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## Patterns of predation on native and invasive alien fish in Mediterranean protected and unprotected areas

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1 **Patterns of predation on native and invasive alien fish in Mediterranean protected and**  
2 **unprotected areas**

3

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29 **Short title:** Predation patterns on native and alien fish in the Mediterranean

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**Abstract**

Worldwide, the loss of predatory fish due to overexploitation has altered the structure of native communities and caused ecosystem shifts. Ecosystems deprived of high-level predators may be more vulnerable to invasive alien species as the latter are subject to reduced predation control. Marine protected areas (MPAs), and particularly no-take reserves where fishing is banned, can be effective tools for the restoration of predatory relationships within their boundaries. We explored whether the restoration of high-level predatory fish populations within Mediterranean MPAs can exert top-down control on alien fish. Fish tethering experiments, including native (*Sardina pilchardus*, *Boops boops*) and alien (*Siganus rivulatus*) dead specimens, were conducted to quantify predation within the no-take zones of three MPAs and in unprotected areas, and to assess potential differences in predation rates and prey type preferences. A subsample of experimental units was filmed to document predation events and related fish behavior. More high-level predators interacted with the tethered fish inside the MPAs than in unprotected areas. Yet we did not find significant differences in the consumption of alien or native fishes between MPAs and unprotected areas. The native *S. pilchardus* was consumed more in comparison to the other tethered fishes, regardless of protection status and location. Interestingly, the alien *S. rivulatus* was consumed by native predators in the western Mediterranean locations where this alien fish is not established. Despite its limitations, our study provides evidence on the ability of some native predators to feed on and potentially control certain alien species without requiring ‘adaptive’ time-lag periods.

**Keywords:** predators; invasive fish; Mediterranean; marine protected areas; no-take marine reserves; tethering experiment

69 **1. Introduction**

70

71 High-level marine predators, including top (or apex) predators, are usually large-bodied long-  
72 living animals that occupy the highest trophic levels in food webs, and have a fundamental  
73 influence on the structure of marine communities and ecosystem functioning (Ray et al.  
74 2013). The effect of predators on prey populations and, via trophic webs, on entire  
75 communities (e.g. trophic cascades; Estes et al. 1998) is a combination of lethal direct  
76 predation and non-lethal risk effects (Heithaus et al. 2008). Many prey species alter their  
77 behavior to avoid predation – the so called non-lethal risk effects – influencing their foraging  
78 patterns, growth and reproduction rates. The combined effects of predation exert top-down  
79 control on mesopredators and their prey, that may be as consequential, if not more so in some  
80 cases, than bottom-up forces (Baum and Worm 2009).

81

82 High-level marine predators, including large finfish and sharks, have been intensively fished  
83 worldwide (Pauly et al. 1998; Christensen et al. 2003), leading to a dramatic decline in  
84 predatory fish biomass in the ocean by two thirds over the past 100 years (Christensen et al.  
85 2014). Depletion of predators has caused serious cascading effects in marine ecosystems,  
86 leading to regime shifts and alternative states of ecosystems (Estes et al. 2011). A classical  
87 example of a trophic cascade is the dramatic increase in sea urchin density and the subsequent  
88 deforestation of kelp beds because of the elimination of the sea-otter's keystone role (Estes et  
89 al. 1998). Another example is the decline of great sharks and the subsequent increase of their  
90 prey, mainly elasmobranchs of lower trophic levels, which in turn caused a decline in the  
91 mesopredators' prey populations, including the bay scallop (*Argopecten irradians*), leading to  
92 the collapse of the fishery (Myers et al. 2007). Thus, the overexploitation of high-level  
93 predators and the reduction (or elimination) of top-down control can have important  
94 ecological, socio-economic and conservation implications as intermediate-level consumers  
95 (herbivores and mesopredators) become dominant, and the recovery of overexploited  
96 predators is jeopardized (Baum and Worm 2009).

