

Learning takes time: Biotic resistance by native herbivores increases through the invasion process.

Jorge Santamaría^{1,2}, Raúl Golo², Jana Verdura^{1,3}, Fiona Tomas⁴, Enric Ballesteros¹,
Teresa Alcoverro¹, Rohan Arthur^{1,5}, Emma Cebrian^{1,2}

¹ Centre d'Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas (CEAB-CSIC), Blanes, Spain

² GRMAR, Institut d'Ecologia Aquàtica, Universitat de Girona, Girona, Spain

³ Université Côte d'Azur, CNRS, UMR. 7035 ECOSEAS, Nice, France

⁴ Marine Ecosystems Dynamics Group – Instituto Mediterráneo de Estudios Avanzados (IMEDEA), Universitat de les Illes Balears (UIB) – Consejo Superior de Investigaciones Científicas (CSIC), Esporles, Balearic Islands, Spain

⁵ Nature Conservation Foundation, Mysore, India

Corresponding Author:

Jorge Santamaría¹

Email address: jsantamaria@ceab.csic.es

Author contributions

J.S. and E.C. conceived the ideas and the experimental design; J.S., R.G., J.V., F.T., E.B., and E.C. carried out the fieldwork experiments and collected the data; J.S. analyzed the data; J.S., T.A., R.A., and E.C. drafted the manuscript and all the authors contributed substantially to revisions and accepted the final version before submission.

Data availability

The raw data and R code supporting the conclusions of this article will be available on the Zenodo repository.

Running title: Herbivore learns to consume an invasive alga

Keywords: alga-herbivore interactions, preference shift, biological invasions, herbivory, biotic resistance increase, temperate system, *Caulerpa cylindracea*

Type of article: Letter

Word count:

Abstract: 150 words

Main text: 4,918

References count: 102. 74 without Materials and Methods

Number of figures: 5

1 **Abstract**

2 As invasive species spread, the ability of local communities to resist invasion depends
3 on the strength of biotic interactions. Evolutionarily unused to the invader, native
4 predators or herbivores may be initially wary of consuming newcomers, allowing them
5 to proliferate. However, these relationships may be highly dynamic, and novel
6 consumer-resource interactions could form as familiarity grows. Here, we explore the
7 development of effective biotic resistance towards a highly invasive alga using multiple
8 space-for-time approaches. We show that the principal native Mediterranean herbivore
9 learns to consume the invader within less than a decade. At recently invaded sites, the
10 herbivore actively avoided the alga, shifting to distinct preference and high
11 consumptions at older sites. This rapid strengthening of the interaction contributed to
12 the eventual collapse of the alga after an initial dominance. Therefore, our results stress
13 the importance of conserving key native populations to allow communities to develop
14 effective resistance mechanisms against invaders.

15 **Introduction**

16 As biological invasions spread across the globe, they are causing a reorganization of
17 community assemblages at rates not experienced before. The curious mélange that
18 results brings together species that often have had little recently shared evolutionary
19 history. These actors have to establish entirely novel interactions with each other,
20 leading to communities with no modern analogs (Hobbs *et al.* 2006; Williams &
21 Jackson 2007). With nothing to compare these novel communities to, it is often difficult
22 to predict exactly how they will transform native systems. The invading species
23 encounters a native community whose network of interactions have been established
24 over long ecological time scales, and how this community adapts to the new entrant will
25 determine the impact and scale of the invasion. Freed from their usual suite of
26 predators, alien species can become wildly successful in the recipient area, rapidly
27 overtaking the native community (Keane & Crawley 2002; Colautti *et al.* 2004).
28 However, native communities with strong biotic interactions can show considerable
29 biotic resistance, where predation or competition by native species prevent invaders
30 from successfully establishing (Levine *et al.* 2004; Theoharides & Dukes 2007).

31 With rates of invasions growing rapidly over the last few decades (Seebens *et al.*
32 2017), case studies show that they can radically alter native ecosystems, making
33 invasions one of the leading drivers of the biodiversity crisis, together with climate
34 change, land use and pollution (Butchart *et al.* 2010; Pyšek *et al.* 2020). Their negative
35 impacts cascade through the ecosystem, affecting normal functioning, and disrupting a
36 host of important ecosystem services (Vilà & Hulme 2017). Clearly then, managing
37 current and future invasions is an urgent global priority (Pyšek *et al.* 2020).

38 Central to the management of invasions is an understanding of how invasive
39 species interact with native communities, and the long-term dynamics of these

interactions (Mitchell *et al.* 2006; Saul & Jeschke 2015). The strength and direction of newly formed interactions – either in the form of competition or predation – is key to how successfully non-native species establish within a recipient assemblage (Colautti *et al.* 2004; Levine *et al.* 2004; Sih *et al.* 2010; Pintor & Byers 2015). If native predators learn quickly to consume novel prey, the strength of the predator-prey interactions can significantly reduce invasion success (Carpenter & Cappuccino 2005; De Rivera *et al.* 2005; Carlsson & Strayer 2009; Carlsson *et al.* 2011; Caselle *et al.* 2018). However, very often, invasive species find themselves in assemblages without natural enemies, or where potential predators have not learnt to consume them. Thus, released from biotic control, invader populations soar dramatically and can rapidly overwhelm native assemblages (Maron & Vilà 2001; Keane & Crawley 2002; Colautti *et al.* 2004; Liu & Stiling 2006). Several factors have been proposed to influence the strength and direction of consumer pressure including the defense capabilities of invaders and their ability to release allelopathic chemicals (Inderjit *et al.* 2011; Mennen & Laskowski 2018), the existence of predator avoidance mechanisms (Strauss *et al.* 2006; Ruland & Jeschke 2020) or the inability of predators to identify an invader as prey – native predator “naïveté” (Verhoeven *et al.* 2009; Sih *et al.* 2010; Kimbro *et al.* 2013).

To date, most studies assessing novel predator-prey interactions typically evaluate the impact of the invader at a particular stage during the invasion. However, the relationship invasive species establish with the recipient community is a rapidly evolving one. It is quite likely that the interaction will shift in strength and in character as each actor adjusts to the other through the invasion process. Native predators, with increased exposure to invaders, can learn their vulnerabilities and develop more effective behavioral adaptations to exploit them more efficiently (e.g., Carroll *et al.* 1998; Phillips & Shine 2004; Carlsson *et al.* 2009). Still, how long it takes for novel

interactions to form and stabilize is a matter of some uncertainty. Several studies report increased consumption and damage on invaders with increasing invasion time (Siemann *et al.* 2006; Carlsson & Strayer 2009; Diez *et al.* 2010; Carlsson *et al.* 2011; Stricker *et al.* 2016). Other studies in contrast, show that time since invasion does not influence consumption or damage rates on invading prey (Carpenter & Cappuccino 2005; Harvey *et al.* 2013; Pintor & Byers 2015). In fact, several factors, apart from time, are likely to influence the development of such novel interactions (Mitchell *et al.* 2006). Importantly, the probability of exposure is as much a function of abundance as it is of time. Prey abundance is an important determinant of the strength of predator-prey relationships (Wellenreuther & Connell 2002; Carbone *et al.* 2011), and predator experience of novel prey will likely increase with prey density (Saul & Jeschke 2015). Despite this, invader abundance has rarely been considered when assessing the development of novel predator-prey interactions (but see: Nelson *et al.* 2011).

Studies that integrate abundance and exposure time while evaluating novel predator-prey interactions will help us understand the long-term dynamics of invasions. Unfortunately, long-term data on most invaders are rare (Strayer *et al.* 2006), thus, making it difficult to draw strong conclusions on how predator-prey interactions develop through time (Strayer *et al.* 2017). The use of space-for-time designs or chronosequences, where populations with different invasion times are studied, provides a cost-effective and valid alternative to long-term monitoring (Thomaz *et al.* 2012; Dostál *et al.* 2013; Gruntman *et al.* 2017). These approaches can enable us to effectively assess if the strength of novel consumer-prey interactions shifts throughout the invasion process in relation to exposure time alone or in combination with other factors such as the invasion intensity. Finally, their use could also provide critical clues that may assist in the management of new and ongoing invasions.

In this study, we explore how a novel interaction between a native keystone fish herbivore (*Sarpa salpa*) and a hyper-successful invasive alga (*Caulerpa cylindracea*) develops over time. We use a space-for-time substitution together with a long-term dataset to assess if the strength of the consumer-resource interaction is mediated by the time since the invasion and/or by the abundance of the invader in the community. Preference and consumption assessments were conducted in populations with contrasting characteristics, to assess if feeding preference, the number of herbivores feeding on the invader and *per capita* consumption rates of the herbivore were influenced by the temporal and numerical characteristics of the invasion. Additionally, electivity for the invader was assessed at three successive sampling times in two populations characterized by their differential exposure time to the invader. In summary, our results provide empirical insights into the development of effective predator-prey interactions between native consumers and invaders, which can intensify the biotic resistance of the native community and lead to the collapse of several invader populations with time. Therefore, this study demonstrates that native communities need time to develop effective resistance mechanisms against invaders and it takes us a step forward in the understanding of invasions dynamics.

Materials and methods

Target species

Caulerpa cylindracea is a siphonaceous green alga native to the Southwestern coast of Australia that is invasive in the Mediterranean Sea and in some regions in the Atlantic and Indian oceans (Klein & Verlaque 2008). In the Mediterranean Sea, *C. cylindracea* is rampantly successful as it has colonized marine communities across the basin in less than 20 years since its first detection (Piazzi *et al.* 2005; Klein & Verlaque 2008).

