Biotic Resistance Against Invasive Macroalgae: The Role of Omnivorous Sparid Fishes in the Herbivory on *Caulerpa Cylindracea*

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Biotic resistance against invasive macroalgae: the role of omnivorous sparid fishes in the herbivory on *Caulerpa cylindracea*

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Abstract

Herbivory has long been considered an important component of biotic resistance against macroalgae invasions in marine habitats. However, most of the studies on herbivory of invasive algae refer only to consumption by strictly herbivorous organisms, whereas consumption by omnivorous organisms has been largely ignored and rarely quantified. In this study, we assess whether the commonest omnivorous sparid species in the Mediterranean Sea are consuming the highly invasive alga, *Caulerpa cylindracea*, and determine its importance in their diet and their electivity towards it as a source of food. Our results confirm that three of the four fish species we studied do, in fact, consume *C. cylindracea*, but in most cases, the importance of *C. cylindracea* in the diet was low. Indeed, the low electivity values confirm that all four fish species avoid feeding on the invasive alga and that it is probably consumed accidentally. However, despite animals and detritus being the main food for these sea bream species, several individual specimens were found to have consumed high amounts of *C. cylindracea*. This suggests a potential role that these fish species may play in controlling, to some extent, the abundance of the invader. We suggest that herbivory by omnivorous fish might complement the higher impact exerted by the strict herbivores, and together, they might contribute to the overall biotic resistance against the invader.

Keywords: fish-alga interactions, herbivory, biotic resistance, marine temperate systems, Sparidae, *Caulerpa cylindracea*

Declarations

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Conflicts of interest

All authors certify that they have no conflicts of interest to declare that are relevant to the content of this article

Data availability

The raw data generated and analyzed for this study is available from the corresponding author upon request.

Authors’ contribution

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

Introduction

In the marine environment, invasive species pose a growing threat to native ecosystems (Bax et al. 2003) and the problem is expected to continue to worsen in the near future, due mainly to ongoing intensification in shipping traffic and climate change (Stachowicz et al. 2002; Seebens et al. 2013). In these systems, macroalgae are one of the most successful and conspicuous groups (Schaffelke et al. 2006), with at least 346 identified taxa worldwide (Thomsen et al. 2016). Marine invasive macroalgae can contribute to the homogenization of marine habitats, affecting the structure of native assemblages by reducing both native species biomass and overall assemblage diversity (Williams and Smith 2007; Schaffelke and Hewitt 2008; Thomsen et al. 2009, 2016). Furthermore, once established, they are extremely difficult to eradicate (Anderson 2007). However, various mechanisms and features – both biotic and abiotic – in the receiving community and habitat, can reduce invasive success and affect the establishment and persistence of marine algae (Dunstan and Johnson 2007; Catford et al. 2009; Thomsen et al. 2009; Kimbro et al. 2013; Papacostas et al. 2017). Among the biotic factors, herbivory has long been considered as a potential biotic resistance mechanism and many studies have been conducted worldwide to assess the role of this mechanism on invasive macroalgae success (references within Kimbro et al. 2013 and Papacostas et al. 2017). Until now, the role of herbivory as a limiting factor for macroalgae invasion has dealt mainly with strictly herbivorous species (e.g., Ruitton et al. 2006, Wikström et al. 2006, Lyons and Scheibling 2008, Britton-Simmons et al. 2011, Cebrian et al. 2011, Tomas et al. 2011b, Hammann et al. 2013), with contrasting results depending on the assemblage and the invasive species considered (Boudouresque et al. 1996; Trowbridge and Todd 1999; Scheibling and Anthony 2001;

Stimson et al. 2001; Davis et al. 2005; Wikström et al. 2006; Monteiro et al. 2009; Steinarsdóttir et al. 2009; Tomas et al. 2011b, a; Nejrup et al. 2012).

