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

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

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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 unskilled or wary of consuming newcomers,
5 allowing them to proliferate. However, these relationships may be highly dynamic, and
6 novel consumer-resource interactions could form as familiarity grows. Here, we explore
7 the development of effective biotic resistance towards a highly invasive alga in the
8 Mediterranean using multiple space-for-time approaches. We show that the principal
9 native Mediterranean herbivore learns to consume the invader species within less than a
10 decade. At recently invaded sites, the herbivore actively avoided the alga, shifting to
11 distinct preference and high consumptions at older sites, even when invader abundance

was low. This rapid strengthening of the interaction contributed to the eventual collapse of the alga after an initial dominance. Therefore, our results stress the importance of conserving key native populations to allow communities to develop effective resistance mechanisms against invaders with time.

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

Introduction

As biological invasions spread across the globe, they are causing a reorganization of community assemblages at rates not experienced in historical times. The curious mélange that results brings together species that often have had little recently shared evolutionary history. These actors have to establish entirely novel interactions with each other, leading to communities with no modern analogs ^{1,2}. With nothing to compare these novel communities to, it is often difficult to predict exactly how they will transform native systems. The invading species encounters a native community whose network of interactions have been established over long ecological time scales, and how this community adapts to the new entrant will determine the impact and scale of the invasion. Freed from their usual suite of predators, alien species can become wildly successful in the recipient area, rapidly overtaking the native community ^{3,4}. However, native communities with strong biotic interactions can show considerable biotic resistance, where predation or competition by native species prevent invaders from successfully establishing ^{5,6}.

With rates of invasions growing rapidly over the last few decades ⁷, case studies show that they can radically alter native ecosystems, making invasions one of the leading drivers of the biodiversity crisis, together with climate change, land use and pollution ^{8,9}. Their negative impacts cascade through the ecosystem, affecting normal

functioning, and disrupting a host of important ecosystem services¹⁰ with consequences for human health and well-being¹¹. Clearly then, managing current and future invasions is an urgent global priority⁹.

Central to the management of invasions is an understanding of how invasive species interact with native communities, and the long-term dynamics of these interactions^{12,13}. 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^{4,5,14,15}. If native predators learn quickly to consume novel prey, the strength of the predator-prey interactions can significantly reduce invasion success^{16–20}. 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^{3,4,21,22}. 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^{23,24}, the existence of predator avoidance mechanisms^{25,26} or the inability of predators to identify an invader as prey – native predator “naïveté”^{15,27,28}.

To date, most studies assessing novel predator-prey interactions typically evaluate the impact of the invader at a particular point in time. 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, and feed more efficiently e.g.,^{29–31}. Still, how long it takes for a novel interaction to form and stabilize is a matter of some

uncertainty. Several studies report increased consumption and damage on invaders with increasing invasion time ^{16,17,32–34}. Other studies in contrast, show that time since invasion does not influence consumption or damage rates on invading prey ^{14,18,35}. In fact, several factors, apart from time, are likely to influence the development of such novel interactions ¹³. 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 ^{36,37}, and predator experience of novel prey will likely increase with prey density ¹². Despite this, invader abundance has rarely been considered when assessing the development of novel predator-prey interactions ^{but see: 38}.

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 ³⁹, thus, making it difficult to draw strong conclusions on how predator-prey interactions develop through time ⁴⁰. The use of space-for-time designs or chronosequences, where populations with different invasion times are studied, provides a cost-effective, viable and valid alternative to long-term monitoring ^{41–43}. 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 exposure times and abundances of the invader, 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, to further disentangle the influence of time since invasion on the strength of the consumer-resource interaction, electivity for the invader was assessed at three successive sampling times in two populations characterized by their differential exposure time to the invader.

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 1, A-B). In fact, the native fish showed an at least 2.5-fold higher preference for *C. cylindracea* over native species (Figure 1, 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 1, C-D).

Consumption assessment

The proportion of fish feeding on *C. cylindracea* varied significantly with the interaction term (p-value < 0.05; Table S1), 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 S2, A), with at least 2 times more fish targeting the invader than in populations where *C. cylindracea* abundance was low (Figure 2, A). Additionally, time since invasion significantly influenced the number of fish targeting the invader in locations with high abundances (Table S2, B), with twice the number of fish consuming *C. cylindracea* in the Old-High populations than in the Recent-High populations (Figure 2, A). In summary, more than 90% of *S. salpa* individuals consumed the invasive alga in populations that had a high abundance of *C. cylindracea* and with 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 2, 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 S3). *Sarpa salpa* consumed significantly more *C. cylindracea* in communities with a high invader abundance, regardless of time since invasion (Figure 2, B; Table S4, 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 S4, 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 the recent populations, whereas in communities with a low abundance of *C. cylindracea*, consumption in old and in recent populations was similarly low (Figure 2, 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 S5), increasing slightly with time (Figure 3-Roses; Table S5). Despite this, no significant differences were detected in the Ivlev's Index values between years (p-value > 0.05, Table S6).