Caulerpa cylindracea is able to form dense mono-specific stands that can quickly overgrow the underlying benthic assemblage, causing strong negative impacts on native communities (Piazzi *et al.* 2001; Klein & Verlaque 2008; Bulleri *et al.* 2017). The species can be considered a hyper-successful invader in the Mediterranean Sea (Klein & Verlaque 2008; Katsanevakis *et al.* 2016) and it is ranked globally as one of the most harmful marine invaders (Anton *et al.* 2019). The reasons for its extraordinary success are linked to its ability to resist herbivores by producing deterrent metabolites, its high growth rates and its mechanisms of vegetative and sexual propagation. Over the last years, however, several assemblages have seen steep declines in *C. cylindracea* abundance after an initial period of successful dominance (Klein & Verlaque 2008; García *et al.* 2016; Santamaría *et al.* 2021b). Our team and others have witnessed several native organisms feeding on the invader, hinting at the possibility that native communities may be developing effective resistance mechanisms against *C. cylindracea* (Ruitton *et al.* 2006; Cebrian *et al.* 2011; Tomas *et al.* 2011; Santamaría *et al.* 2021a). Additionally, the spread of the species is being closely tracked, and there is accurate data available on the progress of *C. cylindracea* invasion in Mediterranean waters, making it an ideal species to study how novel consumer-resource interactions evolve through the invasive process.

The only true herbivorous fish in the study area is the bream *Sarpa salpa* (Verlaque 1990; Gianni *et al.* 2017), which plays an important role in structuring seagrass and macroalgae communities (Tomas *et al.* 2005; Vergés *et al.* 2009). This species is abundant in shallow water communities along the NW Mediterranean Sea (Bell 1983; Reñones *et al.* 1997; Tomas *et al.* 2005) and spends most of its time above 20m (Bell 1983; Tomas *et al.* 2011; Vergés *et al.* 2012), where it feeds on a wide variety of species (Verlaque 1990). *Sarpa salpa* has been observed to regularly consume

C. cylindracea (Ruitton *et al.* 2006; Tomas *et al.* 2011) and a previous study has reported that it even prefers the invasive alga over many native species (Tomas *et al.* 2011).

Study sites

This study was conducted in 3 regions in the NW Mediterranean Sea: Cabrera Archipelago, Menorca Island and the Catalan coast (Figure 1), based on the documented invasion history of *C. cylindracea* in these regions.

In Cabrera Archipelago, *C. cylindracea* was detected in 2003 at 30m deep (Cebrian & Ballesteros 2009) and quickly expanded across the archipelago. Nowadays, it can be found in most benthic habitats at depths between 0 to 65m, where it can be the dominant species (Cebrian *et al.* 2011). However, in the past few years, the abundance of the invasive alga has declined in the archipelago, mainly at shallow depths (Santamaría *et al.* 2021b).

In Menorca, *C. cylindracea* was first detected in 2006, in photophilic assemblages (\approx 20m deep) from the south of the island (Pons-Fàbregas *et al.* 2007), and then it started shifting northwards and to shallower depths. From 2010 to date, *C. cylindracea* can be found at many locations around the island, sometimes dominating the benthic assemblages between 5 to 45m deep (Massutí *et al.* 2015), although recent reports show that its abundance is decreasing, predominantly in the shallow assemblages (Cefali *et al.* 2020).

Along the Catalan coast, *C. cylindracea* was first detected in 2008 in the south, at depths between 20 to 50m (Ballesteros *et al.* 2008). The invasive alga maintained this restricted distribution until 2013, when it was detected further north, in Blanes (García

et al. 2016). Since then, it has expanded northwards and can now be found in several locations in northern Catalonia (authors' personal observations).

Preference assessment

To assess if *S. salpa*'s preference for *C. cylindracea* changed in relation to time since invasion and/or in relation to the abundance of the invader in the assemblage, paired-choice feeding experiments were performed at different locations in Menorca and the Catalan coast. These regions were chosen to represent different times since the invasion and because it was possible to find locations within them with contrasting abundances of the invader.

To determine time since invasion at each location, long-term records of *C. cylindracea* presence, available with the research team, were consulted. Based on year since first detection, locations were classified as "Old" when *C. cylindracea* was first detected more than 5 years ago, "or "Recent" when *C. cylindracea* was detected less than 5 years ago. The abundance of *C. cylindracea* at depths between 10 to 15m was assessed underwater on SCUBA. At each location, thirty 25 x 25cm quadrats, divided into 25 subquadrats of 5 x 5cm (Sala & Ballesteros 1997) were randomly located on the benthos and the number of subquadrats where *C. cylindracea* was present was used as a unit of abundance. Mean *C. cylindracea* abundance was calculated for each location. Using this measure, if the abundance of *C. cylindracea* was higher than 30%, locations were classified as "High", whereas if the abundance was lower than 30%, locations were classified as "Low".

A total of 8 locations were chosen for the preference assessment and were classified following the previous criteria as follows: 4 locations from Menorca – Illa del Aire (Old-High), Sa Mola (Old-Low), Porros (Old-High) and Sa Llosa (Recent-Low);

and 4 locations from the Catalan coast – Sant Francesc (Old-Low), Roses 2019 (Recent-Low), Roses 2018 (Recent-High) and Ses Negres (Recent-Low) (Table S1; Figure S1).

At each location, paired-choice feeding experiments were performed to compare the relative palatability of *C. cylindracea* vs. two native macroalgae species: *Cystoseira compressa*, an important habitat-forming alga that is highly palatable (Vergés *et al.* 2009) and commonly found in *S. salpa*'s diet (Verlaque 1990); and *Padina pavonica*, a photophilic alga commonly found in the sublittoral zone of warm-temperate coasts (Sala 1997) and also a usual food source for *S. salpa* (Verlaque 1990) (Figure S1). Thus, we used 2 treatments: i) *Caulerpa* – *Cystoseira* and ii) *Caulerpa* – *Padina*; and 3 controls, one per algal species. Each replicate in each treatment was composed of native and invasive algal fragments held by two clothespins attached to each other with zip-tie, weighted down to keep them in place underwater. Controls were composed of algal fragments of each of the studied species, held by a single clothespin protected from herbivores with a plastic mesh cage (0.5cm mesh size) and were similarly weighted down.

A total of 7 replicates for each treatment and 5 replicates for each control were deployed at each of the eight locations in patches chosen carefully to reduce confounding effects. We maintained the same depth ($\approx 10\text{m}$) for all replicates and chose areas where there were no sea urchins. In addition, we ensured that the patches had similar macroalgae assemblages to guarantee that fishes were choosing between the offered food choices always under the same conditions. The replicates were carefully placed on the sea floor to guarantee that the clothespins were in an upright position and that all algae were easily accessible to fishes. Replicate pairs were placed 1m apart from each other, whereas treatments were placed less than 20m apart to maintain constant environmental conditions between them. All the experiments were conducted at the end

of summer (in 2018 and in 2019), the period of the year when *S. salpa* feeds most intensively to accumulate reserves for winter and prepare for reproduction (Peirano *et al.* 2001). Samples were deployed in the morning and collected after 24h. Before and after deployment, every algal fragment was pad-dried of excess water and wet weighed to the nearest 0.01g. In all pairs, similar initial weights for each alga were offered to herbivores.

Biomass consumption was estimated with the formula:

$$\left(H_i \times \frac{C_f}{C_i}\right) - H_f$$

where H_i and H_f were the initial and final wet weights of algae exposed to herbivory and C_i and C_f were initial and final mean wet weights of the controls (Parker & Hay 2005; Tomas *et al.* 2011). Consumption values were then standardized to percentage of consumed algae.

Consumption assessment

To assess if i) the percent of fish feeding on the invader and ii) the *per capita* consumption rates on the invasive alga (total amount consumed), change in relation to time since invasion and/or in relation to the abundance of the invader in the assemblage, fish fecal pellets were collected in the field (on SCUBA) from the same locations where preference assessments were done (Figure S1). At each location, the day after completion of the preference experiment, we followed schools of *S. salpa* across their depth range and collected fecal pellets from the water column in individual zip bags while swimming below the fish. Between 30 and 50 pellets were collected per location. The pellets were preserved in buffered 4% formaldehyde-seawater for later analysis. This non-invasive method was used to diminish impacts on the study areas; and it has earlier been used to reliably characterize *S. salpa*'s feeding patterns (Tomas *et al.* 2011).

We determined the presence (% of fish feeding in the invader) and abundance (*per capita* consumption rates) of *C. cylindracea* by examining fecal pellets in a reticulated Petri dish under a stereomicroscope Stemi 2000-C (Carl Zeiss, Berlin, Germany). Pellet content was spread uniformly on the dish and the relative abundance of *C. cylindracea* in each pellet was estimated as the mean percentage cover that it occupied in relation to the other content.

Assessment of the electivity towards *Caulerpa cylindracea* throughout the invasion

To assess if *S. salpa*'s electivity towards *C. cylindracea* changed in relation to time since invasion, the Ivlev's Electivity Index (E) (Ivlev 1961) was calculated in two locations: Roses, at the 2nd, 3rd and 4th year after *C. cylindracea* invasion – first record in 2016; and Cabrera Archipelago, at the 4th, 5th and 17th year after the arrival of *C. cylindracea* – first record in 2003 (Figure S1).

To determine E at each location for each time period, the following formula was used:

$$E = \frac{(d_i - a_i)}{(d_i + a_i)}$$

where d_i = % of *C. cylindracea* in the fecal pellets of *S. salpa* (see the consumption assessment section) and a_i = % of *C. cylindracea* available in the environment (see the preference assessment section). The values of Ivlev's Index (E) range from -1 (complete avoidance) to +1 (exclusive selection), with positive values indicating that the food item is selected and eaten more than it is encountered by chance in the environment (Ivlev 1961).

