


LETTER

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

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

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

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

³Université Côte d'Azur, CNRS, ECOSEAS, Nice, France

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

⁵Nature Conservation Foundation, Mysore, India

Correspondence

Jorge Santamaría, Centre d'Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas (CEAB-CSIC), Blanes, Spain.
Email: jsantamaría@ceab.csic.es

Funding information

“la Caixa” Foundation, Grant/Award Number: ID 100010434 under agreement LCF/BQ/DE17/11600001; Executive Agency for Small and Medium Enterprise (EASME) and European Maritime and Fisheries fund (EMFF), Grant/Award Number: AFRIMED project (EASME/EMFF/2017/1.2.1.12/S4/01/SI; Organismo Autónomo de Parques Nacionales, Grant/Award Number: BIGPARK project (2017-2462); Secretaría de Estado de Investigación, Desarrollo e Innovación, Grant/Award Number: ANIMA Project (CGL2016-76341-R) and BES-2017-079907; ‘La Caixa’ Foundation, Grant/Award Number: DE17; European Union, Grant/Award Number: CGL2016-76341-R; Instituto Español de Oceanografía

Editor: Adrian Stier

Abstract

As invasive species spread, the ability of local communities to resist invasion depends on the strength of biotic interactions. Evolutionarily unused to the invader, native predators or herbivores may be initially wary of consuming newcomers, allowing them to proliferate. However, these relationships may be highly dynamic, and novel consumer–resource interactions could form as familiarity grows. Here, we explore the development of effective biotic resistance towards a highly invasive alga using multiple space-for-time approaches. We show that the principal native Mediterranean herbivore learns to consume the invader within less than a decade. At recently invaded sites, the herbivore actively avoided the alga, shifting to distinct preference and high consumptions at older sites. 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.

KEYWORDS

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

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. *Ecology Letters* published by John Wiley & Sons Ltd.

INTRODUCTION

The global spread of biological invasions is causing an unprecedented reorganisation of community assemblages. The curious *mélange* that results requires species with little recently shared evolutionary history having to form entirely novel interactions, leading to communities with no modern analogs (Hobbs et al., 2006; Williams & Jackson, 2007). With nothing to compare these novel communities to, it is difficult to predict exactly how they will transform native systems. How native communities, whose network of interactions have been established over long ecological time scales, adapt to new entrants will determine the impact and scale of invasions. Freed from their usual predators, alien species can become wildly successful in recipient areas, rapidly overtaking native communities (Colautti et al., 2004; Keane & Crawley, 2002). However, native communities with strong biotic interactions can show considerable biotic resistance, where predation or competition by native species prevent invaders from successfully establishing (Levine et al., 2004; Theoharides & Dukes, 2007).

The alarming rate of recent invasions (Seebens et al., 2017), is resulting in radically altered native ecosystems, making invasions a leading driver of the biodiversity crisis, together with climate change, land use and pollution (Butchart et al., 2010; Pyšek et al., 2020). Invasions have cascading ecosystem-wide impacts, affecting normal functioning and disrupting ecosystem services (Vilà & Hulme, 2017). Managing invasive species requires an understanding of their interactions with native communities and the long-term dynamics of these interactions (Mitchell et al., 2006; Saul & Jeschke, 2015). The strength and direction of novel interactions – competition, predation or facilitation – is key to the successful establishment of non-native species (Colautti et al., 2004; Levine et al., 2004; Pintor & Byers, 2015; Sih et al., 2010). If native predators learn quickly to consume novel prey, the strength of predator–prey interactions can significantly reduce invasion success (Carlsson et al., 2011; Caselle et al., 2018; De Rivera et al., 2005). Often though, invaders find themselves in assemblages without natural enemies, or where potential predators have not learnt to consume them. Released from biotic control, invader populations can soar dramatically, rapidly overwhelming native assemblages (Colautti et al., 2004; Keane & Crawley, 2002; Liu & Stiling, 2006; Maron & Vilà, 2001). Several factors influence the strength and direction of consumer pressure including invader anti-predator defences and avoidance mechanisms (Mennen & Laskowski, 2018; Ruland & Jeschke, 2020; Strauss et al., 2006), their ability to release allelopathic chemicals (Inderjit et al., 2011), or predator ‘naïveté’ (Kimbrow et al., 2013; Sih et al., 2010; Verhoeven et al., 2009).

Studies assessing novel predator–prey interactions are often single snapshots of invader impacts. However,

the relationship invasive species establish with recipient communities is highly dynamic. It is quite likely that interactions shift in strength and 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 behavioural adaptations to exploit them more efficiently (e.g. Carlsson et al., 2009; Carroll et al., 1998; Phillips & Shine, 2004). How long it takes for novel interactions to form and stabilise is a matter of some uncertainty. While several studies report increased consumption and damage on invaders with increasing invasion time (Carlsson et al., 2011; Carlsson & Strayer, 2009; Diez et al., 2010; Siemann et al., 2006; Stricker et al., 2016), others indicate no such change (Carpenter & Cappuccino, 2005; Harvey et al., 2013; Pintor & Byers, 2015). The most careful studies assessing the dynamic nature of herbivore–novel plant interactions have focused on tight insect–host relationships (Carpenter & Cappuccino, 2005; Carroll et al., 2005; Strauss et al., 2006), characterised by short-lived herbivores with narrow dietary breadths (Bernays, 1992; Strong et al., 1984). Their specialisation likely limits their ability to target new hosts and, once they switch hosts, it requires several generations for new behaviours or adaptations to stabilise in the population (Carroll et al., 1998; Carroll & Boyd, 1992; Siemann et al., 2006). While these short-lived specialists characterise many terrestrial ecosystems (Bernays, 1989), terrestrial habitats like savannahs, and most aquatic systems, are dominated instead by long-lived generalist herbivores (Du Toit & Cumming, 1999; Steneck et al., 2017) that often show complex social behaviours and have large per capita consumption rates. Being generalists they can more easily target novel plants (Morrison & Hay, 2011; Parker et al., 2006) and can have a greater overall influence on plant population dynamics since they are not dependent on a single species (Lubchenco, 1978; Olf & Ritchie, 1998). In addition, their sociality can lead to rapid learning within a population (Heyes & Galef Jr, 1996; Laland, 2004). Understanding how interactions between social herbivores and novel plants develop over time is therefore fundamental for a fuller understanding of invasion dynamics in terrestrial and aquatic systems.

The probability of exposure is as much a function of abundance as of time. Prey abundance is an important determinant of interaction strength (Carbone et al., 2011; Wellenreuther & Connell, 2002), since predator experience of novel prey will likely increase with prey density (Saul & Jeschke, 2015). Yet, invader abundance has rarely been considered when assessing how novel predator–prey interactions evolve (but see: Nelson et al., 2011). Unfortunately, reliable long-term data are rare for most invading species (Strayer et al., 2006), making it difficult to draw strong conclusions on how novel interactions change with time (Strayer et al., 2017). In their absence, space-for-time designs or chronosequences, where populations with different invasion times are studied, provides

a pragmatic alternative to long-term monitoring (Dostál et al., 2013; Gruntman et al., 2017; Thomaz et al., 2012). Chronosequences can help in effectively assessing if the strength of consumer–prey interactions changes with exposure time alone or in combination with other factors such as the invasion intensity. They could also help suggest effective strategies in managing new and ongoing invasions.

Here we explore how a novel interaction between a dominant native 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 invader abundance. We conducted preference and consumption assessments in populations with contrasting invasion features, to assess if feeding preference, the number of herbivores feeding on the invader, and per capita consumption were influenced by the temporal and numerical characteristics of the invasion. Additionally, we assessed electivity for the invader at three successive periods in two populations characterised by differential exposure time to the invader. Our results empirically show how effective predator–prey interactions between native consumers and invaders develop with time, intensifying the biotic resistance and leading to the eventual collapse of invader populations. This study demonstrates that native communities need time to develop effective resistance mechanisms against invaders but that learning can spread rapidly in social herbivores.