The Mediterranean Sea is currently considered a hot-spot for invasive algae (Williams and Smith 2007; Thomsen et al. 2016) and the number of potential invaders arriving is on the rise due to the intensification of marine traffic and to the widening of the Suez Canal (Katsanevakis et al. 2013; Galil et al. 2017). In the Mediterranean Sea, the most successful and widespread invasive macroalga is *Caulerpa cylindracea* (Klein and Verlaque 2008; Katsanevakis et al. 2016), a green alga native of the Southwestern coast of Australia that was first detected in Mediterranean waters in Libya in 1990 (Nizamuddin 1991). Since then, it has colonized marine communities throughout the entire Mediterranean basin (Piazzi et al. 2005; Klein and Verlaque 2008), where it can exert strong detrimental effects on native communities (Piazzi et al. 2001; Klein and Verlaque 2008; Bulleri et al. 2016, 2017). However, despite its rampant success, several *Caulerpa cylindracea* meadows have suffered sudden steep declines in abundance (Klein and Verlaque 2008; García et al. 2016), which may indicate the existence of certain resistance mechanisms against this invasive species. Among these, herbivory on *C. cylindracea* has been described and assessed, although mainly in relation to the strictly herbivorous species present in the Mediterranean Sea, such as the fishes, *Sarpa salpa* and *Siganus luridus* (Azzurro et al. 2004; Ruitton et al. 2006; Tomas et al. 2011b), and the sea urchins, *Paracentrotus lividus*, *Sphaerechinus granularis* and *Arbacia lixula* (Ruitton et al. 2006; Bulleri et al. 2009; Cebrian et al. 2011; Tomas et al. 2011a). In fact, *S. salpa* and *P. lividus* are keystone herbivores in the Mediterranean, and both show a preference towards the invasive alga (Tomas et al. 2011a, b). Nevertheless, there are omnivorous fish species, such as *Diplodus sargus*, *Boops boops*, and *Spondyllosoma cantharus*, that have been observed feeding on *C. cylindracea*

before (Ruitton et al. 2006; Box et al. 2009; Terlizzi et al. 2011). Unfortunately, information is scarce on whether *C. cylindracea* is a common food source for these fish species, or whether these, and other omnivorous fish actively elect to feed on it. Since some of these species are the dominant fish in the shallow, infralittoral rocky habitats in the Mediterranean Sea (García-Rubies 1997; Sala and Ballesteros 1997), information on their consumption of *C. cylindracea* is needed to determine whether they can play a role in the biotic resistance to the invasive process – by contributing to the control of *C. cylindracea* abundance – and to determine how important such a role might be.

In this study, the diets of four omnivorous sea bream species (Sparidae) were examined in order to determine (i) whether they feed on the invasive alga *C. cylindracea*, (ii) whether *C. cylindracea* is important in their diet and (iii) whether they actively select or avoid *C. cylindracea* as a source of food

Materials and methods

Study area

The samples for this study were collected in the Cabrera Archipelago National Park (North-Western Mediterranean Sea; 39°12'21" N, 2°58'44" E) (Fig. 1) in 2008. This marine-terrestrial protected area was established in 1991 and since then it has maintained an exceptional conservation status for its marine habitats (Sala et al. 2012; Coll et al. 2013; Guidetti et al. 2014). *Caulerpa cylindracea* was recorded for the first time in the National Park in 2003 at a depth of 30-35 m and since then its distribution has expanded to cover most of its benthic communities at depths of between 0 and 65m (Cebrian and Ballesteros 2009).

Analysis of *Caulerpa cylindracea* consumption

To determine whether non-strictly herbivorous fish species consume *C. cylindracea*, specimens for this study were captured by long-lines and gillnets on several occasions during June and July 2008, at different sites across the Archipelago, close to the localities of Ses Rates and the Foradada Islet (Fig. 1). The main fishes targeted belong to the family Sparidae: white sea bream (*Diplodus sargus*), annular sea bream (*Diplodus annularis*), two-banded sea bream (*Diplodus vulgaris*) and black sea bream (*Spondyllosoma cantharus*). These species were chosen because they are common representatives of the fish assemblages found in the Western Mediterranean, they are not herbivorous but can feed on macroalgae (Sala and Ballesteros 1997) and some of them have been observed feeding on *C. cylindracea* before (Box et al. 2009; Terlizzi et al. 2011). These four species have different abundances within the National Park, the least abundant of them being *S. cantharus*, with 0.2 individuals per 250 m², and the most abundant being *D. vulgaris*, with up to 6 individuals per 250 m² (Reñones et al. 1997).