Statistical analysis

To assess if *S. salpa* preference changes with time since invasion and/or with the abundance of *C. cylindracea* in the assemblage, the data from the paired-choice assays was analyzed in the statistical environment R (R version 3.6.3) (R Core Team 2018), with paired Student t-tests when data was normal and homoscedastic, and with Wilcoxon signed-ranks paired tests when data was not normal or was heteroscedastic. Replicates in which fish did not feed on any of the algae were discarded from the statistical analyses, because they do not provide any information on preference.

To evaluate the effect of time since invasion and abundance of the invader on the proportion of fish feeding on the invader and on *per capita* consumption rates of *C. cylindracea*, generalized linear models (GLMs) were fitted to fecal pellet data. In this case, binomial models were used because the response variables were measured either as 0-1 data (presence/absence of *C. cylindracea* in the pellets – % of fishes feeding on the invader) or as proportions (abundance of *C. cylindracea*: % of the pellet content that corresponded to the invader – *per capita* consumption rates) and could be approximated to a logistic distribution. Two models were fitted in R, one for the presence/absence data and the other for the abundance data. In both models, the factors “time since invasion” and “abundance of the invader” were included, together with their interaction when it was significant. Tukey post-hoc tests were performed using the functions “pairs” and “emmeans” from the *emmeans* package (Lenth 2018) to compare effects in the time since invasion factor (“recent” and “old”) at each level of abundance (“high” and “low”) when the interaction between the factors was significant.

To assess whether there were differences in the Ivlev’s Electivity Index values between years at each of the studied locations (Roses and Cabrera Archipelago), Kruskal-Wallis tests were performed due to the lack of normality in the data (Kruskal &

Wallis 1952). Then, to compare effects between years, Dunn's post-hoc tests (Dunn 1964) were performed using the *FSA* package in R (Ogle *et al.* 2020), correcting p-values with the Benjamini-Hochberg method (Benjamini & Hochberg 1995).

Results

Preference assessment

The preference of *Sarpa salpa* towards *Caulerpa cylindracea* was influenced by time since invasion but not by the abundance of the invader in the community. Within five years of the invasion, *S. salpa* developed a distinct preference for *C. cylindracea*, regardless of the abundance of the invader (Figure 2, A-B). In fact, the native fish showed an at least 2.5-fold higher preference for *C. cylindracea* over native species (Figure 2, A-B), both in places with high and with low abundance of the invader.

In contrast, in recently invaded locations, *S. salpa* did not exhibit a preference for the invader regardless of the abundance of *C. cylindracea* in the community (Figure 2, C-D).

Consumption assessment

The proportion of fish feeding on *C. cylindracea* varied significantly with the interaction term (p-value < 0.05; Table S2), indicating that the number of *S. salpa* feeding on *C. cylindracea* was dependent both on the time since the invasion and on the abundance of the invader in the community. At each level of exposure time to the invader, the number of fish eating *C. cylindracea* was significantly higher at high invader abundance (Table S3, A), with at least 2 times more fish targeting the invader than in populations where *C. cylindracea* abundance was low (Figure 3, A). Additionally, time since invasion significantly influenced the number of fish targeting

the invader in locations with high abundances (Table S3, B), with twice the number of fish consuming *C. cylindracea* in the Old-High populations than in the Recent-High populations (Figure 3, A). In summary, more than 90% of *S. salpa* individuals consumed the invader in populations that had a high abundance of *C. cylindracea* and a long history of invasion; whereas only 21% of *S. salpa* individuals consumed *C. cylindracea* in populations that were recently invaded and where the abundance of the invader was low (Figure 3, A).

Similarly, *per capita* consumption rates of *C. cylindracea* by *S. salpa* were also dependent on both, the time since invasion and the abundance of the invader in the community (significant interaction term; Table S4). *Sarpa salpa* consumed significantly more *C. cylindracea* in communities with a high invader abundance, regardless of time since invasion (Figure 3, B; Table S5, A). However, a significant difference in *per capita* consumption rates of *C. cylindracea* between old and recent populations was only detected under high abundances of the invader (Table S5, B). In particular, in communities with a high abundance of *C. cylindracea*, consumption of the invader was almost 7 times higher in old populations than in recent populations, whereas in communities with a low abundance of *C. cylindracea*, consumption in old and in recent populations was similarly low (Figure 3, B).

Electivity assessment

In Roses, where the invasion of *C. cylindracea* began very recently (first detection in 2016), *S. salpa* showed a negative electivity for *C. cylindracea* (Table S6), increasing slightly with time (Figure 4-Roses; Table S6). Despite this, no significant differences were detected in the Ivlev's Index values between years ($p\text{-value} > 0.05$, Table S7).

In Cabrera Archipelago, where *C. cylindracea* has been well established for a long time (~17 years), the electivity of *S. salpa* towards *C. cylindracea* increased throughout the invasion; and native fish now show a clear positive electivity for the invader (Figure 4-Cabrera; Table S6), confirmed by significantly different Ivlev Index values (Kruskal-Wallis test, p-value < 0.05, Table S7). Post-hoc tests indicate that electivity was significantly higher in 2020 than in 2007 and 2008 (p-value < 0.05, Table S8).

Overall, we observe that independent of invader abundance, the number of fish showing a total avoidance of *C. cylindracea* (-1 values; Figure 4 – first years of the invasion) decreases through the invasion and a higher proportion of fish show a positive electivity for the invader (positive values; Figure 4 – year 17 after the invasion).

Discussion

The success of exotic invaders is frequently attributed to a release from biotic control in the communities in which they find themselves (Maron & Vilà 2001; Keane & Crawley 2002; Colautti *et al.* 2004; Liu & Stiling 2006). With no shared evolutionary history with native species, invaders often do much better in recipient communities than they do in their native habitats, where they belong to assemblages that have formed over long ecological time scales. While this initial unfamiliarity drives their rapid spread, does it guarantee long-term success, once the novelty of the species has worn off? Our results indicate that with time, as native species become increasingly familiar with the invader, the strength of consumer-resource interactions increases. Eventually, this may serve to intensify the biotic resistance of native communities as native consumers learn to handle and consume the invader, potentially leading to a control of their populations.

As recorded in Tomas *et al.* 2011, we show that *Sarpa salpa* prefers *Caulerpa cylindracea* to native algae, although the preference for the invasive alga takes several years to develop. In the first few years of the invasion, *S. salpa* is much more wary of the invader, and it is not until around 6 years of exposure that the herbivore starts developing a clear preference for it. In populations with a more recent exposure to *C. cylindracea*, no preference for the invader was detected. Remarkably, preference for the invader in the old populations was maintained even at low abundances of *C. cylindracea*. This is noteworthy since both *Padina pavonica* and *Cystoseira compressa* are highly palatable and are commonly found in *S. salpa*'s diet (Verlaque 1990; Vergés *et al.* 2009). The fact that *S. salpa* becomes increasingly partial to *C. cylindracea* may be linked to nutritive or morphological traits of the invader, as has been described for other generalist herbivores (Lodge 1991; Cronin 1998; Schädler *et al.* 2003). In fact, the softer and more aqueous species like *C. cylindracea* are more easily removed, handled and masticated, making them prime targets for herbivores (Cronin *et al.* 2002; Vergés *et al.* 2007; Morrison & Hay 2011).

The probability of encounter between consumer and resource is a function of time as well as abundance. In our study, the proportion of herbivorous fish feeding on the invader and *per capita* consumption rates were influenced both by exposure time and by availability of the invader in the community. There appears to be a certain threshold of abundance that triggers significant increases both in the proportion of individuals feeding as well as in *per capita* consumption rates, particularly evident at sites historically invaded compared with recent invaded areas. This shows that although exposure time is essential for novel consumer-resource interactions to form (Carlsson & Strayer 2009; Carlsson *et al.* 2011; Schultheis *et al.* 2015), other characteristics of the invasion (e.g. availability of the invader) and synergies between them, strongly

influence their development. However, we cannot ignore that low invader abundances (< 30%) could partially mask the effect of time for the development of novel consumer-resource interactions, since low encounter rates could inherently reduce consumption even if fish populations were highly experienced with the invader. In actual fact, the electivity of *S. salpa* towards *C. cylindracea* increased through time, even when the abundance of the invader had declined considerably (Figure 4). Therefore, it appears that once *S. salpa* has learnt that *C. cylindracea* is a valuable food source and incorporates it in its diet, it seeks out the invader even when it becomes increasingly rare.

On the face of it, the extraordinary preference that develops over time for *C. cylindracea* is *a priori* unexpected, given that the alga produces caulerpenyne, a secondary metabolite with known herbivore deterrent properties (Paul *et al.* 2007). However, *C. cylindracea* produces this metabolite in relatively low concentrations (Box *et al.* 2010). In addition, native *Caulerpa prolifera*, a common food-source for *S. salpa* (Marco-Méndez *et al.* 2017), also produces caulerpenyne, therefore, *S. salpa* may have developed a fair tolerance to the compound, making it an ineffective deterrent. Even though invaders themselves may be novel to the community, their phylogenetic affinities with resident natives may help consumers adjust their behaviors and physiologies much more quickly to the invader and form strong biotic interactions with them. Actually, this could well be an important mechanism explaining the failure of some invasive plants to escape naïve herbivores (Hill & Kotanen 2009; Pearse *et al.* 2013).