MATERIALS AND METHODS

Target species

The Australian siphonaceous green alga *C. cylindracea* is invasive in the Mediterranean Sea and parts of the Atlantic and Indian Oceans (Klein & Verlaque, 2008). In the Mediterranean, *C. cylindracea* is rampantly successful, having spread across the basin in less than 20 years since its first detection (Klein & Verlaque, 2008; Piazzini et al., 2005). *C. cylindracea* can form dense mono-specific stands, quickly overgrowing the benthos, causing strong negative impacts on native communities (Bulleri et al., 2017; Klein & Verlaque, 2008; Piazzini et al., 2001). As a hyper-successful invader in the Mediterranean (Katsanevakis et al., 2016; Klein & Verlaque, 2008), *C. cylindracea* is ranked among the most harmful marine invaders globally (Anton et al., 2019). Its extraordinary success is linked to its ability to resist herbivores by producing deterrent metabolites, high growth rates and mechanisms of vegetative and sexual propagation (Ceccherelli & Piazzini, 2001; Klein & Verlaque, 2008). Recently, however, some locations have seen steep declines in *C. cylindracea* after an initial period of successful

dominance (García et al., 2016; Klein & Verlaque, 2008; Santamaría, Tomas, Ballesteros, Ruiz, et al., 2021). Our team and others have witnessed several native organisms feeding on the invader, suggesting that native communities may be developing effective resistance mechanisms against *C. cylindracea* (Cebrian et al., 2011; Ruitton et al., 2006; Santamaría, Tomas, Ballesteros, & Cebrian, 2021; Tomas et al., 2011). Additionally, trends in the species are closely monitored, with accurate data available on its progress in Mediterranean waters, making it an ideal species to study the evolution of consumer–resource interactions.

The only true herbivorous fish in the study area (NW Mediterranean) is the bream *S. salpa* (Gianni et al., 2017; Verlaque, 1990), which plays an important role in structuring macrophyte communities (Tomas et al., 2005; Vergés et al., 2009). This species is abundant across the NW Mediterranean (Bell, 1983; Reñones et al., 1997; Tomas et al., 2005), spending most of its time in waters shallower than 20 m (Bell, 1983; Tomas et al., 2011; Vergés et al., 2012), where it feeds on a wide variety of species (Verlaque, 1990), including, in recent years, *C. cylindracea* (Ruitton et al., 2006; Tomas et al., 2011). An earlier study reports that it may even prefer the invasive alga over natives (Tomas et al., 2011).

Study sites

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

In Cabrera, *C. cylindracea* was first detected in 2003 at 30 m deep (Cebrian & Ballesteros, 2009), quickly expanding across the archipelago. It was found in most benthic habitats between 0 and 65 m, often as the dominant species (Cebrian et al., 2011). Recently however, its abundance has declined mainly at shallow depths (Santamaría, Tomas, Ballesteros, Ruiz, et al., 2021).

In Menorca, *C. cylindracea* was first detected in 2006, in photophilic assemblages (≈20 m deep) in the south of the island (Pons-Fàbregas et al., 2007), from where it shifted northwards and to shallower depths (Cefali et al., 2020; Massutí et al., 2015). From 2010 to date, *C. cylindracea* is ubiquitous in Menorca, sometimes dominating benthic assemblages between 5 and 45 m deep (Massutí et al., 2015). Recent reports, however, indicate declines, predominantly in shallow assemblages (Cefali et al., 2020).

Along the Catalan coast, *C. cylindracea* was first detected in 2008 in the south, at depths between 20 and 30 m (Ballesteros et al., 2008). The 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

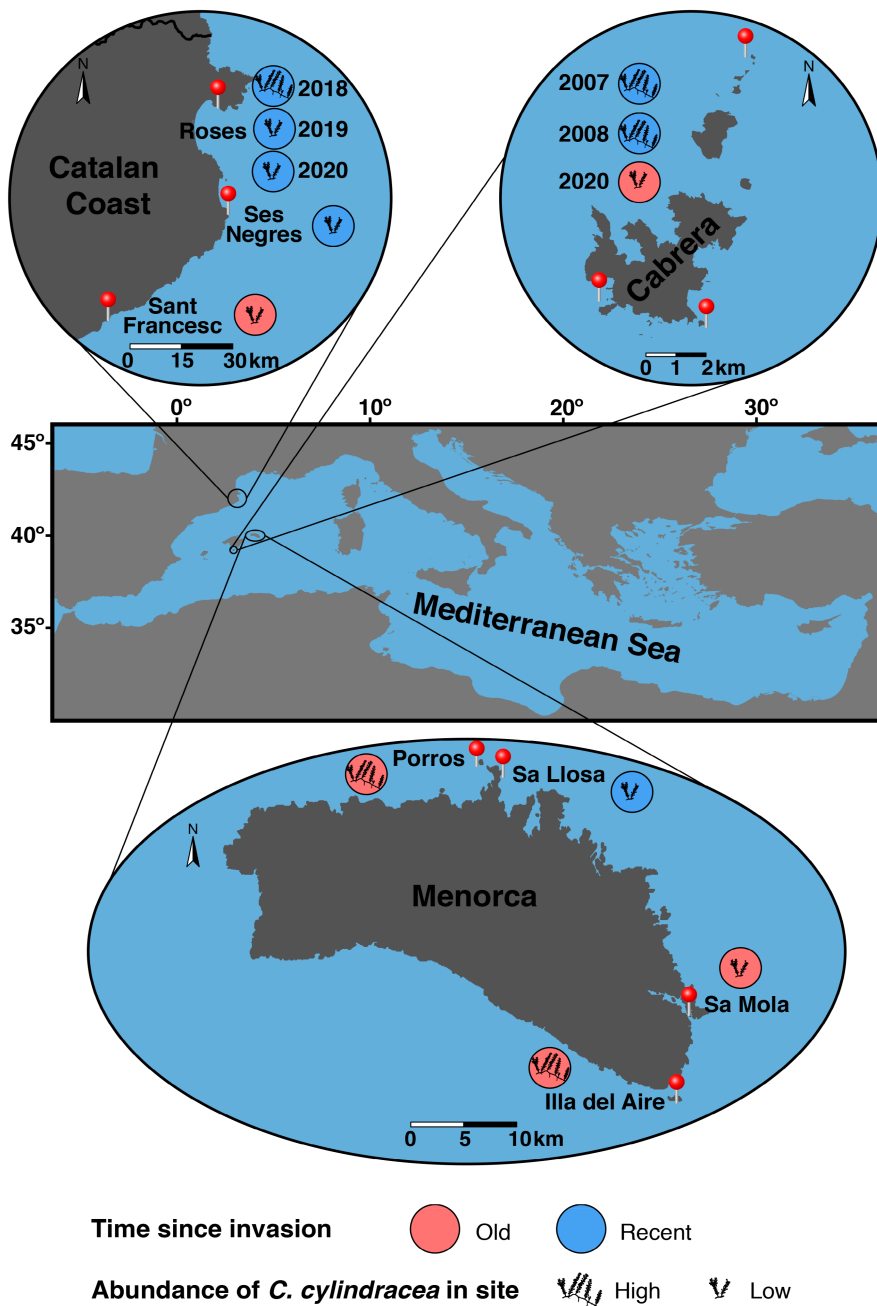


FIGURE 1 Location of the three regions where the study was carried (the Catalan Coast, the Cabrera Archipelago and Menorca). The points show the specific locations where different assessments were done (see Figure S1 for details) while the circle next to each location depicts its features. Red circle: Old invasion; blue circle: Recent invasion; big *Caulerpa*: High abundance; small *Caulerpa*: Low abundance. 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 (GSHHG) database and for the Cabrera archipelago downloaded from www.miteco.gob.es.

found in several locations in northern Catalonia (García et al., 2017 and authors' personal observations).

Preference assessment

To assess if *S. salpa*'s preference for *C. cylindracea* changed with exposure time and/or in relation to the abundance of the invader in the assemblage, we

conducted paired-choice feeding experiments at different locations in Menorca and the Catalan coast. These regions were chosen since it was possible to find locations within them with contrasting times since the invasion and with contrasting abundances of the invader (Table S1).

We consulted our long-term monitoring records of *C. cylindracea* to determine the time since invasion at each location. Based on year since first detection, locations

were classified as ‘Old’ when *C. cylindracea* was first detected >5 years ago, or ‘Recent’ when *C. cylindracea* was detected <5 years ago (Table S1). At each location, we assessed *C. cylindracea* abundance at 10–15 m depth on SCUBA within thirty 25 x 25 cm quadrats, divided into 25 subquadrats of 5 x 5 cm (Sala & Ballesteros, 1997). The quadrats were randomly located on the sea bottom and the number of subquadrats where *C. cylindracea* was present was used as a unit of abundance. We then calculated the mean *C. cylindracea* abundance for each location. Locations were classified as ‘High’ (>30% cover) or ‘Low’ (<30% cover) based on the abundance of *C. cylindracea* (Table S1).

