niche as Serpulinae and would be more likely to be competitively excluded by them. Yet, despite the reduction of its cover between June and August, this species seems to be the least affected one since it still covers 7-44% in August. Thus, the observed differences between June and August might not be attributed to biotic interaction among fouling species or to succession, but rather to external abiotic factors impacting the communities at all three locations. This external factor seems to be highly detrimental to *B. neritina*, *B. schlosseri* and *D. listerianum*, negatively affecting *S. pseudomilitaris*, while less affecting Serpulinae, which could increase their cover in this time. A possible explanation could be a larger scale environmental disturbance, affecting all locations of the harbor, changing between June and August. Serpulinae could have a quicker recolonization rate due to their opportunistic nature and/or be more resistant to this disturbance than the other species. The genus *Salmacina* Claparède, 1870 for instance is capable of asexual reproduction by budding and is capable of quickly forming larger pseudo-colonies (Nishi & Nishihira 1994, Pernet 2001). Many of the present Serpulinae species also have gregarious recruiting strategies (Nishi & Nishihira 1994, Walters et al. 1997, Diaz-Castaneda 2000). The other species in the harbor however may also quickly settle and grow which is especially the case for *B. neritina* which can reach a surface cover of over 20% within one week in warm waters (Lord 2017a). Serpulinae may also have a lag period that could last up to 48 days before (re)settling on the substrate (Diaz-Castaneda 2000). This might favor the idea Serpulinae may have resisted a disturbance occurring in the studied harbor that was highly detrimental to the other species. Strong precipitations may be excluded since less than 3 mm of rain were recorded between June and August 2019 in Marseille (Infoclimat, Accessed 30/03/20). Other climatic variables however could better explain the observed shift in the community. Water temperature has been subject to high variations between June and August with three successive heat waves, the longest lasting 13 days over 25°C, and a thermal maximum of over 28°C. Research on the potential impacts of climate change show a drastic impact of rising temperature on communities (Lejeusne et al. 2010, Sorte et al. 2010b, Smale et al. 2011, 2017). High temperature events with surface temperatures

reaching over 26.5°C in 1997, 1999 and 2003 have led to mass mortalities of multiple sessile species in the studied region (Perez et al. 2000, Garrabou et al. 2009, Lejeune et al. 2010). Heat waves, as short term events highly impact community composition in natural and artificial environments (Sorte et al. 2010b, Pansch et al. 2018, Lonhart et al. 2019). Climate change and heat waves may also favor introduced species which might be more resistant to heat (Kelley 2014, Lord 2017a, Kenworthy et al. 2018a). *Hydroides elegans*, one of the most abundant Serpulinae in our samples, has been shown to be highly resistant to temperatures up to 30°C and seems even to profit from higher temperatures due to faster development if this is not negated by other associated variables (Qiu & Qian 1997, 1998). Moreover, the observed high temperatures should be highly detrimental to *B. schlosseri* and *D. listerianum* which show lower survival of recruits and adults at temperatures over 25°C, and highly decrease their competitiveness (Brunetti et al. 1980, Lord et al. 2015, Lord 2017a b). The case of *B. neritina* is more complex to analyze: it has been shown to be very tolerant to high temperatures up to 30°C which is above the temperatures of the observed heatwaves (Hitoshi & Kazutsugu 1984, Lord 2017a b); conversely, another study conducted on US coasts showed a 50% mortality for *B. neritina* between 24.4°C and 26.4°C, but also revealed a high difference (2°C) of the thermal tolerance of this species between the East and West USA coasts (Sorte et al. 2011). Such differences of responses might be explained by local adaptation and/or maternal effects which have been shown to exist in *B. neritina* facing high temperature and copper pollution (Piola & Johnston 2006b, Marshall 2008, Burgess & Marshall 2011, Sorte et al. 2011). Here, the population of *B. neritina* in the Vieux port de Marseille might not be able to cope with the observed temperatures, hence the die-off.

It is likely that the observed differences between June and August are somewhat temperature mediated as it would be concordant with previously cited literature. They could however not be solely due to the temperature itself but also be caused by its combined action with other factors. By increasing the metabolic rate of marine ectotherms, higher temperature may greatly influence their energy consumption and nutrient uptake (Alava et al. 2017). This notably allows them to increase the uptake of POPs and ETMs (Sokolova & Lannig 2008, Alava et al. 2017) which together with the higher temperature can synergistically impact the present organisms (Cabral et al. 2019). This might in the present case even lead to lethal effects on the local fauna especially considering the remarkably high contaminant concentrations that were measured in the harbor sediments. This would however not explain why mortality was homogenous everywhere, while pollutant concentrations in sediment varied in space. Furthermore, some Serpulinae, which were able to increase their population thus suggesting a certain tolerance to the observed disturbance, have been shown to be very sensitive to ETM pollution in their larval stage and larvae avoid settling in zones with high PAHs concentrations



(Paul et al. 1998, Xie et al. 2005, Thilagam et al. 2008). Their increased population would thus challenge the idea of a synergistic effect between ambient pollution and the heat waves.

Here we exposed an unexpected change in the community of the Vieux port de Marseille associated to a sharp decrease in biodiversity and a decline of most species except Serpulinae, eclipsing the effects of local variation of environmental factors and thus leading to the homogenization of the biodiversity within the harbor. Human activities and climate change have led to the worldwide homogenization of ecosystems in natural and urban habitats (Mckinney & Lockwood 1999, Qian & Ricklefs 2006, Clavel et al. 2011, Magurran et al. 2015). Heat waves are predicted to increase with climate change, both in frequency and intensity (Meehl et al. 2000, Holl 2009, Stott 2016). If heat waves show the same detrimental effects on small scale variability of harbor communities as they did in our experiment, biodiversity homogenization could even occur on a smaller scale than previously considered. Regular heat waves might favor altered communities characterized by highly opportunistic and/or resistant species, which in our case were Serpulinae. These species would benefit from the available space and reduced competitiveness after a die-off. With reduced occupied niches these communities could exert less biotic resistance and thus increase their invasibility (Arenas et al. 2006, Bulleri et al. 2016). Introduced species are generally considered to be more tolerant to higher temperatures and temperature variability (Sorte et al. 2010b, Kelley 2014, Lejeune et al. 2014, Kenworthy et al. 2018a). The introduced Serpulinae species *H. elegans*, that has already been identified in previous studies in the Vieux Port de Marseille (Zibrowius 1972, 1973), has for instance settled on our plates. This species might have directly taken advantage of the heat wave and the associated die-off due to its high temperature tolerance and due to its opportunistic nature (Qiu & Qian 1997, 1998, Walters et al. 1997). Heat waves and the associated homogenization could thus benefit twice to introduced species by selecting only species resistant to high temperature (maybe more frequently introduced species) and by driving communities to a state of high invasibility by reducing diversity and occupied niches.

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## **Chapter IV Investigating the Spatial Variability of Fouling Communities in Multiple Marinas with different disturbance intensities**

Draft version for the present manuscript

## Pollution gradients lead to small-scale spatial variability of communities in marinas with different disturbance intensity

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**Abstract.** As eco-evolutionary feedbacks drive species adaptation in anthropic habitats, high disturbance habitats like marinas constitute a particular challenge for local organisms. Artificial structures in marinas are intended to reduce hydrodynamism, resulting in disturbance gradients, often due to pollution. A previous study has been able to demonstrate that at small spatial scale (< 100 m), a disturbance gradient can cause differences in community structure and lead to local adaptation. This result concerns however only one marina and it is yet unclear if the processes observed in this marina may be generalized. The present study aimed to extend the previously established protocol to six diverse marinas, to verify if the results are replicable. We observed an almost systematic difference between the communities of the entrance and inner locations of the marinas. This difference was proportional to the supposed disturbance gradient intensity. A reciprocal transplant experiment allowed us to confirm the observations made from the previous study and to causally link a hypothetical disturbance gradient with community structure in for four out of six studied marinas. In a fifth marina no influence of transplantation was observed, likely due to very low disturbance, which remains consistent with our hypothesis. These results suggest that local variability of community structure in marinas is caused by disturbance gradients and potentially leads to local adaptation in many marinas, even if disturbance is weak or intermediate.

**Keywords:** local adaptation, fouling, pollution, marinas,

## Introduction

Considering the ever increasing impact of humans on ecosystems, understanding how eco-evolutionary feedbacks drive species adaptation in habitats subjected to anthropic disturbance seems crucial (Alberti 2015). Numerous species have been demonstrated to adapt and evolve with anthropic impacts (Roesijadi et al. 1984, Atwell et al. 2012, Donihue & Lambert 2014, Brans et al. 2017, Martin et al. 2021) and adaptation seems to be the major driver of resistance to press type disturbance exerted by urbanization (Nimmo et al. 2015). Marinas constitute paragon models to study adaptation to urban environments, as they combine multiple facets of anthropogenic disturbances (Bulleri 2006, Bulleri & Chapman 2010). Artificial structures in marinas are intended to modify/reduce hydrodynamism leading to increased pollution (Owen & Sandhu 2000, Tolun et al. 2001, Schiff et al. 2007, Mohammed et al. 2011, Aly et al. 2013), often organized along a gradient (Schiff et al. 2007, Ryu et al. 2011, Kenworthy et al. 2018a). Where a disturbance gradient is observed, species and populations with higher resistance may be selected along this gradient (Nimmo et al. 2015). Local differences of selective pressures may even lead to local adaptation if genetic drift and dispersion are low (Kawecki & Ebert 2004). This might include Non-Indigenous Species (NIS) which seem favored in disturbance regimes (Hobbs & Huenneke 1992, Altman & Whitlatch 2007, Bulleri et al. 2016) and which seem to expose higher resistance to pollutants (Piola & Johnston 2006a, Dafforn et al. 2008, Osborne & Poynton 2019). This may explain why NIS tend to be more prevalent in the inner parts of marinas, even if this prevalence may be variable in time (Chapter I, Chapter II, Kenworthy et al. 2018b).

We have been previously able to expose that community structure correlates with disturbance gradients in marinas (Chapter I, Chapter II, and Chapter III B July), an observation which has been made by multiple authors in the past (Je et al. 2004, Ryu et al. 2011, Kenworthy et al. 2018b). Our reciprocal transplant experiments revealed the causal relationship between a disturbance gradient and community structure in the Château marina (Brest, France), which is associated with local adaptation (Chapter II). Exposing local adaptation on such small spatial scale has far reaching implications when considering contemporary evolution of species to anthropic influences. Understanding the processes of local adaptation might help understanding contemporary evolution in introduced species (Colautti & Lau 2015). Accordingly, it seems crucial to test if the processes observed previously exist in other marinas as well. This is especially important when considering that the intensity of the disturbance gradient might not be equal in all marinas. Indeed, in some marinas no differences in community structure can be observed between locations (Lam & Todd 2013), which may be caused by harbor design (Floerl & Inglis 2003) and the parameters (intensity, frequency, duration) of the considered disturbances.

The aim of our present study was, thus, to identify in different marinas if community composition was affected by anthropogenic disturbance gradients. We studied several marinas in two regions, to obtain a variety of disturbance intensities. As the entrance of a marina is supposed to be comparatively less disturbed, a marina with higher disturbance at the inner location should have a steeper disturbance gradient. We hypothesized that community structure would correlate with disturbance gradients and that differences between communities at the inner and entrance locations of marinas would be greater in more disturbed marinas (*i.e.*, marinas having a steeper gradient). Using reciprocal transplant experiments we aimed to expose the causal link between community structure and the disturbance gradients. The effect of the transplant should be greater when the disturbance gradient is steeper.

## Material and Methods

### Study site

The study was conducted in two distinct biogeographic regions, the NE Atlantic Ocean (Finistère close to Brest, Brittany, France) and the NW Mediterranean Sea (Bouches-du-Rhône close to Marseille, Provence, France). For each region, we aimed to integrate marinas with different levels of disturbance, which was approximated through a combination of their size and their surrounding anthropogenic activities. Thus, a total of six marinas were selected, three for each region. In the Atlantic region, the three study sites were the Marina du Château in Brest (hereafter Château; 48°22'43.4"N; 4°29'22.1"W), the southern basin of the Marina du Moulin Blanc in Brest (hereafter Moulin; 48°23'26.4"N 4°25'53.6"W) and the Marina du Notic in Camaret-sur-Mer (hereafter Camaret; 48°16'48.0"N 4°35'44.6"W). In the Mediterranean region the three study sites were the Vieux-Port de Marseille (hereafter Vieux-Port; 43°17'40.4"N 5°22'06.3"E), the Marina of Carry-le-Rouet (hereafter Carry; 43°19'46.7"N 5°09'08.6"E) and the Marina du Frioul (hereafter Frioul; 43°16'46.5"N 5°18'32.2"E). The disturbance levels of the marinas can be approximated by the diversity and intensity of anthropogenic activities found within each marina (**Erreur ! Source du renvoi introuvable.**). This led us to assume that Château and Vieux-Port would be the most disturbed sites, Camaret and Frioul to be the least disturbed sites and Moulin and Carry intermediate.

Within each of the six sampled marinas, we assumed the presence of a disturbance gradient as previously observed for some of them (Chapter II; Chapter III B; Kenworthy et al. 2018) and also reported from the literature (Je et al. 2004, Ryu et al. 2011). These gradients should vary in intensity, depending on the disturbance level of the considered marina which. To confirm this, we monitored different types of pollutants (POPs, MTEs) similar to analyses in Chapter II and Chapter III, but these data are still being acquired at the time of writing of this manuscript. More disturbed marinas should present a stronger heterogeneity of their communities than the less disturbed ones. Within each

marina and according to the hypothetical disturbance gradient, two study locations were chosen, one at the entrance of the marina and one in the inner parts.

### **Reciprocal transplant experiment**

At each of the two locations of each marina (Tab. 17), 15 black polyethylene panels (Correx, 0,2 m x 0,2 m, 3 mm thick) were deployed at the end of June 2020 in the Mediterranean and two weeks later (early July) in the Atlantic region. After 8 weeks in the field (August/September), all panels were gathered and photographed *in situ* and 5 panels per location were sampled for analysis. Remaining panels were randomly assigned to either be transplanted to the other location within the same marina (5 panels), or to stay in place as control (5 panels). Each panel could thus be identified according to its marina and its treatment depending on its origin (*i.e.*, where initial recruitment happened before transplant) and its destination (*i.e.*, where it was transplanted to). We use the following code for treatment identification: 'I' Inner, 'E' Entrance; '>' indicates the direction of transplant (*e.g.*, I>E corresponds to panels originating at the inner location and transplanted to the entrance E); C control (*e.g.*, IC is the inner control which stayed in place the whole experiment). After 7 additional weeks (October) the panels were transported in sea-water containers and stored in aquaria facilities (max. 1 week) for analysis and taxonomic determination of communities.

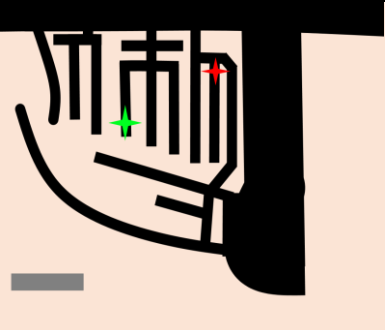
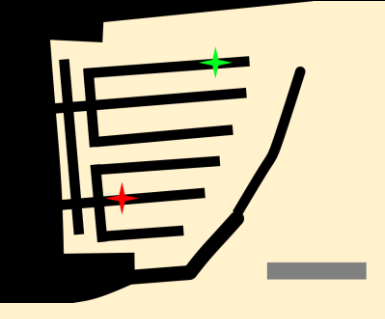
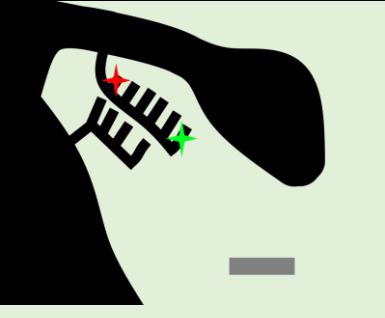
### **Environmental assessment**

For all locations in the marinas, water temperature and salinity were recorded with 1h interval at 1 m depth (panel depth) during the whole experiment using a HOBO® (Onset®) Pendant Temp/Light, 8K logger, and an Odyssey® Conductivity and Temperature recorder, respectively. The salinity logger data was corrected with an in-house calibration curve to account for differences between loggers (11% - 18%) and to account for overestimation of salinity. Temperature was recorded during the whole duration of the experiment, while salinity was recorded only after transplant for the Atlantic marinas and 2 weeks after transplant for the Mediterranean marinas.

At the end of the experiment, surface sediments were sampled by divers at each location in each marina. Five samples of 400g each were taken for MTE quantification and 3 replicates of 400g each for POPs. The samples are still under analysis and not presented here.



Tab. 17: Relevant information on the marinas at the Atlantic (first part) and the Mediterranean (second part) region. Marinas are sorted according to their disturbance level (high to low) The grey bar on the map is a 100 m scale. Green star: entrance location; Red star: inner location. Population data: INSEE.

Marina	Nb of moorings	Surface area	Ships ha <sup>-1</sup>	City and habitants	Situation and activities	Map
<b>Marina du Chateau</b>	600	10 ha	60	Brest, 139 602	<ul style="list-style-type: none"> <li>• Inside large marine urban zone</li> <li>• Close to large military and commercial harbors</li> <li>• Water discharge at the inner part</li> <li>• Dry dock and cleaning area</li> <li>• Semi-Enclosed</li> </ul>	
<b>Marina du Moulin Blanc (South)</b>	530	8.5 ha	62	Brest, 139 602	<ul style="list-style-type: none"> <li>• Close to estuary</li> <li>• Water discharge at the inner part</li> <li>• Dry dock and cleaning area</li> <li>• Floating breakwater</li> </ul>	
<b>Marina de Camaret-Sur-Mer</b>	370	~ 20 ha (no clear limits)	18.5	Camaret-Sur-Mer; 2 504	<ul style="list-style-type: none"> <li>• Close to village center</li> <li>• Close to fishing harbor</li> <li>• Open bay</li> </ul>	

Marina	Nb of moorings	Surface area	Ships ha <sup>-1</sup>	City and habitants	Situation	Map
Vieux-Port de Marseille	3200	25 ha	130	Marseille, 851 420	<ul style="list-style-type: none"> <li>• Inside large marine urban zone</li> <li>• Close to the center of large city</li> <li>• Close to large ferry and commercial harbors</li> <li>• Multiple water discharges</li> <li>• Large dry dock and cleaning area</li> <li>• Fishing and short distance ferries</li> <li>• Semi-Enclosed</li> </ul>	
Marina de Carry-le-Rouet	590	3.2 ha	170	Carry-le-Rouet, 5900	<ul style="list-style-type: none"> <li>• Close to city center</li> <li>• Water discharge at the inner part</li> <li>• Dry dock and cleaning area</li> <li>• Semi-enclosed</li> </ul>	
Marina du Frioul	700	22 ha	31	~ 150	<ul style="list-style-type: none"> <li>• Island 5 km away from shore</li> <li>• Short distance ferries</li> <li>• Semi-enclosed</li> </ul>	

### **Community taxonomic assessment**

The analysis procedure for photos before transplant, for panels sampled before transplant and for panels sampled at end of the experiment is described in Chapter II. For all treatments within a marina, the total species richness, mean Shannon diversity index and mean Pielou evenness were calculated. Panel photos were used to check for community homogeneity within each location before transplant, thus allowing to exclude experimental artifacts resulting from initial differences between control and transplanted panels. A PERMANOVA ( $10^4$  permutations) was conducted on a Bray-Curtis dissimilarity matrix testing for the effect of panel origin and its experimental assignment (being transplanted or control) with the 'vegan' package (version 2-4.6; Oksanen et al. 2018). Afterwards we fully focused on the analysis of panels sampled at the two timepoints of the experiment. For panels sampled before transplant, a PERMANOVA ( $10^4$  permutations) tested for differences among locations. For panels sampled at the end of the experiment, a PERMANOVA ( $10^4$  permutations) was conducted, testing for the effect of panel Origin, panel destination as well as their interaction. This was followed by a pairwise PERMANOVA from the 'pairwiseAdonis' R package (version 0.3; Martinez Arbizu 2019) including a Benjamini-Hochberg correction (Benjamini & Hochberg 1995). A Principal Coordinates Analysis (PCoA) was conducted and visualized using the R package 'ggplot2' (version 3.3.3; Wickham 2016). The 'envfit' function was used to overlay vectors (species), which were significantly correlated to the ordination (with  $p < 0.05$  and  $R^2 > 0.3$ ).

