

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

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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.

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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 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

40 interactions (Mitchell *et al.* 2006; Saul & Jeschke 2015). The strength and direction of
41 newly formed interactions – either in the form of competition or predation – is key to
42 how successfully non-native species establish within a recipient assemblage (Colautti *et*
43 *al.* 2004; Levine *et al.* 2004; Sih *et al.* 2010; Pintor & Byers 2015). If native predators
44 learn quickly to consume novel prey, the strength of the predator-prey interactions can
45 significantly reduce invasion success (Carpenter & Cappuccino 2005; De Rivera *et al.*
46 2005; Carlsson & Strayer 2009; Carlsson *et al.* 2011; Caselle *et al.* 2018). However,
47 very often, invasive species find themselves in assemblages without natural enemies, or
48 where potential predators have not learnt to consume them. Thus, released from biotic
49 control, invader populations soar dramatically and can rapidly overwhelm native
50 assemblages (Maron & Vilà 2001; Keane & Crawley 2002; Colautti *et al.* 2004; Liu &
51 Stiling 2006). Several factors have been proposed to influence the strength and direction
52 of consumer pressure including the defense capabilities of invaders and their ability to
53 release allelopathic chemicals (Inderjit *et al.* 2011; Mennen & Laskowski 2018), the
54 existence of predator avoidance mechanisms (Strauss *et al.* 2006; Ruland & Jeschke
55 2020) or the inability of predators to identify an invader as prey – native predator
56 “naïveté” (Verhoeven *et al.* 2009; Sih *et al.* 2010; Kimbro *et al.* 2013).

57 To date, most studies assessing novel predator-prey interactions typically
58 evaluate the impact of the invader at a particular stage during the invasion. However,
59 the relationship invasive species establish with the recipient community is a rapidly
60 evolving one. It is quite likely that the interaction will shift in strength and in character
61 as each actor adjusts to the other through the invasion process. Native predators, with
62 increased exposure to invaders, can learn their vulnerabilities and develop more
63 effective behavioral adaptations to exploit them more efficiently (e.g., Carroll *et al.*
64 1998; Phillips & Shine 2004; Carlsson *et al.* 2009). Still, how long it takes for novel

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

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

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

107 **Materials and methods**

108 Target species

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

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

132 The only true herbivorous fish in the study area is the bream *Sarpa salpa*
133 (Verlaque 1990; Gianni *et al.* 2017), which plays an important role in structuring
134 seagrass and macroalgae communities (Tomas *et al.* 2005; Vergés *et al.* 2009). This
135 species is abundant in shallow water communities along the NW Mediterranean Sea
136 (Bell 1983; Reñones *et al.* 1997; Tomas *et al.* 2005) and spends most of its time above
137 20m (Bell 1983; Tomas *et al.* 2011; Vergés *et al.* 2012), where it feeds on a wide
138 variety of species (Verlaque 1990). *Sarpa salpa* has been observed to regularly consume

139 *C. cylindracea* (Ruitton *et al.* 2006; Tomas *et al.* 2011) and a previous study has
140 reported that it even prefers the invasive alga over many native species (Tomas *et al.*
141 2011).

142 Study sites

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

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

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

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

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

164 Preference assessment

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

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

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

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

188 At each location, paired-choice feeding experiments were performed to compare
189 the relative palatability of *C. cylindracea* vs. two native macroalgae species: *Cystoseira*
190 *compressa*, an important habitat-forming alga that is highly palatable (Vergés *et al.*
191 2009) and commonly found in *S. salpa*'s diet (Verlaque 1990); and *Padina pavonica*, a
192 photophilic alga commonly found in the sublittoral zone of warm-temperate coasts (Sala
193 1997) and also a usual food source for *S. salpa* (Verlaque 1990) (Figure S1). Thus, we
194 used 2 treatments: i) *Caulerpa* – *Cystoseira* and ii) *Caulerpa* – *Padina*; and 3 controls,
195 one per algal species. Each replicate in each treatment was composed of native and
196 invasive algal fragments held by two clothespins attached to each other with zip-tie,
197 weighted down to keep them in place underwater. Controls were composed of algal
198 fragments of each of the studied species, held by a single clothespin protected from
199 herbivores with a plastic mesh cage (0.5cm mesh size) and were similarly weighted
200 down.