The long-lines and gillnets, two gears commonly used in artisanal fishing, were deployed at depths of between 10 and 30 m. Every time a targeted fish species was hauled in, it was gutted and its stomach was stored and preserved in buffered 4% formaldehyde-seawater solution for later analysis of its content. Once in the laboratory, the species composition and abundance of the food items in each fish stomach was determined under a Stemi 2000-C stereomicroscope (Carl Zeiss, Berlin, Germany). The content of each stomach was spread onto a reticulated Petri dish and the food items were classified to the lowest taxonomic level possible. Both surface area and weight measurements can reflect the dietary contribution of food items (Hyslop 1980; Macdonald and Green 1983), but in this case, and to avoid biases that could be derived

from the small quantities present in the stomach contents, surface area measurements were preferred over weight measurements to quantify the dietary contribution of each food item. As such, the abundance of a particular food item was estimated as the percentage cover on the reticulated fields of the Petri dish in relation to the cover of the whole stomach content. When a species had a minimal presence and its cover could not be determined, a value of 0.1% of relative coverage was assigned.

When calculating the relative measures of prey quantity (RMPQ), and to avoid possible biases caused by each prey (or food item) being identified to different taxonomic levels, the stomach contents were divided into the following five prey categories: *Caulerpa cylindracea*, Algal content, Plants, Animal content and Detritus. Subsequently, for each fish species, the percentage frequency of occurrence of each prey category (FO_i) was calculated as:

$$FO_i = (S_i / S_t) \cdot 100$$

where S_i is the number of stomachs containing the prey category and S_t is the total number of stomachs analyzed for that particular fish species. The FO_i value is a measure of the consistency with which a species selects a given prey category and was used to calculate two dietary indices that allow to compare the diets between species: the Combined Index (Q_i) and the Geometric Index of Importance (GII).

The combined index, Q_i, was chosen to assess the relative importance of each prey category for each fish species. This index standardizes the abundance of each category and increases the importance of frequent smaller items while reducing the importance of occasional larger items (Nilssen et al. 2019). It was calculated as:

$$Q_i = \frac{V_i \cdot FO_i}{\sum_{i=1}^m (V_i \cdot FO_i)}$$

where V_i refers to the percentage surface of a prey category, FO_i refers to the frequency of occurrence of the given prey category, and m is the total number of prey categories.

On the other hand, the Geometric Index of Importance, GII, represents the degree of feeding specialization on a particular prey type (Assis 1996; Preti et al. 2001) and allows us to classify the prey categories as: “Preferential prey”, “Secondary prey” and “Occasional prey” in relation to the larger discontinuities in a decreasing sequence of values (Assis 1996; Tripp-Valdez et al. 2015). It was calculated as:

$$GII_j = \frac{\sum_{i=1}^n (V_i + FO_i)}{\sqrt{2}}$$

Finally, the degree to which the four fish species tend to elect to feed on *C. cylindracea*, was assessed by Ivlev’s electivity Index (E) (Ivlev 1961). This Index was determined by:

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

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

Assessment of the abundance of *Caulerpa cylindracea* in the community

The abundance of *C. cylindracea* at the sampling sites where the fish specimens were captured was assessed by means of scuba diving. At each site, a perpendicular transect to shore was done, at depths of 10 to 30 m, so as to cover the same bathymetric range as that of the fishing gear used to collect fish samples. To estimate *C. cylindracea* abundance, a total of thirty quadrats measuring 25 x 25 cm² were randomly positioned

within each 10 m-depth range (total of 90 quadrats per sampling site). These quadrats were divided into 25 subquadrats of 5 x 5 cm² and the number of subquadrats where *C. cylindracea* appeared was used as the unit of abundance (Sala and Ballesteros 1997; Cebrian and Ballesteros 2004; Sant et al. 2017). Subsequently, the average *C. cylindracea* abundance for the study area was calculated and this value was used in the calculation of the Ivlev's electivity Index.