97

98 The loss of high-level predators not only produces effects that propagate throughout the food  
99 web, affecting native communities, but also makes ecosystems more vulnerable to invasion by  
100 alien species (Carlsson et al. 2009; Estes et al. 2011). A key factor that allows alien species to  
101 successfully invade their new environment is the absence of their original predators and their  
102 release from top-down control (Kolar and Lodge 2001). Yet alien species may become prey of

103 native predators in their new environment; these predators may adapt their diet to include  
104 alien prey, either rapidly, via existing phenotypic plasticity, or more slowly, via natural  
105 selection (Carlsson et al. 2009 and references therein). Alien species often become extremely  
106 abundant, outcompete and displace native prey species, and may eventually come to  
107 constitute the largest prey resource available to native predators. Native predators that feed on  
108 alien species will present a fitness advantage and might contribute to controlling both the  
109 abundance and distribution of alien species (Carlsson et al. 2009). For example, the alien  
110 European green crab (*Carcinus maenas*) is limited by the predation of two different native  
111 crab species, *Callinectes sapidus* in eastern North America (deRivera et al. 2005) and *Cancer*  
112 *productus* in southwestern North America (Jensen et al. 2007). Thus, overharvesting native  
113 predators may compromise their ability to regulate invasive species either through predation,  
114 if the invasive species are of lower trophic levels, or through competition in the case of  
115 invasive and native species occupying the same trophic level.

116  
117 Marine protected areas (MPAs), and particularly no-take marine reserves where extractive  
118 uses are not allowed, can be an effective tool for the recovery of large predatory fishes and the  
119 restoration of predatory relationships within their boundaries (Shears and Babcock 2002;  
120 Micheli et al. 2004; Guidetti 2006; Bond et al. 2012). A recent meta-analysis shows that the  
121 biomass of whole fish assemblages in no-take marine reserves is, on average, 6.7 times  
122 greater than in adjacent unprotected areas (Sala and Giakoumi 2018). This wide difference in  
123 biomass is primarily caused by the presence of large-bodied high-level predators inside  
124 marine reserves. The recovery of high-level predators in marine reserves and in other MPAs  
125 has the potential to control alien species spreading within their boundaries (Francour et al.  
126 2010). In the Caribbean Sea, the density and biomass of the invasive lionfishes (*Pterois*  
127 *volitans*, *P. miles*) have been found to be lower in MPAs than in unprotected areas (Mumby et  
128 al. 2011; Hackerott et al. 2013). However, it remains unclear whether more and larger grouper  
129 inside the MPAs were able to control the lionfish populations (Mumby et al. 2011), or  
130 whether management measures adopted within MPAs, i.e. selective removal of lionfishes,  
131 was responsible for the lower values recorded therein (Hackerott et al. 2013). Experimental  
132 work that will complement observational studies is necessary to test the hypothesis of whether  
133 the restoration of high-level predators within MPAs and predator-driven biotic resistance can  
134 control biological invasions. So far, the available evidence is too limited to draw robust  
135 conclusions (Burfeind et al. 2013; Giakoumi and Pey 2017).

136

137 Within this context, the Mediterranean Sea is a particularly interesting system because: 1) it  
138 has one of longest recorded histories of marine resources overexploitation in the world  
139 (Guidetti and Micheli 2011), 2) high-level predators show clear signs of recovery in well-  
140 enforced Mediterranean MPAs (Garcia Rubies et al. 2013; Guidetti et al. 2014; Giakoumi et  
141 al. 2017), and 3) it is a marine bioinvasion hotspot (Rilov and Galil 2009), with approx. 1000  
142 marine alien species recorded (Zenetos et al. 2012). The Mediterranean Sea is currently  
143 characterized by a paucity of top predators compared to historical times (Ferretti et al. 2008);  
144 nevertheless, in long-established well-enforced marine reserves, the populations of large  
145 piscivorous fishes have been found to recover (Prato et al. 2013), which could exert top-down  
146 control on both native and invasive alien fish populations. The aim of the present study,  
147 therefore, is to provide evidence on whether the restoration of high-level fish predator  
148 populations within Mediterranean MPAs can trigger top-down control on invasive alien  
149 fishes.