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

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

217 Biomass consumption was estimated with the formula:

$$218 \left(H_i \times \frac{C_f}{C_i} \right) - H_f$$

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

223 Consumption assessment

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

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

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

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

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

$$249 \quad E = \frac{(d_i - a_i)}{(d_i + a_i)}$$

250 where d_i = % of *C. cylindracea* in the fecal pellets of *S. salpa* (see the consumption
251 assessment section) and a_i = % of *C. cylindracea* available in the environment (see the
252 preference assessment section). The values of Ivlev's Index (E) range from -1 (complete
253 avoidance) to +1 (exclusive selection), with positive values indicating that the food item
254 is selected and eaten more than it is encountered by chance in the environment (Ivlev
255 1961).

256 Statistical analysis

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

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

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

281 Wallis 1952). Then, to compare effects between years, Dunn's post-hoc tests (Dunn
282 1964) were performed using the *FSA* package in R (Ogle *et al.* 2020), correcting p-
283 values with the Benjamini-Hochberg method (Benjamini & Hochberg 1995).

284 **Results**

285 Preference assessment

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

292 In contrast, in recently invaded locations, *S. salpa* did not exhibit a preference
293 for the invader regardless of the abundance of *C. cylindracea* in the community (Figure
294 2, C-D).

295 Consumption assessment

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

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

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

322 Electivity assessment

323 In Roses, where the invasion of *C. cylindracea* began very recently (first detection in
324 2016), *S. salpa* showed a negative electivity for *C. cylindracea* (Table S6), increasing
325 slightly with time (Figure 4-Roses; Table S6). Despite this, no significant differences
326 were detected in the Ivlev's Index values between years (p -value > 0.05 , Table S7).

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

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

338 **Discussion**

339 The success of exotic invaders is frequently attributed to a release from biotic control in
340 the communities in which they find themselves (Maron & Vilà 2001; Keane & Crawley
341 2002; Colautti *et al.* 2004; Liu & Stiling 2006). With no shared evolutionary history
342 with native species, invaders often do much better in recipient communities than they
343 do in their native habitats, where they belong to assemblages that have formed over long
344 ecological time scales. While this initial unfamiliarity drives their rapid spread, does it
345 guarantee long-term success, once the novelty of the species has worn off? Our results
346 indicate that with time, as native species become increasingly familiar with the invader,
347 the strength of consumer-resource interactions increases. Eventually, this may serve to
348 intensify the biotic resistance of native communities as native consumers learn to handle
349 and consume the invader, potentially leading to a control of their populations.

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

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

375 influence their development. However, we cannot ignore that low invader abundances
376 (< 30%) could partially mask the effect of time for the development of novel consumer-
377 resource interactions, since low encounter rates could inherently reduce consumption
378 even if fish populations were highly experienced with the invader. In actual fact, the
379 electivity of *S. salpa* towards *C. cylindracea* increased through time, even when the
380 abundance of the invader had declined considerably (Figure 4). Therefore, it appears
381 that once *S. salpa* has learnt that *C. cylindracea* is a valuable food source and
382 incorporates it in its diet, it seeks out the invader even when it becomes increasingly
383 rare.