Mechanisms such as learning and social transmission can influence the way predators adapt to novel prey at different time scales, from days to years and even generations since they are first encountered (Cox 2004; Strauss *et al.* 2006; Carlsson *et*

400 *al.* 2009). The mechanisms by which *S. salpa* begins to consume *C. cylindracea* remain
401 speculative, but they potentially follow steps similar to the predation cycle (Saul &
402 Jeschke 2015) (Figure 5), which involves *S. salpa* identifying *C. cylindracea* as a
403 suitable resource and developing a taste for the newcomer. Therefore, after an initial
404 wariness because of the unfamiliarity with the invader, the first few interactions may be
405 completely accidental and involve very few individuals (low proportion of individuals
406 consume the invader in recent populations) (Figure 3-A; Figure 5). However, after these
407 individuals come to discover the high nutritional value of the invader, they begin to
408 target it, soon followed by the rest of the population when it learns that the invader is
409 safe to eat and nutritious. In non-solitary fish, social learning, where individuals learn
410 behaviors and acquire information such as what to eat and what to avoid through
411 observation, is an important foraging mechanism (Brown & Laland 2003; Warburton &
412 Hughes 2011). This may help explain the fast transmission of search images between
413 demonstrators, those that know how to feed on novel prey, and bystanders, those that
414 observe and learn to target the new prey (White & Gowan 2014). Actually, *S. salpa* is a
415 highly social species that feeds in large cooperative shoals which show complex feeding
416 behaviors (Buñuel *et al.* 2020). Within a population, behavioral syndromes could play
417 an important role in determining how quickly consumers take novel resources, with
418 bolder phenotypes less reticent to try unfamiliar food items than shy individuals
419 (Sundström *et al.* 2004), based on their past experiences (Frost *et al.* 2007). How
420 rapidly this learning spreads through the population could, as our results suggest, be
421 influenced by invader abundance, because the formation and transmission of search
422 images likely increases with encounter rates of novel resources (Allen 1988; Saul &
423 Jeschke 2015).

Our study suggests that as the invasion progresses, novel predator-prey interactions can become an effective biotic resistance mechanism against invaders. However, given that this resistance does not develop immediately, invaders can escape biotic control at the start of the invasion, allowing them to become hyper-successful and to impact native communities, as has been observed for *C. cylindracea* (Piazzi *et al.* 2001; Klein & Verlaque 2008; Katsanevakis *et al.* 2016). In fact, many invading species experience major population outbreaks when they first arrive in an area, that can be linked to a release from natural enemies (Strayer *et al.* 2017). But then, as encounters increase and native communities learn to deal with the novel species, invader populations could well experience a crash after a period of successful dominance (Carlsson *et al.* 2009, 2011; Strayer *et al.* 2017). Shifts in the strength of biotic resistance through time may be the main factor in the steep declines several hyper-successful invaders have suffered with time (e.g., Simberloff & Gibbons 2004; De Rivera *et al.* 2005; Carlsson *et al.* 2011; Santamaría *et al.* 2021b). Thus, biotic resistance against an invader is not static and changes dynamically through the invasion, increasing its strength as the invasion progresses (Hawkes 2007; Diez *et al.* 2010; Carlsson *et al.* 2011; Stricker *et al.* 2016). For this reason, snapshot studies that only focus on a specific point in time give us only a limited view of the importance of biotic resistance towards an invader since the outcomes may vary greatly influenced across time. This may help explaining contradictory patterns in the ability of native communities to develop effective biotic resistance mechanisms towards invaders (Maron & Vilà 2001; Colautti *et al.* 2004; Mitchell *et al.* 2006; Kimbro *et al.* 2013). Therefore, long-term studies or chronosequences should be favored to help us understand the long-term dynamics of certain invaders (Strayer 2012; Strayer *et al.*

2017). In addition, given how important encounter rate is, studies need to assess how these interactions develop at different densities of the invader as well.

Our study adds to the growing literature showing that native communities need time to develop resistance mechanisms against invasive species (e.g., Lankau *et al.* 2009; Diez *et al.* 2010; Carlsson *et al.* 2011; Stricker *et al.* 2016). Studies in terrestrial ecosystems refer to time exposures of decades or even centuries for effective resistance mechanisms to develop through time (Siemann *et al.* 2006; Hawkes 2007; Diez *et al.* 2010; Dostál *et al.* 2013; Stricker *et al.* 2016). In our system, however, an exposure time of less than a decade was enough to observe an increase in the biotic resistance of the native community and a decline in invader populations (Santamaría *et al.* 2021b). This represents roughly one generation of the herbivore *S. salpa* (Méndez-Villamil *et al.* 2002), indicating that a population-level preference for the invader spread within the lifetime of the herbivore. However, a decade is sufficient time for an initially successful invader to trigger catastrophic and potentially irreversible shifts in ecosystems. As global change continues to extend its reach across the world's oceans, the swell of novel species entering native waters is only going to increase. Therefore, considering that it takes time for native assemblages to learn to adjust to these new entrants; in managing invasions, it is imperative to ensure that native communities are maintained in as healthy a state as possible until any potential biotic resistance to novel species can develop.

References

- Allen, J.A. (1988). Frequency-dependent selection by predators. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 319, 485–503.
- Anton, A., Geraldi, N.R., Lovelock, C.E., Apostolaki, E.T., Bennett, S., Cebrian, J., *et al.* (2019). Global ecological impacts of marine exotic species. *Nat. Ecol. Evol.*, 3, 787–800.
- Ballesteros, E., García, M. & Weitzmann, B. (2008). *Informe: Detecció de Caulerpa racemosa var. cylindracea a la costa de Vilanova i la Geltrú.*
- Bell, J.D. (1983). Effects of depth and marine reserve fishing restrictions on the structure of a rocky reef fish assemblage in the North-Western Mediterranean Sea. *J. Appl. Ecol.*, 20, 357–369.
- Benjamini, Y. & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B*, 57, 289–300.
- Box, A., Sureda, A., Tauler, P., Terrados, J., Marbà, N., Pons, A., *et al.* (2010). Seasonality of caulerpenyne content in native *Caulerpa prolifera* and invasive *C. taxifolia* and *C. racemosa var. cylindracea* in the western Mediterranean Sea. *Bot. Mar.*, 53, 367–375.
- Brown, C. & Laland, K.N. (2003). Social learning in fishes: A review. *Fish Fish.*, 4, 280–288.
- Bulleri, F., Benedetti-Cecchi, L., Ceccherelli, G. & Tamburello, L. (2017). A few is enough: A low cover of a non-native seaweed reduces the resilience of Mediterranean macroalgal stands to disturbances of varying extent. *Biol. Invasions*, 19, 2291–2305.
- Buñuel, X., Alcoverro, T., Pagès, J.F., Romero, J., Ruiz, J.M. & Arthur, R. (2020). The dominant seagrass herbivore *Sarpa salpa* shifts its shoaling and feeding strategies

493 as they grow. *Sci. Rep.*, 10, 106222.
 494 Butchart, S.H.M., Walpole, M., Collen, B., van Strien, A., Scharlemann, J.P.W.,
 495 Almond, R.E.A., *et al.* (2010). Global Biodiversity: Indicators of Recent Declines.
 496 *Science* (80-.), 328, 1164–1168.
 497 Carbone, C., Pettorelli, N. & Stephens, P.A. (2011). The bigger they come, the harder
 498 they fall: Body size and prey abundance influence predator-prey ratios. *Biol. Lett.*,
 499 7, 312–315.
 500 Carlsson, N.O.L., Bustamante, H., Strayer, D.L. & Pace, M.L. (2011). Biotic resistance
 501 on the increase: native predators structure invasive zebra mussel populations.
 502 *Freshw. Biol.*, 56, 1630–1637.
 503 Carlsson, N.O.L., Sarnelle, O. & Strayer, D.L. (2009). Native predators and exotic prey
 504 - An acquired taste? *Front. Ecol. Environ.*, 7, 525–532.
 505 Carlsson, N.O.L. & Strayer, D.L. (2009). Intraspecific variation in the consumption of
 506 exotic prey - a mechanism that increases biotic resistance against invasive species?
 507 *Freshw. Biol.*, 54, 2315–2319.
 508 Carpenter, D. & Cappuccino, N. (2005). Herbivory, time since introduction and the
 509 invasiveness of exotic plants. *J. Ecol.*, 93, 315–321.
 510 Carroll, S.P., Klassen, S.P. & Dingle, H. (1998). Rapidly evolving adaptations to host
 511 ecology and nutrition in the soapberry bug. *Evol. Ecol.*, 12, 955–968.
 512 Caselle, J.E., Davis, K. & Marks, L.M. (2018). Marine management affects the invasion
 513 success of a non-native species in a temperate reef system in California, USA.
 514 *Ecol. Lett.*, 21, 43–53.
 515 Cebrian, E. & Ballesteros, E. (2009). Temporal and spatial variability in shallow- and
 516 deep-water populations of the invasive *Caulerpa racemosa* var. *cylindracea* in the
 517 Western Mediterranean. *Estuar. Coast. Shelf Sci.*, 83, 469–474.

518 Cebrian, E., Ballesteros, E., Linares, C. & Tomas, F. (2011). Do native herbivores
519 provide resistance to Mediterranean marine bioinvasions? A seaweed example.
520 *Biol. Invasions*, 13, 1397–1408.

521 Cefali, M.E., Movilla, J., Bolado, I., Mallol, S., Reñones, O., Díaz, D., *et al.* (2020).
522 *Informe final 2016-2020. Convenio de colaboración para la consolidación de la*
523 *estación de investigación “Jaume Ferrer” (La Mola, Menorca).*

524 Colautti, R.I., Ricciardi, A., Grigorovich, I.A. & MacIsaac, H.J. (2004). Is invasion
525 success explained by the enemy release hypothesis? *Ecol. Lett.*, 7, 721–733.

526 Cox, G.W. (2004). *Alien species and evolution: The evolutionary ecology of exotic*
527 *plants, animals, microbes, and interacting native species.* Island Press.

528 Cronin, G. (1998). Influence of macrophyte structure, nutritive value, and chemistry on
529 the feeding choices of a generalist crayfish. In: *The Structuring Role of Submerged*
530 *Macrophytes in Lakes* (eds. Jeppesen, E., Søndergaard, M., Søndergaard, M. &
531 Christoffersen, K.). Springer New York, New York, NY, pp. 307–317.