Eight locations were chosen for preference assessments to represent conditions of invasion time and abundance. In Menorca these were: Illa del Aire (Old-High), Sa Mola (Old-Low), Porros (Old-High) and Sa Llosa (Recent-Low); along the Catalan coast these were as follows: Sant Francesc (Old-Low), Roses 2019 (Recent-Low), Roses 2018 (Recent-High) and Ses Negres (Recent-Low) (Table S1; Figure S1). Additionally, to guarantee site independence between locations, and to reduce the possibility of *S. salpa* moving between conditions, locations were chosen such that no *C. cylindracea* was present within a 3 km buffer area and that locations were separated by at least 4 km. This allows for site independence, since *S. salpa* shows high site fidelity and its home range rarely exceeds 150 ha, normally staying within 1 km from its resting area (Jadot et al., 2002, 2006; Pagès et al., 2013).

At each location, we conducted paired-choice feeding experiments to compare the relative palatability of *C. cylindracea* vs. two native macroalgae species: *Cystoseira compressa*, a highly palatable habitat-forming alga (Vergés et al., 2009) and commonly found in *S. salpa*'s diet (Verlaque, 1990); and *Padina pavonica*, a common photophilic alga in warm-temperate sublittoral waters (Sala, 1997) and also a usual food source for *S. salpa* (Verlaque, 1990) (Figure S1). Thus, we used two 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 with two clothespins attached to each other with a zip-tie, weighted down to keep them in place underwater. Controls comprised 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 similarly weighted down.

A total of seven replicates for each treatment and five 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 without sea urchins. In addition, we ensured that the patches had similar macroalgal assemblages, dominated by the photophilic species *Halopteris scoparia* together with *P. pavonica*,

Cladostephus hirsutus and *Dictyota dichotoma*, along with turf-forming algae such as *Corallina elongata* and with an encrusting layer dominated by *Lithophyllum incrustans*. This was done 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, while treatments were placed less than 20 m apart to maintain constant environmental conditions between them. All the experiments were conducted in 2018 and 2019 at the end of summer, when *S. salpa* feeds most intensively to accumulate winter reserves and prepare for reproduction (Peirano et al., 2001). Samples were deployed in the morning and collected after 24 h. Before and after deployment, every algal fragment was pat-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 (Parker & Hay, 2005). Consumption values were then standardised 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 faecal 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 faecal 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 characterise *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 faecal pellets in a reticulated Petri dish under a stereomicroscope Stemi 2000-C (Carl Zeiss). 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 exposure time, 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 faecal 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 analysed 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*, generalised linear models were fitted to faecal 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 with 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 *S. salpa* towards *C. cylindracea* was influenced by time since invasion but not by the abundance of the invader in the community. Within 5 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 2a,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 2c,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 S3A), with at least two times more fish targeting the invader than in populations where *C. cylindracea* abundance was low (Figure 3a). Additionally, time since invasion significantly influenced the number of fish targeting the invader in locations with high abundances (Table S3B), with twice the number of fish consuming *C. cylindracea* in the Old-High populations than in the Recent-High populations (Figure 3a). In summary, more than 90%

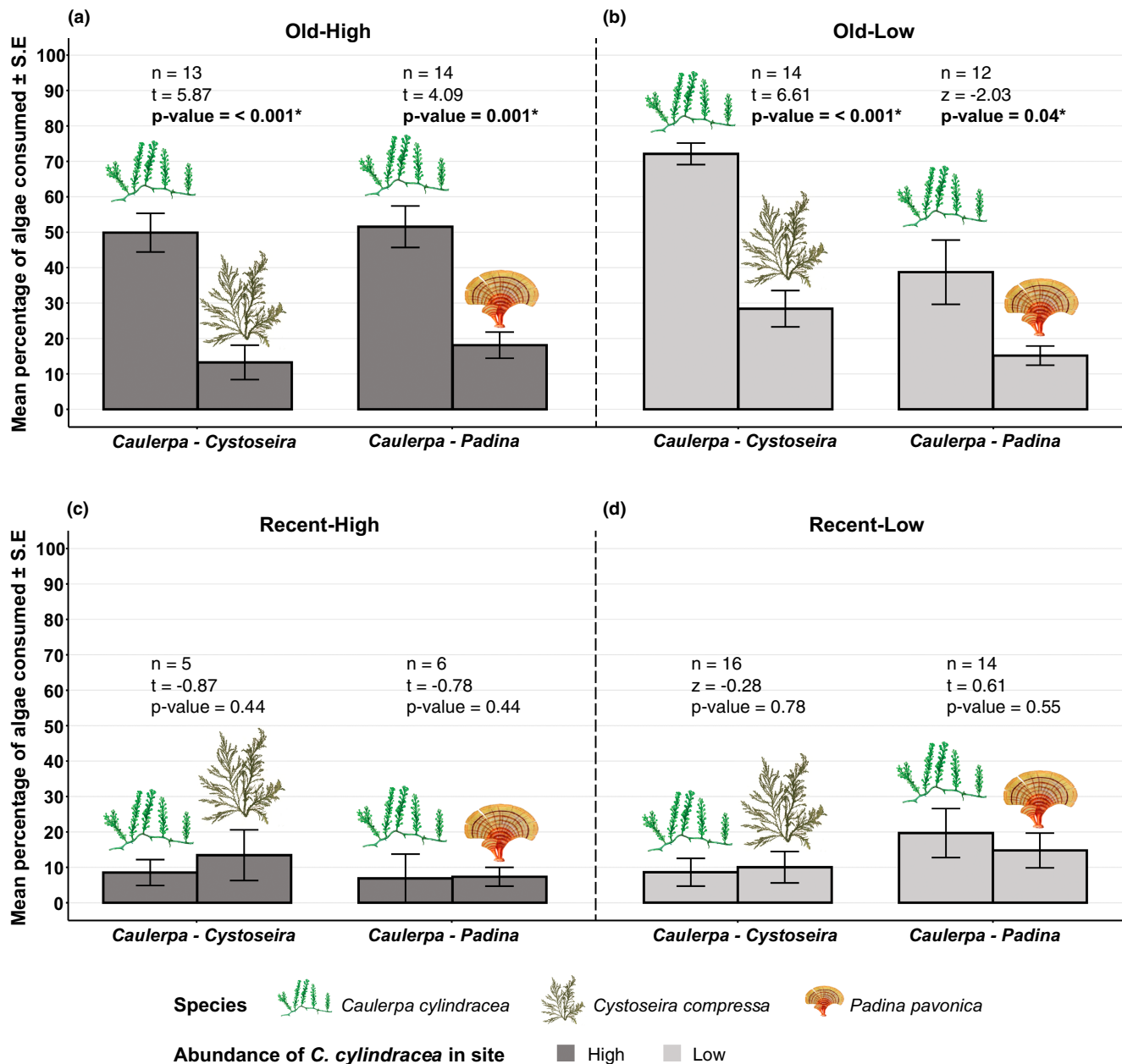


FIGURE 2 Results from the preference assessment. Paired-choice feeding experiments were performed at: (a, b) locations that have been invaded by *Caulerpa cylindracea* for more than 5 years (old locations) and where the abundance of the invader in the community was either (a) high (dark grey bars) or (b) low (light grey bars); and (c, d) locations that have been invaded by *C. cylindracea* for less than 5 years (recent locations) and where the abundance of the invader in the community was either (c) high (dark grey 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 (*C. 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 and followed by the * 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]).

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 3a).