150

## 151 **2. Methods**

152

### 153 2.1. Experiment locations

154

155 To test the hypothesis of whether the restoration of top-down regulation inside no-take MPAs  
156 enables the control of the spread of alien fish species, fish tethering experiments were  
157 conducted to quantify relative predation rates inside the no-take zones of three MPAs and in  
158 adjacent unprotected areas (i.e. open to professional and recreational fishing, according to  
159 national legislation). Two MPAs were situated in the Western Mediterranean, the Cabo de  
160 Palos MPA in Spain and the Tavolara MPA in Italy (Fig. 1), where invasive fishes rarely  
161 occur (Guidetti et al. 2014; Giakoumi et al. 2019a). The third MPA was situated in the Eastern  
162 Mediterranean, the Kaş - Kekova MPA in Turkey (Fig. 1), where invasive alien fishes are  
163 abundant (Giakoumi et al. 2019a). This experimental approach enabled us to reconcile the  
164 contrasting facts that while the oldest and most effective MPAs are situated in the Western  
165 Mediterranean, the species richness and abundance of invasive fishes is much higher in the  
166 Eastern Mediterranean due to more favorable environmental conditions (Guidetti et al. 2014;  
167 Giakoumi et al. 2019a). Evidence from previous studies (Guidetti et al. 2014; Hackradt et al.  
168 2014; Giakoumi et al. 2017, 2019a) shows that the biomass and size of high-level predators  
169 are higher inside the no-take zone of the selected MPAs than in adjacent unprotected areas.

170 The experiments were performed within a narrow time window, in August and September  
171 2017, to minimize temporal effects.

172

## 173 2.2. Experimental design

174

175 For each combination of 'location' (Cabo de Palos, Kaş - Kekova, or Tavolara) and 'protection  
176 level' (no-take vs unprotected area), we deployed 18 replicates of tethering experimental units.  
177 In each experimental unit, three dead specimens were tethered: two specimens of native  
178 species (the sardine *Sardina pilchardus* and the bogue *Boops boops*) and one specimen of an  
179 alien species (the marbled spinefoot *Siganus rivulatus*). The species selected are part of the  
180 diet of native and invasive high-level predators (Andarolo and Pipitone 1997; Marengo et al.  
181 2014; Lopez and i Orvay 2005; Kalogirou et al. 2007; Bariche et al. 2009). The size of all  
182 specimens used in the experiment ranged between 8 and 12 cm TL, and in each unit the size  
183 of specimens of the three species was roughly equal. Fishes were bought from fish markets  
184 and transported frozen to the experimental locations. Experimental units were deployed two  
185 hours before sunset as at that time of day, some high-level predators are known to be  
186 particularly active (Harmelin-Vivien 1982).

187

188 A subsample of experimental units was equipped with HD cameras to get footage of the  
189 predation events and general fish behavior around the tethered fish (see example in the video  
190 provided as supplementary material). Overall, six replicates of video recordings were  
191 deployed for each location and protection level combination. Although the replication of  
192 video recordings was low due to logistical constraints, this complementary approach enabled  
193 us to get more precise information on the predators' identity, behavior and time of arrival.

194

## 195 2.3. Statistical analyses

196

### 197 2.3.1. Tethered fish consumption

198 *In situ* visual observations of the tethered fish enabled us to assess their status, 120 min after  
199 the deployment of the experimental units, as either consumed (i.e. we did not recover  
200 anything or less than half the tethered specimen) or not consumed (i.e. we recovered the  
201 whole specimen or more than half the tethered specimen). This provided a basis for  
202 assessment of the relative consumption rate. Video recordings enabled us to assess the  
203 tethered fish status from the 1<sup>st</sup> minute until the 90<sup>th</sup> minute after deployment of the

204 experimental unit. The duration of the video recordings was constrained by the autonomy of  
205 the camera battery. The tethered fish status based on the video footage was assessed as: (1)  
206 unvisited, (2) visited, (3) partially consumed, or (4) completely consumed. The two latter  
207 categories (partially and completely consumed) were grouped within the status 'consumed'.  
208 Respectively, the visiting fish behaviors observed within the experimental setup (during the  
209 90 minutes) were classified into four categories: (1) present (i.e. species present around the  
210 experimental setup without showing specific interest in the tethered fishes), (2) interested (i.e.  
211 species being specifically interested in one or more tethered fish), (3) partial consumer (i.e.  
212 species biting one or more tethered fish), (4) consumer (i.e. species eating completely a  
213 tethered fish). Examples of each visiting fish behavior type are provided in Supplementary  
214 Video S1. Overall, 54 hours of video footage were recorded and analyzed.