532 Cronin, G., Lodge, D.M., Hay, M.E., Miller, M., Hill, A.M., Horvath, T., *et al.* (2002).
533 Crayfish feeding preferences for freshwater macrophytes: The influence of plant
534 structure and chemistry. *J. Crustac. Biol.*, 22, 708–718.

535 Diez, J.M., Dickie, I., Edwards, G., Hulme, P.E., Sullivan, J.J. & Duncan, R.P. (2010).
536 Negative soil feedbacks accumulate over time for non-native plant species. *Ecol.*
537 *Lett.*, 13, 803–809.

538 Dostál, P., Müllerová, J., Pyšek, P., Pergl, J. & Klinerová, T. (2013). The impact of an
539 invasive plant changes over time. *Ecol. Lett.*, 16, 1277–1284.

540 Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics*, 6, 241–252.

541 Frost, A.J., Winrow-Giffen, A., Ashley, P.J. & Sneddon, L.U. (2007). Plasticity in
542 animal personality traits: Does prior experience alter the degree of boldness? *Proc.*

543 *R. Soc. B Biol. Sci.*, 274, 333–339.

544 García, M., Weitzmann, B., Pinedo, S., Cebrian, E. & Ballesteros, E. (2016). First
 545 report on the distribution and impact of marine alien species in coastal benthic
 546 assemblages along the Catalan coast. *Handb. Environ. Chem.*, 43, 249–270.

547 Gianni, F., Bartolini, F., Pey, A., Laurent, M., Martins, G.M., Airoidi, L., *et al.* (2017).
 548 Threats to large brown algal forests in temperate seas: the overlooked role of native
 549 herbivorous fish. *Sci. Rep.*, 7, 6012.

550 Gruntman, M., Segev, U., Glauser, G. & Tielbörger, K. (2017). Evolution of plant
 551 defences along an invasion chronosequence: Defence is lost due to enemy release –
 552 but not forever. *J. Ecol.*, 105, 255–264.

553 Harvey, K.J., Nipperess, D.A., Britton, D.R. & Hughes, L. (2013). Does time since
 554 introduction influence enemy release of an invasive weed? *Oecologia*, 173, 493–
 555 506.

556 Hawkes, C. V. (2007). Are invaders moving targets? The generality and persistence of
 557 advantages in size, reproduction, and enemy release in invasive plant species with
 558 time since introduction. *Am. Nat.*, 170, 832–843.

559 Hill, S.B. & Kotanen, P.M. (2009). Evidence that phylogenetically novel non-
 560 indigenous plants experience less herbivory. *Oecologia*, 161, 581–590.

561 Hobbs, R.J., Arico, S., Aronson, J., Baron, J.S., Bridgewater, P., Cramer, V.A., *et al.*
 562 (2006). Novel ecosystems: Theoretical and management aspects of the new
 563 ecological world order. *Glob. Ecol. Biogeogr.*, 15, 1–7.

564 Inderjit, Wardle, D.A., Karban, R. & Callaway, R.M. (2011). The ecosystem and
 565 evolutionary contexts of allelopathy. *Trends Ecol. Evol.*, 26, 655–662.

566 Ivlev, V.S. (1961). *Experimental ecology of the feeding of fishes*. Yale University Press,
 567 New Haven, Connecticut, USA.

568 Katsanevakis, S., Tempera, F. & Teixeira, H. (2016). Mapping the impact of alien
 569 species on marine ecosystems: The Mediterranean Sea case study. *Divers. Distrib.*,
 570 22, 694–707.

571 Keane, R.M. & Crawley, M.J. (2002). Exotic plant invasions and the enemy release
 572 hypothesis. *Trends Ecol. Evol.*, 17, 164–170.

573 Kimbro, D.L., Cheng, B.S. & Grosholz, E.D. (2013). Biotic resistance in marine
 574 environments. *Ecol. Lett.*, 16, 821–833.

575 Klein, J. & Verlaque, M. (2008). The *Caulerpa racemosa* invasion: A critical review.
 576 *Mar. Pollut. Bull.*, 56, 205–225.

577 Kruskal, W.H. & Wallis, W.A. (1952). Use of ranks in one-criterion variance analysis.
 578 *J. Am. Stat. Assoc.*, 47, 583–621.

579 Lankau, R.A., Nuzzo, V., Spyreas, G. & Davis, A.S. (2009). Evolutionary limits
 580 ameliorate the negative impact of an invasive plant. *Proc. Natl. Acad. Sci. U. S. A.*,
 581 106, 15362–15367.

582 Lenth, R. (2018). emmeans: Estimated Marginal Means, aka Least-Squares Means. R
 583 package version 1.3.0.

584 Levine, J.M., Adler, P.B. & Yelenik, S.G. (2004). A meta-analysis of biotic resistance
 585 to exotic plant invasions. *Ecol. Lett.*, 7, 975–989.

586 Liu, H. & Stiling, P. (2006). Testing the enemy release hypothesis: a review and meta-
 587 analysis. *Biol. Invasions*, 8, 1535–1545.

588 Lodge, D.M. (1991). Herbivory on freshwater macrophytes. *Aquat. Bot.*, 41, 195–224.

589 Marco-Méndez, C., Ferrero-Vicente, L.M., Prado, P. & Sánchez-Lizaso, J.L. (2017).
 590 Epiphytes and nutrient contents influence *Sarpa salpa* herbivory on *Caulerpa* spp.
 591 vs. seagrass species in Mediterranean meadows. *Estuar. Coast. Shelf Sci.*, 184, 54–
 592 66.

593 Maron, J.L. & Vilà, M. (2001). When do herbivores affect plant invasion? Evidence for
 594 the natural enemies and biotic resistance hypotheses. *Oikos*, 95, 361–373.

595 Massutí, E., Sales, M., Reñones, O., Cuadros-Casado, I.A., Vidal-Cejuela, E.M., Sintés,
 596 J., *et al.* (2015). *Informe final 2010-2015. Convenio de colaboración para la*
 597 *puesta en marcha y el desarrollo científico de la estación de investigación “Jaume*
 598 *Ferrer” (La Mola, Menorca).*

599 Méndez-Villamil, M., Lorenzo, J.M., Pajuelo, J.G., Ramos, A. & Coca, J. (2002).
 600 Aspects of the life history of the salema, *Sarpa salpa* (Pisces, Sparidae), off the
 601 Canarian archipelago (Central-East Atlantic). *Environ. Biol. Fishes*, 63, 183–192.

602 Mennen, G.J. & Laskowski, K.L. (2018). Defence is the best offence: Invasive prey
 603 behaviour is more important than native predator behaviour. *Anim. Behav.*, 138,
 604 157–164.

605 Mitchell, C.E., Agrawal, A.A., Bever, J.D., Gilbert, G.S., Hufbauer, R.A., Klironomos,
 606 J.N., *et al.* (2006). Biotic interactions and plant invasions. *Ecol. Lett.*, 9, 726–740.

607 Morrison, W.E. & Hay, M.E. (2011). Herbivore preference for native vs. exotic plants:
 608 Generalist herbivores from multiple continents prefer exotic plants that are
 609 evolutionarily naïve. *PLoS One*, 6, e17227.

610 Nelson, D.W.M., Crossland, M.R. & Shine, R. (2011). Foraging responses of predators
 611 to novel toxic prey: Effects of predator learning and relative prey abundance.
 612 *Oikos*, 120, 152–158.

613 Ogle, D.H., Wheeler, P. & Dinno, A. (2020). FSA: Fisheries Stock Analysis.

614 Parker, J.D. & Hay, M.E. (2005). Biotic resistance to plant invasions? Native herbivores
 615 prefer non-native plants. *Ecol. Lett.*, 8, 959–967.

616 Paul, V., Arthur, K.E., Ritson-Williams, R., Ross, C. & Sharp, K. (2007). Chemical
 617 defenses: from compounds to communities. *Biol. Bull.*, 213, 226–251.

618 Pearse, I.S., Harris, D.J., Karban, R. & Sih, A. (2013). Predicting novel herbivore-plant
 619 interactions. *Oikos*, 122, 1554–1564.

620 Peirano, A., Niccolai, I., Mauro, R. & Bianchi, C.N. (2001). Seasonal grazing and food
 621 preference of herbivores in a *Posidonia oceanica* meadow. *Sci. Mar.*, 65, 367–374.

622 Phillips, B.L. & Shine, R. (2004). Adapting to an invasive species: Toxic cane toads
 623 induce morphological change in Australian snakes. *Proc. Natl. Acad. Sci. U. S. A.*,
 624 101, 17150–17155.

625 Piazzì, L., Ceccherelli, G. & Cinelli, F. (2001). Threat to macroalgal diversity: effects of
 626 the introduced green alga *Caulerpa racemosa* in the Mediterranean. *Mar. Ecol.*
 627 *Prog. Ser.*, 210, 149–159.

628 Piazzì, L., Meinesz, A., Verlaque, M., Akçali, B., Antolić, B., Argyrou, M., *et al.*
 629 (2005). Invasion of *Caulerpa racemosa* var. *cylindracea* (Caulerpales,
 630 Chlorophyta) in the Mediterranean Sea: An assessment of the spread. *Cryptogam.*
 631 *Algol.*, 26, 189–202.

632 Pintor, L.M. & Byers, J.E. (2015). Do native predators benefit from non-native prey?
 633 *Ecol. Lett.*, 18, 1174–1180.

634 Pons-Fàbregas, C., Sales, M., Canals, A. & Borràs, R. (2007). Primera cita de *Caulerpa*
 635 *racemosa* var. *cylindracea* (Caulerpales, Clorophyta) a Menorca, Mediterrània
 636 Occidental. *Bolleti la Soc. d'Historia Nat. les Balear.*, 50, 21–26.