The Ash Free Dry Mass (AFDM) was determined after the community analyses in the laboratory were finished by drying the communities of each panel for one week at 60°C, weighting them, and burning them at 520°C for 6h to allow to calculate the AFDM. The AFDM and the relative contribution of Non-Indigenous Species (NIS) to the total cover (NIS cover / Total cover) for each treatment were assessed. An ANOVA testing for the effect of treatment on these two variables was conducted in 'R' (version 3.6.1; R Core Team 2020). Residual normality was verified with a Shapiro test and residual homoscedasticity was assessed with a Bartlett test. If both were respected we used the result of the ANOVA and conducted a subsequent Tukey Honest Significant Differences test (Tukey 1949). If one of both criteria was not respected, we conducted a Kruskal-Wallis test followed by a Dunn test. A Benjamini-Hochberg correction for the p value was applied in both cases (Benjamini & Hochberg 1995).

### **Assessment of Biological function**

At the end of the experiment, community respiration was measured *in situ* following the protocol of Chapter II. For the marinas in the Atlantic (not enough biomass in the Mediterranean), 5 samples of *Bugula neritina* (Linnaeus, 1758) per treatment in each marina were taken for quantifying MTE.

Additionally, 5 colonies of *B. neritina* per panel were taken for metabolomic analyses. These analyses are still ongoing and not represented here.

## Results

For each region, over 50 species were identified (Tab. 18). For the Mediterranean region, a total of 9 Non-Indigenous Species (NIS; 16%; 7 for Vieux-Port, 4 for Carry and Frioul) has been identified, while for the Atlantic region it was 12 NIS (22 %; 9 for Chateau, 11 for Moulin, 9 for Camaret). All of these species have been already described in the region or even the same marinas (Zibrowius 1973, Zibrowius & Bianchi 1981, Minchin et al. 2013, Vieira et al. 2014, Porter et al. 2017, Ulman et al. 2017, Kenworthy et al. 2018b, Reverter-gil & Souto 2019).

Species richness and biodiversity indices were mostly higher in the Atlantic marinas compared to their Mediterranean counterpart (Fig. 36). While species richness was higher in the two less disturbed marinas (Camaret and Frioul) compared to the other marinas of their region, the lowest species number was not always reached in the disturbed marinas (Chateau, Vieux-Port). The lowest species richness overall was reached in Carry. Interestingly, very low values for the Shannon index and Pielou evenness were reached in Camaret, while this marina had the highest species richness. Transplanted panels often had often slightly higher diversity than their origin with however overall similar values.

AFDM was one to two orders of magnitude higher in Atlantic marinas compared to Mediterranean ones. Similar patterns can be observed for the relative contribution of NIS to the total cover. The ratio of NIS never reached 10% in the Mediterranean marinas, while it was always over 25% and even reached over 75% in the Atlantic. When significant differences of AFDM were observable between treatments within a marina, it was always higher at the entrance location of a marina compared to its inner location (Fig. 36). However, no clear pattern concerning the transplant were observable for AFDM. NIS relative contribution to total cover did only vary as expected in the Chateau marina, with higher NIS contribution at the inner location compared to the EC and E>I (Fig. 36). In the Chateau I>E had an intermediate NIS contribution; however, this treatment had the highest NIS contribution in the Vieux-Port and Frioul (Fig. 36).

The photo analysis of panels before transplant revealed no significant effect of their experimental assignment (transplanted vs control) for each location (for each pairwise PERMANOVA;  $R^2 < 0.14$ ;  $p > 0.161$ ), excluding experimental artifacts. Before transplant, significant differences were however found between the entrance and the inner locations of all marinas (PERMANOVA;  $R^2 > 0.32$ ;  $p < 0.016$ ).

Tab. 18: Species list for the Mediterranean and Atlantic study region. Non-Indigenous Species (NIS) are indicated.

Mediterranean	Atlantic
<b>Annelida</b>	<b>Annelida</b>
<i>Circeis spirillum</i> (Linnaeus, 1758)	<i>Circeis armoricana</i> Saint-Joseph, 1894
<i>Hydroides elegans</i> (Haswell, 1883) NIS	<i>Circeis spirillum</i> (Linnaeus, 1758)
<i>Janua heterostropha</i> (Montagu, 1803)	<i>Janua heterostropha</i> (Montagu, 1803)
<i>Pileolaria berkeleyana</i> (Rioja, 1942) NIS	<i>Neodexiospira brasiliensis</i> (Grube, 1872) NIS
<i>Pileolaria militaris</i> Claparède, 1870	<i>Paralaeospira malardi</i> Caullery & Mesnil, 1897
<i>Salmacina incrustans</i> Claparède, 1870	<i>Pileolaria militaris</i> Claparède, 1870
<i>Serpula vermicularis</i> Linnaeus, 1767	<i>Sabella pavonina</i> Savigny, 1822
<i>Spirobranchus lamarcki</i> (Quatrefages, 1866)	<i>Salmacina incrustans</i> Claparède, 1870
<i>Spirobranchus triqueter</i> (Linnaeus, 1758)	<i>Serpula vermicularis</i> Linnaeus, 1767
<i>Spirorbis marioni</i> Caullery & Mesnil, 1897 NIS	<i>Spirobranchus triqueter</i> (Linnaeus, 1758)
<b>Arthropoda</b>	<i>Spirorbis rupestris</i> Gee, Knight-Jones, 1962
<i>Perforatus perforatus</i> (Bruguère, 1789)	<b>Arthropoda</b>
<b>Urochordata</b>	<i>Austrominius modestus</i> (Darwin, 1854) NIS
<i>Ascidia conchilega</i> Müller, 1776	<i>Perforatus perforatus</i> (Bruguère, 1789)
<i>Ascidia scabra</i> (Müller, 1776)	<b>Urochordata</b>
<i>Botrylloides</i> sp. Milne Edwards, 1841	<i>Ascidia aspersa</i> (Müller, 1776)
<i>Botryllus schlosseri</i> (Pallas, 1766)	<i>Ascidia scabra</i> (Müller, 1776)
<i>Ciona intestinalis</i> (Linnaeus, 1767)	<i>Asterocarpa humilis</i> (Heller, 1878) NIS
<i>Clavelina dellavallei</i> (Zirpolo, 1925)	<i>Botrylloides</i> sp. Milne Edwards, 1841
<i>Clavelina lepadiformis</i> (Müller, 1776)	<i>Botryllus schlosseri</i> (Pallas, 1766)
<i>Diplosoma listerianum</i> (Milne-Edwards, 1841) NIS	<i>Ciona intestinalis</i> (Linnaeus, 1767)
<i>Perophora listeri</i> Wiegman, 1835	<i>Ciona robusta</i> Hoshino & Tokioka, 1967 NIS
<i>Pyura</i> sp. Molina, 1782	<i>Clavelina lepadiformis</i> (Müller, 1776)
<b>Bryozoa</b>	<i>Corella eumyota</i> Traustedt, 1882 NIS
<i>Aetea sica</i> (Couch, 1844)	<i>Didemnum coriaceum</i> (Drasche, 1883)
<i>Amathia verticillata</i> (delle Chiaje, 1822) NIS	<i>Didemnum vexillum</i> Kott, 2002 NIS
<i>Bugula neritina</i> (Linnaeus, 1758) NIS	<i>Diplosoma listerianum</i> (Milne-Edwards, 1841)
<i>Bugulina simplex</i> (Hincks, 1886)	<i>Lissoclinum perforatum</i> (Giard, 1872)
<i>Bugulina stolonifera</i> (Ryland, 1960)	<i>Molgula citrina</i> Alder & Hancock, 1848
<i>Bugulina turbinata</i> (Alder, 1857)	<i>Morchellium argus</i> (Milne Edwards, 1841)
<i>Celleporaria brunnea</i> (Hincks, 1884) NIS	<i>Perophora japonica</i> Oka, 1927 NIS
<i>Cradoscrupocellaria bertholletii</i> (Audouin, 1826)	<i>Perophora listeri</i> Wiegman, 1835
<i>Crisia denticulata</i> (Lamarck, 1816)	<i>Phallusia mammillata</i> (Cuvier, 1815)
<i>Crisia ramosa</i> Harmer, 1891	<i>Styela clava</i> Herdman, 1881 NIS
<i>Crisia sigmoidea</i> Waters, 1916	<b>Byozoa</b>
<i>Crisularia aperta</i> (Hincks, 1886)	<i>Bugula neritina</i> (Linnaeus, 1758) NIS
<i>Crisularia plumosa</i> (Pallas, 1766)	<i>Bugulina fulva</i> (Ryland, 1960)
<i>Cryptosula pallasiana</i> (Moll, 1803)	<i>Cellepora pumicosa</i> (Pallas, 1766)

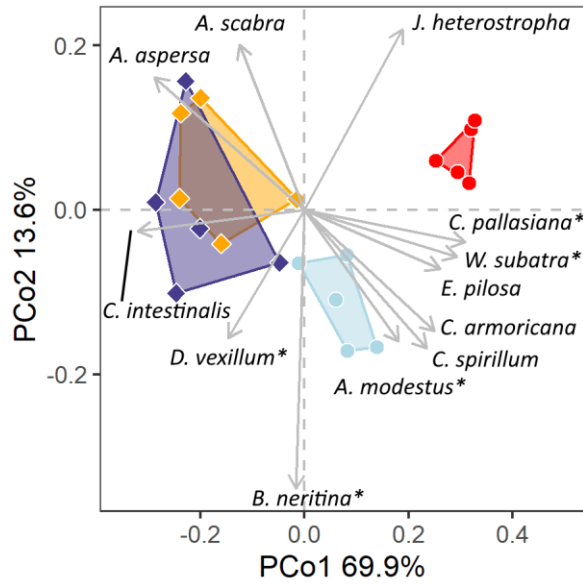
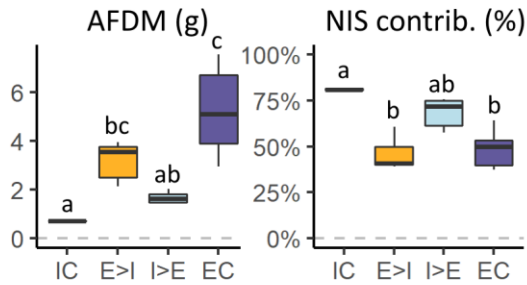
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Tab. 18 continued

<i>Filicrisia geniculata</i> (Milne Edwards, 1838)	<i>Cryptosula pallasiana</i> (Moll, 1803)	NIS
<i>Savignyella lafontii</i> (Audouin, 1826)	<i>Electra pilosa</i> (Linnaeus, 1767)	
<i>Schizoporella errata</i> (Waters, 1878)	<i>Tricellaria inopinata</i> d'Hondt & Occhipinti Ambrogi, 1985	NIS
<i>Scrupocellaria scruposa</i> (Linnaeus, 1758)	<i>Watersipora subatra</i> (Ortmann, 1890)	NIS
<i>Watersipora subtorquata</i> (Ortmann, 1890)	NIS	
<b>Cnidaria</b>	<b>Cnidaria</b>	
<i>Clytia hemisphaerica</i> (Linnaeus, 1767)	<i>Laomedea angulata</i> Hincks, 1861	
<i>Eudendrium ramosum</i> (Linnaeus, 1758)	<i>Laomedea flexuosa</i> Alder, 1857	
<i>Haleciidae</i> Hincks, 1868	<i>Plumularia setacea</i> (Linnaeus, 1758)	
<i>Laomedea flexuosa</i> Alder, 1857	<i>Tubularia indivisa</i> Linnaeus, 1758	
<i>Plumularia setacea</i> (Linnaeus, 1758)	<b>Mollusca</b>	
<i>Scyphozoa polyp</i>	<i>Anomia ephippium</i> Linnaeus, 1758	
<b>Mollusca</b>	<i>Mimachlamys varia</i> (Linnaeus, 1758)	
<i>Anomia ephippium</i> Linnaeus, 1758	<i>Modiolus barbatus</i> (Linnaeus, 1758)	
<i>Chlamys varia</i> (Linnaeus, 1758)	<i>Musculus subpictus</i> (Cantraine, 1835)	
<i>Crassostrea gigas</i> (Thunberg, 1793)	<i>Mytilus edulis</i> Linnaeus, 1758	NIS
<i>Mytilus sp.</i> Linnaeus, 1758	<i>Ostrea edulis</i> Linnaeus, 1758	
<i>Parvicardium pinnulatum</i> (Conrad, 1831)	<b>Porifera</b>	
<b>Porifera</b>	<i>Clathrina sp.</i> Gray, 1867	
<i>Batzella inops</i> (Topsent, 1891)	<i>Halichondria bowerbanki</i> Burton, 1930	
<i>Clathrina coriacea</i> (Montagu, 1814)	<i>Mycale macilenta</i> (Bowerbank, 1866)	
<i>Sycon ciliatum</i> (Fabricius, 1780)	<i>Sycon ciliatum</i> (Fabricius, 1780)	
<i>Sycon raphanus</i> Schmidt, 1862		

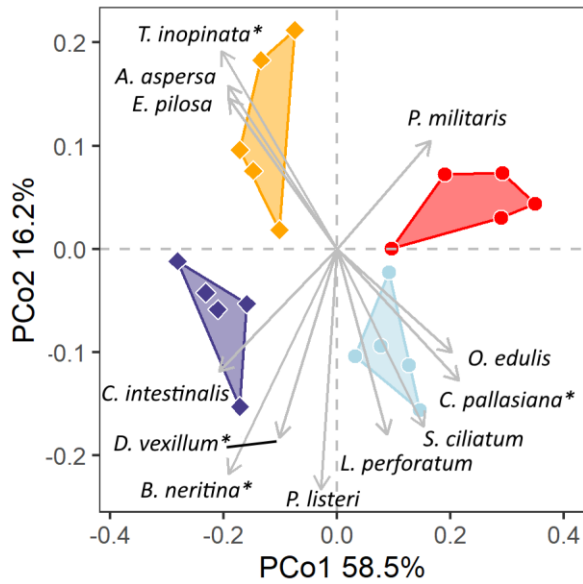
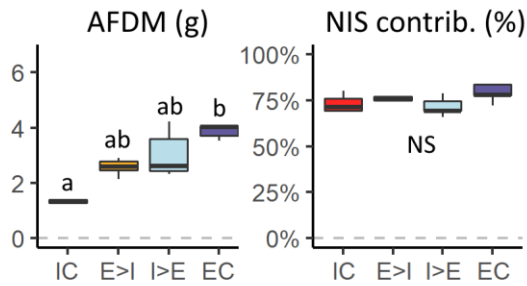
## Chateau Marina

Treatment	Shannon	Evenness	S
IC	2,57	0,59	28
E>I	2,56	0,63	25
I>E	2,68	0,63	25
EC	2,55	0,64	23



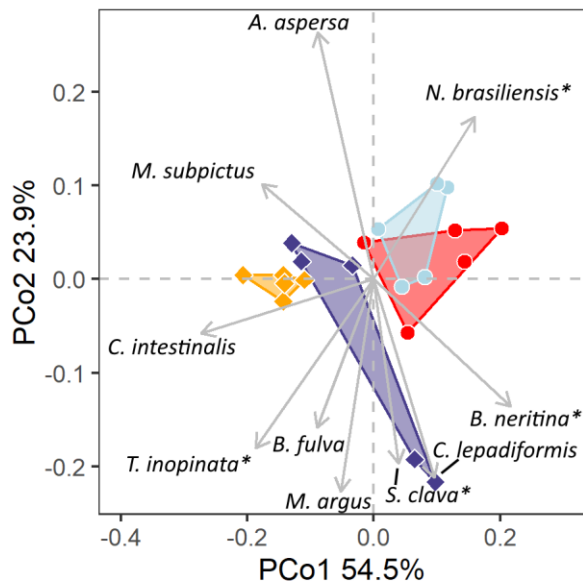
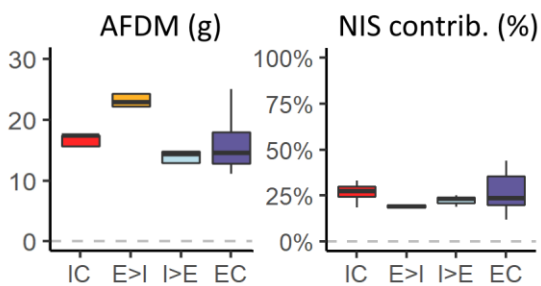
## Moulin Marina

Treatment	Shannon	Evenness	S
IC	2,89	0,67	28
E>I	2,71	0,63	28
I>E	2,97	0,68	29
EC	2,69	0,61	25



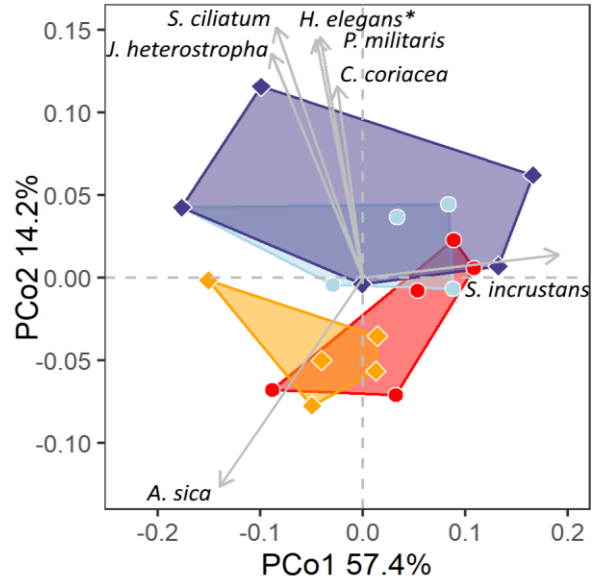
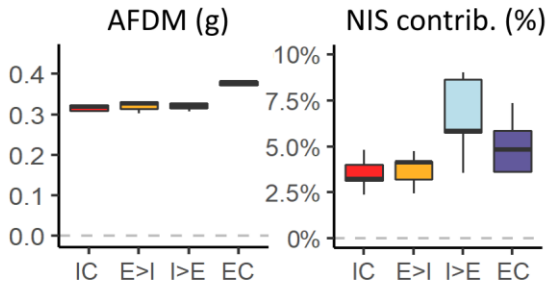
## Camaret-sur-Mer Marina

Treatment	Shannon	Evenness	S
IC	2,32	0,52	35
E>I	2,04	0,49	30
I>E	2,18	0,5	29
EC	2,2	0,52	31