215

216 To compare the consumption rate of tethered specimens (using the data either from *in situ*  
217 visual observations or from the video recordings) among locations and between protection  
218 levels, we used Generalized Linear Models for Multivariate Binomial Data (Wang et al.  
219 2012). Terms included in the models were the factor 'protection' (fixed, 2 levels: NTZ vs  
220 OUT), the factor 'location' (fixed, 3 levels: CAB, KAS, TAV) and the interaction term (two-  
221 way crossed design). The factor 'location' was fixed to allow for the comparison of patterns  
222 among the three locations distributed along a longitudinal gradient. Significance of terms was  
223 assessed using analyses of deviance. P-values were calculated using 999 resampling iterations  
224 via PIT-trap resampling (to account for correlation in testing). Models and tests were carried  
225 out using the mvabund R package (Wang et al. 2018). Binomial confidence intervals of  
226 probabilities (of tethered fish being consumed) were calculated using Bayesian inference  
227 (Gelman et al. 1997) with the binom R package (Dorai-Raj 2014).

228

### 229 2.3.2. *Predator arrival time and interaction with tethered fish*

230 When collecting data from the video footages, we also recorded the exact time of the first  
231 presence (arrival time) of a species within the experimental setup (i.e. within the camera field  
232 of view), and the time of the first predator-tethered fish interaction (i.e. when a visiting fish  
233 was interested, or a partial consumer or a consumer). For these two metrics (time of arrival  
234 and time of interaction), we produced accumulation curves and boxplots of the number of  
235 species within 90 mins from the deployment of the experimental unit.

236



237 To compare the arrival time and the time of predator-tethered fish interaction between  
238 protection levels and among locations, we performed univariate and multivariate  
239 Permutational Multivariate Analysis of Variance (PERMANOVA). The response variables  
240 used in the multivariate tests were: 1) the number of fish species sighted in the camera field of  
241 view at least once within 30min, 60min, and 90min, and 2) the number of fish species  
242 observed to interact with a tethered specimen (or multiple specimens) at least once within  
243 30min, 60min and 90min, distinguishing visiting fish species by trophic level. We also ran the  
244 second analysis exclusively for Kaş, categorizing visiting fish species by trophic level and  
245 origin (native vs alien). Univariate tests were carried out for each response variable (i.e. each  
246 time period) separately. Euclidean distance was used in all tests. The design was two-way  
247 crossed, with the factor 'protection' (fixed, 2 levels: NTZ vs OUT) and the factor 'location'  
248 (fixed, 3 levels: CAB, KAS, TAV). The factor 'location' was fixed to allow for the  
249 comparison of patterns among the three locations distributed along a longitudinal gradient.

250

### 251 **3. Results**

252

#### 253 3.1. Tethered fish consumption

254

255 Analyses of both *in situ* visual data and video footage did not reveal statistically significant  
256 differences in the mean consumption rates of the three tethered fish species between no-take  
257 zones and unprotected areas (Tables 1 & 2). Based on *in situ* visual observations, specimens  
258 of *S. pilchardus* presented the highest consumption rate in both protected and unprotected  
259 sites (Fig. 2). The video footage revealed that native species were selected (partially or  
260 completely consumed) first, regardless of protection status and location (Fig. 2).

261

262 Based on the *in situ* visual observations, the mean consumption rate differed across locations  
263 (Dev = 18.113,  $p = 0.021$ , Table 1). More specifically, the consumption of *S. pilchardus* (the  
264 most consumed species) differed according to the factor 'location' (Dev = 11.628,  $p = 0.014$ ,  
265 Table 1), and was particularly high at Tavolara.

266

267 Based on the video footage, the consumption rate of a specimen (i.e. partially or completely  
268 consumed) also differed among locations (Table 2, Fig. 3). Specimens of the three species  
269 tend to have higher consumption rates at Kaş, regardless of the protection status. Yet, this was  
270 statistically significant only for *B. boops*. The consumption rate of *B. boops* was significantly

271 higher at Kaş for all time periods examined (i.e. within first 30mins, 60mins, or 90mins; Table  
272 2).