637 Pyšek, P., Hulme, P.E., Simberloff, D., Bacher, S., Blackburn, T.M., Carlton, J.T., *et al.*
 638 (2020). Scientists' warning on invasive alien species. *Biol. Rev.*, 95, 1511–1534.

639 R Core Team. (2018). R: a language and environment for statistical computing.

640 Reñones, O., Moranta, J., Coll, J. & Morales-Nin, B. (1997). Rocky bottom fish
 641 communities of Cabrera Archipelago National Park (Mallorca, Western
 642 Mediterranean). *Sci. Mar.*, 61, 495–506.

643 De Rivera, C.E., Ruiz, G.M., Hines, A.H. & Jivoff, P. (2005). Biotic resistance to
644 invasion: Native predator limits abundance and distribution of an introduced crab.
645 *Ecology*, 86, 3364–3376.

646 Ruitton, S., Verlaque, M., Aubin, G. & Boudouresque, C.F. (2006). Grazing on
647 *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) in the
648 Mediterranean Sea by herbivorous fishes and sea urchins. *Vie Milieu*, 56, 33–41.

649 Ruland, F. & Jeschke, J.M. (2020). How biological invasions affect animal behaviour:
650 A global, cross-taxonomic analysis. *J. Anim. Ecol.*, 89, 2531–2541.

651 Sala, E. (1997). The role of fishes in the organization of a Mediterranean sublittoral
652 community. II: Epifaunal communities. *J. Exp. Mar. Bio. Ecol.*, 212, 45–60.

653 Sala, E. & Ballesteros, E. (1997). Partitioning of space and food resources by three fish
654 of the genus *Diplodus* (Sparidae) in a Mediterranean rocky infralittoral ecosystem.
655 *Mar. Ecol. Prog. Ser.*, 152, 273–283.

656 Santamaría, J., Tomas, F., Ballesteros, E. & Cebrian, E. (2021a). Herbivory on the
657 invasive alga *Caulerpa cylindracea*: The role of omnivorous fishes. *Front. Mar.*
658 *Sci.*, 8, 1055.

659 Santamaría, J., Tomas, F., Ballesteros, E., Ruiz, J.M., Bernardeau-Esteller, J., Terrados,
660 J., *et al.* (2021b). The role of competition and herbivory in biotic resistance against
661 invaders: a synergistic effect. *Ecology*, 102, e03440.

662 Saul, W.C. & Jeschke, J.M. (2015). Eco-evolutionary experience in novel species
663 interactions. *Ecol. Lett.*, 18, 236–245.

664 Schädler, M., Jung, G., Auge, H. & Brandl, R. (2003). Palatability, decomposition and
665 insect herbivory: Patterns in a successional old-field plant community. *Oikos*, 103,
666 121–132.

667 Schultheis, E.H., Berardi, A.E. & Lau, J.A. (2015). No release for the wicked: Enemy

668 release is dynamic and not associated with invasiveness. *Ecology*, 96, 2446–2457.

669 Seebens, H., Blackburn, T.M., Dyer, E.E., Genovesi, P., Hulme, P.E., Jeschke, J.M., *et*
670 *al.* (2017). No saturation in the accumulation of alien species worldwide. *Nat.*
671 *Commun.*, 8, 14435.

672 Siemann, E., Rogers, W.E. & Dewalt, S.J. (2006). Rapid adaptation of insect herbivores
673 to an invasive plant. *Proc. R. Soc. B Biol. Sci.*, 273, 2763–2769.

674 Sih, A., Bolnick, D.I., Luttbeg, B., Orrock, J.L., Peacor, S.D., Pintor, L.M., *et al.*
675 (2010). Predator-prey naïveté, antipredator behavior, and the ecology of predator
676 invasions. *Oikos*, 119, 610–621.

677 Simberloff, D. & Gibbons, L. (2004). Now you see them, now you don't! – population
678 crashes of established introduced species. *Biol. Invasions*, 6, 161–172.

679 Strauss, S.Y., Lau, J.A. & Carroll, S.P. (2006). Evolutionary responses of natives to
680 introduced species: What do introductions tell us about natural communities? *Ecol.*
681 *Lett.*, 9, 357–374.

682 Strayer, D.L. (2012). Eight questions about invasions and ecosystem functioning. *Ecol.*
683 *Lett.*, 15, 1199–1210.

684 Strayer, D.L., D'Antonio, C.M., Essl, F., Fowler, M.S., Geist, J., Hilt, S., *et al.* (2017).
685 Boom-bust dynamics in biological invasions: Towards an improved application of
686 the concept. *Ecol. Lett.*, 20, 1337–1350.

687 Strayer, D.L., Eviner, V.T., Jeschke, J.M. & Pace, M.L. (2006). Understanding the
688 long-term effects of species invasions. *Trends Ecol. Evol.*, 21, 645–651.

689 Stricker, K.B., Philip, F., Goss, E.M. & Flory, S.L. (2016). Emergence and
690 accumulation of novel pathogens suppress an invasive species. *Ecol. Lett.*, 19,
691 469–477.

692 Sundström, L.F., Petersson, E., Höjesjö, J., Johnsson, J.I. & Järvi, T. (2004). Hatchery

693 selection promotes boldness in newly hatched brown trout (*Salmo trutta*):
694 implications for dominance. *Behav. Ecol.*, 15, 192–198.

695 Theoharides, K.A. & Dukes, J.S. (2007). Plant invasion across space and time: Factors
696 affecting nonindigenous species success during four stages of invasion. *New*
697 *Phytol.*, 176, 256–273.

698 Thomaz, S.M., Agostinho, A.A., Gomes, L.C., Silveira, M.J., Rejmánek, M., Aslan,
699 C.E., *et al.* (2012). Using space-for-time substitution and time sequence
700 approaches in invasion ecology. *Freshw. Biol.*, 57, 2401–2410.

701 Tomas, F., Cebrian, E. & Ballesteros, E. (2011). Differential herbivory of invasive algae
702 by native fish in the Mediterranean Sea. *Estuar. Coast. Shelf Sci.*, 92, 27–34.

703 Tomas, F., Turon, X. & Romero, J. (2005). Seasonal and small-scale spatial variability
704 of herbivory pressure on the temperate seagrass *Posidonia oceanica*. *Mar. Ecol.*
705 *Prog. Ser.*, 301, 95–107.

706 Vergés, A., Alcoverro, T. & Ballesteros, E. (2009). Role of fish herbivory in structuring
707 the vertical distribution of canopy algae *Cystoseira* spp. in the Mediterranean Sea.
708 *Mar. Ecol. Prog. Ser.*, 375, 1–11.

709 Vergés, A., Becerro, M.A., Alcoverro, T. & Romero, J. (2007). Variation in multiple
710 traits of vegetative and reproductive seagrass tissues influences plant-herbivore
711 interactions. *Oecologia*, 151, 675–686.

712 Vergés, A., Tomas, F. & Ballesteros, E. (2012). Interactive effects of depth and marine
713 protection on predation and herbivory patterns. *Mar. Ecol. Prog. Ser.*, 450, 55–65.

714 Verhoeven, K.J.F., Biere, A., Harvey, J.A. & Van Der Putten, W.H. (2009). Plant
715 invaders and their novel natural enemies: Who is naïve? *Ecol. Lett.*, 12, 107–117.

716 Verlaque, M. (1990). Relations entre *Sarpa salpa* (Linnaeus, 1758) (Téléostéen,
717 Sparidae), les autres poissons broyeurs et le phytobenthos algal méditerranéen.

718 *Oceanol. Acta*, 13, 373–388.

719 Vilà, M. & Hulme, P.E. (2017). *Impact of biological invasions on ecosystem services*.
720 Springer.

721 Warburton, K. & Hughes, R. (2011). Learning of foraging skills by fish. In: *Fish*
722 *Cognition and Behavior* (eds. Brown, C., Laland, K.N. & Krause, J.). Blackwell
723 Publishing Ltd, pp. 10–35.

724 Wellenreuther, M. & Connell, S.D. (2002). Response of predators to prey abundance:
725 Separating the effects of prey density and patch size. *J. Exp. Mar. Bio. Ecol.*, 273,
726 61–71.

727 White, S.L. & Gowan, C. (2014). Social learning enhances search image acquisition in
728 foraging brook trout. *Environ. Biol. Fishes*, 97, 523–528.

729 Williams, J.W. & Jackson, S.T. (2007). Novel climates, no-analog communities, and
730 ecological surprises. *Front. Ecol. Environ.*, 5, 475–482.

731 **Acknowledgements**

732 We thank the managers and rangers from the Cabrera Archipelago National Park for
733 sampling permissions and helping with field logistics and people at the Jaume Ferrer
734 Marine Station (Instituto Español de Oceanografía) for providing us with technical and
735 facility support. Funding for this Project was obtained from the Spanish Ministry
736 Project ANIMA (CGL2016-76341-R, MINECO/FEDER, UE), the European Union's
737 EMFS Program, AFRIMED (No. EASME/EMFF/2017/1.2.1.12/S4/01/SI2.789059),
738 and the Spanish Autonomous Organism of National Parks through the project
739 BIGPARK (project 2017-2462). J.S. received the support of a fellowship from “La
740 Caixa” Foundation (ID 100010434) under agreement LCF/BQ/DE17/11600001, and
741 R.G benefitted from a pre-doctoral fellowship from the Spanish Ministry of Science

(ref. BES-2017-079907). This is a contribution from the Consolidated Research Group “Medrecover; www.medrecover.org”; SGR2017-1521 (Govern de Catalunya).

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

Figure legends

Figure 1. Location of the 3 regions where the study was carried (the Catalan Coast, the Cabrera Archipelago and Menorca). The points show the specific locations where different assessments were done (see Figure S1 for details). Shapefile for the Mediterranean Sea downloaded from www.naturalearthdata.com, for the Catalan Coast and Menorca downloaded from the Global Self-consistent, Hierarchical, High-resolution Geography Database (GSHHG) and for the Cabrera Archipelago downloaded from www.miteco.gob.es.