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 5-Cabrera; Table S5), confirmed by significantly different Ivlev Index values (Kruskal-Wallis test, p-value < 0.05, Table S6). Post-hoc tests indicate that electivity was significantly higher in 2020 than in 2007 and 2008 (p-value < 0.05, Table S7).

Overall, we observe that independent of invader abundance, the number of fish showing a total avoidance of *C. cylindracea* (-1 values; Figure 3 – 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 3 – 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^{3,4,21,22}. 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.* ⁴⁴, 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 ^{45,46}. 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 ⁴⁷⁻⁴⁹. In fact, fast growing species like *C. cylindracea* ⁵⁰, tend to be more palatable because they allocate resources to re-growth rather than chemical or mechanical defenses ⁵¹⁻⁵³. In general, softer and more aqueous species such as *C. cylindracea* are more easily removed, handled and masticated, making them prime targets for herbivores ⁵⁴⁻⁵⁶.

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

182 sites historically invaded compared with recent invaded areas. This shows that although
183 exposure time is essential for novel consumer-resource interactions to form ^{16,17,57}, other
184 characteristics of the invasion (e.g. availability of the invader) and synergies between
185 them, strongly influence their development. However, we cannot ignore that low
186 invader abundances (< 30%) could partially mask the effect of time for the development
187 of novel consumer-resource interactions, since low encounter rates could inherently
188 reduce consumption even if fish populations were highly experienced with the invader.
189 In actual fact, the electivity of *S. salpa* towards *C. cylindracea* increased through time,
190 even when the abundance of the invader had declined considerably (Figure 3).
191 Therefore, it appears that once *S. salpa* has learnt that *C. cylindracea* is a valuable food
192 source and incorporates it in its diet, it seeks out the invader even when it becomes
193 increasingly rare.

194 On the face of it, the extraordinary preference that develops over time for *C.*
195 *cylindracea* by a native herbivore is *a priori* unexpected, given that the alga produces
196 caulerpenyne, a secondary metabolite with known herbivore deterrent properties ⁵⁸.
197 However, *C. cylindracea* produces this metabolite in relatively low concentrations ⁵⁹. In
198 addition, native *Caulerpa prolifera*, a common food-source for *S. salpa* ⁶⁰, also
199 produces caulerpenyne, therefore, *S. salpa* may have developed a fair tolerance to the
200 compound, making it an ineffective deterrent. Even though invaders themselves may be
201 novel to the community, their phylogenetic affinities with resident natives may help
202 consumers adjust their behaviors and physiologies much more quickly to the invader
203 and form strong biotic interactions with them. Actually, this could well be an important
204 mechanism explaining the failure of some invasive plants to escape naïve herbivores
205 ^{61,62}.

206 Mechanisms such as learning and social transmission can influence the way
207 predators adapt to novel prey at different time scales, from days to years and even
208 generations since they are first encountered ^{25,29,63}. The mechanisms by which *S. salpa*
209 begins to consume *C. cylindracea* remain speculative, but they potentially follow steps
210 similar to the predation cycle ¹² (Figure 6), which involves *S. salpa* identifying *C.*
211 *cylindracea* as a suitable resource and developing a taste for the newcomer. Therefore,
212 after an initial wariness because of the unfamiliarity of the invader, the first few
213 interactions may be completely accidental and involve very few individuals (low
214 proportion of individuals consume the invader in recent populations) (Figure 2-A;
215 Figure 4). However, after these individuals come to discover the high nutritional value
216 of the invader, they begin to target it, soon followed by the rest of the population when
217 it learns that the invader is safe to eat and nutritious. In non-solitary fish, social
218 learning, where individuals learn behaviors and acquire information such as what to eat
219 and what to avoid through observation, is an important foraging mechanism ^{64,65}. This
220 may help explain the fast transmission of search images between demonstrators, those
221 that know how to feed on novel prey, and bystanders, those that observe and learn to
222 target the new prey ⁶⁶. Actually, *Sarpa salpa* is a highly social species that feeds in large
223 cooperative shoals which show complex feeding behaviors ⁶⁷. Within a population,
224 behavioral syndromes could play an important role in determining how quickly
225 consumers take to novel resources, with bolder phenotypes less reticent to try unfamiliar
226 food items than shy individuals ⁶⁸, based on their past experiences ⁶⁹. How rapidly this
227 learning spreads through the population could, as our results suggest, be influenced by
228 invader abundance, because the formation and transmission of search images likely
229 increases with encounter rates of novel resources ^{12,70}.