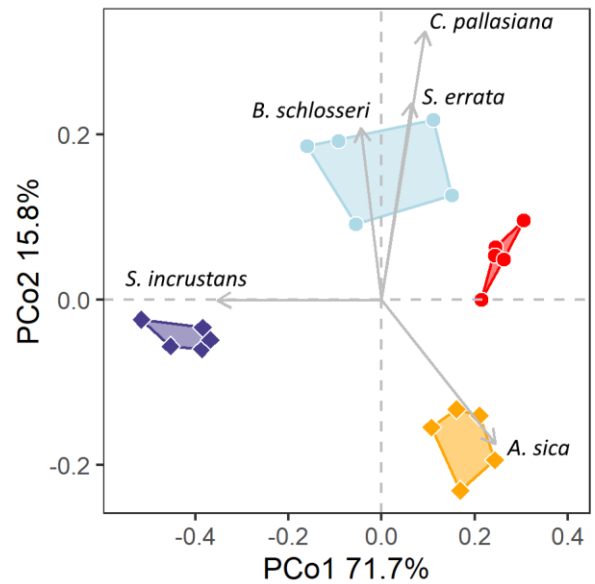
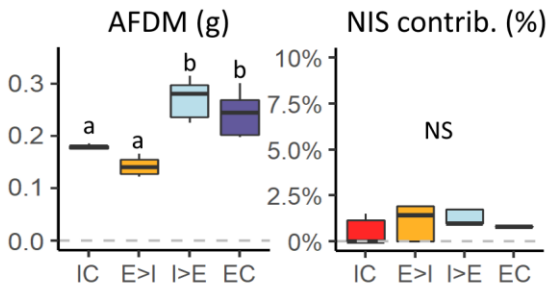
### Vieux Port Marseille

Treatment	Shannon	Evenness	S
IC	2,33	0,62	19
E>I	2,35	0,64	22
I>E	2,53	0,63	24
EC	2,43	0,64	19



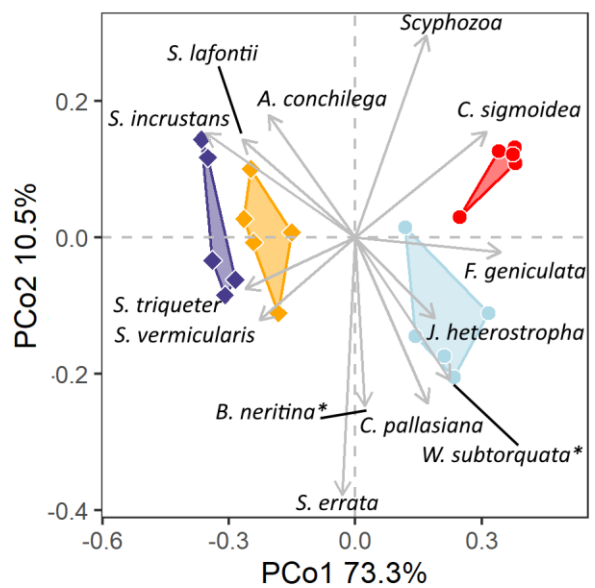
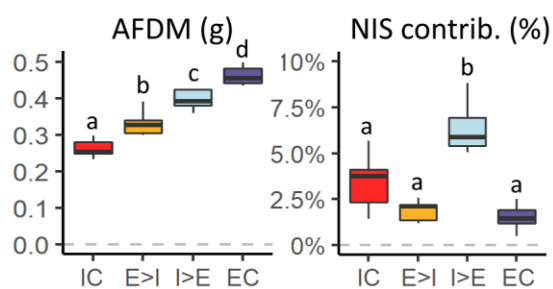
### Carry le Rouet Marina

Treatment	Shannon	Evenness	S
IC	2,15	0,64	15
E>I	1,81	0,57	17
I>E	2,45	0,65	24
EC	1,68	0,5	17



### Frioul Marina

Treatment	Shannon	Evenness	S
IC	3,17	0,74	24
E>I	2,84	0,64	24
I>E	3,15	0,72	23
EC	2,31	0,54	25





*Fig. 36: Diversity indices (mean Shannon and Pielou evenness and total species richness) for each treatment (top left); boxplot of Ash Free Dry Mass (AFDM) and Non-Indigenous contribution to total cover for each treatment (bottom left); and Principal Coordinate Analysis (PCoA) of the communities for each treatment (right) for the six studied Marinas. Treatments are labelled according to their origin followed (>) by their destination upon transplant (I: inner, E: entrance; C: controls i.e. identical Origin and Destination) and indicated in the PCoA according to their destination (colors: red = inner; blue = entrance) and their origin (shapes: circle= inner, diamond = entrance). Color hues in the table indicate higher (green) or lower (diversity) compared to all sites from the same region. Letters in the boxplot indicate significant differences identified by Tuckey Honest Significant Differences Test or Dunn Test. Vector overlay indicates species with a positive correlation ( $p < 0.05$ ;  $R^2 > 0.3$ ) with the axes. Non-Indigenous species are indicated with an asterisk.*

At the end of the experiment, for Chateau, Moulin, Carry and Frioul, community structure of the IC was significantly different from the EC (Pairwise PERMANOVA;  $R^2 > 0.75$ ,  $p < 0.05$ ; Tab. 19) and appeared separated along the PCo1 axis (58.5% - 73.3% of the variability; Fig. 36). All marinas except the Vieux-Port had a significant origin effect (PERMANOVA;  $R^2 > 0.26$ ;  $p < 0.001$ ; Tab. 19). In the Chateau, Moulin, Carry and Frioul a significant effect of panel destination was observed (PERMANOVA;  $R^2 > 0.1$ ;  $p < 0.004$ ; Tab. 19). This effect did however not always apply in the same manner. For these marinas the PCo2 axis (10.2% - 16.2% of variability) did often represent the effect of the transplant, excepted for Moulin where both PCo axes may represent a part of the treatment. For Moulin and Carry, the destination had the strongest effect (Fig. 36; Tab. 19), while a lower effect of the transplant is present at the Frioul Marina. For Chateau the transplant had only an effect when panels were transplanted I>E (Fig. 36, Tab. 3). No effect of the transplant was noted for the Vieux-Port and Camaret.

Tab. 19: Statistics associated to the PERMANOVA testing for differences between the entrance and inner location of each marina before transplant and PERMANOVA testing for the effect of Origin, Destination, and their interaction on community structure within each marina after transplant. The results of the pairwise PERMANOVA are indicated in an infographic for each marina. Arrows show significant pairwise differences ( $R^2 > 0.4$ ;  $p < 0.05$ ). Treatments are labelled according to their origin followed (>) by their destination upon transplant (I: inner, E: entrance; C: controls i.e. identical Origin and Destination)

Before transplant				
PERMANOVA (I vs E)	Df	R <sup>2</sup>	p value	
Château Marina (Atlantic)	1	0.92	0.008 **	
Moulin Marina (Atlantic)	1	0.83	0.009 **	
Camaret-sur-Mer (Atlantic)	1	0.32	0.010 *	
Vieux-Port (Mediterr.)	1	0.80	0.005 **	
Carry-le-Rouet (Mediterr.)	1	0.79	0.007 **	
Frioul Marina (Mediterr.)	1	0.70	0.016 *	
After transplant PERMANOVA				
<b>Château Marina (Atlantic)</b>	<b>Df</b>	<b>R<sup>2</sup></b>	<b>p value</b>	<b>Pairwise</b>
Origin	1	0,54	<0,001 ***	
Destination	1	0,13	0,002 **	
Origin:Destination	1	0,06	0,04 *	
<b>Moulin Marina (Atlantic)</b>	<b>Df</b>	<b>R<sup>2</sup></b>	<b>p value</b>	<b>Pairwise</b>
Origin	1	0,49	<0,001 ***	
Destination	1	0,17	0,004 **	
Origin:Destination	1	0,05	0,038 *	
<b>Camaret-sur-Mer (Atlantic)</b>	<b>Df</b>	<b>R<sup>2</sup></b>	<b>p value</b>	<b>Pairwise</b>
Origin	1	0,35	<0,001 ***	
Destination	1	0,03	0,445	
Origin:Destination	1	0,09	0,058	
<b>Vieux-Port (Mediterr.)</b>	<b>Df</b>	<b>R<sup>2</sup></b>	<b>p value</b>	<b>Pairwise</b>
Origin	1	0,08	0,167	
Destination	1	0,08	0,188	
Origin:Destination	1	0,04	0,438	
<b>Carry-le-Rouet (Mediterr.)</b>	<b>Df</b>	<b>R<sup>2</sup></b>	<b>p value</b>	<b>Pairwise</b>
Origin	1	0,26	<0,001 ***	
Destination	1	0,49	<0,001 ***	
Origin:Destination	1	0,07	0,011 *	
<b>Frioul Marina (Mediterr.)</b>	<b>Df</b>	<b>R<sup>2</sup></b>	<b>p value</b>	<b>Pairwise</b>
Origin	1	0,67	<0,001 ***	
Destination	1	0,1	0,003 **	
Origin:Destination	1	0,04	0,051	

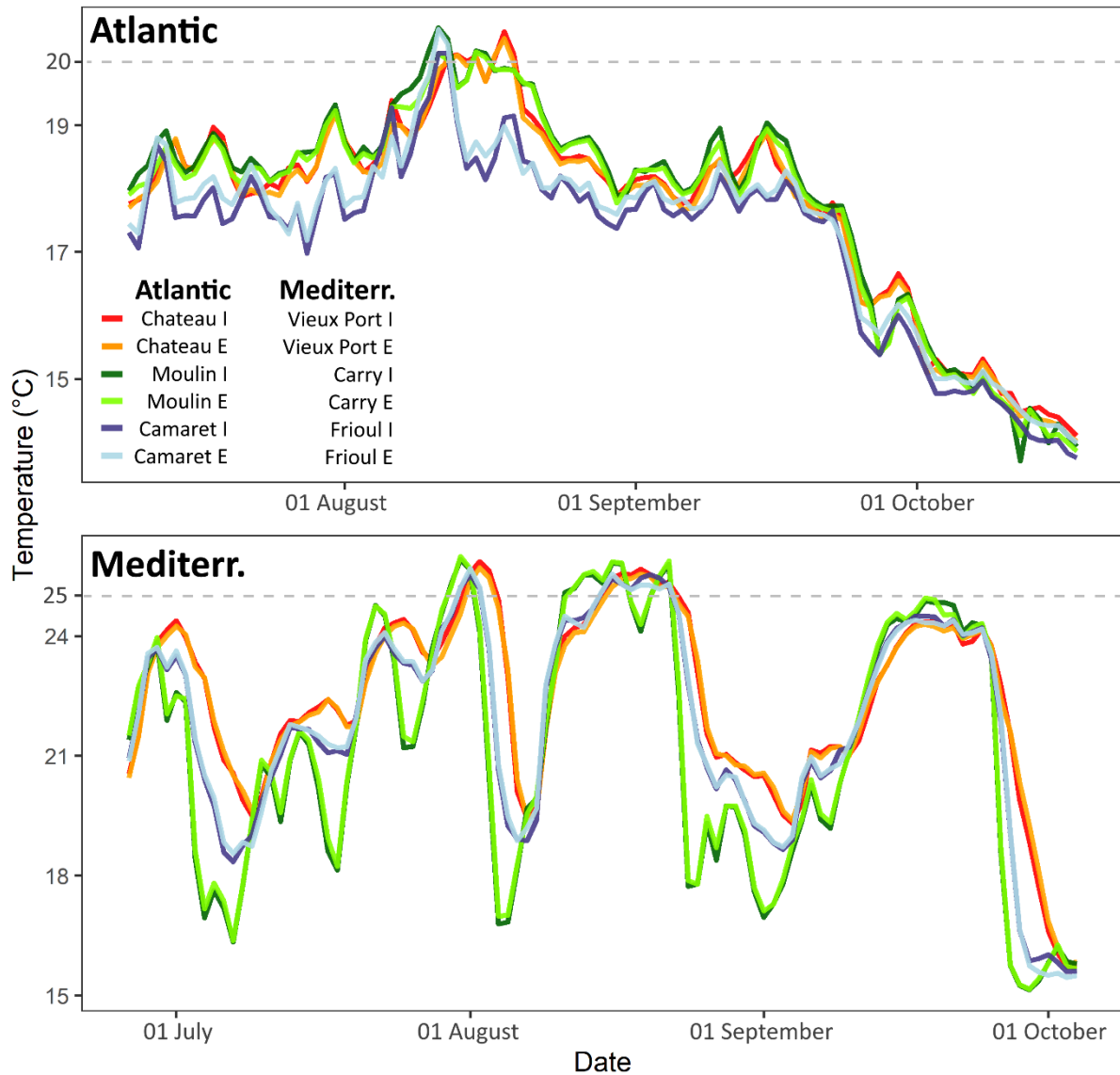


Fig. 37: Water temperature monitoring for the six marinas during the duration of the experiment. Colors are attributed depending on the hypothetical disturbance level of a marina. Red hues: Chateau or Vieux-Port; Green hues: Moulin or Carry; Blue hues: Camaret or Frioul. The darker hue is always associated to the inner location while the lighter hue is associated to the entrance. Treatments in the legend are labelled according to their Marina and location (I: inner, E: entrance).

No Particular extreme temperature event can be noted in any of the marinas (Fig. 37). Differences in temperature between the inner and entrance location of the marinas are below values expected to cause effects on communities. In the Atlantic, the temperature of Camaret is slightly cooler than the two other marinas. In the Mediterranean the Vieux-Port has the highest temperature, followed by the Frioul marina and then the Carry marina with the lowest temperature. The temperature difference between Carry and the Vieux-Port can reach over 3°C when as Carry cools down rapidly compared to the Vieux-Port, which seems to have more inertia. Salinity was notably higher in the inner location of the Moulin and Camaret marina, while it was higher at the entrance for the Frioul marina. Two dips in

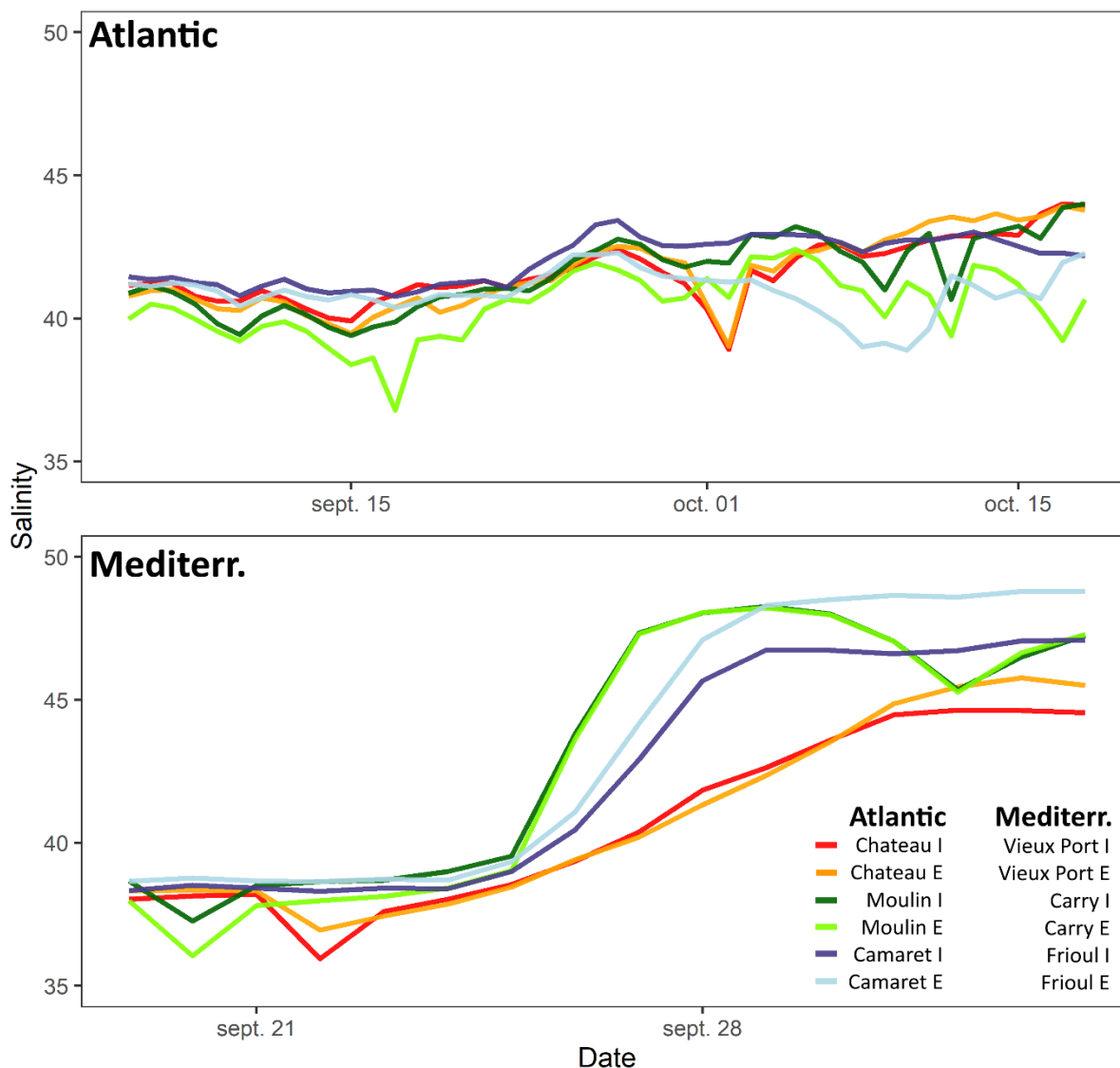


Fig. 38: Water salinity monitoring for the six marinas during the duration of the experiment. Colors are attributed depending on the hypothetical disturbance level of a marina. Red hues: Chateau or Vieux-Port; Green hues: Moulin or Carry; Blue hues: Camaret or Frioul. The darker hue is always associated to the inner location while the lighter hue is associated to the entrance. Treatments in the legend are labelled according to their Marina and location (I: inner, E: entrance).

salinity can be seen at the Atlantic marinas, one for the Moulin marina on September 17<sup>th</sup> (entrance location only) and one for the Chateau on October 2<sup>nd</sup> (both locations; Fig. 38). For all the Mediterranean marinas a strong increase in salinity can be noted from mid to end of September (Fig. 38). Two notable dips in salinity can be seen for the Carry marina (Sept 20<sup>th</sup> and Oct 3<sup>rd</sup>), while one dip is present for the Vieux-Port (Sept 22<sup>nd</sup>).

## Discussion

In previous studies, local variability of communities has been correlated with disturbance gradients in marinas (Je et al. 2004, Ryu et al. 2011, Kenworthy et al. 2018b; Chapter I, Chapter II, Chapter III B). In earlier transplant attempts, we were able to confirm the causal effect of environmental conditions on community structure and to demonstrate the presence of local adaptation in the Chateau marina (Chapter II), but not in the Vieux-Port, due to the impacts of heatwaves (Chapter III B). Exposing the causal link between local conditions and community composition and exposing local adaptation on such small spatial scale is important for understanding the processes of contemporary evolution, especially considering that non-indigenous species (NIS) may be favored in disturbance regimes (Hobbs & Huenneke 1992, Altman & Whitlatch 2007, Bulleri et al. 2016) due to their higher resistance (Piola & Johnston 2006a, Dafforn et al. 2008, Lejeusne et al. 2014, Kenworthy et al. 2018a, Osborne & Poynton 2019). It is however difficult to generalize from one tested marina, as the processes applicable in one may be highly different from elsewhere. It was thus required to conduct a larger scale study with multiple marinas, ideally with different disturbance levels.