Statistical analyses

Differences in the specific composition of stomach contents between fish species were assessed through multivariate techniques such as non-metric multi-dimensional scaling plots (NMDS plots), analysis of similarities (ANOSIM) and similarity percentage analysis (SIMPER). All of these techniques were performed within the *vegan* package (Oksanen et al. 2018) in the R environment (R Core Team 2018). First, in order to visualize and represent stomach content composition, an NMDS (Clarke and Warwick 1994; Cox and Cox 2000) based on the Bray-Curtis dissimilarity matrix of the square-root transformed data was plotted and the most important species that determine the least stressful ordination were detected using the *envfit* function within the *vegan* package. Then, the statistical differences in the food items consumed by the fish species were assessed using ANOSIM (Clarke 1993), applied to the Bray-Curtis dissimilarity matrix, with fish species as a fixed factor. Additionally, to perform the pairwise comparison between the fish species, a pairwise ANOSIM was performed by modifying the *pairwise.adonis* function (<https://github.com/pmartinezarbizu/pairwiseAdonis>) and the R-values obtained were used as an indication of diet similarity, with values near 1 indicating separation in diet composition and values near 0 indicating diet similarity (Rogers et al. 2012). Finally, a SIMPER analysis based on the Bray-Curtis dissimilarity

index was used to assess the relative contribution of each food item to the overall differences between fish species diets.

Results and discussion

During the sampling events, a total of 93 fishes were captured, with *D. sargus* being the most abundant (51 individuals) and *D. annularis* the scarcest (7 individuals). All the stomachs examined contained ingested material of some kind, which, as a whole, was composed of a high diversity of taxonomic groups, with 73 different prey items identified, 32 of them to the species level (Table S1). Differences in stomach content were observed between species in terms of the dominant prey categories, although detritus and animal content were certainly prominent in all four species (Table 1). In this sense, the Combined Index (Qi) and the Geometric Index of Importance (GII), identified the category “Detritus” as the preferential food item for *S. cantharus*, while the category “Animal content” was the preferential prey for the other three fish species (Figs. 2 and 3). These findings agree with previous studies in which the predominant food type in the diet of these sea breams was observed to be animals or detritus (Sala and Ballesteros 1997; Gonçalves and Erzini 1998; Pita et al. 2002; Box et al. 2009; Terlizzi et al. 2011; Fellingine et al. 2012, 2017), although they have also been found to feed on considerable amounts of algae (Sala and Ballesteros 1997; Pita et al. 2002; Box et al. 2009; Terlizzi et al. 2011; Fellingine et al. 2012, 2017). In our case, all the fish species studied had consumed algae and seagrasses; in particular, the stomach contents of both *D. sargus* and *D. annularis* were rich in algae, with values of around 18% and 30% respectively (Table 1), which are higher than previously reported results for sea breams (Sala and Ballesteros 1997; Sánchez-Jerez et al. 2002; Box et al. 2009).

Considering the whole diet of the four sampled sea bream species, the graphical ordination suggests that there might be some overlap between diets (Fig. 4); however, according to the ANOSIM, the stomach content composition was significantly different between fish species (p-value < 0.05; Table 2), with two exceptions: *D. sargus* – *D. vulgaris* and *D. sargus* – *D. annularis* (p-value > 0.05; Table 2). In this sense, the greatest dissimilarities in diet were between *D. annularis* and *S. cantharus* (R=0.63) and between *D. vulgaris* and *S. cantharus* (R=0.47) (Table 2), which may be due to the fact that the diet of *S. cantharus* is more homogeneous than those of the other species, being dominated by detritus rather than animal items (Fig. 4). Actually, the SIMPER analysis identified the food item ‘organic detritus’ as the biggest contributor to the diet dissimilarities between the four fish species, with values of between 19% and 40% (Table 3).