Our study suggests that as the invasion progresses, novel predator-prey interactions can become an effective biotic resistance mechanism against an invader. 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 the invasion of *C. cylindracea*^{50,71,72}. 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 enemies⁴⁰. 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^{17,29,40}. 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.,^{17,19,73,74}. Thus, biotic resistance against an invader is not static and changes dynamically through the invasion, increasing its strength as the invasion progresses^{17,33,34,75}. 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^{4,13,22,27}. Therefore, long-term studies or chronosequences should be favored to help us understand the long-term dynamics of certain invaders^{40,76}. 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.,^{17,33,34,77}. Studies in terrestrial ecosystems refer to time exposures of decades or even centuries for effective resistance mechanisms to develop through time^{32–34,42,75}. 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 ⁷⁴. This represents roughly one generation of the herbivore *S. salpa* ⁷⁸, 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.

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 Ocean and the Indian Ocean ⁷¹. 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 ^{71,79}. *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 ^{50,71,80}. The species can be considered a hyper-successful invader in the Mediterranean Sea ^{71,72} and it is ranked globally as one of the most harmful marine invaders, causing widespread negative ecological impacts wherever it spreads ⁸¹. 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^{71,74,82}. 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*^{44,83–85}. 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 time and to assess whether the strength of the interaction shifts throughout the course of the invasion and/or in relation to the abundance of the invader at the study site.

The only true herbivorous fish in the study area is the bream *Sarpa salpa*^{46,86}, which plays an important role in structuring seagrass and macroalgae communities^{45,87}. This species is abundant in shallow water communities along the NW Mediterranean Sea^{87–89} and spends most of its time above 20 m^{44,88,90}, where it feeds on a wide variety of species⁴⁶. *Sarpa salpa* has been observed to regularly consume *C. cylindracea*^{44,83} and a previous study has reported that it even prefers the invasive alga over many native species⁴⁴.

Study sites

This study was conducted in 3 regions in the NW Mediterranean Sea: Cabrera Archipelago, Menorca Island and the Catalan coast (Figure 5), based on the documented invasion history of *C. cylindracea* in these regions.

In Cabrera Archipelago, *C. cylindracea* was detected in 2003 at 30 m deep⁹¹ and quickly expanded across the archipelago. Nowadays, it can be found in most

benthic habitats at depths between 0 to 65 m, where it can be the dominant species ⁸⁴. However, in the past few years, the abundance of the invasive alga has experienced a decline in the archipelago, mainly at shallow depths ⁷⁴.

In Menorca, *C. cylindracea* was first detected in 2006, in photophilic assemblages (≈ 20 m deep) from the south of the island (Illa de l'Aire) ⁹², 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 at depths between 5 to 45 m ⁹³, although recent reports show that its abundance is decreasing, predominantly in the shallow assemblages ⁹⁴.

Along the Catalan coast, *C. cylindracea* was first detected in 2008 in its southern waters, at depths between 20 to 50 m ⁹⁵. The invasive alga maintained this restricted distribution until 2013, when it was detected further north, in Blanes ⁸². 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 15 m was assessed underwater on SCUBA. At each location, thirty 25 x 25 cm quadrats, divided into 25 subquadrats of 5 x 5 cm ⁹⁶ 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 S8; 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 ⁴⁵ and commonly found in *S. salpa*’s diet ⁴⁶; and *Padina pavonica*, a photophilic alga commonly found in the sublittoral zone of warm-temperate coasts ⁹⁷ and also a usual food source for *S. salpa* ⁴⁶ (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 (0.5 cm mesh size)

and were similarly weighted down. The clothespins were used to hold the algal fragments and avoid losses due to currents.