273

274 In consistency with the fact that alien fish have rarely been recorded in the Western  
275 Mediterranean, we found that the estimated consumption rates of the tethered fish by alien  
276 species differed markedly among locations, being very high at Kaş, whereas at Cabo de Palos  
277 and Tavolara it was almost null (Table 3, Fig. 4a). Conversely, estimated consumption rates  
278 of tethered fish by native species did not vary among locations, except for *B. boops* for which  
279 the probability of being consumed by a native species was higher at Tavolara and Kaş. As a  
280 result, at Kaş, the consumption rates of all tethered specimens by alien predators (especially  
281 alien low-medium level predators; non-overlapping confidence interval in Fig. 4) were higher  
282 than the consumption rates by native predator fishes.

283

### 284 3.2. Predator arrival time and interaction with tethered fish

285

286 High-level predator species accumulation was higher in the no-take zone of the MPAs than in  
287 unprotected areas (pseudo-F = 18.365,  $p = 0.0001$ , Table S1; Fig. 5a) and was higher at Cabo  
288 de Palos than in the other two locations (pseudo-F = 3.9444,  $p = 0.0175$ , Table S1; Fig. 5a).  
289 Moreover, in all locations, high-level predator species accumulated (i.e. arrived within the  
290 camera's field of view) faster in MPAs than in unprotected areas (Table S1, Fig. 5a).  
291 Generally, high-level predator richness was higher in MPAs than in unprotected areas (Fig.  
292 5b). Conversely, low- and medium-level predator accumulation was higher and faster in  
293 unprotected areas at Cabo de Palos and Tavolara, whereas the opposite pattern was observed  
294 at Kaş (pseudo-F = 5.2553,  $p = 0.0213$  for protection, pseudo-F = 8.7447,  $p = 0.0005$  for  
295 location, and pseudo-F = 5.8936,  $p = 0.0033$  for their interaction, Table S1, Fig. 6).

296

297 Overall, a total of 19 species interacted with the tethered fishes (i.e. were interested or partial  
298 consumers or consumers). The list of species as well as the respective estimated consumption  
299 rates are provided in Table 4 and Fig. S1. Out of these species, ten were high-level predators  
300 whereas the others were low- and medium-level predators. In accordance with the results  
301 mentioned above, more high-level predators interacted with the tethered fish inside the MPAs,  
302 whereas more low- and medium-level predators interacted with the specimens outside the  
303 MPAs (pseudo-F = 24.331,  $p = 0.0001$  for factor 'protection', Table S2; Fig. 6a).

304

305 In the two western locations (Cabo de Palos and Tavolara), all visiting fish interacting with  
306 the specimens were native species. At Kaş (Eastern Mediterranean), alien species also  
307 interacted with the tethered specimens, two were high-level (*Fistularia commersonii* and  
308 *Lagocephalus sceleratus*) and two medium-level predators (*Sargocentron rubrum* and  
309 *Torquigener flavimaculatus*). At Kaş, native high-level predators interacted more with the  
310 tethered fish inside the MPA than in unprotected areas (Fig. S2) over the entire period of  
311 video recording (pseudo-F = 20.714, p = 0.0019) and for each time interval (within 30mins:  
312 pseudo-F = 10, p = 0.05; within 60mins: pseudo-F = 15, p = 0.01, pseudo-F = 15, p = 0.01).