384 On the face of it, the extraordinary preference that develops over time for *C.*
385 *cylindracea* is *a priori* unexpected, given that the alga produces caulerpenyne, a
386 secondary metabolite with known herbivore deterrent properties (Paul *et al.* 2007).
387 However, *C. cylindracea* produces this metabolite in relatively low concentrations (Box
388 *et al.* 2010). In addition, native *Caulerpa prolifera*, a common food-source for *S. salpa*
389 (Marco-Méndez *et al.* 2017), also produces caulerpenyne, therefore, *S. salpa* may have
390 developed a fair tolerance to the compound, making it an ineffective deterrent. Even
391 though invaders themselves may be novel to the community, their phylogenetic
392 affinities with resident natives may help consumers adjust their behaviors and
393 physiologies much more quickly to the invader and form strong biotic interactions with
394 them. Actually, this could well be an important mechanism explaining the failure of
395 some invasive plants to escape naïve herbivores (Hill & Kotanen 2009; Pearse *et al.*
396 2013).

397 Mechanisms such as learning and social transmission can influence the way
398 predators adapt to novel prey at different time scales, from days to years and even
399 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).

424 Our study suggests that as the invasion progresses, novel predator-prey
425 interactions can become an effective biotic resistance mechanism against invaders.
426 However, given that this resistance does not develop immediately, invaders can escape
427 biotic control at the start of the invasion, allowing them to become hyper-successful and
428 to impact native communities, as has been observed for *C. cylindracea* (Piazzi *et al.*
429 2001; Klein & Verlaque 2008; Katsanevakis *et al.* 2016). In fact, many invading species
430 experience major population outbreaks when they first arrive in an area, that can be
431 linked to a release from natural enemies (Strayer *et al.* 2017). But then, as encounters
432 increase and native communities learn to deal with the novel species, invader
433 populations could well experience a crash after a period of successful dominance
434 (Carlsson *et al.* 2009, 2011; Strayer *et al.* 2017). Shifts in the strength of biotic
435 resistance through time may be the main factor in the steep declines several hyper-
436 successful invaders have suffered with time (e.g., Simberloff & Gibbons 2004; De
437 Rivera *et al.* 2005; Carlsson *et al.* 2011; Santamaría *et al.* 2021b). Thus, biotic
438 resistance against an invader is not static and changes dynamically through the invasion,
439 increasing its strength as the invasion progresses (Hawkes 2007; Diez *et al.* 2010;
440 Carlsson *et al.* 2011; Stricker *et al.* 2016). For this reason, snapshot studies that only
441 focus on a specific point in time give us only a limited view of the importance of biotic
442 resistance towards an invader since the outcomes may vary greatly influenced across
443 time. This may help explaining contradictory patterns in the ability of native
444 communities to develop effective biotic resistance mechanisms towards invaders
445 (Maron & Vilà 2001; Colautti *et al.* 2004; Mitchell *et al.* 2006; Kimbro *et al.* 2013).
446 Therefore, long-term studies or chronosequences should be favored to help us
447 understand the long-term dynamics of certain invaders (Strayer 2012; Strayer *et al.*

448 2017). In addition, given how important encounter rate is, studies need to assess how
449 these interactions develop at different densities of the invader as well.

450 Our study adds to the growing literature showing that native communities need
451 time to develop resistance mechanisms against invasive species (e.g., Lankau *et al.*
452 2009; Diez *et al.* 2010; Carlsson *et al.* 2011; Stricker *et al.* 2016). Studies in terrestrial
453 ecosystems refer to time exposures of decades or even centuries for effective resistance
454 mechanisms to develop through time (Siemann *et al.* 2006; Hawkes 2007; Diez *et al.*
455 2010; Dostál *et al.* 2013; Stricker *et al.* 2016). In our system, however, an exposure time
456 of less than a decade was enough to observe an increase in the biotic resistance of the
457 native community and a decline in invader populations (Santamaría *et al.* 2021b). This
458 represents roughly one generation of the herbivore *S. salpa* (Méndez-Villamil *et al.*
459 2002), indicating that a population-level preference for the invader spread within the
460 lifetime of the herbivore. However, a decade is sufficient time for an initially successful
461 invader to trigger catastrophic and potentially irreversible shifts in ecosystems. As
462 global change continues to extend its reach across the world's oceans, the swell of novel
463 species entering native waters is only going to increase. Therefore, considering that it
464 takes time for native assemblages to learn to adjust to these new entrants; in managing
465 invasions, it is imperative to ensure that native communities are maintained in as
466 healthy a state as possible until any potential biotic resistance to novel species can
467 develop.