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 (≈ 10 m) for all replicates and chose areas where there were no sea urchins. In addition, we ensured that the patches all 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 1 m apart from each other, whereas treatments were placed less than 20 m apart to maintain constant environmental conditions between them. All the experiments were conducted at the end of summer (in 2018 and in 2019), corresponding to the period of the year when *S. salpa* feeds most intensively to accumulate reserves for winter and prepare for reproduction⁹⁸. Samples were deployed in the morning and collected after 24 h; before and after deployment, every algal fragment was pad-dried of excess water and wet weighed to the nearest 0.01 g. 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^{44,99}. Consumption values were then standardized to a 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 ⁴⁴.

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 rest of the 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) ¹⁰⁰ was calculated in two locations: Roses, at the 2nd, 3rd and 4th year after *C. cylindracea* invasion – first record in 2016; and the 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 environment¹⁰⁰.

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)¹⁰¹, 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 species – *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 and if the interaction between them was significant, it was also included in the model. Tukey post hoc tests were performed using the functions “pairs” and “emmeans” from the *emmeans* package¹⁰² 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 the Cabrera Archipelago), Kruskal-Wallis tests were performed due to the lack of normality in the data¹⁰³. Then, to compare effects between years, Dunn’s post hoc tests¹⁰⁴ were performed using the *FSA* package in R¹⁰⁵, correcting p-values with the Benjamini-Hochberg method¹⁰⁶.

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719 **Author contributions**

720 J.S. and E.C. conceived the ideas and the experimental design; J.S., R.G., J.V., F.T.,
721 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.

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.

Data availability

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

Figure legends

Figure 1. 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 *Padina pavonica*). Text on top of the bars refers to the number of replicates (n), statistics from either paired Student T-tests (t) or Wilcoxon signed-ranks paired tests (z) and the probability values for those statistics (p-value). P-values in bold highlight the pairs where significant differences were detected. [Algae illustrations were obtained and

744 modified from the IUCN, Henry Bradbury's original illustration and Greville's original
745 illustration (Algae Britannicae)].

746 **Figure 2.** Results from the consumption assessment. **A)** Percentage of *Sarpa salpa*
747 individuals feeding on *Caulerpa cylindracea* (percentage of pellets with the invader) in
748 old and in recent locations, with high and with low abundances of the invader in the
749 community. **B)** *Per capita* consumption rates of *Sarpa salpa* on *Caulerpa cylindracea*
750 (mean percentage of *C. cylindracea* in pellets $\pm$ S.E.), in old and in recent locations,
751 with high and with low abundances of the invader in the community.

752 **Figure 3.** Results from the electivity assessment. Progress of the Ivlev's Electivity
753 Index towards *Caulerpa cylindracea* through time in two locations that differ in their
754 exposure time to the invader: Cabrera, where *C. cylindracea* has been in the
755 assemblages for 17 years; and Roses, where *C. cylindracea* has been in the assemblages
756 for 4 years. Green points represent the *S. salpa* specimens with an electivity index
757 higher than -1 (specimens that had consumed the invader), whereas the red points
758 represent the *S. salpa* specimens with an electivity index of -1 (specimens that had not
759 eaten the invader). The vertical lines represent the mean Ivlev's Electivity Index $\pm$ S.E.
760 for each year since the invasion and at each particular location. The line connecting the
761 points does not represent any relationship between them and was only added to help
762 interpretation of the figure. Dark grey rectangles highlight the sampling times when the
763 abundance of *C. cylindracea* in the community was high; and light grey rectangles
764 highlight the sampling times when the abundance of *C. cylindracea* in the community
765 was low.

Figure 4. Diagram showing the phases necessary for the development of the effective predator-prey relationship between the native herbivore *Sarpa salpa* and the invasive alga *Caulerpa cylindracea*. In the first phase, consumption of the invader is accidental and will only be done by few individuals. Then, those individuals that accidentally consumed the invader will start to target it and to have a preference towards it due to its highly nutritious value. Following that, the rest of the population will learn by observing the individuals that target the invader and the search image for *C. cylindracea* will be transferred to the entire fish population. Finally, most of the fish population will target the invader, increasing the electivity towards it as the invasion progresses.

Figure 5. 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.

Figures

Figure 1

Figure 1. 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 gray 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 gray 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 *Padina pavonica*). Text on top of the bars refers to the number of replicates (n), statistics from either paired Student T-tests (t) or Wilcoxon signed-ranks paired tests (z) and the probability values for those statistics (p-value). P-values in bold highlight the pairs where significant differences were detected. [Algae illustrations were obtained and modified from the IUCN, Henry Bradbury's original illustration and Greville's original illustration (Algae Britannicae)].

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