Regarding the consumption of *C. cylindracea*, three species, namely *D. annularis*, *D. sargus* and *S. cantharus*, were found to have consumed it. Although both *D. sargus* and *S. cantharus* had already been observed feeding on *C. cylindracea* (Box et al. 2009; Terlizzi et al. 2011; Feline et al. 2012), this is the first evidence of such behavior in *D. annularis*. Furthermore, while only 41% and 45% of the *S. cantharus* and *D. sargus* specimens had consumed *C. cylindracea*, the figure for *D. annularis* specimens was around 85%, which strongly suggests that this invasive alga is a recurrent item in the diet of *D. annularis* in the Cabrera Archipelago. Unfortunately, it must be stressed that only seven specimens of *D. annularis* were captured and examined, so a higher sample size is needed to draw more robust conclusions on the importance of *C. cylindracea* in the diet of *D. annularis*. In contrast with the other three species, there was no evidence that the *D. vulgaris* specimens (n=13) collected at the Cabrera Archipelago for this study had consumed *C. cylindracea*, despite the fact that

D. vulgaris has previously been observed feeding on *C. cylindracea* (Felline et al. 2017).

Overall, although *C. cylindracea* was found in the diet of three of the four sea bream species, its contribution to the total stomach content was generally low. The biggest consumer was *D. annularis*, with nearly 26% of its total stomach content corresponding to *C. cylindracea* (Table 1). In fact, both dietary indexes, Q_i and GII, classified this food item as a preferential prey item for *D. annularis* as it was the second most common prey category in their stomachs and had importance values similar to those of the most common prey category, which was animal content (Figs. 2 and 3). Furthermore, the SIMPER analysis identified *C. cylindracea* as the second most important food item in terms of explaining the diet dissimilarities between *D. annularis* and the other fish species (Table 3). On the other hand, *C. cylindracea* was classified as an occasional food item for both *D. sargus* and *S. cantharus*, but while the algae content in the stomachs of *S. cantharus* was only around 7%, it is worth noting that most of it ($\approx 74\%$) corresponded to *C. cylindracea* (Table 1). These findings are in agreement with the study by Box *et al.* (2009), where *C. cylindracea* was not the main carbon and nitrogen source for *S. cantharus*. On the other hand, in contrast with our results, previous studies have shown *C. cylindracea* to be the most important prey item for *D. sargus* and the second-most for *S. cantharus* (Terlizzi et al. 2011; Felline et al. 2012, 2017). Although these differences could be due to different sampling methodologies or to the site-specific characteristics of the study areas, it is also possible that they are the result of seasonal dynamics in the abundance of the invasive alga. Our samples were taken in early to mid summer, whereas the samples in the studies that found higher percentages of *C. cylindracea* in the stomach contents, were taken at the end of summer and the beginning of autumn (Terlizzi et al. 2011; Felline et al. 2012, 2017), which

corresponds to the season when *C. cylindracea* achieves its peak in abundance and growth (Piazzi et al. 2001; Ruitton et al. 2005; Klein and Verlaque 2008).

Despite the significant contribution of *C. cylindracea* to the diet of *D. annularis*, and despite the fact that it was found to be the dominant algae species in the benthic communities (with mean coverage values close to 55%), the negative values obtained in the Ivlev Electivity Index suggest that all four of the fish species studied tend to avoid feeding on the invasive alga (Table 4). However, it is important to consider that, as is the case with consumption, electivity towards *C. cylindracea* may also change in relation to the season. Indeed, this has already been observed in the strictly herbivorous fish, *Sarpa salpa* (Tomas et al. 2011b). It may therefore be important to assess whether the consumption of *C. cylindracea* by the four sea bream species studied here, and their electivity towards it, is related to *C. cylindracea*'s seasonality in the Mediterranean Sea and whether it increases towards autumn when the invasive alga is the dominant component of many benthic assemblages. In fact, it was at the beginning of autumn when the stomach contents of *S. salpa* were found to be dominated by *C. cylindracea* and when the highest grazing activity on the invasive alga was observed (Ruitton et al. 2005, 2006; Tomas et al. 2011b).