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744 **Competing interests**

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

747 **Figure legends**

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

755 **Figure 2.** Results from the preference assessment. Paired-choice feeding experiments
756 were performed at: **A, B**) locations that have been invaded by *Caulerpa cylindracea* for
757 more than 5 years (Old locations) and where the abundance of the invader in the
758 community was either **A**) high (dark gray bars) or **B**) low (light grey bars); and **C, D**)
759 locations that have been invaded by *Caulerpa cylindracea* for less than 5 years (Recent
760 locations) and where the abundance of the invader in the community was either **C**) high
761 (dark gray bars) or **D**) low (light grey bars). Bars represent the mean percentage of algae
762 consumed \pm S.E.. The illustrations represent the algae species used in the experiments:
763 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)
766 and the probability values for those statistics (p-value). P-values in bold highlight the
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*
774 (mean percentage of *C. cylindracea* in pellets \pm S.E.), in old and in recent locations,
775 with high and with low abundances of the invader in the community.

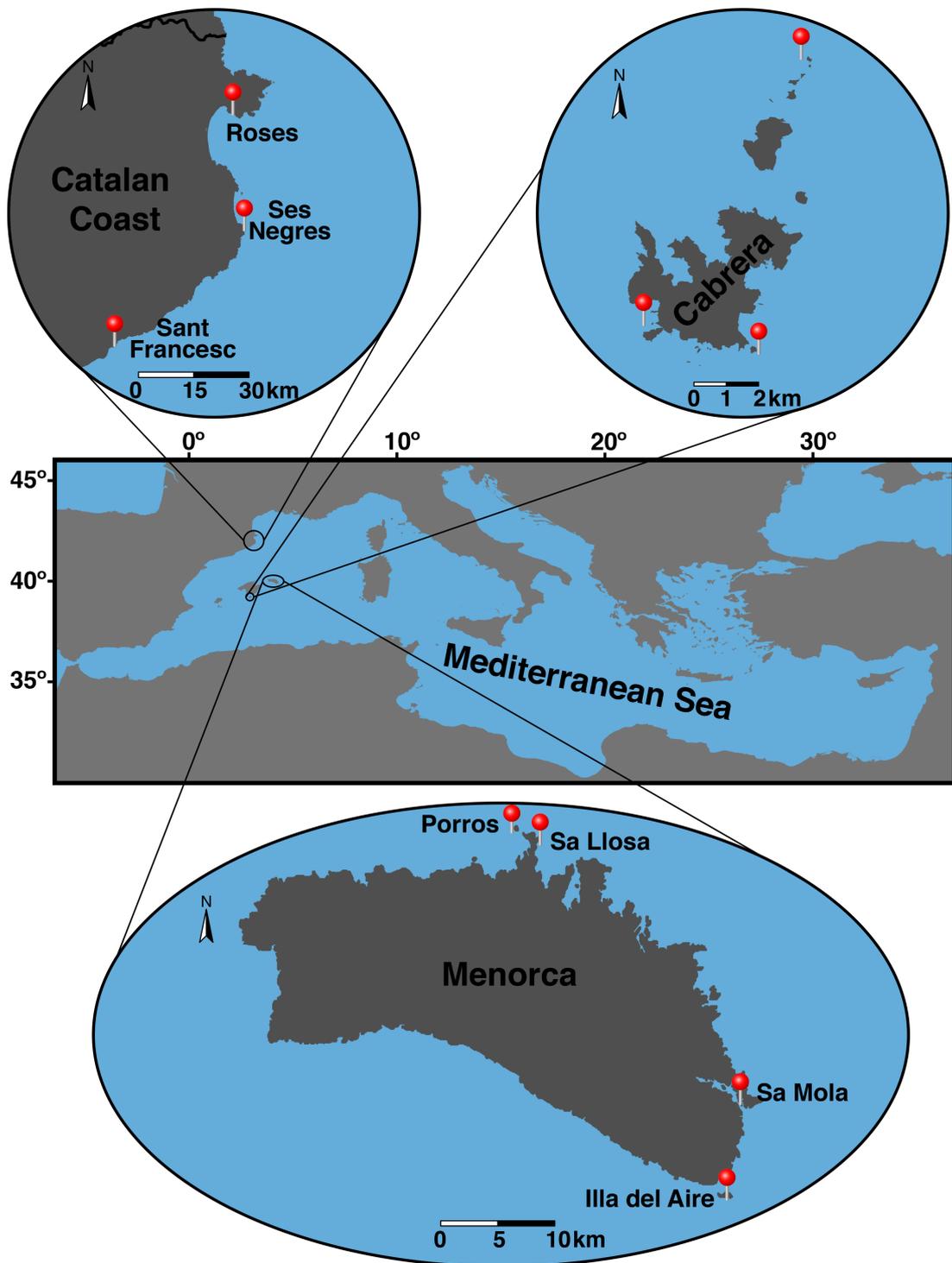
776 **Figure 4.** Results from the electivity assessment. Progress of the Ivlev's Electivity
777 Index towards *Caulerpa cylindracea* through time in two locations that differ in their
778 exposure time to the invader: Cabrera, where *C. cylindracea* has been in the
779 assemblages for 17 years; and Roses, where *C. cylindracea* has been in the assemblages
780 for 4 years. Green points represent the *S. salpa* specimens with an electivity index