The aim of the present study was thus to verify if the causal effect of disturbance gradients on community structure is present in other marinas and if the strength of this effect depends on the putative intensity of the disturbance gradient. We multiplied observations in marinas allowing to have different intensities of disturbance. Although data concerning the pollution levels in sediments and animals of the six marinas is not available yet (ongoing analysis), we believe Chateau and Vieux-Port to be the most disturbed marinas, Camaret and Frioul to be the least disturbed marinas, and Moulin and Carry to be intermediate, based on harbor size, surrounding human activities and structures, and mooring number. Community structure was significantly different between the inner and entrance locations of all marinas before transplant. This confirms the correlation of the disturbance gradient with the community structure. This effect was maintained throughout the whole duration of the experiment for most marinas. Notable exceptions to this were the Vieux-Port and Camaret which had overall homogenous communities at the end of the experiment. For the latter, this is not unexpected, as disturbance might be so low, that no community differences were present. While heat waves have been observed to homogenize the community structure within the Vieux-Port (Chapter III B), no die-off had been observed in 2020 and water temperatures in the marina did not reach 26°C. Furthermore, no strong community shift during the experiment was noted conversely to 2019 (Chapter III B). We have previously shown that pollution is indeed organized as a gradient in this harbor, which is especially the case for Metallic Trace Elements (Chapter III B). Even if we do not know pollution levels for 2020 yet, it is unlikely that these change drastically from 2019 as for instance very similar pollution values have been identified in the Chateau marina between 2016 and 2019 (Kenworthy et al. 2018b;

Chapter II). However, this data gained from sediment samples integrates long term tendencies for pollution and does not account for short term variability. Pulse pollution has been shown to highly impact fouling community structure (Johnston & Keough 2000, 2002, Johnston et al. 2002). It may be possible that a pulse pollution event may have affected the communities, homogenizing community structure without causing a strong mortality and community shift like heat waves. Precipitations may also have contributed to the homogenization, as numerous water discharges exist in the marina and high precipitations (25,8 mm on September 22<sup>nd</sup>; Infoclimat) were present. This coincides with a dip in salinity in the marina. Yet, this dip is comparable to what can be observed in Carry, where no homogenization was present. This may indicate that the water discharge might not have homogenized communities through salinity decrease but rather through an input of pollutants. Numerous human activities are present in this marina, with multiple boat cleaning stations, fishing boats, ferries etc. multiplying the potential sources of pulse disturbance.

The reciprocal transplant revealed an origin effect in 5 of the 6 studied marinas, confirming the important role of historical contingency. Previous transplant studies including ours already demonstrated how primary colonization influences subsequent community structure in marinas (Cifuentes et al. 2010; Chang and Marshall 2016, Chapter II). The reciprocal transplant also revealed a destination effect in 4 out of the 6 marinas indicating a causal effect of environmental conditions on community structure. We expected this effect to be stronger compared to the origin effect in more disturbed marinas due to higher selectivity of the disturbance and to be weaker or absent in less disturbed marinas. This assumption was met in the low disturbance marinas Camaret (where no effect of transplant was noted) and Frioul where origin effect dominated. A stronger effect of the transplant was present in the intermediate marinas (Moulin and Carry). For the Chateau marina, the transplant had a strong effect, but only in one direction (I>E), while E>I communities were similar to their origin control. Conversely to our observations from 2019 (Chapter II) no strong selective effect of the inner location was observable on the community level for this marina. This might be due differences in the experimental setup like shorter time between transplant and the end of the experiment (42 days compared to 70 in 2019). Temperature differences due to our later timing (approx. One month compared to 2019) may also explain these results, as higher temperatures favor the pollutant uptake of marine ectotherms (Sokolova & Lannig 2008, Cabral et al. 2019). But the observed differences may also be explained by annual recruitment patterns of the present species.

AFDW was often higher in EC compared to IC. This may be due to the presence of species with massive recruitments and fast growth rate like ascidians (*e.g.*, *Ciona intestinalis*, *Asciella aspersa* and *Didemnum vexillum*; Fig. 36) for the Atlantic marinas and Serpulinae (*Salmacina incrustans* and *Spirobranchus triqueter*, Fig. 36) in the Mediterranean marinas. Species like *C. intestinalis* which may

monopolize substratum and reduce diversity (Blum et al. 2007) or *S. incrustans* which is capable of asexual reproduction (Nishi & Nishihira 1994, Pernet 2001) may exert high competitive pressure leading to the reduction/exclusion of other species. This might explain the higher biomass and the counterintuitively lower diversity in EC compared to IC. In the Mediterranean more NIS species were associated to the most disturbed marina (Vieux-Port) compared to both others. However, NIS richness seemed constant between the three Atlantic marinas. Generally, higher disturbance marinas had higher NIS cover. This is particularly visible in the IC (which is the most disturbed treatment) and I>E (which was transplanted to less disturbed conditions). This agrees with the assumption that NIS may be favored by disturbance (Hobbs & Huenneke 1992, Altman & Whitlatch 2007, Piola & Johnston 2008, Piola et al. 2009, Bulleri et al. 2016, Kenworthy et al. 2018b). The high NIS prevalence in I>E in most marinas indicates that NIS may establish in disturbed environments and persist, even if the disturbance diminishes afterwards. NIS prevalence did however not always depend on disturbance intensity as Frioul had higher NIS contribution in all treatments than Carry and almost as much as the Vieux-Port for example.

The present study, although yet incomplete, highlights a strong correlation between the supposed disturbance gradient and community structure. We observed an almost systematic difference between the communities of the entrance and inner locations of the marinas. This difference was proportional to the supposed disturbance gradient intensity. The reciprocal transplant experiment showed us that colonization succession plays an important role in shaping the community, but most importantly allowed us to demonstrate the causal action of the disturbance gradient on community structure in most marinas. This confirms previous observations made in the Chateau marina (Chapter II) and extends them to other marinas. The impact of disturbance gradients on community structure and potentially on local adaptation might be universally present in marinas where disturbance is strong enough. The effects of the disturbance gradient and local adaptation may however be masked by temporal variability of other disturbances acting as confounding factors. Further efforts are needed here to quantify pollutants in the sediments of the studied marinas. This will help to confirm assumptions on disturbance intensities in all marinas. Measures of accumulated pollutants and analyses of the metabolome will further help to observe if local adaptation is indeed present in all the marinas.

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# **Chapter V    Prospective Effects of Climate Change and Associated Ocean Warming on Marina Communities**

Draft version for the present manuscript

## Getting used to the heat – Climate change might only favor introduced species climates with regular heat waves.

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**Abstract.** Climate change constitutes a major challenge for marine urban ecosystems and ocean warming will likely strongly affect local communities. Introduced species have often been shown to have higher heat resistance, but *in situ* studies testing how expected future global warming might affect them remain scarce, especially in marine urban environments. Here we used an *in situ* warming experiment in a NW Mediterranean (warm temperate) and a NE Atlantic (cold temperate) marina to see how climate change might affect recruited communities in the near future. In both marinas, warming resulted in significantly different communities, lower biomass, and more empty space compared to control. However, while in the warm temperate marina, introduced species showed an increased surface cover, their surface cover was reduced in the cold temperate one. Metabolomic analyses in the Atlantic marina revealed potential heat stress experienced by the introduced bryozoan *Bugula neritina*. The present results might indicate that in the near future, global warming might not cause identical effects everywhere on the prevalence of introduced species, and some locations where regular heat events are already experienced might have a higher risk at favoring them compared to cooler and more stable climates.

**Keywords:** Non-Indigenous species; experimental climate change; *in situ* warming; marina; hot plates

## Introduction

Climate change constitutes a major challenge for humanity and the situation has incited to strong political engagements to counter it (UN 2015; IPCC 2021). It is however unlikely that efforts suffice to limit global warming to +1.5°C, aimed for by the Paris agreement, and a scenario with a global temperature increase of +3.2°C seems most likely (Raftery et al. 2017). Associated to this, sea surface temperature is also projected to increase with climate change, with mean estimates ranging from +0.7°C to +3°C above preindustrial levels depending on the considered scenario (IPCC 2019). Climate change already poses a significant threat on natural ecosystems (Hanson & Weltzin 2000, Ayres & Lombardero 2000, Hughes et al. 2003, Lejeusne et al. 2010, Waldock et al. 2018, Lonhart et al. 2019), but as urban areas constitute islands of increased heat, climate change effects may be even exacerbated (Chapman et al. 2017). These combined effects of climate and urbanization can be observed in terrestrial, aquatic and marine urban environments and are affecting species characteristics (Luo et al. 2006, Chapman et al. 2017, Brans et al. 2017, Todd et al. 2019). Understanding how climate change influences urban ecosystems, which are already subjected to cumulative anthropic impacts, constitutes a grand challenge of high priority (Borja et al. 2020).

Climate change may substantially modify biological invasion patterns through niche modifications inducing new areas to become available for invasive species (Zhang et al. 2020, Vilizzi et al. 2021). Non indigenous species (NIS) may completely restructure the ecosystem leading to the loss of biodiversity and ecosystem services (Pejchar & Mooney 2009, Johnston et al. 2015b, Walsh et al. 2016). Besides the long term warming, climate change is projected to further increase the frequency of extreme weather events such as heat waves which can cause mass mortalities and restructure communities in natural (Garrahou et al. 2009, Lejeusne et al. 2010, Collier & Waycott 2014) and in urban ecosystems (Smale et al. 2015; Castro et al. 2021; Chapter III B). This effect may directly benefit NIS as many have been shown to have a higher temperature tolerance (Zerebecki & Sorte 2011, Lejeusne et al. 2014, Kenworthy et al. 2018a, Gewing et al. 2019). Increased warming can also exacerbate the effect of pollution on local fauna through accelerating metabolism (Sokolova & Lannig 2008, Cabral et al. 2019, Todd et al. 2019). This may even further advantage NIS as they appear more tolerant to pollution as well (Piola & Johnston 2008, Osborne & Poynton 2019). In the context of invasion, climate change might thus be a particular management challenge as it may increase the impact of NIS. For now, many NIS are established in their introduced range but are not considered invasive as they do not proliferate and do not cause adverse effects on the environment (Occhipinti-Ambrogi & Galil 2004, Blackburn et al. 2011, Ojaveer et al. 2015). Yet some of these NIS may be sleeper species, which are currently impeded in their proliferation by environmental conditions and may become invasive if these conditions change due to climate change (Bradley et al. 2018, Spear et al. 2021). Furthermore, climate

exerts strong selective pressures on native and introduced populations and some species, including NIS, might rapidly adapt to climate change through genetically mediated differences in thermal tolerances (Brans et al. 2017, Sork 2018) or through plastic responses (Zerebecki & Sorte 2011, Sork 2018). Divergences in thermal tolerances of species from different geographical locations may be good indicators of their capacity to adapt to climate change (Sorte et al. 2011). It is thus crucial to foster the understanding of how climate change might impact marine urban communities, especially considering the important place the NIS occupy.

Marinas are often used as a model for studying marine urban communities, since they combine the different disturbances exerted by urbanization and have a high abundance of NIS. Many studies in aquaria and mesocosms have been conducted to understand the effect of global warming and/or heatwaves on a variety of coastal ecosystems (Sorte et al. 2010a b, Smale et al. 2015, Wahl et al. 2015, Pansch et al. 2018, Castro et al. 2021) but completely *in situ* experiments remain rare (examples are Smale et al. 2011, 2017; Ashton et al. 2017), due to technical and experimental difficulties linked to *in situ* warming.

In the present study we thus aim to assess *in situ* the effect of +3°C sea surface warming (RCP8.5; IPCC 2019) in two marinas, one in the NW Mediterranean and one in the NE Atlantic. We deployed indirectly heated hot plates in open systems in two marinas and assessed differences in sessile community characteristics from control panels. We also focused on the metabolism of a common marina species (reported as NIS in both marinas) to see if it was affected under increased heat. The observed results could help to make predictions about the effect of climate change on these two marinas, especially in regard to the prevalence of NIS, which are supposed to be advantaged by increased heat.

## Materials and Methods

### Study sites

The two sites selected for our study were the Marina du Chateau in Brest (henceforth “Chateau”; Finistère, France; 48°22'45.4"N, 4°29'21.2"W) and the Port de Carry-le-Rouet (henceforth “Carry”; Bouches-du-Rhône, France; 43°19'47.0"N, 5°09'09.5"E). Both marinas have similar boating capacities (650 and 590 berths respectively), are semi-enclosed, and subject to anthropic impacts due to their position in their urban area and water runoffs at the inner parts of the marinas. We deployed experimental setups at the entrance of both marinas to avoid a synergetic and confounding effect of strong pollution in the inner parts of marinas (Je et al. 2004, Kenworthy et al. 2018b).

### Experimental setup

For each marina we deployed a structure with 10 settlement panels (Correx Polyethylene, 20 cm x 20 cm; Fig. 39). These recruitment panels were randomly assigned to be either control panels (5 per marina) or being constantly heated at +3°C above the recorded ambient water temperature (5 per marina). To reach a stable water temperature difference with ambient water, the settlement panels were placed at the top of transparent bottom opened acrylic cylinders. Water column warming of the cylinders was achieved through 200W aquaria resistors (approx. 20cm away from the panels) regulated with a built-in computer (Raspberry Pi3+) coupled to thermometers (DS18B20) measuring the outside water temperature (Fig. 39). The water temperature around the heating elements and directly around the panels was regularly monitored to avoid overheating. The design of the experimental system had been preliminarily tested to provide the best warming set up. The experiment lasted 50 days in both marinas and started in mid-August 2020 in Carry and early September for Chateau.



Fig. 39: *in situ* warming structure deployed in the marinas. Photo credit: Wilfried Thomas (CNRS Station Biologique de Roscoff)

### Fauna identification and community analysis

At the end of the experiment all panels were transported to the laboratory where they were maintained in open circuit aquaria until identification by a single observer in less than 36h. A surface of 15 cm x 15 cm was analyzed by superposing a 100-point grid allowing for a resolution of more than 0.4 points per cm<sup>2</sup> necessary for a reliable estimation of the surface cover (Taormina et al. 2020b). At each intersection of the grid all living individuals of any species were counted. Species present on a panel that did not fall within an intersection were counted with 1% cover. Identification went to the lowest possible taxonomical level (Hayward & Ryland 1979, 1985, 1995, 1998, Riedl 1983, Zabala & Maluquer 1988, Brunetti & Mastrototaro 2017b). After analysis, the panels were scraped, and biomass

was dried at 60°C for one week then weighted. To determine ash free dry mass (AFDM) the dry communities were burned at 520°C for 6 h.

The community matrix has been analyzed using the 'vegan' R package (version 2.4-6; Oksanen et al. 2018). The Shannon and Simpson diversity indices as well as Pielou's evenness and species richness were assessed for each panel. They were compared between the control and heated treatment with an ANOVA. A Poisson glm was applied for species richness since it constitutes count data. The community matrix was transformed into a Bray-Curtis dissimilarity matrix and multivariate homogeneity of groups was verified with the 'betadisper' function. A Principal Coordinate Analysis (PCoA) was used to visualize the community of both treatments for the two marinas. A subsequent PERMANOVA (10<sup>5</sup> perm.) was used to assess for significant differences between treatments of each marina. To identify the species contributing to the differences between treatments a SIMPER analysis followed by an Multipattern analysis using the 'indicSpecies' package was conducted (10<sup>4</sup> perm.; ver. 1.7.1; De Cáceres 2013). An ANOVA to determine if significant differences were present between treatments was applied to the AFDM for each marina. The cover occupied by empty space without any species and the cover exerted by NIS, was analyzed via a Poisson glm since they originated from count data (One count = 1% cover).

### **Metabolome identification and analysis**

*Bugula neritina* was chosen as model organism for metabolomic analyses. This species has been the object of previous studies in the Chateau marina and seems to be a good model species for metabolomics. Additionally, this species occurs in both marinas due to its cosmopolitan distribution. Unfortunately, individuals in the Carry marina were absent or too small for Metabolomic sampling, so analyses were only conducted for the Chateau marina. For each panel one large colony of *B. neritina* was sampled. The samples were immediately frozen in dry ice, then stored at -80°C, freeze dried and ground to dust. The total metabolome was extracted out of 5 mg of dry powder with 3 times 1 mL MeOH/DCM (CH<sub>2</sub>Cl<sub>2</sub>; 1:1) and ultrasonication at room temperature during 2 min. Supernatants were filtered on PTFE syringe filters (13 mm, 0.22 µm, Restek®). Two samples (one of each treatment) were excluded due to strong contamination. Before injection into Liquid Chromatography Mass Spectrometry (LC-MS) the samples were resuspended in 500 µL MeOH and a pool sample was created by mixing 30µl of each. Two blank samples, which were submitted to the entirety of the extraction process were additionally produced. LC-MS were performed with UHPLC (Dionex Ultimate 3000 with RS Pump, autosampler, thermostat column and UV diode array, Thermo Scientific®) coupled to a quadrupole Time of Flight spectrometer (qToF) with an ESI source (Impact II, Bruker Daltonics®). We conducted UHPLC separation on an Acclaim™ RSLC 120C18 column (150 mm x 2.1 mm, 2.2 µm,

ThermoScientific®) at 40°C at 0.5 mL min<sup>-1</sup> elution rate. Water (Polar, LC/MS grade, Carlo Erba®) with 0.1% formic acid (A) and acetonitrile (Apolar, LC/MS grade) with 0.1% formic acid (B) were used as chromatographic solvents. Chromatography cycles were set as follows: 1) mix of 5:95 of A:B during 2 min, 2) linear increase up to 100% B during 8 min followed by 100% B during 4 min, 3) return to initial conditions (5:95, A:B) for 3 min (17 min total). We injected 10 µL in MS1+ and MS2+ with N<sub>2</sub> at 3.5 bars as nebulizer gas, N<sub>2</sub> at 12 L min<sup>-1</sup> as dry gas, capillary temp. at 200 °C and voltage at 3500 V for a scan from 50 to 1200 mass units at 2 Hz. A spectrometer calibration with a formate:acetate solution was performed before each sample and the pool sample was injected every 4 samples to correct for source drift. Blank samples and pure solvent were injected to filter for ions linked to the extraction process.

LC-MS profiles were automatically recalibrated with the internal calibration in 'Bruker Compass DataAnalysis' (version 4.3) resulting in a *m/z* precision of 2 ppm. The netCDF data was processed with the 'XCMS' R package (version 3.12; Smith et al. 2006) with peak picking for the detection of features, obiwarp retention time correction, feature grouping (time frame 5 s) and peak filling. Ions were manually normalized in Microsoft Excel (Van Der Kloet et al. 2009) and were filtered to exclude ions originating from the blanks, based on signal/noise ratio (*S/N* = 10), ions with high variability in the pool sample (> 0.2) and ions that were auto-correlated (> 0.8). To increase relevancy, we excluded the first 100 seconds of chromatography of our analysis.

Metabolome analyses were mainly performed in 'Metaboanalyst' (Xia et al. 2009). Our matrix had less than 5000 features and was log transformed and auto scaled. A Principal Correspondence Analysis (PCA) was used to visualize our sample structure. A PPLS-DA driven permutation Model Validation Analysis (MVA) (10<sup>5</sup> perm., 6 components) using the 'RVAideMemoire' package in R (version 0.9-79; Hervé 2021) tested for statistical differences between the treatments. A Volcano plot from 'Metaboanalyst' identified features with a significant foldchange (t-Test, *p* < 0.05; FC > 1.5). These molecules were analyzed with fragmentation mass spectrometry (MS2) spectra. Molecular networking via Global Natural Products Social Molecular Networking (GNPS; Wang et al. 2016) and visualized in 'cytoscape' (version 3.7.2) helped ion identification.