Despite the general avoidance of *Caulerpa cylindracea*, high amounts of it were found in the stomach contents of some individuals. This suggests that it is probably consumed accidentally when the fish are trying to feed on other prey living within the dense meadows of *C. cylindracea*. In fact, polychaetes, mollusks and decapods – which are the preferential prey for most of the sea breams studied (Bauchot and Hureau 1986; Sala and Ballesteros 1997; Gonçalves and Erzini 1998; Pita et al. 2002; Leitão et al. 2007) – have been found to be very abundant under the stolons of *C. cylindracea* (Carriglio et al. 2003; Galil 2007; Box 2008; Klein and Verlaque 2008). Furthermore,

the suggestion that consumption of the alga is accidental is also supported by the low assimilation of *C. cylindracea* in the stomach contents, as in most cases it was found intact and undigested (Fig. 5). However, taking into account that some of the sea breams considered here have small home ranges and show strong site fidelity (D'Anna et al. 2011; March et al. 2011; Alós et al. 2012; Di Lorenzo et al. 2014), it cannot be ruled out that they might also be forced to feed on *C. cylindracea* in heavily colonized areas (Felline et al. 2014), and this may have a negative impact on the physiology of the fish. Actually, previous evidence relate *C. cylindracea* consumption to a decrease in certain essential fatty acids in fish tissues and liver (Felline et al. 2014), an increase in the levels of antioxidants and in pro-oxidant effects (Box et al. 2009; Terlizzi et al. 2011; Felline et al. 2012), a decrease in the condition factor (Terlizzi et al. 2011) and a decrease in the gonadosomatic-index (Felline et al. 2012), all of which may negatively affect the nutritional status and the health of the fish. It is not yet clear what causes these physiological responses, although they could be caused by the accumulation of some of the compounds produced by *C. cylindracea*, such as caulerpenyne, a toxic, secondary metabolite that has herbivore-deterrent properties (Paul et al. 2007). However, considering that *Caulerpa prolifera*, a native species in the Mediterranean Sea, has much higher caulerpenyne concentrations than *C. cylindracea* (Box et al. 2010) and that sea breams can often consume the native *Caulerpa* species (Table S1) (Chaouch et al. 2013, 2014; Marco-Méndez et al. 2017), it is likely that the fish have developed a certain tolerance to the toxic metabolites, as other herbivores have done (Cornell and Hawkins 2003) and they may also have effective detoxification pathways. In any case, more studies are needed to understand the possible long-term consequences of *C. cylindracea* consumption on the health of fish assemblages and whether these consequences could propagate throughout the food-web, potentially affecting the

functioning of the ecosystem through cascading effects. Furthermore, long-term studies that determine whether the consumption of *C. cylindracea* changes with time since invasion and whether the physiological consequences of *C. cylindracea* consumption decrease with time, might provide important information on the potential adaptation of sea breams to the consumption of this invasive alga.

Overall, our findings confirm that the invasion of *Caulerpa cylindracea* in the Mediterranean Sea is not influencing the foraging habits and diets of the strictly herbivorous organisms (Azzurro et al. 2004; Ruitton et al. 2006; Cebrian et al. 2011; Tomas et al. 2011b, a), but also those of omnivorous fish species (Box et al. 2009; Terlizzi et al. 2011; Felling et al. 2014). In this sense, our assessment of *C. cylindracea* consumption by omnivorous species (i.e. non-strict herbivores) is noteworthy since most of the previous research into the effects of herbivory on invasive algae has focused only on the strict herbivores (e.g., Scheibling and Anthony 2001, Davis et al. 2005, Wikström et al. 2006, Ruitton et al. 2006, Lyons and Scheibling 2008, Vermeij et al. 2009, Britton-Simmons et al. 2011, Cebrian et al. 2011, Tomas et al. 2011b, 2011a, Nejrup et al. 2012, Hammann et al. 2013). Such studies have pointed out the potential contribution of some of these herbivores to limiting the abundance of invasive algae (Stimson et al. 2001; Lyons and Scheibling 2008; Britton-Simmons et al. 2011; Tomas et al. 2011b), but our findings suggest that a similar, albeit less important contribution by non-strict herbivores should also be considered. In fact, although it appears that the four omnivorous species studied here tended to avoid *C. cylindracea*, the role of these fish in the biotic control of *C. cylindracea* cannot be disregarded since high amounts of the alga were found in the stomachs of some individuals and these fish do, in fact, dominate the shallow rocky infralittoral habitats in the Mediterranean Sea (García-Rubies 1997; Sala and Ballesteros 1997). As such, it is quite likely that the lower