781 higher than -1 (specimens that had consumed the invader), whereas the red points
782 represent the *S. salpa* specimens with an electivity index of -1 (specimens that had not
783 eaten the invader). The vertical lines represent the mean Ivlev's Electivity Index \pm S.E.
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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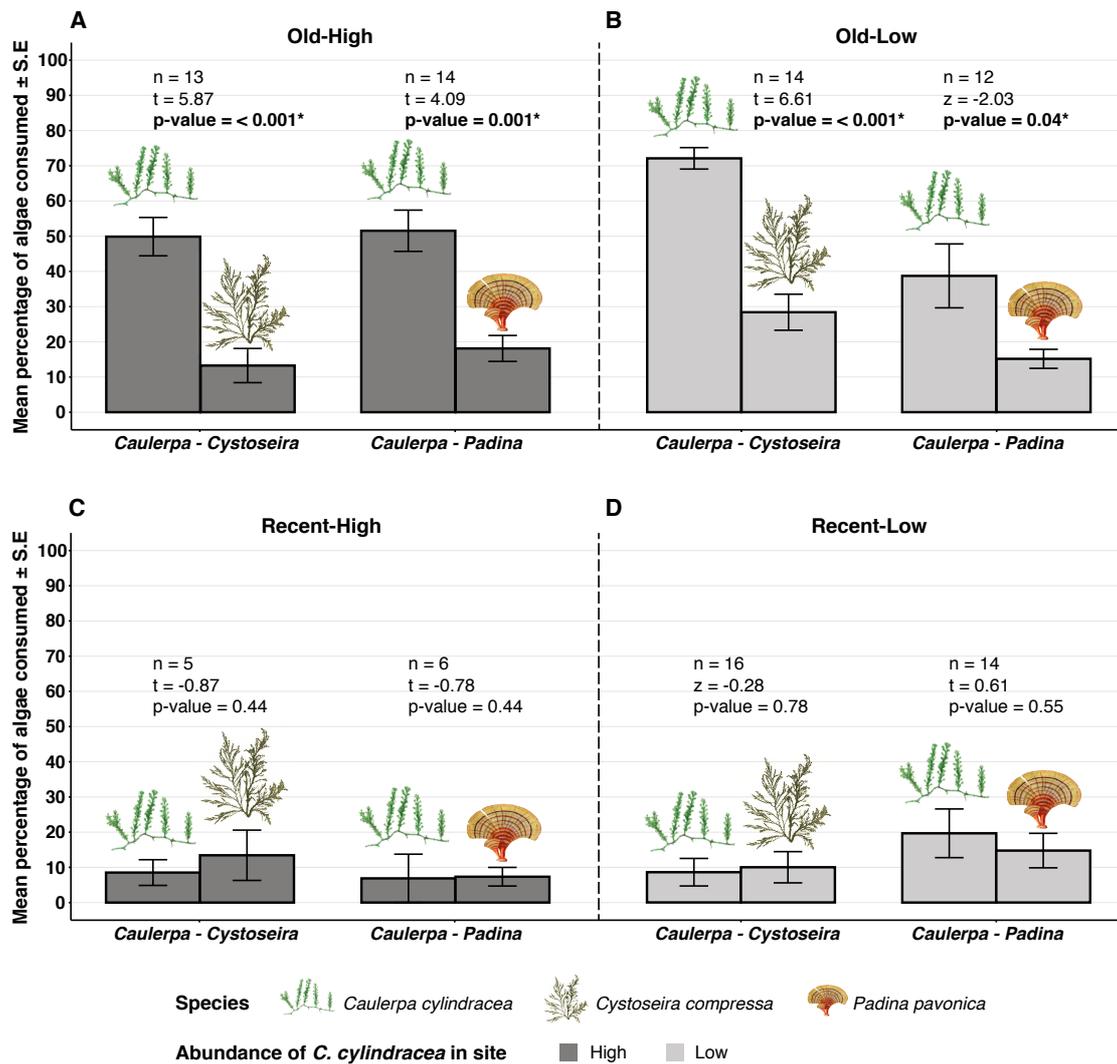
790 **Figure 5.** Diagram showing the phases necessary for the development of the effective
791 predator-prey relationship between the native herbivore *Sarpa salpa* and the invasive
792 alga *Caulerpa cylindracea*. In the first phase, consumption of the invader is accidental
793 and will only be done by few individuals. Then, those individuals that accidentally
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
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799 **Figures**