## Results

None of the biodiversity indices had significant differences between control and heated panels in any marina (Carry:  $H' J' \lambda$ , ANOVA,  $|R^2| < 0.27$ ,  $p > 0.07$ ; S, Poisson glm,  $z = -0.52$ ,  $p = 0.62$ ; Chateau:  $H' J' \lambda$ , ANOVA,  $|R^2| < 0.12$ ;  $p > 0.33$ ; S, Poisson glm,  $z = 0.25$ ,  $p = 0.81$ ). The species list for each marina can be found in Sup. Tab. 4. On the PCoA of the community structure (Fig. 40), panels were distributed according to their treatment (Control vs Heated) on the PCo1 axis, explaining 40.6% of the variability between samples for Carry (Fig. 40 A) and 70.7% for Chateau (Fig. 40 B). The PCo2 axis (< 26.3%) corresponded to intra treatment variability. For both marinas, significant effects of the warming were observable on the communities (PERMANOVA; Carry:  $R^2 = 0.34$ ,  $p = 0.008$ ; Chateau:  $R^2 = 0.54$ ,  $p = 0.008$ ). The SIMPER analysis reveals that in both cases, 5 species contribute to more than 70% of the contrast between the treatments (over 85% for Chateau; Fig. 41; Tab. 20). At the Carry marina, only *Aetea sica* has been identified as indicator species for the control (Tab. 20). In the Chateau marina however, most of the contributors identified by SIMPER analysis were also indicator species of the control treatment (Tab. 20). For this marina two additional species were identified as indicator species for the heated treatment: *Cryptosula pallasiana*, and *Spirobranchus triqueter* (Tab. 20).

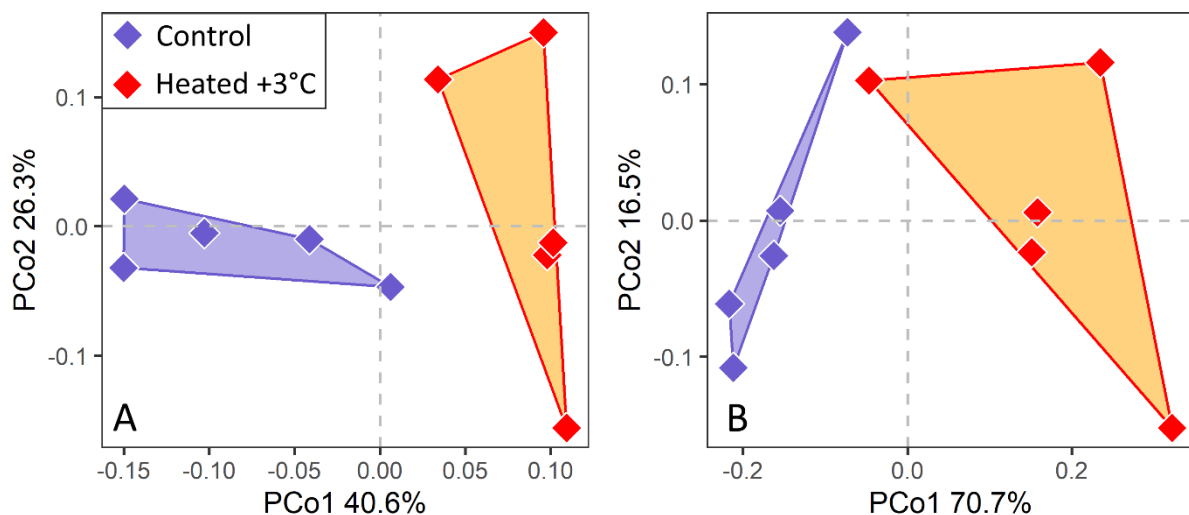


Fig. 40: Principal Coordinate Analysis (PCoA) of the control and heated (+3°C) communities in the A) Carry le Rouet Marina (Mediterranean) and in B) Marina du Chateau (Atlantic). PERMANOVA: Carry ( $R^2 = 0.34$ ,  $p = 0.008$ ); Chateau ( $R^2 = 0.54$ ,  $p = 0.008$ )

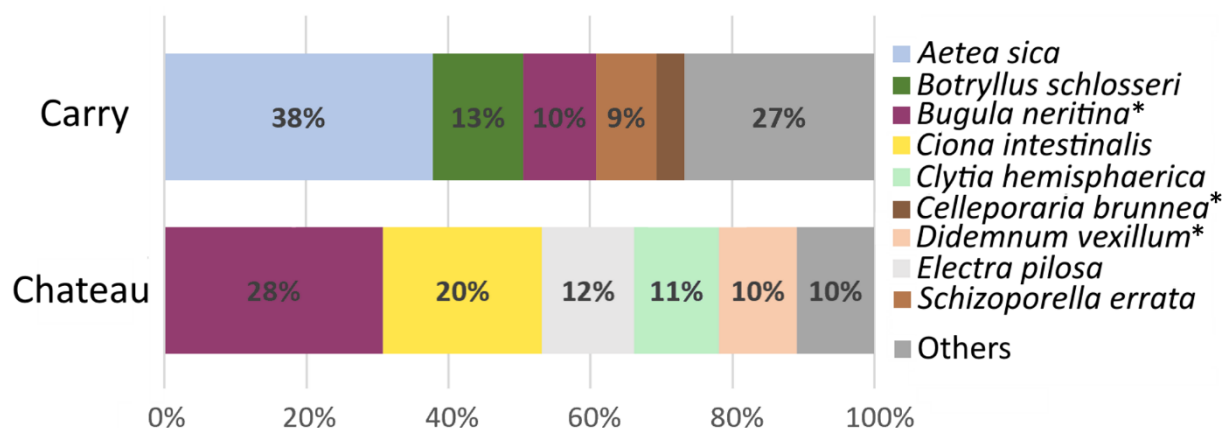


Fig. 41: Cumulated contribution of different species to the contrast between the control and heated (+3°C) communities in the Carry le Rouet and Chateau marinas (SIMPER analysis). Species with an asterisk are non indigenous species.

Tab. 20: Species most contributing to the contrast between the control and heated (+3°C) communities and indicator species for the treatments. Species in bold with an asterisk are non indigenous species. SIMPER analysis score indicates the percentage a species contributes to the contrast between treatments (see also Fig. 3). The direction of the warming effect on the percent cover occupied by these species is indicated. Indicator species for each treatment identified through Multipattern analysis are indicated with their association statistic (Stat.) and the p-value. SIMPER scores in parentheses are scores that are not part of the most important contributors.

Carry le Rouet						
Species	SIMPER Score	Effect of warming	Indicator species contol		Indicator species heated	
			Stat.	p-value	Stat.	p-value
<i>Aetea sica</i>	38%	↘	0.76	0.008		
<i>Botryllus schlosseri</i>	13%	↘				
<b><i>Bugula neritina</i>*</b>	10%	↗				
<i>Schizoporella errata</i>	9%	↘				
<b><i>Celleporaria brunnea</i>*</b>	4%	↗				

Chateau Marina						
Species	SIMPER Score	Effect of warming	Indicator species contol		Indicator species heated	
			Stat.	p-value	Stat.	p-value
<b><i>Bugula neritina</i>*</b>	28%	↘	0.78	0.015		
<i>Ciona intestinalis</i>	20%	↘	0.89	0.007		
<i>Electra pilosa</i>	12%	↘	0.84	0.040		
<i>Clytia hemisphaerica</i>	11%	↗				
<b><i>Didemnum vexillum</i>*</b>	10%	↘	0.90	0.015		
<i>Spirobranchus triqueter</i>	(4%)	↗			0.90	0.047
<b><i>Cryptosula pallasiana</i>*</b>	(3%)	↗			0.91	0.033

Ash free dry mass (AFDM) varied significantly between treatments for both marinas (ANOVA,  $R^2 > 0.56$ ,  $p < 0.005$ ; Fig. 42) with lower biomass in the heated treatment. Accordingly, empty space was significantly higher in the heated treatment for both marinas (Poisson glm,  $z > 2.28$ ,  $p < 0.022$ ; Fig. 42 A B). The absolute cover exerted by NIS as well as the ratio of NIS cover on total cover (proportional cover) was higher in the heated treatment for Carry le Rouet (Poisson glm,  $z = 2.149$ ,  $p = 0.0317$ ; Fig. 42 C; ANOVA,  $R^2 = 0.29$ ,  $p$  value = 0.063). In the Chateau marina the absolute cover was significantly lower in the heated treatment while the proportional cover remained unaffected (Poisson glm,  $z = -5.36$ ,  $p < 0.001$ ; Fig. 42 D; ANOVA, NS).

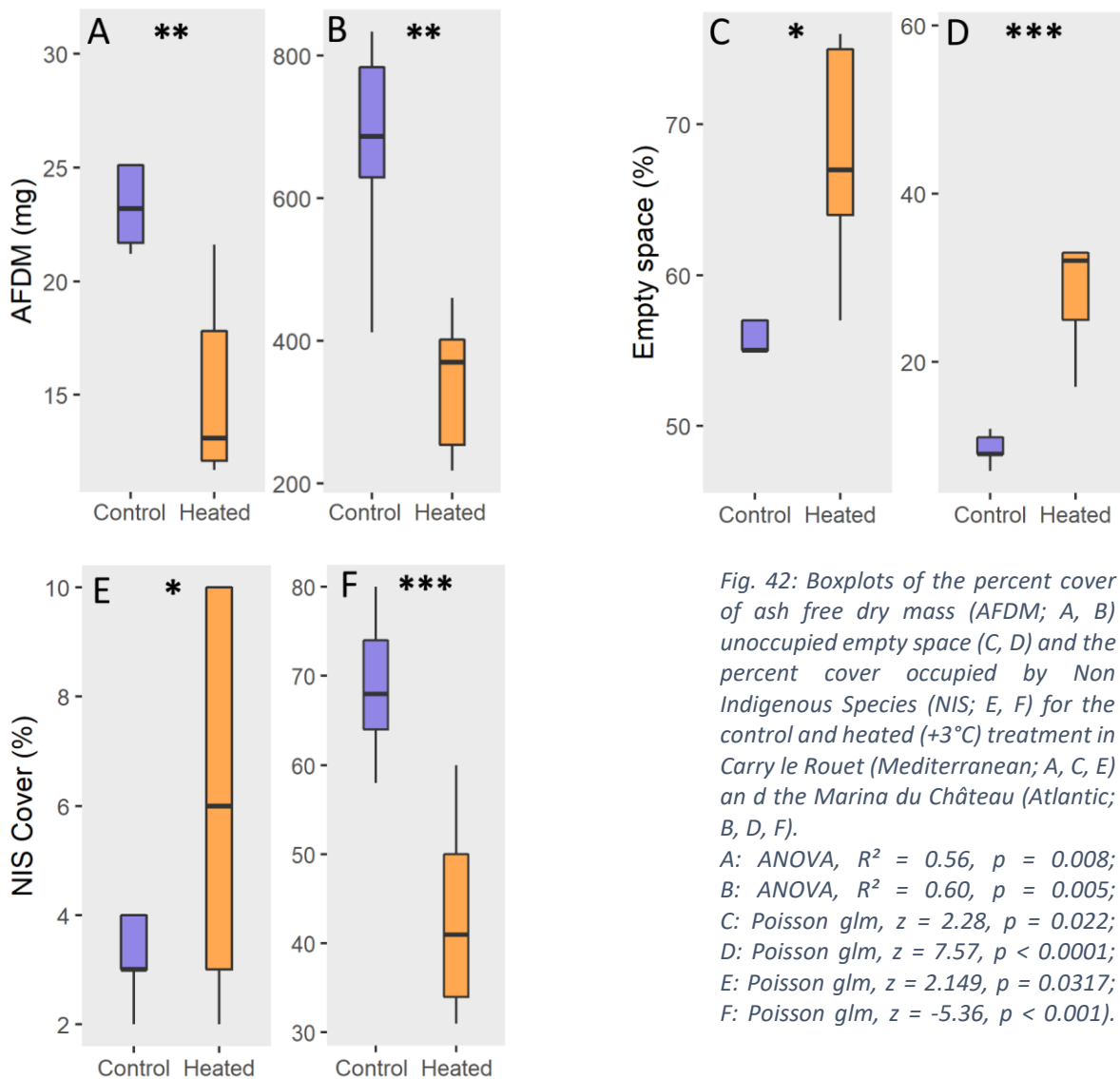


Fig. 42: Boxplots of the percent cover of ash free dry mass (AFDM; A, B) unoccupied empty space (C, D) and the percent cover occupied by Non Indigenous Species (NIS; E, F) for the control and heated (+3°C) treatment in Carry le Rouet (Mediterranean; A, C, E) and the Marina du Château (Atlantic; B, D, F).

A: ANOVA,  $R^2 = 0.56$ ,  $p = 0.008$ ;  
 B: ANOVA,  $R^2 = 0.60$ ,  $p = 0.005$ ;  
 C: Poisson glm,  $z = 2.28$ ,  $p = 0.022$ ;  
 D: Poisson glm,  $z = 7.57$ ,  $p < 0.0001$ ;  
 E: Poisson glm,  $z = 2.149$ ,  $p = 0.0317$ ;  
 F: Poisson glm,  $z = -5.36$ ,  $p < 0.001$ ).

## Metabolome analysis

The metabolome of *Bugula neritina* showed a marginally significant difference between the control and the heated treatment (Fig. 43; MVA: CER = 0.181,  $p$ -value = 0.052). A total of 12 molecules were found to vary significantly between treatments. Seven had a significantly lower fold change while 5 had a significant overexpression (t-Test,  $p < 0.05$ ; Fig. 44). From these molecules, we were able to identify 6 (Tab. 21).

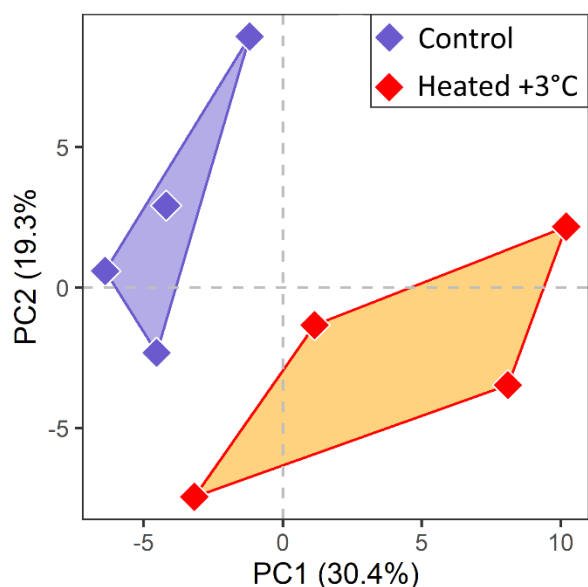


Fig. 43: Principal Correspondence Analysis (PCA) of the metabolome of *Bugula neritina* for the control and heated (+3°C) treatment in the Chateau marina (Atlantic; MVA: CER = 0.181,  $p$ -value = 0.052).

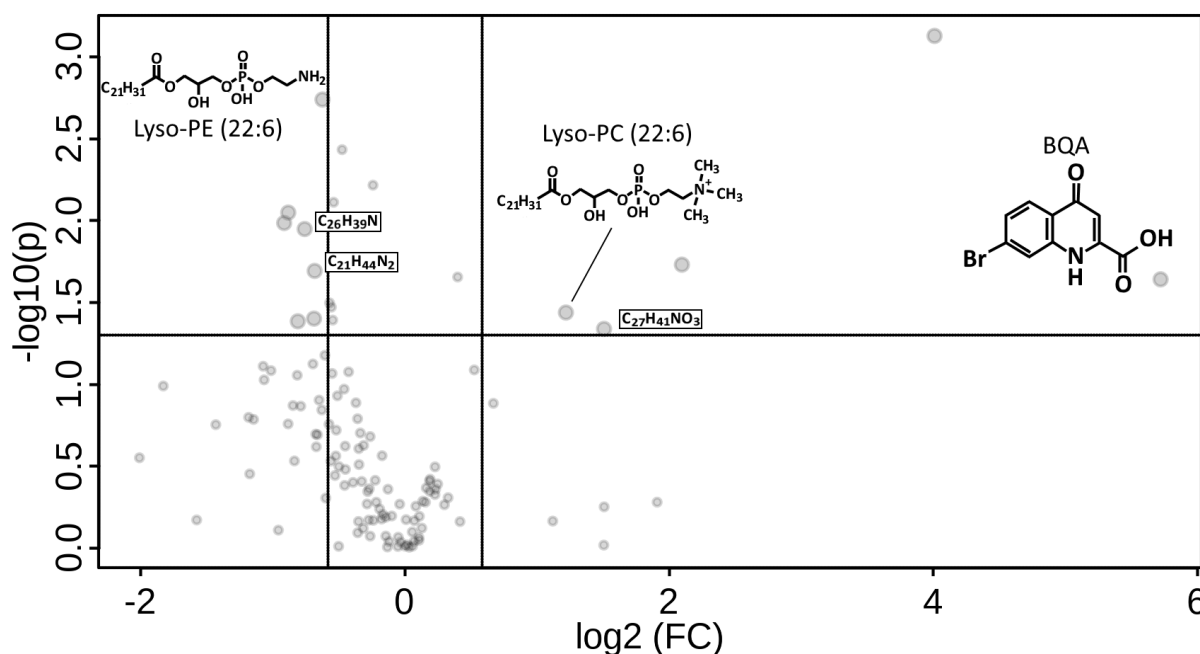


Fig. 44: Volcano plot showing molecules of the metabolome of *Bugula neritina* in the Chateau marina (Atlantic) with a significant (t-test;  $p < 0.05$ ) fold change ( $FC > 1.5$ ) in the heated (+3°C) treatment compared to control (indicated by larger dots on plot). Left: underexpressed molecules compared to control; Right: overexpressed molecules compared to control. The y-intercept corresponds to a  $p$ -value of 0.05. Molecules from Tab. 2 indicated.

Tab. 21: Identified molecules with a significant fold change (FC) between heated vs control treatments in *Bugula neritina* in the Chateau marina. Identification confidence levels are indicated (Schymanski et al. 2014): 5 Exact mass (with tentative formula), 4 Unequivocal molecular formula, 3 Tentative candidate (structure), 2b Probable structure by diagnostic evidence.

Molecule	Formula	m/z	rt	FC	p.val	Confid.
C <sub>21</sub> H <sub>44</sub> N <sub>2</sub>	C <sub>21</sub> H <sub>44</sub> N <sub>2</sub>	325.36	538	0,54	0,01	5
C <sub>27</sub> H <sub>41</sub> NO <sub>3</sub>	C <sub>27</sub> H <sub>41</sub> NO <sub>3</sub>	428.32	769	2,84	0,05	5 to 4
C <sub>26</sub> H <sub>39</sub> N	C <sub>26</sub> H <sub>39</sub> N	366.32	648	0,59	0,01	4
Bromo Oxo Quinoline Carboxylic Acid	C <sub>10</sub> H <sub>6</sub> BrNO <sub>3</sub>	267.96	394	52,73	0,02	3
Lyso-PE (22:6)	C <sub>27</sub> H <sub>44</sub> NO <sub>7</sub> P	526.29	612	0,65	< 0.001	2b
Lyso-PC (22:6)	C <sub>30</sub> H <sub>50</sub> NO <sub>7</sub> P	568.34	623	2,33	0,04	2b

## Discussion

The present results indicate that long-term warming due to climate change may affect marina communities and the metabolism of certain species. We chose to simulate *in situ* a warming of +3°C compared to the control in accordance with the mean estimate of global surface temperature increase projected in RCP8.5 (IPCC 2019). Simulated warming in mesocosms (Sorte et al. 2010b) and *in situ* (Smale et al. 2011, 2017, Ashton et al. 2017) has been shown to strongly impact communities favoring opportunistic species and NIS. In our present case we did in fact observe a significant difference in the recruited communities between heated and control treatments in both marinas, associated to lower biomass (AFDM) and higher empty space in the heated treatment. In the Carry-le-Rouet marina (Carry, Mediterranean Sea) this did coincide with higher absolute and proportional abundance of introduced species in the heated treatment and this despite. In the marina du Chateau de Brest (Chateau, Atlantic) however, they were negatively impacted by warming, just as were other species as shown by the constant proportional abundance. Yet, *Cryptosula pallasiana*, native from the Mediterranean but introduced in the Atlantic, has been identified as an indicator species of the heated treatment in the Chateau marina. While anecdotal, this might indicate that this species, which should be used to the warmer water of the Mediterranean could have profited from our simulated warming.