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 3b; Table S5a). 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

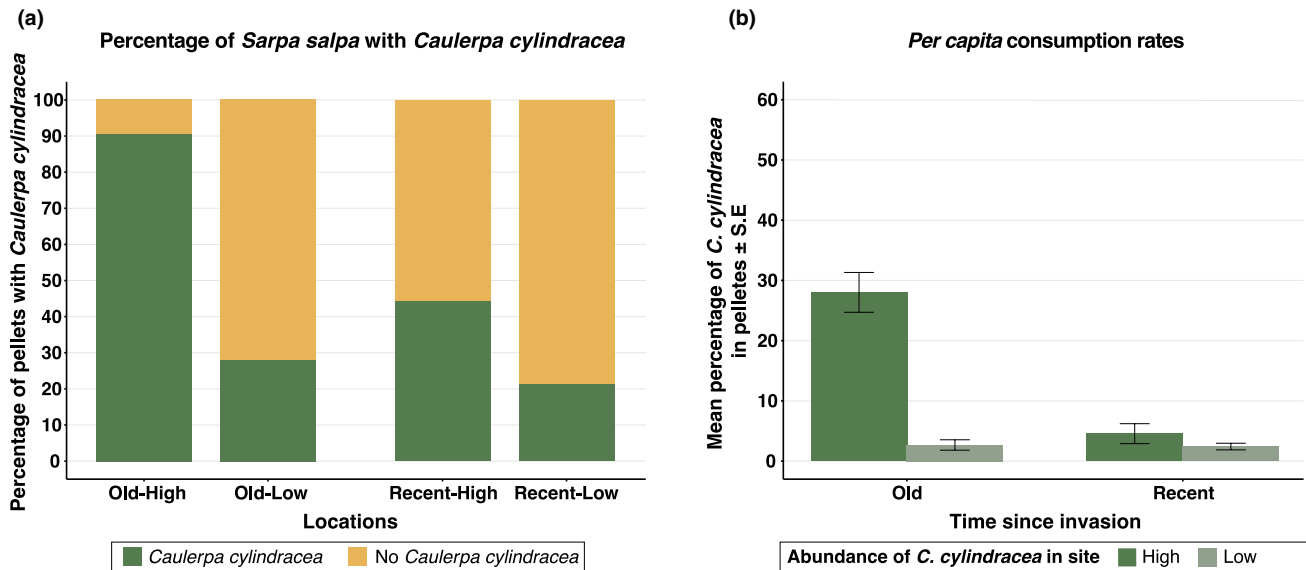


FIGURE 3 Results from the consumption assessment. (a) Percentage of *Sarpa salpa* individuals feeding on *Caulerpa cylindracea* (percentage of pellets with the invader) in old and in recent locations, with high and with low abundances of the invader in the community. (b) per capita consumption rates of *Sarpa salpa* on *Caulerpa cylindracea* (mean percentage of *C. cylindracea* in pellets \pm SE), in old and in recent locations, with high and with low abundances of the invader in the community.

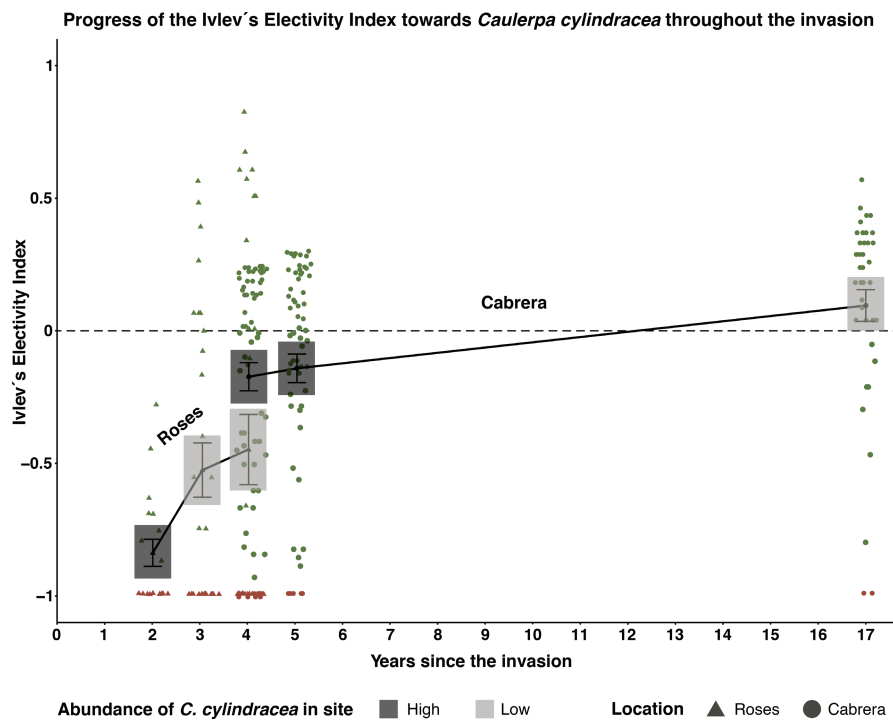


FIGURE 4 Results from the electivity assessment. Progress of the Ivlev's electivity index towards *Caulerpa cylindracea* through time in two locations that differ in their exposure time to the invader: Cabrera, where *C. cylindracea* has been in the assemblages for 17 years; and roses, where *C. cylindracea*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

invader (Table S5B). In particular, in communities with a high abundance of *C. cylindracea*, consumption of the invader was almost seven 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 3b).

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 -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 (Colautti et al., 2004; Keane & Crawley, 2002; Liu & Stiling, 2006; Maron & Vilà, 2001). With no shared evolutionary history with natives, invaders often do much better in recipient communities than they do in their native habitats, where they belong to assemblages 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. Additionally, in highly social species with broad dietary niches, information on palatability and handling can spread rapidly through the population, once learnt. 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 *S. salpa* prefers *C. cylindracea* to native algae, although the preference for the invasive alga takes several years to develop. An initial wariness by *S. salpa* shifts to a clear preference for the invader within around 6 years of exposure. Remarkably, in well-established, old populations, this preference was maintained even at low abundances of *C. cylindracea*. This is noteworthy since both *P. pavonica* and *Cystoseira compressa* are highly palatable and are

commonly found in *S. salpa*'s diet (Vergés et al., 2009; Verlaque, 1990). 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 (Cronin, 1998; Lodge, 1991; Schädler et al., 2003). Species like *C. cylindracea* are likely prime targets for herbivores because they are softer, more aqueous, and are more easily removed, handled and masticated (Cronin et al., 2002; Morrison & Hay, 2011; Vergés et al., 2007).

The probability of encounter between consumer and resource is a function both of time and 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 et al., 2011; Carlsson & Strayer, 2009; Schultheis et al., 2015), other characteristics of the invasion (e.g. invader availability) and synergies between them, strongly influence their development. However, it is possible 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 unexpected, given that this 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 compared to the other *Caulerpa* species in the basin (Box et al., 2010). In fact, native *Caulerpa prolifera*, a common food-source for *S. salpa* (Marco-Méndez et al., 2017), produces the highest amount of caulerpenyne among *Caulerpa* species (Box et al., 2010 and references therein). 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 behaviours and physiologies much more quickly to the invader and form strong biotic interactions with them. This could 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 after they are first encountered (Carlsson et al., 2009; Cox, 2004; Strauss et al., 2006). The mechanisms by which *S. salpa* begins to consume *C. cylindracea* remain speculative, but they potentially follow steps similar to the predation cycle (Saul & Jeschke, 2015) (Figure 5), which involves *S. salpa* identifying *C. cylindracea* as a suitable resource and developing a taste for it. After an initial wariness, the first few interactions may be completely accidental and involve very few individuals (Figure 3a; Figure 5). However, after these individuals accidentally taste the invader and discover its palatability, they begin to target it, soon followed by other individuals in the population when they learn

that the invader is safe to eat and nutritious. In non-solitary fish, social learning, where individuals learn behaviours and acquire information such as what to eat and what to avoid through observation, is an important foraging mechanism (Brown & Laland, 2003; Warburton & Hughes, 2011). This may help explain the fast transmission of search images between demonstrators, those that know how to feed on novel prey, and bystanders, those that observe and learn to target the new prey (White & Gowan, 2014). *S. salpa* is a highly social species that feeds in large cooperative shoals that show complex feeding behaviours (Buñuel et al., 2020). Within a population, behavioural syndromes could play an important role in determining how quickly consumers take novel resources, with bolder phenotypes less reticent to try unfamiliar food items than shy individuals (Sundström et al., 2004), based on past experiences (Frost et al., 2007). How rapidly this learning spreads