800 **Figure 1**

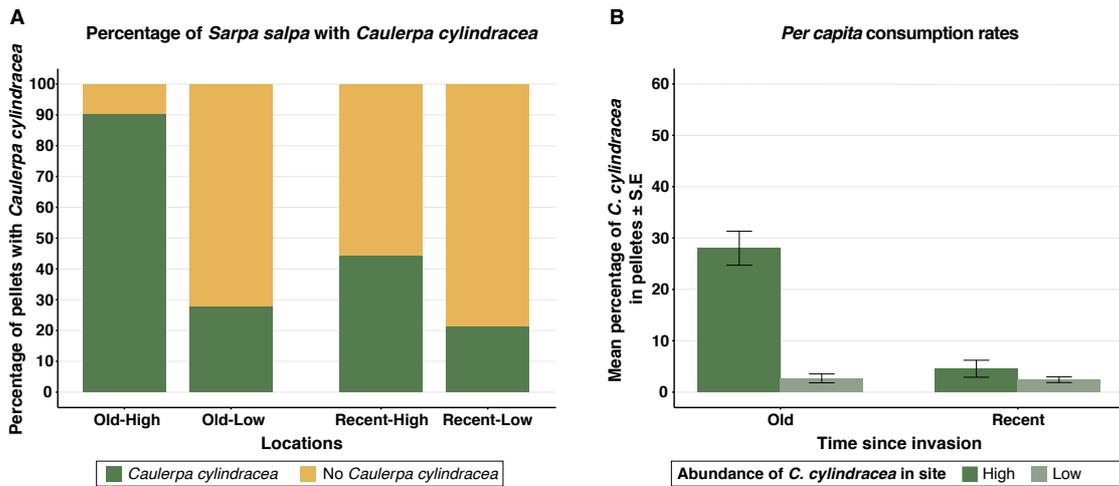


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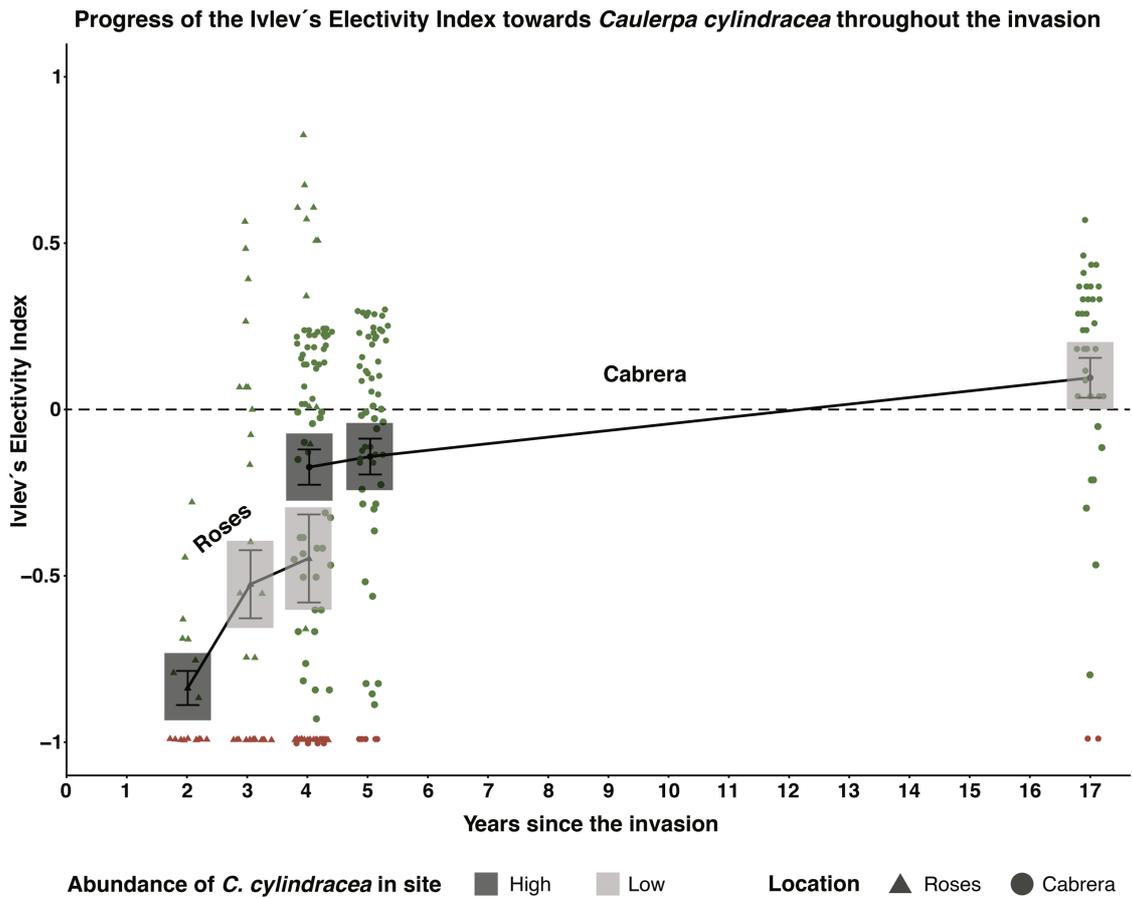