Figure 2. Results from the preference assessment. Paired-choice feeding experiments were performed at: **A, B**) locations that have been invaded by *Caulerpa cylindracea* for more than 5 years (Old locations) and where the abundance of the invader in the community was either **A**) high (dark gray bars) or **B**) low (light grey bars); and **C, D**) locations that have been invaded by *Caulerpa cylindracea* for less than 5 years (Recent locations) and where the abundance of the invader in the community was either **C**) high (dark gray bars) or **D**) low (light grey bars). Bars represent the mean percentage of algae consumed \pm S.E.. The illustrations represent the algae species used in the experiments: invasive species (*Caulerpa cylindracea*), native species (*Cystoseira compressa* and

764 *Padina pavonica*). Text on top of the bars refers to the number of replicates (n),
765 statistics from either paired Student T-tests (t) or Wilcoxon signed-ranks paired tests (z)
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767 pairs where significant differences were detected. [Algae illustrations were obtained and
768 modified from the IUCN, Henry Bradbury's original illustration and Greville's original
769 illustration (Algae Britannicae)].

770 **Figure 3.** Results from the consumption assessment. **A)** Percentage of *Sarpa salpa*
771 individuals feeding on *Caulerpa cylindracea* (percentage of pellets with the invader) in
772 old and in recent locations, with high and with low abundances of the invader in the
773 community. **B)** *Per capita* consumption rates of *Sarpa salpa* on *Caulerpa cylindracea*
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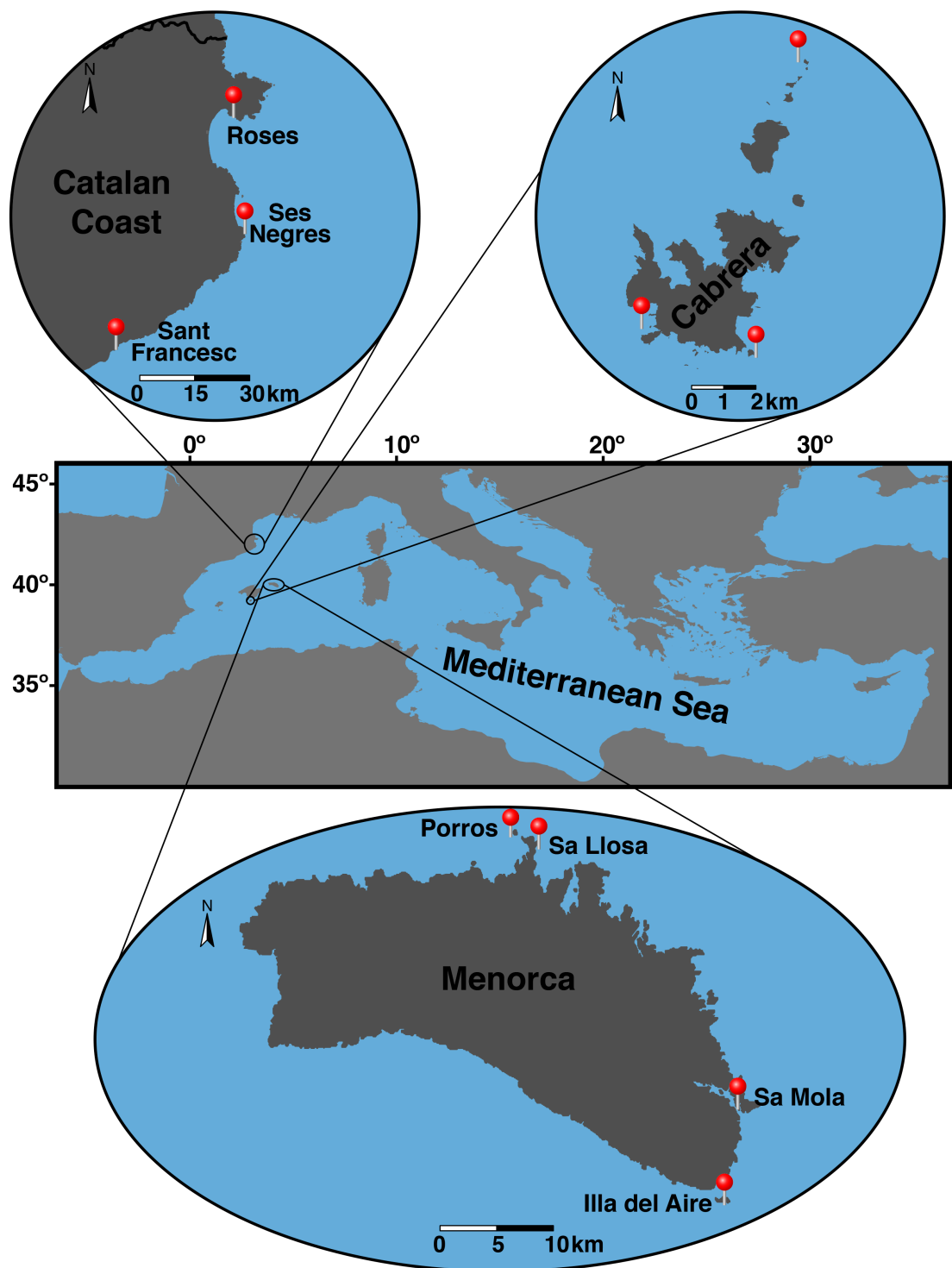
776 **Figure 4.** Results from the electivity assessment. Progress of the Ivlev's Electivity
777 Index towards *Caulerpa cylindracea* through time in two locations that differ in their
778 exposure time to the invader: Cabrera, where *C. cylindracea* has been in the
779 assemblages for 17 years; and Roses, where *C. cylindracea* has been in the assemblages
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781 higher than -1 (specimens that had consumed the invader), whereas the red points
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784 for each year since the invasion and at each particular location. The line connecting the
785 points does not represent any relationship between them and was only added to help
786 interpretation of the figure. Dark grey rectangles highlight the sampling times when the

787 abundance of *C. cylindracea* in the community was high; and light grey rectangles
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789 was low.

790 **Figure 5.** Diagram showing the phases necessary for the development of the effective
791 predator-prey relationship between the native herbivore *Sarpa salpa* and the invasive
792 alga *Caulerpa cylindracea*. In the first phase, consumption of the invader is accidental
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794 consumed the invader will start to target it and to have a preference towards it due to its
795 highly nutritious value. Following that, the rest of the population will learn by
796 observing the individuals that target the invader and the search image for *C. cylindracea*
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799 **Figures**

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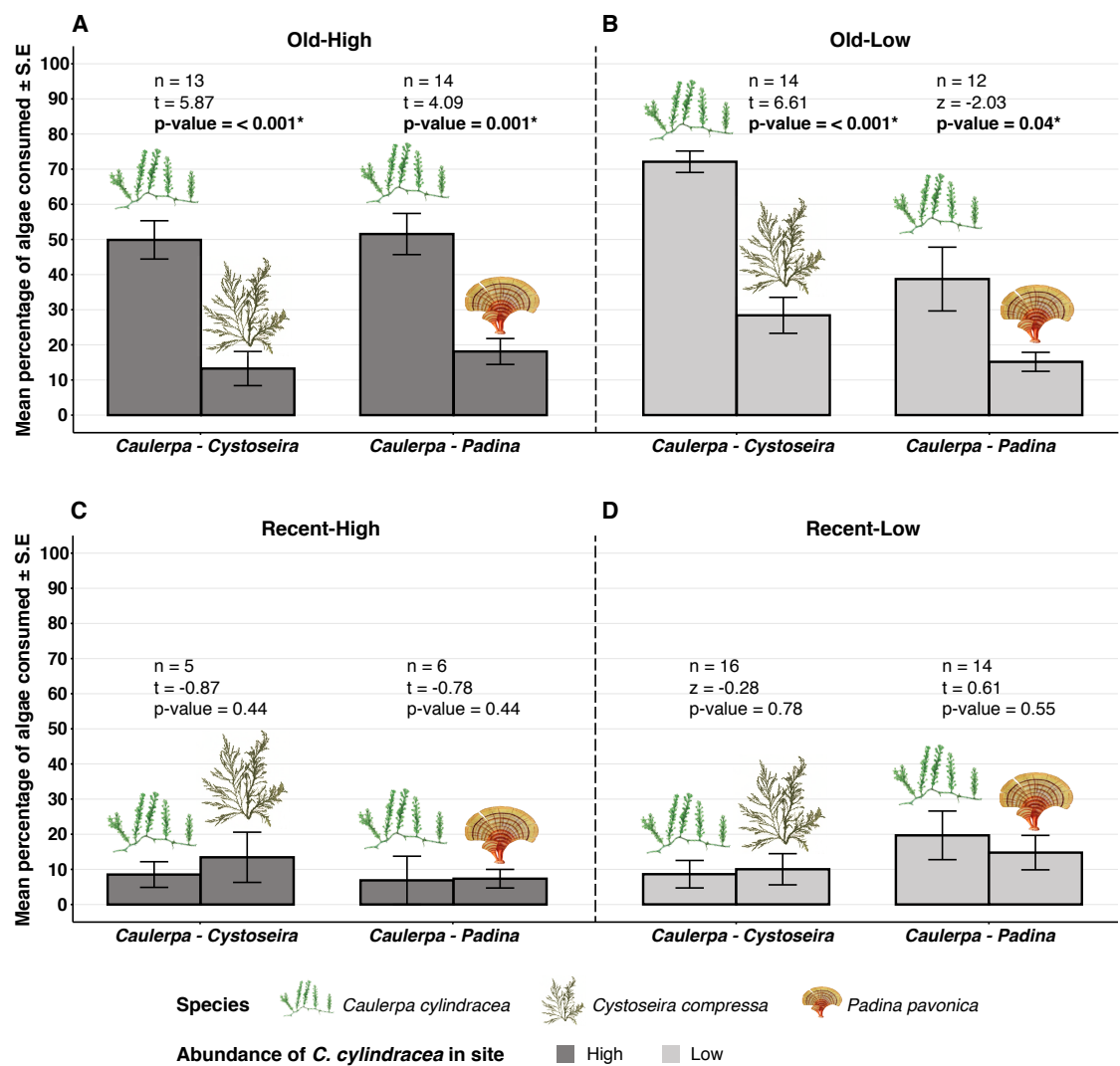
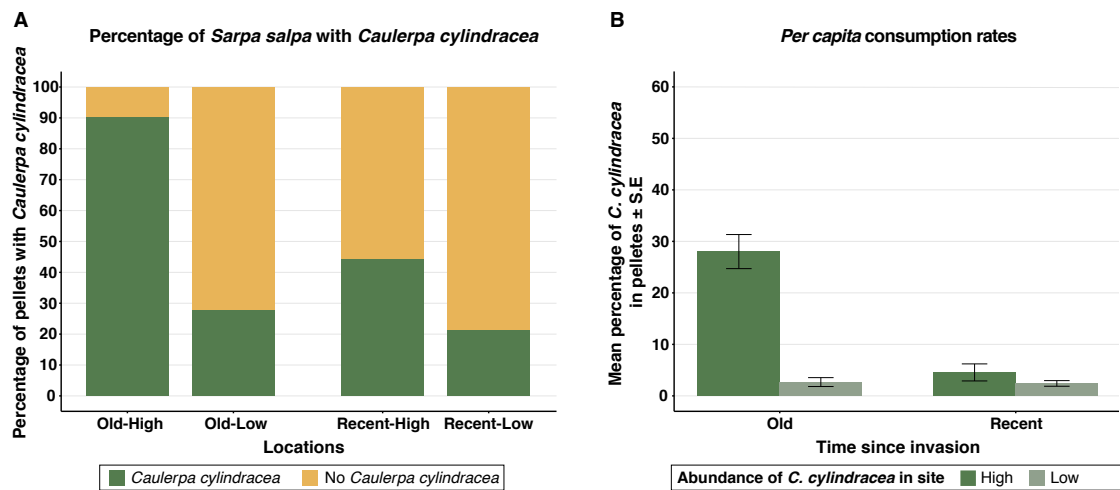