impact exerted by the omnivorous fish can complement the higher impact exerted by the strictly herbivorous organisms and that, taken together, they might significantly reduce the abundance of *C. cylindracea* in shallow habitats. Finally, considering that sea breams are highly targeted by fisheries and have already suffered important declines in the Mediterranean basin (Sala et al. 1998; Coll et al. 2004; Sala 2004; Morales-Nin et al. 2005; Guidetti 2006; Lloret et al. 2008), places that foster their recovery, such as well-enforced marine protected areas (MPAs) (Mosquera et al. 2000; Micheli et al. 2005; Claudet et al. 2006; Guidetti and Sala 2007; Guidetti et al. 2008, 2014; Sala et al. 2012; Coll et al. 2013), might also foster the complementary biotic resistance of native assemblages against the invasion of *C. cylindracea*.

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672

673 **Tables**674 **Table 1.** Summary of the stomach content data for each fish species.

<i>D. annularis</i>	Mean $\pm$ S.E. (%)	<i>D. sargus</i>	Mean $\pm$ S.E. (%)
Algal content	30.41 $\pm$ 13.58	Algal content	18.68 $\pm$ 3.84
<i>Caulerpa cylindracea</i>	25.73 $\pm$ 11.80	<i>Caulerpa cylindracea</i>	3.56 $\pm$ 0.96
Plants	0.26 $\pm$ 0.21	Plants	9.36 $\pm$ 2.84
Animal content	43.28 $\pm$ 16.00	Animal content	48.31 $\pm$ 5.53
Detritus	26.05 $\pm$ 15.03	Detritus	23.64 $\pm$ 5.08
<i>D. vulgaris</i>	Mean $\pm$ S.E. (%)	<i>S. cantharus</i>	Mean $\pm$ S.E. (%)
Algal content	8.80 $\pm$ 6.91	Algal content	7.03 $\pm$ 2.50
<i>Caulerpa cylindracea</i>	0	<i>Caulerpa cylindracea</i>	5.15 $\pm$ 1.94
Plants	0.29 $\pm$ 0.25	Plants	0.40 $\pm$ 0.26
Animal content	54.75 $\pm$ 12.30	Animal content	14.10 $\pm$ 4.81
Detritus	36.16 $\pm$ 11.14	Organic detritus	78.47 $\pm$ 5.12

The ingested food items are grouped into categories and the values given are mean percentages $\pm$ S.E. for each fish species.

675 **Table 2.** Results of the ANOSIM and the pairwise comparisons between fish species to detect diet
676 similarities between species under 999 permutations.

Test	Fish species	R Statistic	Significance
Global test	All species	0.201	0.001*
Pairwise test	<i>D. annularis</i> vs <i>D. sargus</i>	0.184	0.306
	<i>D. annularis</i> vs <i>D. vulgaris</i>	0.398	0.006*
	<i>D. annularis</i> vs <i>S. cantharus</i>	0.625	0.006*
	<i>D. sargus</i> vs <i>D. vulgaris</i>	0.156	0.102
	<i>D. sargus</i> vs <i>S. cantharus</i>	0.171	0.012*
	<i>D. vulgaris</i> vs <i>S. cantharus</i>	0.473	0.006*

The asterisk (*) indicates statistical significance (p-value < 0.05).