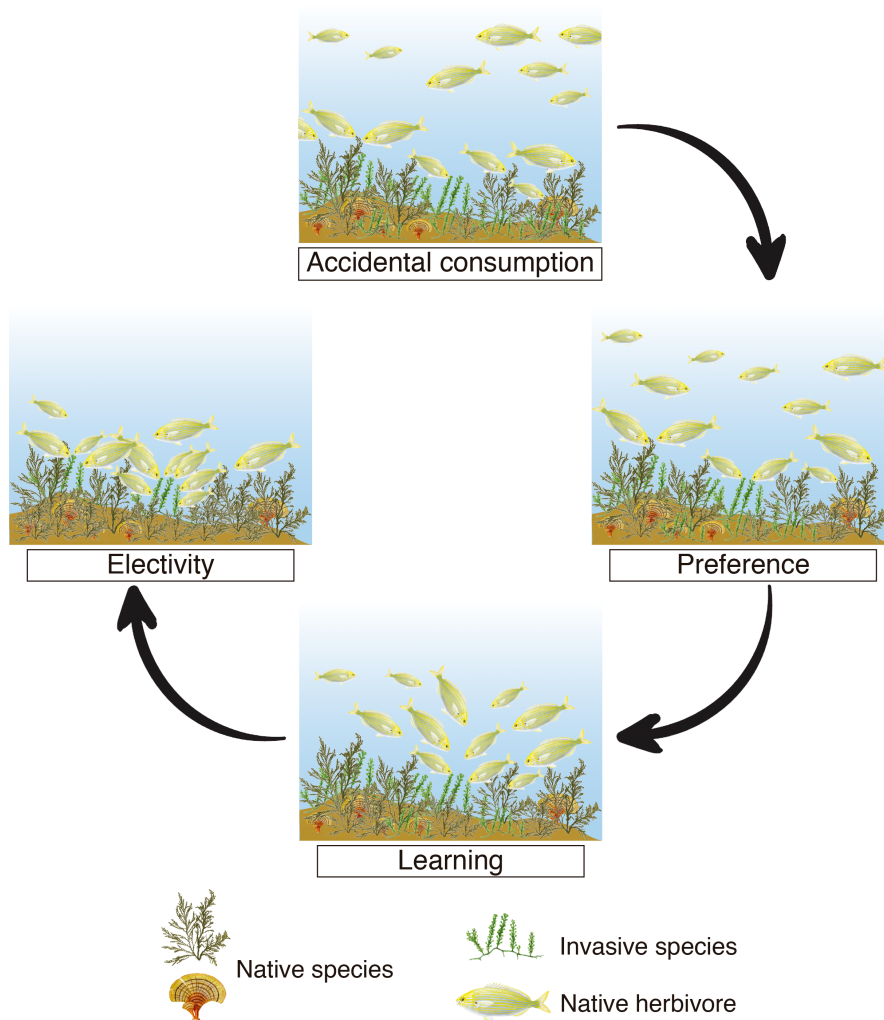


FIGURE 5 Diagram showing the proposed phases necessary for the development of the effective predator–prey relationship between the native herbivore *Sarpa salpa* and the invasive alga *Caulerpa cylindracea*. In the first phase, consumption of the invader is accidental and will only be done by few individuals. 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.

through the population could, as our results suggest, be influenced by invader abundance, because the formation and transmission of search images likely increases with encounter rates of novel resources (Allen, 1988; Saul & 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* (Katsanevakis et al., 2016; Klein & Verlaque, 2008; Piazzini et al., 2001) and for many invaders across ecosystems (Gallardo et al., 2016; Molnar et al., 2008; Pyšek et al., 2020). 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. Carlsson et al., 2011; De Rivera et al., 2005; Santamaría, Tomas, Ballesteros, Ruiz, et al., 2021; Simberloff & Gibbons, 2004). Thus, biotic resistance against an invader is not static and changes dynamically through the invasion, increasing its strength as the invasion progresses (Carlsson et al., 2011; Diez et al., 2010; Hawkes, 2007; Stricker et al., 2016). Additionally, as time progresses, not only do consumers become less wary of invaders and change their foraging behaviour as described here, but other mechanisms could also influence in strengthening the biotic interactions. For instance, over time, invaders may also undergo physiological changes that could reduce either their toxicity or their allelochemical potential (Lankau et al., 2009; Magro et al., 2018; Van Kleunen et al., 2018), thus becoming more susceptible to biological control by competition or herbivory. 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 (Colautti et al., 2004; Kimbro et al., 2013; Maron & Vilà, 2001; Mitchell et al., 2006). Therefore, long-term studies or chronosequences should be favoured 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. Finally, since changes in environmental conditions could also potentially cause population

declines of invasive species (Bradley et al., 2009; Wenger et al., 2011; White et al., 2015), the influence of abiotic conditions on invader dynamics should also be more closely assessed.

Our study adds to the growing literature showing that native communities need time to develop resistance mechanisms against invasive species (e.g. Carlsson et al., 2011; Diez et al., 2010; Lankau et al., 2009; Stricker et al., 2016). Studies in terrestrial ecosystems indicate that it may take decades or even centuries for effective resistance mechanisms to develop (Diez et al., 2010; Dostál et al., 2013; Hawkes, 2007; Siemann et al., 2006; Stricker et al., 2016). This may be in part because most studies have dealt with herbivores with low sociality that may need several generations before adaptations develop through genetic differentiation (Carroll et al., 1998; Carroll & Boyd, 1992; Strauss et al., 2006). In our system, however, 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, Tomas, Ballesteros, Ruiz, et al., 2021). 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, probably because the high sociality of the herbivore allows information to spread rapidly within the population through cultural transmission and imitation (Brown & Laland, 2003; Heyes & Galef Jr, 1996; Laland, 2004). However, a decade is still 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, the swell of novel species entering native ecosystems is only going to increase (Bellard et al., 2013; Diez et al., 2012; García Molinos et al., 2016). Given 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.

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. analysed 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.

ACKNOWLEDGEMENTS

We thank the managers and rangers from the Cabrera Archipelago National Park for sampling permissions and helping with field logistics, people at the Jaume Ferrer Marine Station (Instituto Español de Oceanografía) for providing us with technical and facility support, and Candela Marco-Méndez, Xavier Turón and Adrià

Antich for helping us during field sampling at the Cabrera Archipelago. Funding for this Project was obtained from the Spanish Ministry Project ANIMA (CGL2016-76341-R, MINECO/FEDER, UE), the European Union's EMFS Program, AFRIMED (No. EASME/EMFF/2017/1.2.1.12/S4/01/SI2.789059) and the Spanish Autonomous Organism of National Parks through the project BIGPARK (project 2017-2462). J.S. received the support of a fellowship from 'La Caixa' Foundation (ID 100010434) under agreement LCF/BQ/DE17/11600001, and 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).

FUNDING INFORMATION

"la Caixa" Foundation, Grant/Award Number: ID 100010434 under agreement LCF/BQ/DE17/11600001; Executive Agency for Small and Medium Enterprise (EASME) and European Maritime and Fisheries fund (EMFF), Grant/Award Number: AFRIMED project (EASME/EMFF/2017/1.2.1.12/S4/01/SI; Organismo Autónomo de Parques Nacionales, Grant/Award Number: BIGPARK project (2017-2462); Secretaría de Estado de Investigación, Desarrollo e Innovación, Grant/Award Number: ANIMA Project (CGL2016-76341-R) BES-2017-079907; 'La Caixa' Foundation, Grant/Award Number: DE17; European Union, Grant/Award Number: CGL2016-76341-R; Instituto Español de Oceanografía

CONFLICT OF INTEREST

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.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/ele.14115>.

DATA AVAILABILITY STATEMENT

The data and R code supporting the conclusions of this article are available on the Zenodo repository: <https://doi.org/10.5281/zenodo.7074595> (Santamaría, 2022).