The higher temperature did however not only influence the settlement dynamics on the panels, but also had an impact on the metabolism of our model species. In Carry we were unfortunately unable to sample *B. neritina* since too few individuals were present (mainly in the control). In the Chateau marina however a slight difference between the metabolome of the heated and the control treatment is visible in the PCA and MVA test.

A total of 12 molecules had significant fold changes (FC > 1.5) between the treatments. Most notably two phospholipids (Glycerophosphoethanolamine, Lyso-PE (22:6) and Glycerophosphocholine, Lyso-PC (22:6)) and a molecule we suspect to be a Bromo Quinolinecarboxylic Acid (BQA), were identified

as significantly affected molecules. BQA fragmentation (Sup. Fig. 5) seems compatible with a molecule akin to Caelestine A (BQA) which has been described in the ascidian *Aplidium caelestis* (Yin et al. 2010). Quinolinecarboxylic acids (QA) seem to inhibit the activity of one of the most common protein kinases, Protein Kinase CK2 (Syniugin et al. 2016), which has numerous functions including inhibiting apoptosis and whose activity seems to increase with cellular stress (Manni et al. 2012, Piazza et al. 2012). Caelestines (including BQA) have been shown to have only minor cyto- and bacterio-toxicity at high concentrations (Yin et al. 2010). QA derivatives have rarely been identified in nature (Yin et al. 2010) and their exact function is thus difficult to establish. Considering the very high overexpression of our BQA in the heated treatment and the implication of QAs with the inhibition of CK2, this molecule might be linked to the stress response of *Bugula neritina* to heat stress. While yet speculative the implication of BQA in *B. neritina* heat stress response should be further investigated in future experiments.

Two phospholipids varied between the control and the heated treatment. Phospholipids play a major role in cell membrane fluidity, which changes as a response to ambient temperature (Pruitt 1990, Cuculescu et al. 1995, Sung et al. 2003, Chen et al. 2016). The type of lipids used as well as the number of unsaturation is a key component in this response (Pruitt 1990, Cuculescu et al. 1995). It is interesting to note that in our case, both phospholipids have the same adducts. Lyso-PC (22:6) might result directly from the conversion of Lyso-PE (22:6) through successive methylation by the phosphatidylethanolamine N-methyltransferase (Gibellini & Smith 2010), indicating that this conversion is associated to the increased heat. In the marine algae *Pyropia sp.* long term warming and short term heat shocks modified Lyso-PC and Lyso-PE concentrations generally increasing the first and decreasing the latter (Chen et al. 2016, Song et al. 2018), which corresponds to our observed results. Furthermore, PC and PE of different length including PC (22:6) and PE (22:6) have been shown to vary in *Carcinus maenas* and *Cancer pagurus* in respect to temperature (Cuculescu et al. 1995). The same Lyso-PE has also been previously described in *B. neritina*, varying according to environmental conditions in the Chateau marina (Chapter II). It may constitute a marker of good environmental conditions as Lyso-PE may play a role in gametogenesis in certain marine organisms (Ivanisevic et al. 2011).

It is interesting to note that despite apparent similarities of the effect of +3°C warming in both marinas (Community differences, lower biomass, more empty space) the effect of warming on introduced species were opposite, including *B. neritina*. In the Chateau marina the higher temperature led to lower abundance of introduced species indicating that the generated heat stress may cause a disadvantage. This is further supported by the metabolomic differences between heated and control treatment in the Chateau marina, potentially indicating heat stress in *B. neritina*. Yet, in Carry le Rouet introduced species and *B. neritina* had an increased abundance in the heated treatment. This is more in line with

what has been described in the literature, as opportunistic species and NIS were shown to have higher biomass in warm treatments (Smale et al. 2011, 2017, Ashton et al. 2017). *B. neritina* has been shown to have increased growth under higher temperatures (Lord 2017a). Numerous introduced species, including *B. neritina* are supposed to have a certain heat tolerance (Hitoshi & Kazutsugu 1984, Zerebecki & Sorte 2011, Lejeusne et al. 2014, Lord 2017a, Kenworthy et al. 2018a). However, temperature tolerance of several fouling species including *B. neritina* has been shown to be highly variable (2°C) between populations of different areas (Sorte et al. 2011; Zerebecki and Sorte 2011). This is due to adaptation of populations to their local climate and its variability (Sorte et al. 2011). Tolerance to temperature related stress may be acquired through numerous cellular pathways (Sung et al. 2003). Such tolerance can be increased in an invasive species by the exposure to stress (Clutton et al. 2021) by modifying epigenetics (Ardura et al. 2018) or be modulated by maternal effects (Burgess & Marshall 2011). High temperature heatwaves in a marina close to Carry le Rouet have been shown to lead to summer mass mortality for numerous species including *B. neritina* (Chapter III B). It may be possible that the regular exposure of Mediterranean communities to high temperatures and heat waves (Garrabou et al. 2009; Lejeusne et al. 2010, Chapter III B) increases the thermal tolerance of present introduced species, thus allowing their spread in warmer water. Conversely, the water temperature in the vicinity of the Chateau marina is more constant due to rarer heatwaves and higher water mixing due to higher tides and wave action (less stratification). This may explain why the simulated warming strongly impacted the present introduced species which would not be accustomed to such disturbance.

The present results indicate that in the short term, climate change caused warming might not cause the same effect on the prevalence of introduced species everywhere and that certain locations, where regular heat events are already experienced, might have a higher risk at favoring them compared to cooler and more stable climates. This might be attributed to heat resistant individuals being favored by the intense selection exerted by heat waves. The impact of climate change might thus also depend on how global warming modifies cooler temperate climates and if this increases extreme weather events like heat waves. It is crucial that further studies investigate *in situ* how heat waves and climate change caused temperature rising influence marine urban communities and the impact of introduced species in them.

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## Supplementary Material

### Getting used to the heat – Climate change might only favor introduced species climates with regular heat waves.

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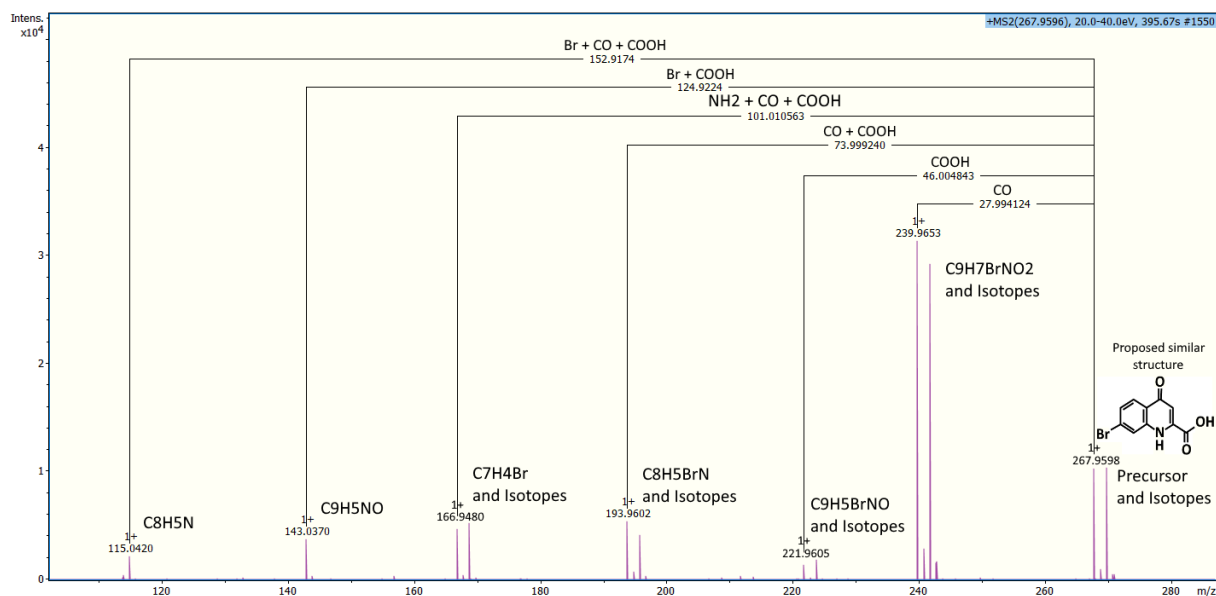
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**Abstract.** Climate change constitutes a major challenge for marine urban ecosystems and ocean warming will likely strongly affect local communities. Introduced species have often been shown to have higher heat resistance, but *in situ* studies testing how expected future global warming might affect them remain scarce, especially in marine urban environments. Here we used an *in situ* warming experiment in a NW Mediterranean (warm temperate) and a NE Atlantic (cold temperate) marina to see how climate change might affect recruited communities in the near future. In both marinas, warming resulted in significantly different communities, lower biomass, and more empty space compared to control. However, while in the warm temperate marina, introduced species showed an increased surface cover, their surface cover was reduced in the cold temperate one. Metabolomic analyses in the Atlantic marina revealed potential heat stress experienced by the introduced bryozoan *Bugula neritina*. The present results might indicate that in the near future, global warming might not cause identical effects everywhere on the prevalence of introduced species, and some locations where regular heat events are already experienced might have a higher risk at favoring them compared to cooler and more stable climates.

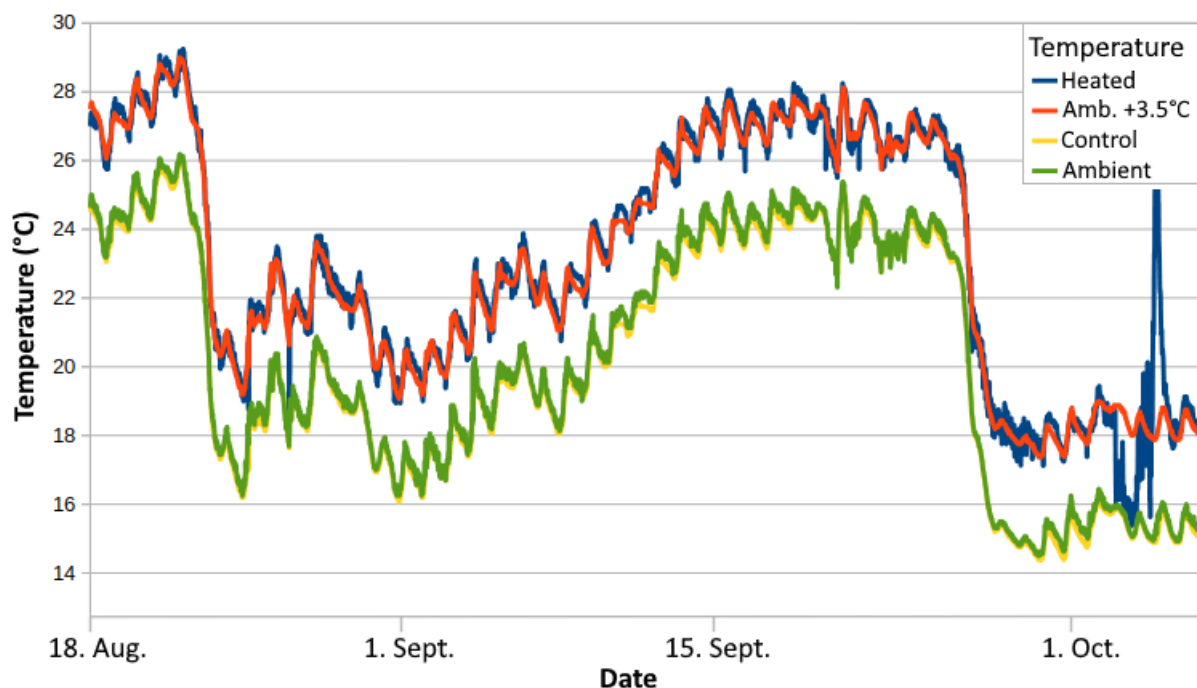
**Keywords:** Non-Indigenous species; experimental climate change; *in situ* warming; marina; hot plates

Sup. Tab. 4: Species list for both studied harbors. Introduced species in the respective harbor are indicated with an asterisk.

Phylum	Marina du Château	Carry le Rouet
Annelida	Circeis armoricana Circeis spirillum Janua pagenstecheri Neodexiospira brasiliensis*	Janua pagenstecheri Pileolaria berkeleyana Pileolaria militaris Salmacina incrustans Spirorbis cuneatus Spirorbis marioni Spirorbis rupestris
Anthozoa	Sagartia elegans	
Arthropoda	Perforatus perforatus	Perforatus perforatus
Asciacea	Ciona intestinalis Didemnum vexillum* Botryllus schlosseri Asciella aspersa Clavelina lepadiformis Asterocarpa humilis* Perophora listeri	Botryllus schlosseri Asciella aspersa
Bryozoa	Bugula neritina* Bugulina fulva Cryptosula pallasiana* Electra pilosa Watersipora subatra*	Aetea sica Bugula neritina* Bugulina simplex Celleporaria brunnea* Cryptosula pallasiana Filicrisia geniculata Schizoporella errata Cradoscrupocellaria bertholletii* Watersipora subatra*
Hydrozoa	Clytia hemisphaerica Obelia geniculata	
Mollusca	Anomia ehippium	Anomia ehippium
Porifera	Sycon ciliatum	Clathrina coriacea Sycon ciliatum Sycon raphanus



Sup. Fig. 5: MS2+ fragments of a molecule we identified as Bromo Quinolinecarboxylic Acid (C10H6BrNO3). Proposed similar structure: Caelestine A from *Aplidium caelestis* (Yin et al. 2010). Exact position of Br, O and COOH is unknown. 'Bruker Compass DataAnalysis' (version 4.3)



Sup. Fig. 6 Example of the temperature curve of one heated (Blue) and one control treatment (Yellow). For reference the ambient temperature (control supposed = ambient; green) and the targeted ambient +3.5°C (Heated supposed = ambient +3.5°C; red) are indicated.



# Discussion

## I Selective Pressure by Disturbance Gradients in Marinas

The present work aimed to understand how disturbance gradients within marinas shape community structure, influence the prevalence of NIS, and how they might cause local adaptation on small spatial scale. Our photographic survey from three years of experiments in the inner, middle and entrance location of one marina (Chapter I) showed that despite heterogeneity in community structure a systematic difference between the entrance and the inner location of the marina exists. This reflects the results of previous studies that aimed to understand the link between pollution gradients and community structure (Je et al. 2004, Ryu et al. 2011, Kenworthy et al. 2018b). Combining these results obtained over three separate years of experiments with previous studies insinuates that a tangible process such as a disturbance gradient might operate at very small spatial scales, driving community composition and potentially causing local adaptation. All these studies, including ours can however only remain putative regarding the causal link between disturbance and community composition as they show correlative results. To expose the causal link between local variability and environmental factors such as disturbance gradients, manipulative experiments like reciprocal transplants with one species or with entire communities were required (Kawecki & Ebert 2004, Angert & Schemske 2005, Thorpe et al. 2005, Chang & Marshall 2016, Sork 2018). The main effort of our work laid thus in the reciprocal transplant experiments (Chapter I; Chapter III B; Chapter IV) that aimed to expose this causal link between local environmental conditions and community structure. In Chapter II, we were able to observe how a pollution gradient affected communities and individual species on different levels of biological organization and function (community structure, community respiration, pollutant uptake of an individual species, metabolomic analyses). The reciprocal transplant affected all these biological functions, exposing the causal link. We later aimed to extend the observations made in one marina to multiple marinas with different putative levels of disturbance. We replicated the reciprocal transplant experiment in a total of six marinas (three in the Mediterranean, three in the NE Atlantic; Chapter IV). This allowed to reveal that the causal relationship between heterogenous disturbance and community structure (and local adaptation) may be a common phenomenon. However, we also show that the situation is more complex than a straightforward 'gradient drives communities'. In Chapter I, Chapter II and Chapter III A, we expose that biotic interactions play an additional major role in community structure as we noticed a strong influence of some very competitive species like *Ciona intestinalis* and the effect of historical contingency (Origin effect in Chapter II and Chapter IV). This is concordant with previous transplant studies which have already demonstrated how primary colonization influences

subsequent community composition (Cifuentes et al. 2010, Chang & Marshall 2016). Yet, competition for space is not the only interaction we observed to influence fouling communities. Predation, as yet another biotic interaction, may be a major driver of community composition in fouling communities (Osman & Whitlatch 1995, Osman & Whitlatch 2004, Oricchio et al. 2016b a). In Chapter III A we exposed that the influence of predation might even surpass the effect of the disturbance gradient, depending on the studied species. Larger scale or stochastic processes may impair local adaptation (Kawecki & Ebert 2004). Biotic interactions like competition and predation might, thus, mask the effect of local adaptation to the disturbance gradient. They may also constitute a different selective pressure which may not necessarily be associated to the first gradient. Indeed, the arms race of biotic interactions has been noted to be a dominant driver of evolution (Darwin & Wallace 1858, Van Valen 1973, Benton 2009). However, we do still observe local adaptation in our experiments, indicating that biotic interactions might not completely mask local adaptation. This somewhat mimics a small scale (spatial and temporal) combination of Red Queen mechanics (biotic drivers) and Court Jester mechanics (abiotic drivers). Court Jester mechanics describe the larger scale (temporal and spatial) adaptation of species to their abiotic environment, while Red Queen mechanics due to the biological arms race act with high pressure on small scales (Barnosky 2001, Benton 2009). Here the temporally relatively stable disturbance gradients constitute abiotic selective pressures driving local adaptation, while biotic pressures drive small scale variability in community composition. When combining these different experiments, it is possible to conceptualize a duality of two opposing selection gradients (Fig. 48). On the one side, it is possible to distinguish a disturbance gradient with its maximum in the inner part of the marinas, which selects resistant species (including many NIS) and causes local adaptation. On the other side, from the entrance of the marina, a biotic resistance gradient with higher competition and higher predation exerts a pressure opposing the disturbance gradient. Although we have explicitly shown that biotic resistance may not always work against NIS (Chapter III A), it has yet been shown to be often important in regulating them (Dumont et al. 2011b a, Kimbro et al. 2013, Gestoso et al. 2018, Leclerc et al. 2019, Yorisue et al. 2019), sometimes even driving them into refuges (Dumont et al. 2011b). These opposing gradients are somewhat similar to what can be observed in intertidal habitats. Tide caused emersion, salinity increase or heat increase causes major physiological stress for marine organisms (Montagnes et al. 2002, Contreras-Porcia et al. 2017), which can be compared to the physiological stress that is constituted by pollution and other disturbances in the inner parts of marinas. The abiotic disturbance gradient constituted by the tide may determine the upper distribution levels of organism in these habitats, depending on the emersion length, frequency and the resistance of the present species (Peres & Picard 1964, Laborel 1986). However, predation and competition may be the major drivers of the lower distribution limits of many intertidal species (Peres & Picard 1964, Rilov & Schiel 2006). These species are thus often organized in clearly defined strips