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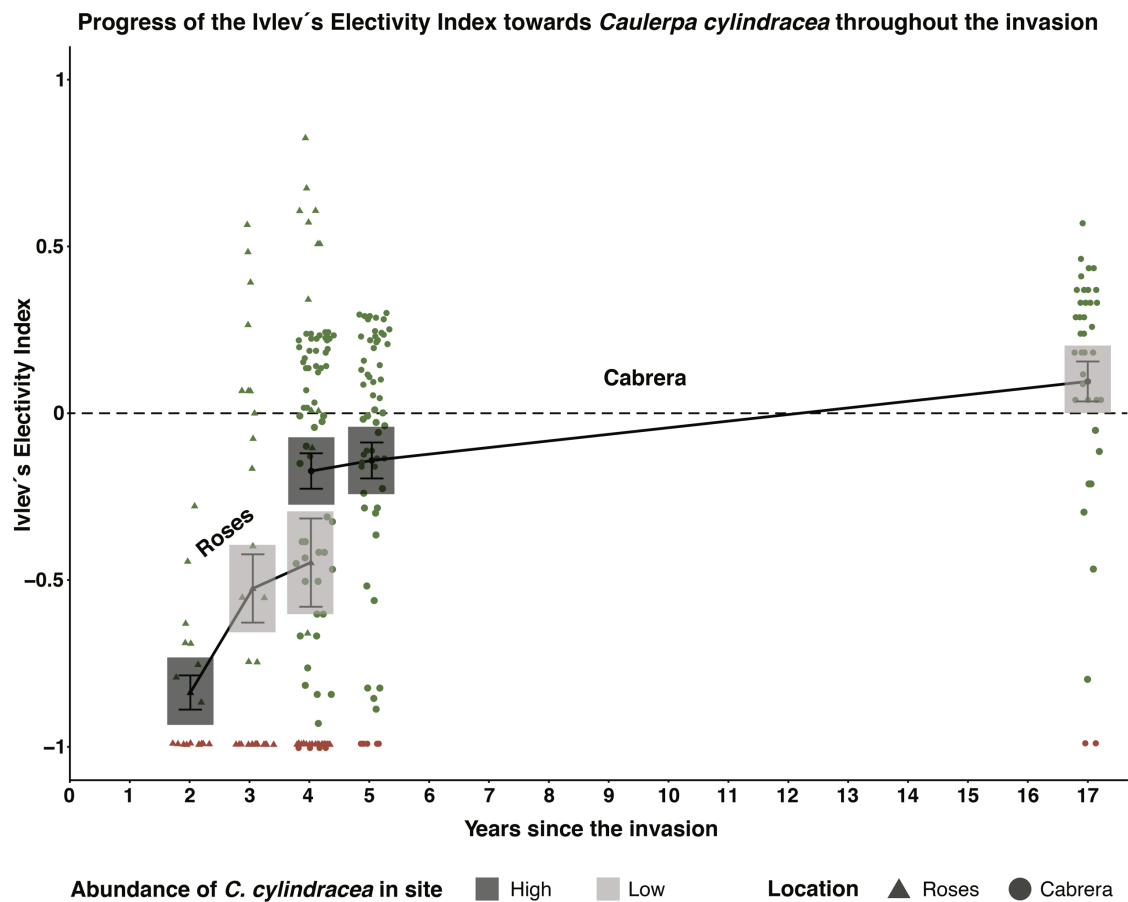
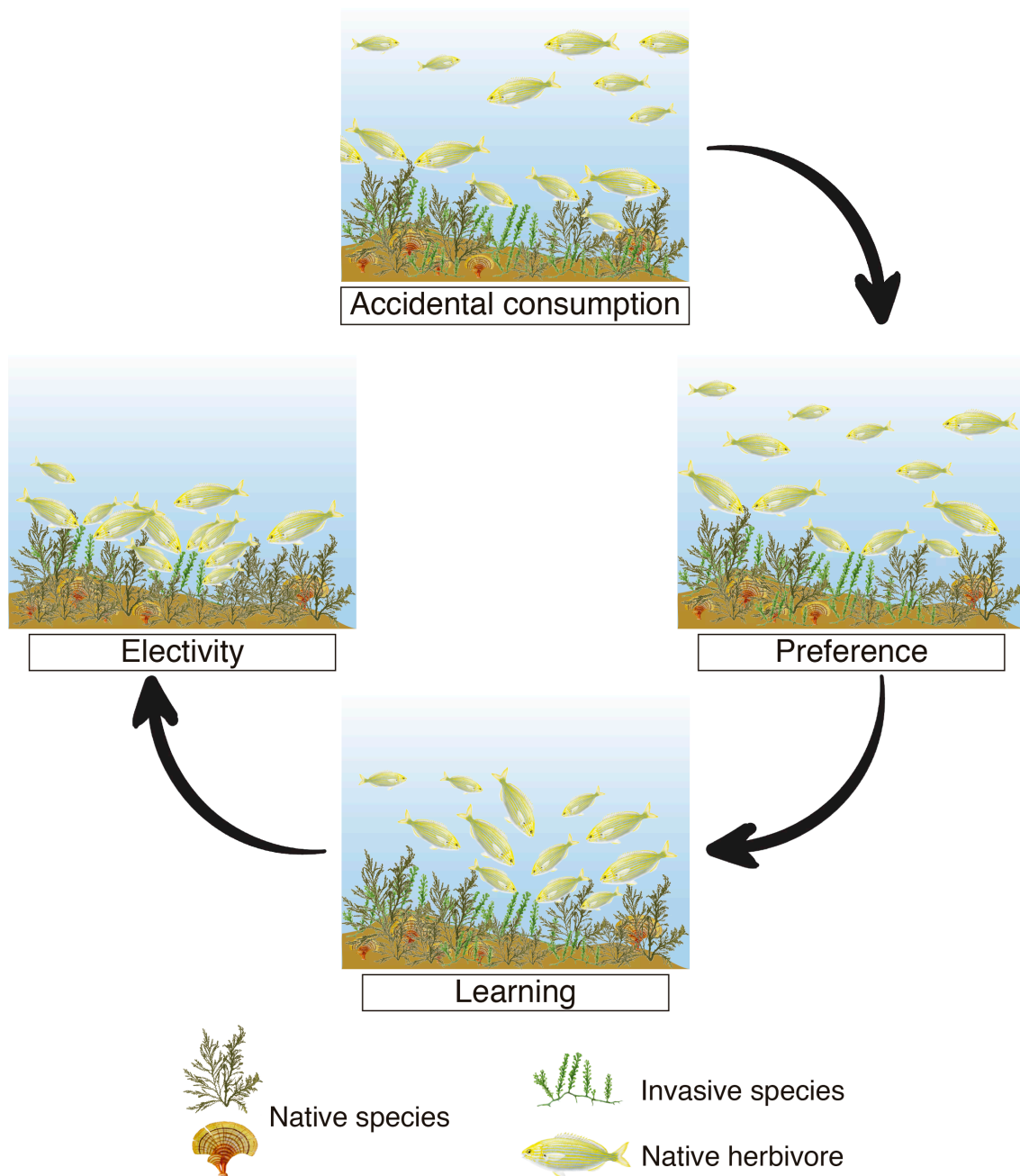


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