ORCID

Jorge Santamaría  <https://orcid.org/0000-0003-4425-6297>

REFERENCES

- Allen, J.A. (1988) Frequency-dependent selection by predators. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 319, 485–503.
- Anton, A., Gheraldi, N.R., Lovelock, C.E., Apostolaki, E.T., Bennett, S., Cebrian, J. et al. (2019) Global ecological impacts of marine exotic species. *Nature Ecology and Evolution*, 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. *Journal of Applied Ecology*, 20, 357–369.
- Bellard, C., Thuiller, W., Leroy, B., Genovesi, P., Bakkenes, M. & Courchamp, F. (2013) Will climate change promote future invasions? *Global Change Biology*, 19, 3740–3748.
- Benjamini, Y. & Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society, Series B*, 57, 289–300.
- Bernays, E.A. (1989) Host range in phytophagous insects: the potential role of generalist predators. *Evolutionary Ecology*, 3, 299–311.
- Bernays, E.A. (1992) Interaction of insects and plants. *Science Progress (1933-)*, 76, 247–271.
- 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. *Botanica Marina*, 53, 367–375.
- Bradley, B.A., Oppenheimer, M. & Wilcove, D.S. (2009) Climate change and plant invasions: restoration opportunities ahead? *Global Change Biology*, 15, 1511–1521.
- Brown, C. & Laland, K.N. (2003) Social learning in fishes: a review. *Fish and Fisheries*, 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. *Biological 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 as they grow. *Scientific Reports*, 10, 106222.
- Butchart, S.H.M., Walpole, M., Collen, B., van Strien, A., Scharlemann, J.P.W., Almond, R.E.A. et al. (2010) Global biodiversity: indicators of recent declines. *Science (80-)*, 328, 1164–1168.
- Carbone, C., Pettolelli, N. & Stephens, P.A. (2011) The bigger they come, the harder they fall: body size and prey abundance influence predator-prey ratios. *Biology Letters*, 7, 312–315.
- Carlsson, N.O.L., Bustamante, H., Strayer, D.L. & Pace, M.L. (2011) Biotic resistance on the increase: native predators structure invasive zebra mussel populations. *Freshwater Biology*, 56, 1630–1637.
- Carlsson, N.O.L., Sarnelle, O. & Strayer, D.L. (2009) Native predators and exotic prey - an acquired taste? *Frontiers in Ecology and the Environment*, 7, 525–532.
- Carlsson, N.O.L. & Strayer, D.L. (2009) Intraspecific variation in the consumption of exotic prey - a mechanism that increases biotic resistance against invasive species? *Freshwater Biology*, 54, 2315–2319.
- Carpenter, D. & Cappuccino, N. (2005) Herbivory, time since introduction and the invasiveness of exotic plants. *Journal of Ecology*, 93, 315–321.
- Carroll, S.P. & Boyd, C. (1992) Host race radiation in the soapberry bug: natural history with the history. *Evolution (N. Y.)*, 46, 1052–1069.
- Carroll, S.P., Klassen, S.P. & Dingle, H. (1998) Rapidly evolving adaptations to host ecology and nutrition in the soapberry bug. *Evolutionary Ecology*, 12, 955–968.
- Carroll, S.P., Loye, J.E., Dingle, H., Mathieson, M., Famula, T.R. & Zalucki, M.P. (2005) And the beak shall inherit - evolution in response to invasion. *Ecology Letters*, 8, 944–951.
- Caselle, J.E., Davis, K. & Marks, L.M. (2018) Marine management affects the invasion success of a non-native species in a temperate reef system in California, USA. *Ecology Letters*, 21, 43–53.
- Cebrian, E. & Ballesteros, E. (2009) Temporal and spatial variability in shallow- and deep-water populations of the invasive *Caulerpa*

- racemosa* var. *cylindracea* in the Western Mediterranean. *Estuarine, Coastal and Shelf Science*, 83, 469–474.
- Cebrian, E., Ballesteros, E., Linares, C. & Tomas, F. (2011) Do native herbivores provide resistance to Mediterranean marine bioinvasions? A seaweed example. *Biological Invasions*, 13, 1397–1408.
- Ceccherelli, G. & Piazzini, L. (2001) Dispersal of *Caulerpa racemosa* fragments in the Mediterranean: lack of detachment time effect on establishment. *Botanica Marina*, 44, 209–213.
- Cefali, M.E., Movilla, J., Bolado, I., Mallol, S., Reñones, O., Díaz, D., et al. (2020). Informe final 2016–2020. Convenio de colaboración para la consolidación de la estación de investigación “Jaume Ferrer” (La Mola, Menorca)
- Colautti, R.I., Ricciardi, A., Grigorovich, I.A. & MacIsaac, H.J. (2004) Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, 7, 721–733.
- Cox, G.W. (2004) *Alien species and evolution: the evolutionary ecology of exotic plants, animals, microbes, and interacting native species*. Washington: Island Press.
- Cronin, G. (1998) Influence of macrophyte structure, nutritive value, and chemistry on the feeding choices of a generalist crayfish. In: Jeppesen, E., Søndergaard, M., Søndergaard, M. & Christoffersen, K. (Eds.) *The structuring role of submerged macrophytes in lakes*. New York: Springer, pp. 307–317.
- Cronin, G., Lodge, D.M., Hay, M.E., Miller, M., Hill, A.M., Horvath, T. et al. (2002) Crayfish feeding preferences for freshwater macrophytes: the influence of plant structure and chemistry. *Journal of Crustacean Biology*, 22, 708–718.
- De Rivera, C.E., Ruiz, G.M., Hines, A.H. & Jivoff, P. (2005) Biotic resistance to invasion: native predator limits abundance and distribution of an introduced crab. *Ecology*, 86, 3364–3376.
- Diez, J.M., D'Antonio, C.M., Dukes, J.S., Grosholz, E.D., Olden, J.D., Sorte, C.J.B. et al. (2012) Will extreme climatic events facilitate biological invasions? *Frontiers in Ecology and the Environment*, 10, 249–257.
- Diez, J.M., Dickie, I., Edwards, G., Hulme, P.E., Sullivan, J.J. & Duncan, R.P. (2010) Negative soil feedbacks accumulate over time for non-native plant species. *Ecology Letters*, 13, 803–809.
- Dostál, P., Müllerová, J., Pyšek, P., Pergl, J. & Klínerová, T. (2013) The impact of an invasive plant changes over time. *Ecology Letters*, 16, 1277–1284.
- Du Toit, J.T. & Cumming, D.H.M. (1999) Functional significance of ungulate diversity in African savannas and the ecological implications of the spread of pastoralism. *Biodiversity and Conservation*, 8, 1643–1661.
- Dunn, O.J. (1964) Multiple comparisons using rank sums. *Technometrics*, 6, 241–252.
- Frost, A.J., Winrow-Giffen, A., Ashley, P.J. & Sneddon, L.U. (2007) Plasticity in animal personality traits: does prior experience alter the degree of boldness? *Proceedings of the Royal Society B: Biological Sciences*, 274, 333–339.
- Gallardo, B., Clavero, M., Sánchez, M.I. & Vilà, M. (2016) Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology*, 22, 151–163.
- García, M., Jordana, E., Pinedo, S., Satta, M.P. & Torras, X. (2017). Seguiment de l'indicador macroalgues de la DMA (2000/60/CE) a les aigües costaneres de Catalunya per a l'obtenció de l'índex de qualitat biològica CARLIT i control d'espècies invasores bentòniques al litoral de Catalunya. CTN1500528.
- García, M., Weitzmann, B., Pinedo, S., Cebrian, E. & Ballesteros, E. (2016) First report on the distribution and impact of marine alien species in coastal benthic assemblages along the Catalan coast. *The Handbook of Environmental Chemistry*, 43, 249–270.
- García Molinos, J., Halpern, B.S., Schoeman, D.S., Brown, C.J., Kiessling, W., Moore, P.J. et al. (2016) Climate velocity and the future global redistribution of marine biodiversity. *Nature Climate Change*, 6, 83–88.
- Gianni, F., Bartolini, F., Pey, A., Laurent, M., Martins, G.M., Airoidi, L. et al. (2017) Threats to large brown algal forests in temperate seas: the overlooked role of native herbivorous fish. *Scientific Reports*, 7, 6012.
- Gruntman, M., Segev, U., Glauser, G. & Tielbörger, K. (2017) Evolution of plant defences along an invasion chronosequence: defence is lost due to enemy release—but not forever. *Journal of Ecology*, 105, 255–264.
- Harvey, K.J., Nipperess, D.A., Britton, D.R. & Hughes, L. (2013) Does time since introduction influence enemy release of an invasive weed? *Oecologia*, 173, 493–506.
- Hawkes, C.V. (2007) Are invaders moving targets? The generality and persistence of advantages in size, reproduction, and enemy release in invasive plant species with time since introduction. *The American Naturalist*, 170, 832–843.
- Heyes, C.M. & Galef, B.G., Jr. (1996) *Social learning in animals: the roots of culture*. Amsterdam: Elsevier.
- Hill, S.B. & Kotanen, P.M. (2009) Evidence that phylogenetically novel non-indigenous plants experience less herbivory. *Oecologia*, 161, 581–590.
- Hobbs, R.J., Arico, S., Aronson, J., Baron, J.S., Bridgewater, P., Cramer, V.A. et al. (2006) Novel ecosystems: theoretical and management aspects of the new ecological world order. *Global Ecology and Biogeography*, 15, 1–7.
- Inderjit, W.D.A., Karban, R. & Callaway, R.M. (2011) The ecosystem and evolutionary contexts of allelopathy. *Trends in Ecology & Evolution*, 26, 655–662.
- Ivlev, V.S. (1961) *Experimental ecology of the feeding of fishes*. New Haven: Yale University Press.
- Jadot, C., Donnay, A., Acolas, M.L., Cornet, Y. & Bégout Anras, M.L. (2006) Activity patterns, home-range size, and habitat utilization of *Sarpa salpa* (Teleostei: Sparidae) in the Mediterranean Sea. *ICES Journal of Marine Science*, 63, 128–139.
- Jadot, C., Ovidio, M. & Voss, J. (2002) Diel activity of *Sarpa salpa* (Sparidae) by ultrasonic telemetry in a *Posidonia oceanica* meadow of Corsica (Mediterranean Sea). *Aquatic Living Resources*, 15, 343–350.
- Katsanevakis, S., Tempera, F. & Teixeira, H. (2016) Mapping the impact of alien species on marine ecosystems: the Mediterranean Sea case study. *Diversity and Distributions*, 22, 694–707.
- Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, 17, 164–170.
- Kimbro, D.L., Cheng, B.S. & Grosholz, E.D. (2013) Biotic resistance in marine environments. *Ecology Letters*, 16, 821–833.
- Klein, J. & Verlaque, M. (2008) The *Caulerpa racemosa* invasion: a critical review. *Marine Pollution Bulletin*, 56, 205–225.
- Kruskal, W.H. & Wallis, W.A. (1952) Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association*, 47, 583–621.
- Laland, K.N. (2004) Social learning strategies. *Animal Learning & Behavior*, 32, 4–14.
- Lankau, R.A., Nuzzo, V., Spyreas, G. & Davis, A.S. (2009) Evolutionary limits ameliorate the negative impact of an invasive plant. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 15362–15367.
- Lenth, R. (2018). Emmeans: estimated marginal means, aka least-squares means. R package version 1.3.0.
- Levine, J.M., Adler, P.B. & Yelenik, S.G. (2004) A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters*, 7, 975–989.
- Liu, H. & Stiling, P. (2006) Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions*, 8, 1535–1545.
- Lodge, D.M. (1991) Herbivory on freshwater macrophytes. *Aquatic Botany*, 41, 195–224.
- Lubchenco, J. (1978) Plant species diversity in a marine intertidal community: importance of herbivore food preference and algal competitive abilities. *The American Naturalist*, 112, 23–39.