677 **Table 3.** Results of the SIMPER analysis to detect the most important prey items contributing to the diet
678 dissimilarities between fish species.

Fish species	Item	Cumulative contribution to dissimilarities
<i>D. annularis</i> vs. <i>D. sargus</i>	Organic detritus	0.187
	<i>Caulerpa cylindracea</i>	0.327
	Eggs	0.466
<i>D. annularis</i> vs. <i>D. vulgaris</i>	Organic detritus	0.215
	<i>Caulerpa cylindracea</i>	0.359
	Eggs	0.495
<i>D. annularis</i> vs. <i>S. cantharus</i>	Organic detritus	0.399
	<i>Caulerpa cylindracea</i>	0.568
	Eggs	0.732
<i>D. sargus</i> vs. <i>D. vulgaris</i>	Organic detritus	0.214
	Gasteropoda	0.394
	Crustacea	0.518
<i>D. sargus</i> vs. <i>S. cantharus</i>	Organic detritus	0.394
	Gasteropoda	0.503
	Non-identified invertebrate	0.554
<i>D. vulgaris</i> vs. <i>S. cantharus</i>	Organic detritus	0.382
	Gasteropoda	0.548
	Crustacea	0.695

Only the 3 most important food items are shown.

679 **Table 4.** Mean $\pm$ S.E. values for Ivlev's electivity Index (E), as a measure of the electivity of the four
680 sparid fish species studied towards the invasive alga *C. cylindracea*.

$E_{Caulerpa}$	
<i>D. annularis</i>	-0.52 ± 0.18
<i>D. sargus</i>	-0.90 ± 0.02
<i>D. vulgaris</i>	-1
<i>S. cantharus</i>	-0.86 ± 0.05

An E value approaching -1 indicates that the food item is avoided; whereas an E value approaching 1 indicates the species only feeds on that item.

681 Figures

682 **Fig. 1:** Map of Cabrera Archipelago with the locations where the fishing was done. Shapefile for the
 683 Mediterranean Sea downloaded from www.naturalearthdata.com and for the Cabrera Archipelago
 684 National Park from www.miteco.gob.es.

685 **Fig. 2.** Combined Index (Q) for each fish species. Each color represents one of five prey item categories
 686 (Detritus, Animals, Plants, Algae and *Caulerpa*).

Fig. 3. Geometric Importance Index (GII) for each fish species. Prey items are classified as: “Preferential prey”, “Secondary prey” or “Occasional prey” according to their relative importance to the diet of each fish species. The line connecting the points was added to help interpretation of the figure.

690 **Fig. 4.** Non-metric MDS for the stomach content of the different fish species. Each ellipse surrounds the
691 points for one fish species and the black arrows represent the most significant food items determining the
692 ordination. For these variables, only the ones with a p-value lower or equal to 0.001 were represented.

693 **Fig. 5.** Macroscopic view of *Caulerpa cylindracea* fragments, as found in the stomach contents.

Figures

Figure 1

Map of Cabrera Archipelago with the locations where the fishing was done. Shapefile for the Mediterranean Sea downloaded from www.naturalearthdata.com and for the Cabrera Archipelago National Park from www.miteco.gob.es. Note: The designations employed and the presentation of the

material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Combined Index (Q) for each fish species. Each color represents one of five prey item categories (Detritus, Animals, Plants, Algae and Caulerpa).

Figure 3

Geometric Importance Index (GII) for each fish species. Prey items are classified as: “Preferential prey”, “Secondary prey” or “Occasional prey” according to their relative importance to the diet of each fish species. The line connecting the points was added to help interpretation of the figure.

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Non-metric MDS for the stomach content of the different fish species. Each ellipse surrounds the points for one fish species and the black arrows represent the most significant food items determining the ordination. For these variables, only the ones with a p-value lower or equal to 0.001 were represented.

Figure 5

Macroscopic view of *Caulerpa cylindracea* fragments, as found in the stomach contents.

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