313

#### 314 **4. Discussion**

315

316 The present study did not reveal differences in the average consumption rates of tethered alien  
317 or native fishes between the no-take zones of MPAs and adjacent unprotected areas. However,  
318 we found that more high-level predators interacted with the tethered fish inside the MPAs,  
319 whereas more low- and medium-level predators interacted with the tethered specimens  
320 outside the MPAs. This observation reinforces findings of previous studies demonstrating that  
321 the density and biomass of high-level predators in the no-take zones of the MPAs considered  
322 herein is higher than in adjacent fished sites (e.g. Hackradt et al. 2014; Guidetti et al. 2014;  
323 Giakoumi et al. 2019a). The so called 'reserve effect' (i.e. increasing levels of density and size  
324 of individuals inside the reserve) of the Cabo de Palos and Tavolara MPAs, is further  
325 supported by the fact that the accumulation of high-level predator species was higher and  
326 faster in the MPAs, whereas low- and medium-level predators' accumulation was higher and  
327 faster in unprotected areas. This was not the case at Kaş, where the density and biomass of  
328 high-level predators is much lower than in the two western MPAs. The Kaş - Kekova MPA is  
329 relatively new, as it was only designated in 2012, and enforcement mechanisms are not well-  
330 established yet (Giakoumi et al. 2019a). In this MPA, large piscivorous predatory populations  
331 might need more time and more effective protection in order to increase in numbers and  
332 individual size that will allow them to exert top-down control on native and alien prey.

333

334 Furthermore, our study showed a preference towards the native *S. pilchardus*, which was  
335 consumed more in comparison to the other tethered fish, regardless of protection status and  
336 location. Nevertheless, alien rabbitfish specimens were consumed by native predators (mostly  
337 by *Muraena helena* but also by *Dentex dentex*) in the locations where the alien species has  
338 never or rarely been observed (i.e. Cabo de Palos and Tavolara). This indicates that some

339 native predators can feed on the alien species probably without requiring an 'adaptive' time-  
340 lag period (see Carlsson et al. 2009). The fact that the tethered fish were dead specimens may  
341 explain partly why some other predators were interested in the alien specimens (as well as in  
342 the native tethered fish) but did not bite or consume them. Piscivorous fish have predation  
343 strategies that may involve live fish to be ambushed, chased, stalked and caught (Hobson  
344 1979); thus, dead fish may be less attractive. In the recordings, we observed individuals of the  
345 species *Seriola dumerili* and *D. dentex* 'blowing water' on the tethered fish, potentially to test  
346 whether the 'prey' would try to escape (watch video provided as supplementary material).  
347 Moreover, some of the interested predators might need to slightly change their behavior, life  
348 history or morphology to incorporate alien species into their diet. Phenotypic plasticity can  
349 occur during the lifetime of an individual or across generations (Padilla and Savedo 2013),  
350 and thus the potential for control of the alien population can occur either within short or  
351 longer time periods (Carlsson et al. 2009). In more southeastern locations in the  
352 Mediterranean Sea, such as Lebanon, where *S. rivulatus* has been present for almost a century  
353 (Golani 1998), specimens have been found as items in the stomachs of several native high-  
354 level predator fishes including grouper (*Epinephelus* spp.), the greater amberjack (*Seriola*  
355 *dumerili*), and the bluefish (*Pomatomus saltatrix*) (Guidetti P. and Fatfat S., unpublished  
356 data). This evidence suggests that native predators could potentially exert predation pressure  
357 and regulate invasive fish populations.

358

359 The population of invasive alien species could also be regulated by the predatory activity of  
360 higher trophic level alien species that have been introduced in the recipient environment at the  
361 same or a different time period. According to the video recordings data, the consumption  
362 probability of the tethered specimen (both native and alien) differed across locations and was  
363 generally higher at Kaş, triggered by the predation activity of alien predators (especially, *L.*  
364 *sceleratus* and *T. flavimaculatus*). Evidence from studies exploring the difference in the  
365 impact of native and alien predators on native terrestrial vertebrates shows that alien predators  
366 can have a more severe impact on native species populations compared to native predators  
367 (Salo et al. 2007). In the marine environment, the invasive lionfish *Pterois volitans* appears to  
368 have a stronger ecological impact on native prey in northwestern Atlantic coral reefs than  
369 native piscivores of similar size (Albins 2013). Our recordings demonstrate that the invasive  
370 pufferfish *L. sceleratus* and *T. flavimaculatus* are voracious predators that consume  
371 indistinctively native and alien fishes. These two species appear to be well adapted to their  
372 new environment, and their predation activity may have a major impact on both native and