- Magro, A., Ramon-Portugal, F., Facon, B., Ducamp, C. & Hemptinne, J.L. (2018) The evolution of chemical defenses along invasion routes: *Harmonia axyridis* Pallas (Coccinellidae: coleoptera) as a case study. *Ecology and Evolution*, 8, 8344–8353.
- Marco-Méndez, C., Ferrero-Vicente, L.M., Prado, P. & Sánchez-Lizaso, J.L. (2017) Epiphytes and nutrient contents influence *Sarpa salpa* herbivory on *Caulerpa* spp. vs. seagrass species in Mediterranean meadows. *Estuarine, Coastal and Shelf Science*, 184, 54–66.
- Maron, J.L. & Vilà, M. (2001) When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos*, 95, 361–373.
- Massutí, E., Sales, M., Reñones, O., Cuadros-Casado, I.A., Vidal-Cejuela, E.M., Sintes, J., et al. (2015). Informe final 2010–2015. Convenio de colaboración para la puesta en marcha y el desarrollo científico de la estación de investigación “Jaume Ferrer” (La Mola, Menorca)
- Méndez-Villamil, M., Lorenzo, J.M., Pajuelo, J.G., Ramos, A. & Coca, J. (2002) Aspects of the life history of the salema, *Sarpa salpa* (Pisces, Sparidae), off the Canarian archipelago (central-East Atlantic). *Environmental Biology of Fishes*, 63, 183–192.
- Mennen, G.J. & Laskowski, K.L. (2018) Defence is the best offence: invasive prey behaviour is more important than native predator behaviour. *Animal Behaviour*, 138, 157–164.
- Mitchell, C.E., Agrawal, A.A., Bever, J.D., Gilbert, G.S., Hufbauer, R.A., Klironomos, J.N. et al. (2006) Biotic interactions and plant invasions. *Ecology Letters*, 9, 726–740.
- Molnar, J.L., Gamboa, R.L., Revenga, C. & Spalding, M.D. (2008) Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment*, 6, 485–492.
- Morrison, W.E. & Hay, M.E. (2011) Herbivore preference for native vs. exotic plants: generalist herbivores from multiple continents prefer exotic plants that are evolutionarily naïve. *PLoS One*, 6, e17227.
- Nelson, D.W.M., Crossland, M.R. & Shine, R. (2011) Foraging responses of predators to novel toxic prey: effects of predator learning and relative prey abundance. *Oikos*, 120, 152–158.
- Ogle, D.H., Wheeler, P. & Dinno, A. (2020). FSA: fisheries stock analysis.
- Olf, H. & Ritchie, M.E. (1998) Effects of herbivores on grassland plant diversity. *Trends in Ecology & Evolution*, 13, 261–265.
- Pagès, J.F., Bartumeus, F., Hereu, B., López-Sanz, À., Romero, J. & Alcoverro, T. (2013) Evaluating a key herbivorous fish as a mobile link: a Brownian bridge approach. *Marine Ecology Progress Series*, 492, 199–210.
- Parker, J.D., Burkepile, D.E. & Hay, M.E. (2006) Opposing effects of native and exotic herbivores on plant invasions. *Science* (80-.), 311, 1459–1461.
- Parker, J.D. & Hay, M.E. (2005) Biotic resistance to plant invasions? Native herbivores prefer non-native plants. *Ecology Letters*, 8, 959–967.
- Paul, V., Arthur, K.E., Ritson-Williams, R., Ross, C. & Sharp, K. (2007) Chemical defenses: from compounds to communities. *The Biological Bulletin*, 213, 226–251.
- Pearse, I.S., Harris, D.J., Karban, R. & Sih, A. (2013) Predicting novel herbivore-plant interactions. *Oikos*, 122, 1554–1564.
- Peirano, A., Niccolai, I., Mauro, R. & Bianchi, C.N. (2001) Seasonal grazing and food preference of herbivores in a *Posidonia oceanica* meadow. *Scientia Marina*, 65, 367–374.
- Phillips, B.L. & Shine, R. (2004) Adapting to an invasive species: toxic cane toads induce morphological change in Australian snakes. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 17150–17155.
- Piazzi, L., Ceccherelli, G. & Cinelli, F. (2001) Threat to macroalgal diversity: effects of the introduced green alga *Caulerpa racemosa* in the Mediterranean. *Marine Ecology Progress Series*, 210, 149–159.
- Piazzi, L., Meinesz, A., Verlaque, M., Akçali, B., Antolić, B., Argyrou, M. et al. (2005) Invasion of *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) in the Mediterranean Sea: an assessment of the spread. *Cryptogamie Algologie*, 26, 189–202.
- Pintor, L.M. & Byers, J.E. (2015) Do native predators benefit from non-native prey? *Ecology Letters*, 18, 1174–1180.
- Pons-Fàbregas, C., Sales, M., Canals, A. & Borràs, R. (2007) Primera cita de *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Clorophyta) a Menorca, Mediterrània Occidental. *Bolletti la Soc. d'Historia Nat. les Balear.*, 50, 21–26.
- Pyšek, P., Hulme, P.E., Simberloff, D., Bacher, S., Blackburn, T.M., Carlton, J.T. et al. (2020) Scientists' warning on invasive alien species. *Biological Reviews*, 95, 1511–1534.
- R Core Team. (2018). *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Reñones, O., Moranta, J., Coll, J. & Morales-Nin, B. (1997) Rocky bottom fish communities of Cabrera archipelago National Park (Mallorca, Western Mediterranean). *Scientia Marina*, 61, 495–506.
- Ruitton, S., Verlaque, M., Aubin, G. & Boudouresque, C.F. (2006) Grazing on *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) in the Mediterranean Sea by herbivorous fishes and sea urchins. *Vie Milieu*, 56, 33–41.
- Ruland, F. & Jeschke, J.M. (2020) How biological invasions affect animal behaviour: a global, cross-taxonomic analysis. *The Journal of Animal Ecology*, 89, 2531–2541.
- Sala, E. (1997) The role of fishes in the organization of a Mediterranean sublittoral community. II: epifaunal communities. *Journal of Experimental Marine Biology and Ecology*, 212, 45–60.
- Sala, E. & Ballesteros, E. (1997) Partitioning of space and food resources by three fish of the genus *Diplodus* (Sparidae) in a Mediterranean rocky infralittoral ecosystem. *Marine Ecology Progress Series*, 152, 273–283.
- Santamaría, J. (2022). JorgeSantamaría/Herbivory_Learning: First release of data and code to assess learning by herbivores.
- Santamaría, J., Tomas, F., Ballesteros, E. & Cebrian, E. (2021) Herbivory on the invasive alga *Caulerpa cylindracea*: the role of omnivorous fishes. *Frontiers in Marine Science*, 8, 1055.
- Santamaría, J., Tomas, F., Ballesteros, E., Ruiz, J.M., Bernardeau-Esteller, J., Terrados, J. et al. (2021) The role of competition and herbivory in biotic resistance against invaders: a synergistic effect. *Ecology*, 102, e03440.
- Saul, W.C. & Jeschke, J.M. (2015) Eco-evolutionary experience in novel species interactions. *Ecology Letters*, 18, 236–245.
- Schädler, M., Jung, G., Auge, H. & Brandl, R. (2003) Palatability, decomposition and insect herbivory: patterns in a successional old-field plant community. *Oikos*, 103, 121–132.
- Schultheis, E.H., Berardi, A.E. & Lau, J.A. (2015) No release for the wicked: enemy release is dynamic and not associated with invasiveness. *Ecology*, 96, 2446–2457.
- Seebens, H., Blackburn, T.M., Dyer, E.E., Genovesi, P., Hulme, P.E., Jeschke, J.M. et al. (2017) No saturation in the accumulation of alien species worldwide. *Nature Communications*, 8, 14435.
- Siemann, E., Rogers, W.E. & Dewalt, S.J. (2006) Rapid adaptation of insect herbivores to an invasive plant. *Proceedings of the Royal Society B: Biological Sciences*, 273, 2763–2769.
- Sih, A., Bolnick, D.I., Luttbeg, B., Orrock, J.L., Peacor, S.D., Pintor, L.M. et al. (2010) Predator-prey naïveté, antipredator behavior, and the ecology of predator invasions. *Oikos*, 119, 610–621.
- Simberloff, D. & Gibbons, L. (2004) Now you see them, now you don't!—population crashes of established introduced species. *Biological Invasions*, 6, 161–172.
- Steneck, R.S., Bellwood, D.R. & Hay, M.E. (2017) Herbivory in the marine realm. *Current Biology*, 27, R484–R489.
- Strauss, S.Y., Lau, J.A. & Carroll, S.P. (2006) Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? *Ecology Letters*, 9, 357–374.