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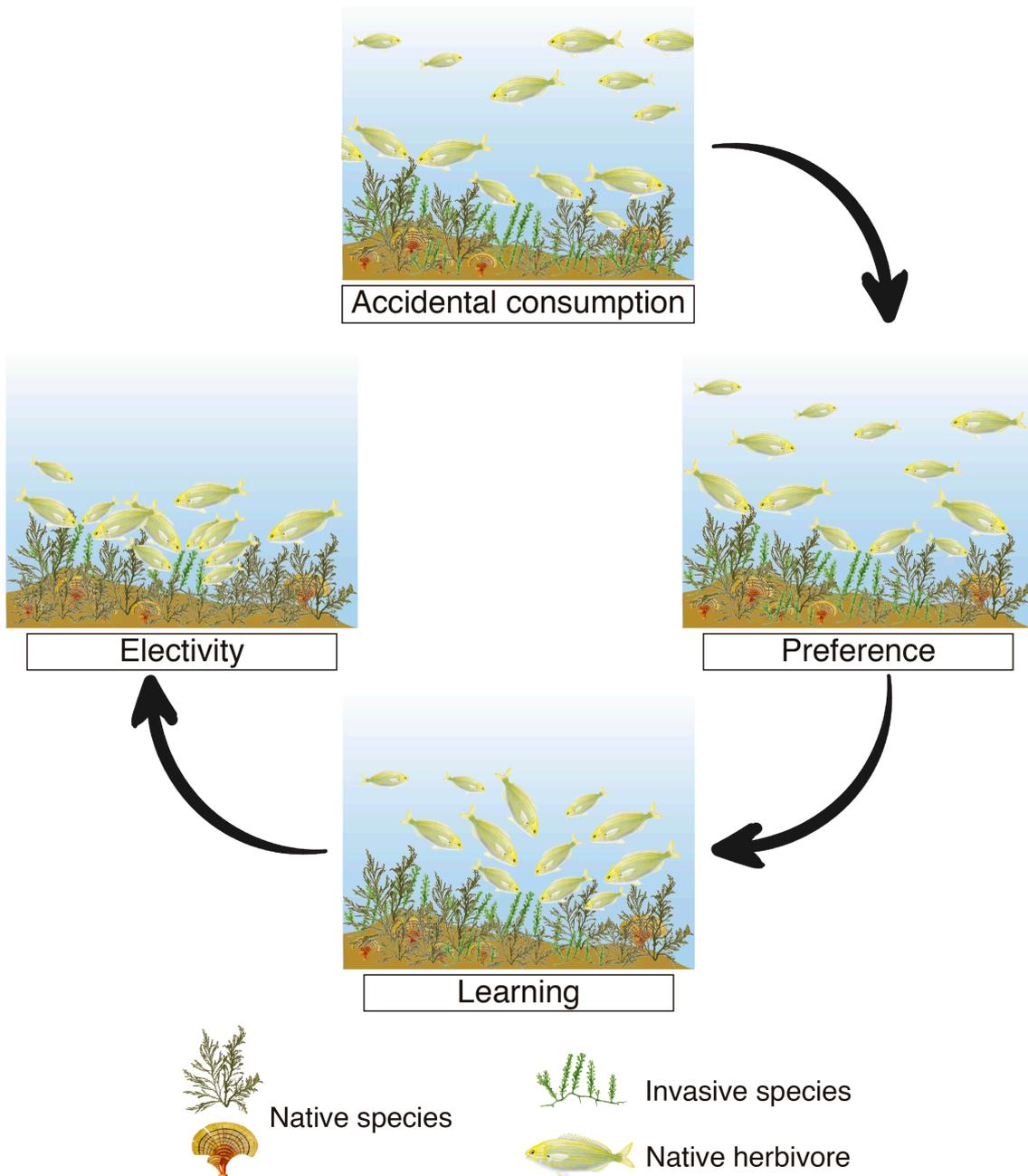


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