(Peres and Picard 1964; Laborel 1986; Rilov and Schiel 2006). While their fundamental niche would be larger since only limited by abiotic factors, their realized niche is much smaller due to biotic pressures (Hutchinson 1957). This drives many species into refuges dependent on their capacity to escape biotic pressures by colonizing zones with high abiotic pressure (Robles & Desharnais 2002). Marinas and harbors have a very high percentage of NIS, which is sometimes attributed to their resistance to abiotic stress like pollution (Piola & Johnston 2008, Marins et al. 2010, Kenworthy et al. 2018b, Osborne & Poynton 2019). Many NIS are even completely exclusive to marinas (Marins et al. 2010, Ferrario et al. 2017). While some NIS may slowly infiltrate natural habitats, most of them remain exclusive to anthropogenic structures due to yet unknown processes (Simkanin et al. 2012). It may be possible, that akin to intertidal habitats, established NIS in marinas may use these habitats as a refuge, as they might be very resistant to abiotic disturbance, but less to biotic interactions. Should a NIS use the marina as refuges, it likely depends on its selection strategy. While the  $r/K$  model is not really applicable here as almost all the studied species were  $r$ -selection species (fast growth and high reproduction; MacArthur and Wilson 1967; Pianka 1970), it is possible to apply the  $C$ -,  $S$ - and  $R$ - selection models (competitive,  $C$ -selection with fast growth favoring own survival and competitiveness towards other species; stress-tolerant,  $S$ -selection for increased resistance to unfavorable conditions, stress and disturbance; ruderal,  $R$ -selection with all resources invested in reproduction; Grime 1977). Long duration (or permanent) disturbances like the pollution gradient here are known to select resistant phenotypes ( $S$ -selection; Grime 1977; Lake 2013; Nimmo et al. 2015). Species present in the inner location of the marina might thus have a  $S$ - $R$ -selection while the species at the entrance might adopt more a  $C$ - $R$ -selection strategy. For the numerous NIS present at the inner locations, this might be attributed to preadaptation (see Schlaepfer et al. 2010; MacDougall et al. 2018) as the species surviving introduction might already be adapted to physiologically challenging conditions. This may be further stabilized or even exacerbated by subsequent local adaptation. Pollution resistance by detoxification depends on costly metabolic pathways that require energy investment trade-offs (Handy et al. 1999; Pook et al. 2009). Being adapted to polluted environments could thus further drive NIS into  $S$ - $R$  strategies and impair their competitiveness in less polluted habitats due to wasted energy. Eco-evolutionary feedback loops are a major driver of microevolution in urban habitats (Alberti 2015) and many species have been demonstrated to adapt and evolve with anthropic impacts (Roesijadi et al. 1984, Atwell et al. 2012, Donihue & Lambert 2014, Brans et al. 2017, Martin et al. 2021). Integrating humans and their actions into a new definition of the Hutchinson's realized niche is a challenge of major importance (Alberti et al. 2003) as it may help to conceptualize how species exist and evolve in anthropic habitats. With the insights gained from confirming local adaptation in marinas, we may argue that the observed process is applicable to whole marine urban areas, as the disturbance gradient and

the presence of NIS exceeds the limits of our study area. Considering this might help to conceive management strategies to mitigate NIS (see II).

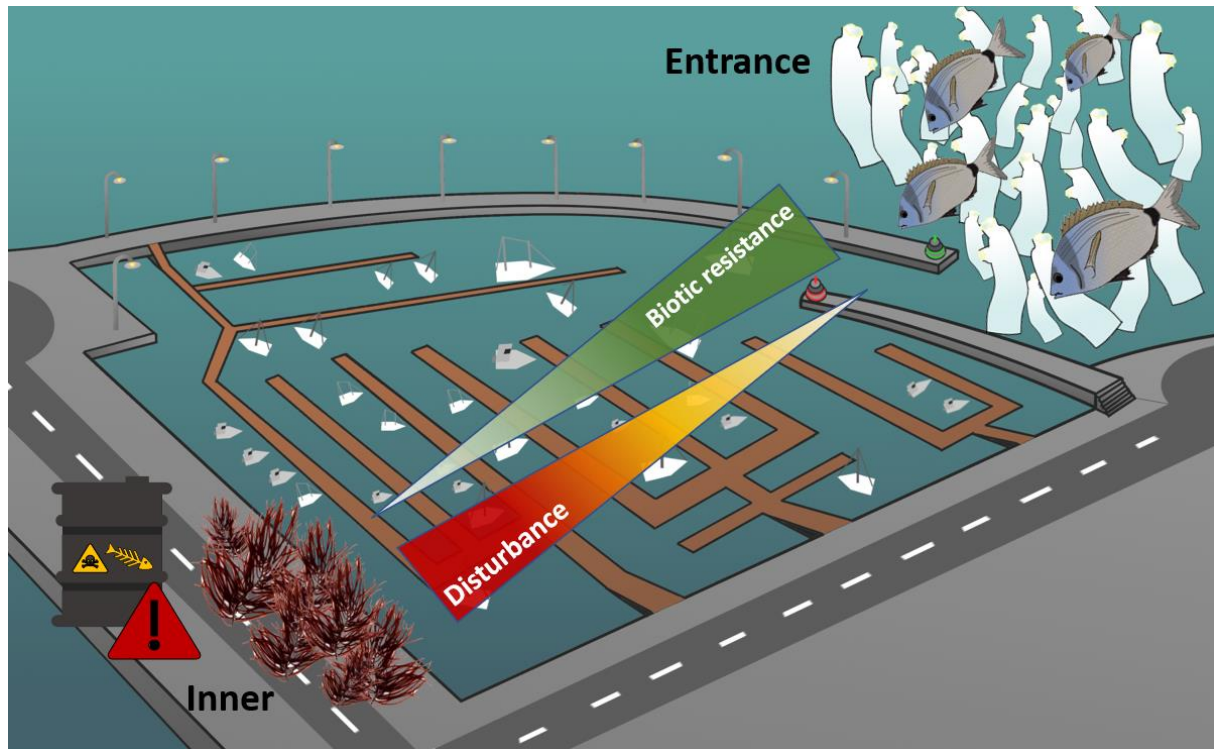


Fig. 45: Proposed opposing gradient framework developed from our works. At the left (inner marina), disturbance like pollution, temperature variations etc. is highest due to lower water mixing. Only stress resistant species can thrive at the inner marina (S-Selection; Grime 1977). This may include NIS, which seem to be more stress resistant. At the entrance and beyond, biotic interaction such as predation and competition are highest, with abundant C-Selection species, potentially exerting biotic resistance against NIS. For this reason, marinas might act as a spatial refuge for NIS.

The disturbance gradients, which in our experiments may have constituted press type disturbances, might have a stabilizing effect on some NIS through local adaptation, restricting them to the inner locations of marinas to escape competition. However, these gradients themselves may not always be stable: pollution may be variable and occur in pulses due to water discharges and changes in hydrodynamics (Johnston & Keough 2000, 2002, Johnston et al. 2002). Such pulse pollution has been shown to decrease otherwise dominant competitive species like ascidians (Johnston et al. 2002). Depending on the frequency of such pulse events, NIS may overcome the biotic resistance exerted by other species in otherwise less polluted habitats. As an example, the middle of a marina may be a highly variable environment as seen in Chapter I. Origin effects observed at this location with *Bugula neritina* and *Watersipora subatra* constituting indicator species with higher abundance (Chapter II), or higher NIS prevalence in communities transplanted from disturbed to less disturbed locations (Chapter IV) indicate that once these species gain a foothold, they may resist environmental change, which might be of concern in environments with variable pollution. Other pulse disturbances may also give



an advantage to NIS. As such, extreme climate events have been shown to have profound effect on many ecosystems (Garrabou et al. 2009, Smale et al. 2015, 2019, Pansch et al. 2018, Lonhart et al. 2019, Aoki et al. 2020, Strydom et al. 2020) and NIS seem to profit from these events due to yet again, higher resistance (Sorte et al. 2010a, Kenworthy et al. 2018a, Castro et al. 2021). With reduced occupied niches, heat stressed communities could exert less biotic resistance and thus further have their invasibility increased (Arenas et al. 2006, Bulleri et al. 2016). One of our experiments (Chapter III B), which originally aimed to demonstrate the local variation of community structure according to the disturbance gradient (like in Chapter II) was affected by such event, which allowed us to observe the effect of larger scale pulse disturbance on community structure. Our lucky sampling timing allowed us to confirm a spatial variability of communities between the inner, middle and entrance location of the studied marina (Vieux-Port of Marseille) and to correlate it with a pollution gradient before disturbance. However, at the end of the experiment, no such variability was observable anymore, with a strong die-off of the few originally dominant species and the multiplication of one single sub-family. We were able to attribute this to the impact of three subsequent heat waves during our experiment. Larger scale stochastic disturbances may mask/erase the effect of local adaptation as they select phenotypes independently of the original conditions (Kawecki & Ebert 2004). Regular heat waves might result in altered communities characterized by highly opportunistic and/or resistant species, which may include NIS. They would benefit from the available space and reduced competitiveness after a die-off. This further illustrates that NIS in harbors might benefit from pulse disturbances as they may be able to colonize environments originally not accessible to them due to higher competition (Fig. 46).

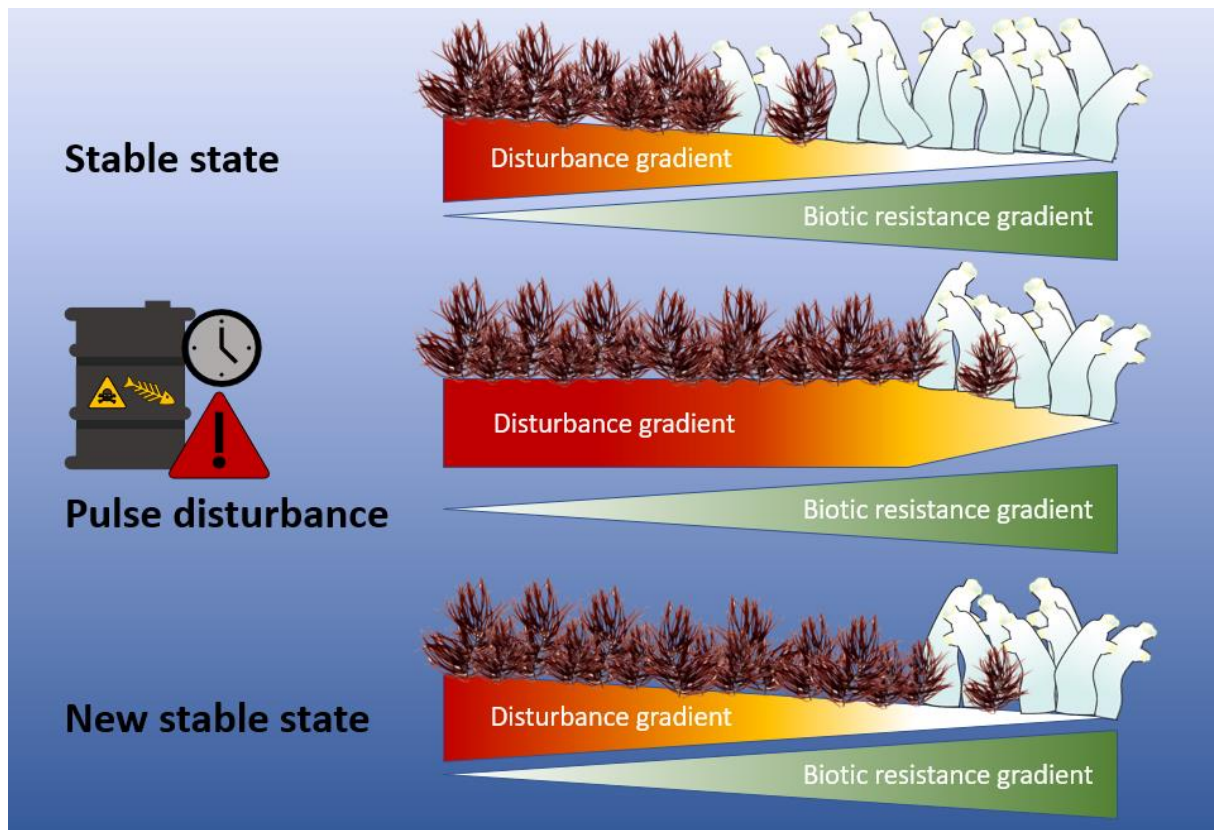


Fig. 46: Illustration of how pulse disturbance might impact the balance of the opposing gradients of disturbance and biotic resistance. On the top, a stable equilibrium exists between resistant species like many NIS in the disturbed inner part of the marina (left) and competitive species at the entrance of the marina (right). A pulse disturbance such as pollution might increase the impact of the disturbance gradient, giving a window of opportunity for resistant species to establish in previously inaccessible locations. After the end of the pulse disturbance a new stable equilibrium with a higher persistence of the resistant species has been reached. If these resistant species are non-indigenous, pulse disturbance might constitute an opportunity to spread.

## II Mitigation Methods of NIS in Marine Urban Environments

NIS, if they become invasive, can have drastic ecological consequences by restructuring ecosystems, leading to the loss of biodiversity and ecosystem services (Pejchar & Mooney 2009, Johnston et al. 2015b, Walsh et al. 2016). This may cause high economic impacts (Lovell et al. 2006, Olson 2006, Jardine & Sanchirico 2018). NIS are abundant in marinas which has been linked to ambient pollution, especially due to anti fouling compounds and reduced competition (Piola & Johnston 2008, Marins et al. 2010, Kenworthy et al. 2018b, Osborne & Poynton 2019). This is further contributed by ships, which are the major cause of the spread of NIS through hull fouling and ballast waters but also a major source of pollution (Piola et al. 2009, Canning-Clode et al. 2011, Dafforn et al. 2011, Sylvester et al. 2011, Ferrario et al. 2017). This might imply that an 'easy solution' to mitigate these species would be to reduce pollution to take away the competitive advantage of NIS. However, decreasing pollution might exacerbate the effects of NIS. When the anti-foulant tributyltin was forbidden on most leisure ships and mitigated due to its high environmental impact (Voulvoulis et al. 1999, Dafforn et al. 2008), an increase in NIS as has been observed in certain areas (Minchin & Gollasch 2010). Very careful planning is thus required when considering mitigating pollution in marinas (Dafforn et al. 2011). Our results indicate that the disturbance gradient, mainly composed by pollution, might be the reason why many NIS are restricted to marinas (and other artificial structures). It is however unclear if in our opposing gradient framework, the mitigation of disturbance would result in exclusion of NIS or their spread. While at first, they would have a clear competitive disadvantage as competitive species from the less disturbed parts of marinas could colonize the inner parts, this would force evolution in a different direction, and NIS might rapidly adapt and reallocate resources from pollution resistance towards other functions like growth or competitiveness. The Evolution of Improved Competitive Ability (EICA) hypothesis associates NIS success in invaded areas with a reallocation of resources originally intended for predation defense due to enemy release (Blossey & Notzold 1995, Joshi & Vrieling 2005). Here, diminished pollution might result in a reallocation of resources and cause an EICA due to stress release which could trigger the invasiveness of numerous NIS in marinas.

Another option to mitigate NIS might be to increase the biotic resistance instead. This might be achieved by reducing the effects of urbanization, and increasing conservation in peri-urban areas but also through eco-engineering to increase diversity (Lai et al. 2015, Dafforn 2017, Evans et al. 2019, Airoidi et al. 2021). As anthropic structures often lack complexity (*e.g.*, smooth plastic of pontoons or vertical smooth walls), one strategy might consist in increasing their complexity by including crevices (Strain et al. 2020a b) or water-retaining features (Firth et al. 2013, 2016a, Hall et al. 2018; Fig. 47). Using holes and grooves on intertidal rock fortifications against erosion has, for instance, been shown

to increase biodiversity and abundance of organisms while not giving an increased advantage to NIS (Hall et al. 2018). In a same manner, artificial tide pools may increase patchiness and thus local biodiversity (Firth et al. 2016a). However increased complexity does not always increase diversity and biotic and abiotic factors play a major role on the success of eco-engineering (Firth et al. 2016a, Strain et al. 2020b). Using seeded oysters to increase biodiversity on artificial structures might be a complementary approach (Strain et al. 2020a, Vozzo et al. 2021; Fig. 47) as these structures have the added benefit of filtering the water (Dafforn 2017, Vozzo et al. 2021) which perfectly integrates into the approach of making marine urban structures multifunctional (Dafforn et al. 2015, Firth et al. 2016b, Dafforn 2017). We exposed that local adaptation was present on very small scale within marinas. Marinas however just constitute a practical model representing urbanization of marine habitats. Urban areas generate a multitude of disturbances (reviewed in State of the Art III.B) organized as gradient (Airoldi et al. 2021). By extension we may propose that the eco-evolutionary processes observed here may be applicable to the larger urban area. We may propose that the best way to mitigate NIS in marine urban ecosystems might not be to start at the most disturbed sites where they are abundant and adapted to; but to slowly increase the quality of less disturbed sites through mitigation and eco-engineering, increasing the biotic resistance gradient, driving NIS further into their refuge. It might then be possible to mitigate them at the most disturbed sites by regulating pollution or by eradication campaigns.