- Strayer, D.L. (2012) Eight questions about invasions and ecosystem functioning. *Ecology Letters*, 15, 1199–1210.
- Strayer, D.L., D'Antonio, C.M., Essl, F., Fowler, M.S., Geist, J., Hilt, S. et al. (2017) Boom-bust dynamics in biological invasions: towards an improved application of the concept. *Ecology Letters*, 20, 1337–1350.
- Strayer, D.L., Eviner, V.T., Jeschke, J.M. & Pace, M.L. (2006) Understanding the long-term effects of species invasions. *Trends in Ecology & Evolution*, 21, 645–651.
- Stricker, K.B., Philip, F., Goss, E.M. & Flory, S.L. (2016) Emergence and accumulation of novel pathogens suppress an invasive species. *Ecology Letters*, 19, 469–477.
- Strong, D.R., Lawton, J.H. & Southwood, S.R. (1984) *Insects on plants*. Community patterns and mechanisms: Blackwell Scientific Publications.
- Sundström, L.F., Petersson, E., Höjesjö, J., Johnsson, J.I. & Järvi, T. (2004) Hatchery selection promotes boldness in newly hatched brown trout (*Salmo trutta*): implications for dominance. *Behavioral Ecology*, 15, 192–198.
- Theoharides, K.A. & Dukes, J.S. (2007) Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion. *The New Phytologist*, 176, 256–273.
- Thomaz, S.M., Agostinho, A.A., Gomes, L.C., Silveira, M.J., Rejmánek, M., Aslan, C.E. et al. (2012) Using space-for-time substitution and time sequence approaches in invasion ecology. *Freshwater Biology*, 57, 2401–2410.
- Tomas, F., Cebrian, E. & Ballesteros, E. (2011) Differential herbivory of invasive algae by native fish in the Mediterranean Sea. *Estuarine, Coastal and Shelf Science*, 92, 27–34.
- Tomas, F., Turon, X. & Romero, J. (2005) Seasonal and small-scale spatial variability of herbivory pressure on the temperate seagrass *Posidonia oceanica*. *Marine Ecology Progress Series*, 301, 95–107.
- Van Kleunen, M., Bossdorf, O. & Dawson, W. (2018) The ecology and evolution of alien plants. *Annual Review of Ecology, Evolution, and Systematics*, 49, 25–47.
- Vergés, A., Alcoverro, T. & Ballesteros, E. (2009) Role of fish herbivory in structuring the vertical distribution of canopy algae *Cystoseira* spp. in the Mediterranean Sea. *Marine Ecology Progress Series*, 375, 1–11.
- Vergés, A., Becerro, M.A., Alcoverro, T. & Romero, J. (2007) Variation in multiple traits of vegetative and reproductive seagrass tissues influences plant-herbivore interactions. *Oecologia*, 151, 675–686.
- Vergés, A., Tomas, F. & Ballesteros, E. (2012) Interactive effects of depth and marine protection on predation and herbivory patterns. *Marine Ecology Progress Series*, 450, 55–65.
- Verhoeven, K.J.F., Biere, A., Harvey, J.A. & Van Der Putten, W.H. (2009) Plant invaders and their novel natural enemies: who is naïve? *Ecology Letters*, 12, 107–117.
- Verlaque, M. (1990) Relations entre *Sarpa salpa* (Linnaeus, 1758) (Téléostéen, Sparidae), les autres poissons broyeurs et le phyto-benthos algal méditerranéen. *Oceanologica Acta*, 13, 373–388.
- Vilà, M. & Hulme, P.E. (2017) *Impact of biological invasions on ecosystem services*. New York: Springer.
- Warburton, K. & Hughes, R. (2011) Learning of foraging skills by fish. In: Brown, C., Laland, K.N. & Krause, J. (Eds.) *Fish cognition and behavior*. Hoboken: Blackwell Publishing Ltd, pp. 10–35.
- Wellenreuther, M. & Connell, S.D. (2002) Response of predators to prey abundance: separating the effects of prey density and patch size. *Journal of Experimental Marine Biology and Ecology*, 273, 61–71.
- Wenger, S.J., Isaak, D.J., Luce, C.H., Neville, H.M., Fausch, K.D., Dunham, J.B. et al. (2011) Flow regime, temperature, and biotic interactions drive differential declines of trout species under climate change. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 14175–14180.
- White, J.D., Hamilton, S.K. & Sarnelle, O. (2015) Heat-induced mass mortality of invasive zebra mussels (*Dreissena polymorpha*) at sublethal water temperatures. *Canadian Journal of Fisheries and Aquatic Sciences*, 72, 1221–1229.
- White, S.L. & Gowan, C. (2014) Social learning enhances search image acquisition in foraging brook trout. *Environmental Biology of Fishes*, 97, 523–528.
- Williams, J.W. & Jackson, S.T. (2007) Novel climates, no-analog communities, and ecological surprises. *Frontiers in Ecology and the Environment*, 5, 475–482.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Santamaría, J., Golo, R., Verdura, J., Tomas, F., Ballesteros, E. & Alcoverro, T. et al. (2022) Learning takes time: Biotic resistance by native herbivores increases through the invasion process. *Ecology Letters*, 25, 2525–2539. Available from: <https://doi.org/10.1111/ele.14115>