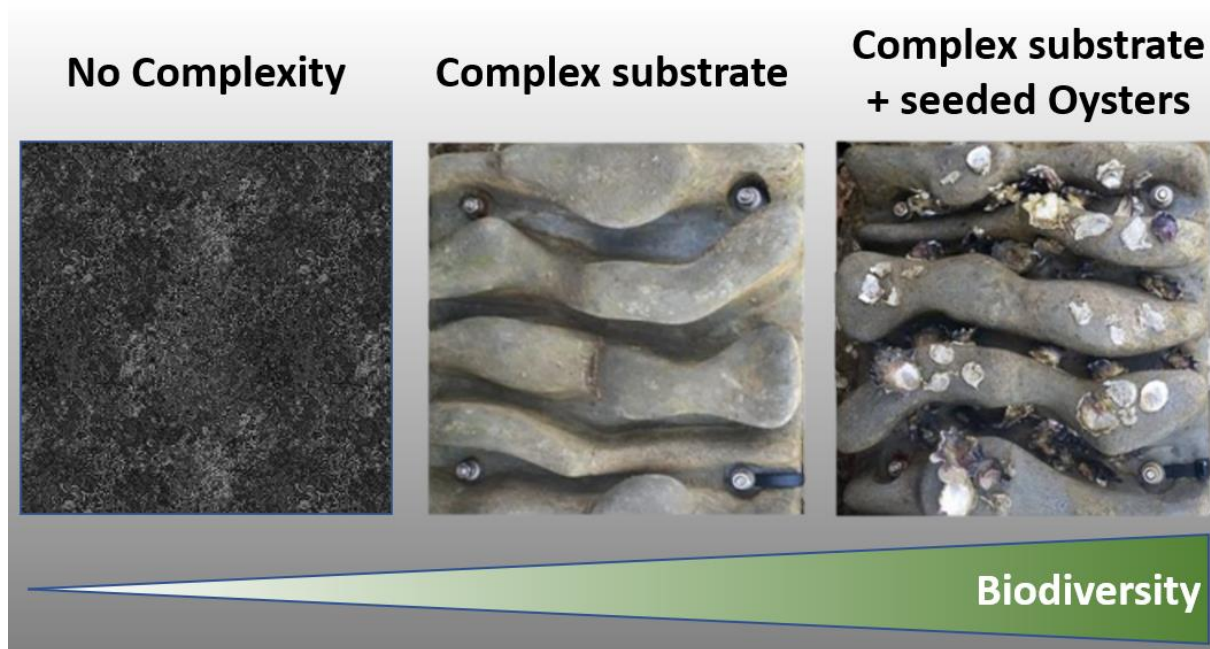


Fig. 47: How Ecoengineering might increase biodiversity (and potentially biotic resistance against NIS) in relation to substrate complexity. Left, straight concrete with low complexity and low biodiversity. Middle, complexification of substrate by ridges. Right, complex substrate with seeded oysters increases biodiversity. Photos © Strain et al. (2020a)

### III Climate Change and NIS – A Ticking Timebomb?

Due to the combustion of fossil fuels and other human activities, atmospheric CO<sub>2</sub> levels have drastically increased (Malhi 2002, Laxis et al. 2010, Cook et al. 2016), leading to climate change (Cook et al. 2016, Powell 2019, IPCC 2021). The global average temperature has already surpassed +1°C compared to preindustrial times (IPCC 2021) making it unlikely that mitigation promises will be able to limit global heating to +1.5°C. A scenario with a global temperature increase of +3.2°C seems more likely (Raftery et al. 2017). Climate change already strongly affects ecosystems with shifts in community composition, range changes in multiple species, and changes in trophic function of ecosystems (Hanson & Weltzin 2000, Ayres & Lombardero 2000, Hughes et al. 2003, Lejeusne et al. 2010, Waldock et al. 2018, Lonhart et al. 2019). This highlights a complex ensemble of changes that may potentially exacerbate the effect of NIS. Climate change may modulate invasions as new areas become available to NIS due to ocean warming in previously too cold areas (Occhipinti-Ambrogi 2007, Zhang et al. 2020, Vilizzi et al. 2021). Many invasive species could already potentially colonize areas larger than their current distribution, suggesting that they might invade new areas soon (Zhang et al. 2020). In the Mediterranean this process is particularly visible, as Lessepsian species (species originating from the red sea and introduced through the Suez Canal) like the fishes *Siganus* spp. and *Pterois miles* already extend their introduced range due to climate change, which is predicted to further increase in the future (Lejeusne et al. 2010, Chartosia & Menicou 2012, D’Amen & Azzurro 2020) Climate change could modify environmental conditions enough so that many established but not yet invasive NIS become invasive (sleepers species; Bradley et al. 2018; Spear et al. 2021). Marinas and harbors contain a disproportionately greater concentration of NIS than other areas (Mineur et al. 2008, Sylvester et al. 2011, Clarke Murray et al. 2012), and many seem to be restrained to these habitats (Simkanin et al. 2012), which might be caused by the opposing gradients developed earlier. A significant portion of these might be sleeper species that will become invasive if environmental conditions are affected by climate change (Bradley et al. 2018, Spear et al. 2021). It is however until now unclear if and how many of NIS in marinas might be sleeper species. In Chapter V we simulated climate change *in situ* to observe how it may affect marinas and how it might provide an advantage for NIS. We deployed heating structures at the entrance location in two marinas, where resistant species, including NIS, were supposed to be less abundant and more competitive native species present instead (Chapter I; Chapter II; Chapter IV). Increased heat should be expected to increase NIS prevalence, since they seem to have higher thermal resistance (Sorte et al. 2010b, Kelley 2014, Lord 2017a, Kenworthy et al. 2018a). While we did indeed observe this for the Mediterranean marina, NIS were impacted like all other species in the Atlantic marina with only one benefiting NIS. Temperature tolerance seems to be dependent on the studied population (Sorte et al. 2011; Zerebecki and Sorte 2011). This might be due to genetic

differences in the populations and due to large scale local adaptation (Mediterranean vs Atlantic), however other processes may also be at play. It was hypothesized that the regular exposure of Mediterranean communities to high temperatures and heat waves (Garrabou et al. 2009; Lejeune et al. 2010) constitutes a filter selecting heat-resistant individuals within the population. This selection would not occur in the Atlantic marina due to a more stable temperature. Indeed, we did ourselves observe that heat waves could constitute high mortality events for fouling communities, masking the effect of local adaptation (Chapter III B). This might mean that currently stable climates are less vulnerable to the spread of NIS from marinas due to climate change. However, along with temperature increase, climate change is projected to increase the frequency of heat waves (Meehl et al. 2000, Holl 2009, Stott 2016). This might also apply to currently stable climates like in the north Atlantic, as temperature records have been beaten twice since 2019 in Brest (Brittany) for instance (Infoclimat). As climate change constitutes a disturbance and as it increases the impact of extreme weather disturbances it may alter the already present disturbance gradient in marinas. Warmer temperatures have synergistic effects with other disturbances such as pollution (Sokolova & Lannig 2008, Alava et al. 2017, Cabral et al. 2019) which could exacerbate the effect of the disturbance gradient. On the other hand, increased heat wave intensity and frequency might mask the effects of the disturbance gradient (Chapter III B). In both experiments and Chapter III B we observe a significant effect of heat which coincided with lower biomass and lower recruitments of the total community (or a die-off in the case of the heatwave). Reduced biotic resistance due to depressed diversity may increase invasibility (Kimbrow et al. 2013, Bulleri et al. 2016). Furthermore, heat-stressed communities have been shown to exert lower biotic resistance (Castro et al. 2021). Climate change might thus act on both sides of our opposing gradient framework as it might increase the disturbance gradient while diminishing the biotic resistance gradient on the other side.

## IV Future Challenges

While our results here provide strong indications for local adaptation at small spatial scale, our approach has yet some limitations. Local adaptation requires strong selective pressures (Williams 1966, Kawecki & Ebert 2004), which is the case for the disturbance gradients. However, gene flow or dispersion may restrict differentiation and thus local adaptation (Brown & Pavlovic 1992, Kisdi 2002, Kawecki & Ebert 2004, Savolainen et al. 2007). For numerous species in marinas the distance of propagule dispersion is poorly known. Ascidian and Bryozoan are the main contributors to marina communities. Their lecithotrophic larvae have very short pelagic larval durations, lasting between minutes and hours (more rarely days) with realized dispersion distances measuring in meters to hundreds of meters (up to 1km for *C. intestinalis*; Keough and Chernoff 1987; Svane and Havenhand 1993; Marshall and Keough 2003; Shanks et al. 2003; Collin et al. 2013). *B. neritina* for which we had the best indicators for local adaptation, has a realized dispersion distance of less than 100 m (Keough & Chernoff 1987, Shanks et al. 2003), which might favor local adaptation. This might however not always be the case as other species like *W*

Reciprocal transplant experiments are the main staple for exposing local adaptation (Kawecki & Ebert 2004, Angert & Schemske 2005, Leimu & Fischer 2008, Colautti & Lau 2015, Sork 2018, Martin et al. 2021). However, some adaptations might not be genotypic but rather associated to adaptive plasticity (Kawecki & Ebert 2004). Many organisms are able to acclimate to their environment and current conditions through regulation of gene expression (Marshall 2008, Burgess & Marshall 2011, Lasky et al. 2014, Marie et al. 2017, Ardura et al. 2018, Clark et al. 2018). Here we transplanted established communities and individuals between locations to assess local adaptation. While we gave enough time for transplanted communities to acclimate to their new environment (one must consider that fouling communities are rather short-lived), it might be possible that they retained certain phenotypic traits from their original location. Some types of phenotypic plasticity can even be hereditary like maternal effects and epigenetics (Marshall 2008, Burgess & Marshall 2011, Lasky et al. 2014). However due to the high potential of hereditary plasticity and epigenetics in adaptive processes and driving evolution (Mousseau & Fox 1998, Richards et al. 2010a, Klironomos et al. 2013, Cushman 2014, Moler et al. 2018, Sork 2018), and due to the scale of our studies, we may include such hereditary adaptive phenotypes in local adaptation. Yet, the situation does not allow us to conclude on the genomic basis of the observed local adaptation pattern. This could be remediated with a common garden experiment. Common garden experiments allow to conclude on genomic differences between two populations by reproducing them separately in identical conditions and then challenging them with the same environment (Kawecki & Ebert 2004, Thorpe et al. 2005, Moloney et al. 2009, De Villemereuil et al.

2016). This allows to exclude hereditary adaptive plasticity and to conclude on the genomic basis of local adaptation (Kawecki & Ebert 2004, Thorpe et al. 2005). Upon confirming local adaptation in a marina with a reciprocal transplant experiment akin to ours, it could be possible to sample individuals of one or two species at the entrance and the inner location of the studied marina. Both populations of the species would need to be kept separated, but in similar conditions and ideally reproduced for multiple generations (Moloney et al. 2009). This poses however a significant challenge, as the studied organism do need to not only be maintained in laboratory conditions, but also successfully reproduced. This requires a profound knowledge of the studied organisms and their life cycle which is rarely the case for fouling organisms. In Chapter III A we were able to show how laboratory reproduction of two species in our marina can be achieved. While we used wild animals for reproduction the laboratory, the techniques used originated from the reproduction facility at our laboratory. However, *C. intestinalis*, despite having shown slight indicators for local adaptation (metabolome), or other solitary ascidians might not be the best model species for testing local adaptation as they have a larger dispersion distance ( $\sim 1$  km; Collin et al. 2013). Using *B. neritina* might provide better (and more interesting) results as this species has a very low dispersion distance, has been shown to have multiple indicators for local adaptation (Chapter II) and is a NIS introduced almost worldwide. Maintenance of adult brood stock and the raising of colonies from larvae seems achievable (Kitamura & Hirayama 1984, Dahms et al. 2007) by feeding them with *Rhodomonas* or *Oxyrrhis marina* (Kitamura & Hirayama 1984). At maturity, larvae release can be triggered by strong fluorescent light (Marshall & Keough 2003, Dahms et al. 2007). *B. neritina* from the inner and the entrance of a marina could thus be kept in aquaria, reproduced for two to three generations, and then exposed together to two treatments: clean water and water from the inner location. If local adaptation is present, the entrance population should have a fitness disadvantage compared to the inner population in the marina water and the inner population a fitness disadvantage in the 'clean' water. Fitness could be measured by survival which should be highly affected in the marina water. However, in the clean water the fitness disadvantage of the inner individuals might not be as tangible, as they might only have a metabolic tradeoff for detoxification. Metabolomics have been shown here to be an excellent tool to quantify metabolic responses of *B. neritina* to environmental factors. The analysis could among others target Lyso-PE which seem to be a fitness marker for this species (Chapter II). Such experiment would help to validate the observations made in this present work and to unambiguously expose how the disturbance gradient causes evolution on the genomic level, resulting in local adaptation of harbor communities, including NIS.



## Conclusion

Anthropic disturbances have a high impact on communities and synergize with each other. These disturbances are concentrated in marinas leading to strong spatial variability of the environment. Marinas have become a privileged environment for studying ecological processes in marine urban habitats since they represent, on a smaller scale, how urban processes affect biodiversity and ecosystems. By extension, results on eco-evolutionary dynamics obtained in marinas might be applied to the larger urban area. The present work aimed to understand how a disturbance gradient (mostly pollution) from the inner parts to the entrance of marinas shaped community structure and how it caused local adaptation on small spatial scale. We confirmed previous correlative works noticing a link between community composition and environmental gradients in marinas (Je et al. 2004, Ryu et al. 2011, Kenworthy et al. 2018b) but also exposed through an experimental approach that on very small-scale, differences in community composition are caused by local environmental conditions in multiple marinas. Local adaptation seems to indeed exist at this small spatial scale as we were able to expose the 'foreign vs local' criterium of local adaptation (Kawecki & Ebert 2004). However, we also demonstrated that other processes might affect this dynamic like biotic interactions and pulse disturbances like heat waves or pulse pollution. We extend our previous hypothesis and now include not one disturbance gradient, structuring communities, but two gradients (biotic resistance vs disturbance) that both act on the distribution of species in the marina (Fig. 48). While, more competitive species will colonize the entrance, resistant species will favor the inner parts of the marina. This includes NIS which are more prevalent in the inner parts of marinas (Kenworthy et al. 2018b). This insight is important considering that many NIS are regarded as particularly resistant (Gröner et al. 2011, Lejeune et al. 2014, Marie et al. 2017, Kenworthy et al. 2018a) and may help to develop future NIS mitigation strategies based on increasing biotic resistance (Fig. 48). However, quick action is required as climate change might profoundly impact the balance in marinas, increasing the prevalence of NIS and potentially awakening the numerous sleeper species among them (Bradley et al. 2018, Spear et al. 2021; Fig. 48)

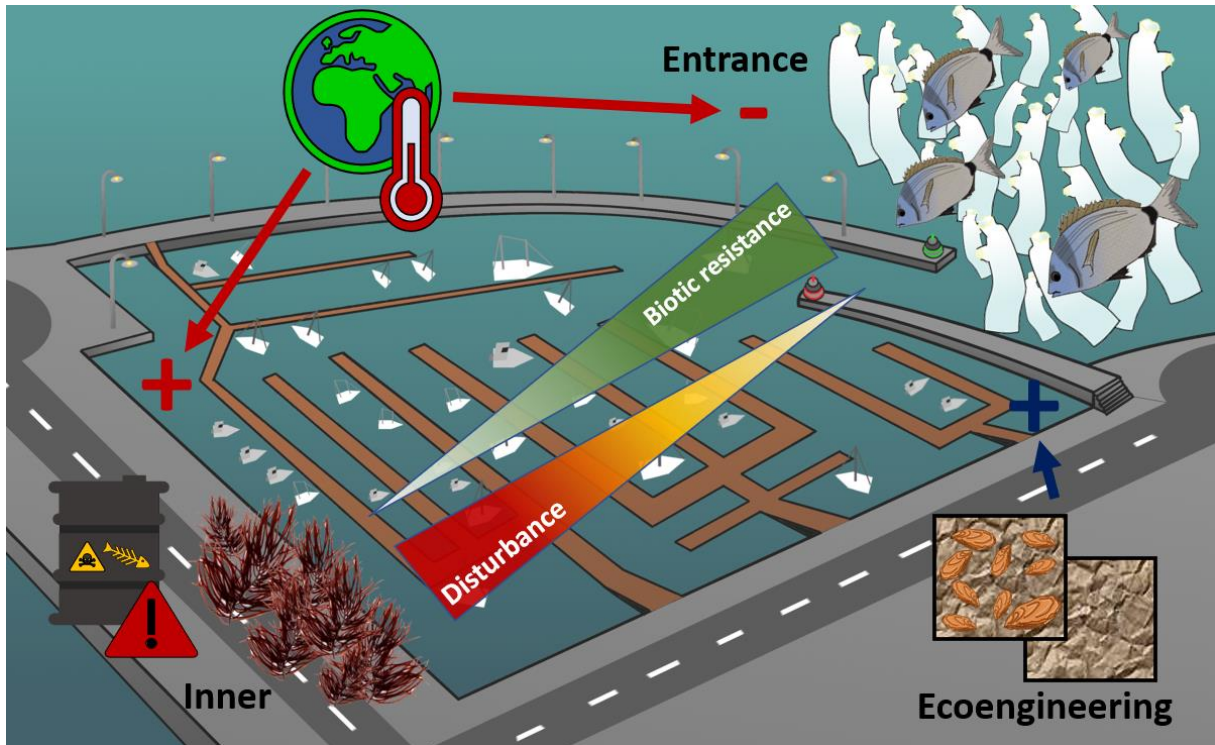


Fig. 48: Proposed opposing gradient framework developed from our works accounting for climate change and ecoengineering. At the left (inner marina), disturbance like pollution, temperature variations etc. is highest due to lower water mixing. Only stress resistant species can thrive at the inner marina (S-Selection; Grime 1977). This may include NIS, which seem to be more stress resistant. At the entrance and beyond, biotic interaction such as predation and competition are highest, with abundant C-Selection species, potentially exerting biotic resistance against NIS. For this reason, marinas might act as a spatial refuge for NIS. Ecoengineering through complexification of substrate or through deploying substrates seeded with oysters might increase biodiversity and potentially biotic resistance. However, climate change might impact this framework as it may exacerbate disturbance (See Discussion III), especially at the inner marina and since it may diminish biotic resistance by stressing native communities.

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*Diversity and Functioning of Harbor Communities - Influence on Non-Indigenous Species in a Climate Change Driven Context*

*Robin Gauff – These de Doctorat Ecologie Marine*

**Résumé :**

Les infrastructures marines offrent des nouveaux substrats, colonisés par divers organismes (*biofouling*), mais qui diffèrent des habitats naturels par leurs filtres environnementaux et processus écologiques, leur composition biotique et leur grande diversité/abondance d'espèces non indigènes (ENI). L'artificialisation dans les marinas modifie l'hydrodynamisme, favorisant la concentration de perturbations humaines (p. ex. polluants). Des gradients peuvent être présents, avec une intensité plus élevée des perturbations dans les parties les plus internes des marinas, constituant ainsi un filtre sélectif pour les organismes. Ce travail visait à comprendre la diversité et le fonctionnement des communautés sessiles des marinas en étudiant l'impact des gradients de perturbation sur leurs structures de l'entrée vers le fond plus perturbé. Constituant un défi sociétal important, les ENI étaient au cœur du sujet. Une approche expérimentale (transplantation réciproque) dans six marinas de deux régions (Méditerranée, Atlantique) a révélé que les différences dans la structure des communautés est causée par les niveaux locaux de pollution, associées à de l'adaptation locale à petite échelle spatiale (<100m). D'autres processus peuvent affecter ces dynamiques comme les interactions biotiques (prédation) et les perturbations sporadiques (vagues de chaleur). Dans le contexte du changement climatique, nous avons testé comment un réchauffement prévu de la mer (+3°C) impacte les communautés des marinas et favoriserait les ENI. Ces résultats visent à comprendre comment les perturbations dans les écosystèmes urbains marins affectent la biodiversité et en particulier la prévalence des ENI.

**Mot clefs :** Espèces introduites, urbanisation, pollution, adaptation locale, changement climatique

**Abstract:**

Marine infrastructures offer new substrates, colonized by a variety of organisms (biofouling), but are different from natural habitats since environmental filters and ecological processes differ, biotic composition differs from natural habitats, and they are characterized by a high diversity and abundance of non-indigenous species (NIS). Artificialization in marinas modifies hydrodynamism, affecting the water column characteristics like temperature and favoring the concentration of anthropic disturbances like pollutants. Environmental gradients may be present, with higher levels of disturbance in the innermost parts of marinas, acting as selective filters for organisms. The present work aimed to understand the diversity and functioning of sessile communities in marinas by studying how disturbance gradients shape community structure and function between the entrances of marinas and their more disturbed inner parts. A particular focus laid on NIS, which are common in marinas and constitute an important societal challenge. An experimental approach (reciprocal transplant) in six marinas from two regions (Mediterranean, Atlantic), revealed that differences in community structure are caused by local pollution levels, associated to local adaptation at small spatial scale (<100m). However, other processes might affect these dynamics like biotic interactions (predation) and pulse disturbances (heat waves). In the context of climate change, we also tested how projected seawater warming (+3°C) may impact marina communities and might favor NIS. These results might help understanding how disturbances in marine urban ecosystems drive biodiversity and especially the prevalence of NIS.

**Keywords:** Introduced species, urbanization, pollution, local adaptation, climate change