373 alien species in the host environment. Evidence shows that the high-level predator *L.*  
374 *sceleratus* can cause a major negative impact on native communities (Kalogirou 2013),  
375 fisheries (Ünal et al. 2015) and human health (Beköz et al. 2013). Despite its negative  
376 ecological and socio-ecological impact, *L. sceleratus* could control other invasive species  
377 populations through predation or competition. The ability of *L. sceleratus* to resist the venom  
378 of its prey (Kalogirou 2013) makes it a fit predator for many invasive alien species, including  
379 rabbitfish and lionfish that are venomous. The cornet fish *F. commersonii* is another invasive  
380 high-level predator that feeds on rabbitfish, among other alien species (Bariche et al. 2009),  
381 and thus could exert control on alien species of lower trophic levels. Besides exploring the  
382 impact of alien high-level predators on native fishes, future research should also investigate  
383 the direct impact of alien high-level predators on alien species and associated trophic cascades  
384 (see Kindinger and Albins 2017).

385

386 Our study presents some limitations in assessing the predation rates inside MPAs *versus*  
387 adjacent unprotected areas, and thus in providing evidence on whether the restoration of high-  
388 level predator populations within Mediterranean MPAs can potentially exert top-down control  
389 on invasive alien fish. First, we fully acknowledge that this type of experiment cannot be used  
390 to estimate predation rates as they occur in nature, but rather to compare relative predation  
391 rates under different conditions; here, to compare predation rates in areas under different  
392 fishing regulations. Moreover, our experiment suffers from the bias associated with all  
393 tethering experiments regarding alterations in predator behavior and, most importantly,  
394 reduction in the effectiveness of the escape responses of the prey (Aronson et al. 2001; Mills  
395 et al. 2008). In our experiment, these important sources of bias were even greater as the  
396 tethered fish were dead specimens. Logistical, legal, and ethical issues ruled out the use of  
397 living specimens; introducing living alien specimens into an MPA where the species does not  
398 occur would be at least reckless, if not unethical. The exposure time of the tethered fish might  
399 also have been shorter than appropriate, but logistical reasons (e.g. camera battery autonomy,  
400 transfer to remote locations, loss of equipment at sites with high diving activity) prevented us  
401 from further extending the duration of the experiment. Lastly, short-term snapshot studies  
402 may be insufficient to assess the long-term regulatory potential of native predators as there  
403 may be a long time-lag before native predators become effective at controlling an alien  
404 population (Carlsson et al. 2009).

405

406 Despite its limitations, the present study contributes to a research topic – the relationships  
407 between MPAs and biological invasions – for which empirical evidence is scarce (Burfeind et  
408 al. 2013; Giakoumi and Pey 2017). Our finding that some native predators consumed  
409 specimens of *S. rivulatus* shows a potential ability of native predator populations to control  
410 certain alien species populations. The approach we followed, i.e. complementing the tethering  
411 experiments with video recordings, enabled us to observe species interactions that could be  
412 suggestive of potentially higher predation rates within MPAs. The higher density, size and  
413 biomass of high-level (including piscivorous) predators in the no-take zones of the MPAs than  
414 in adjacent unprotected sites (as documented by Hackradt et al. 2014; Guidetti et al. 2014;  
415 Giakoumi et al. 2019a) can result from different predation behavior patterns and/or higher  
416 predation pressure (Vergès et al. 2012). The potential higher predation pressure exerted in the  
417 MPAs may lead to higher consumption of alien species within their boundaries than in  
418 unprotected areas. However, as there are plentiful and diverse food resources in well-enforced  
419 MPAs (especially in no-take zones), the native predators might prefer native over alien  
420 species (as our results indicate), and thus consume alien species in smaller quantities. To  
421 support or reject the hypothesis that higher predation pressure can trigger alien population  
422 control, experimental studies and stomach content analyses should be conducted examining  
423 prey preferences of native high-level predators. Finally, our findings suggest that the  
424 predation activity of alien predators may play an important role in shaping food webs in the  
425 host environment. Currently, evidence on the impact of invasive alien predators on both  
426 native and (particularly) alien species is very limited (Katsanevakis et al. 2014). Filling such  
427 knowledge gaps is critical for the effective control of alien populations (Giakoumi et al.  
428 2019b) and for marine conservation decision-making.

429

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438

439

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598  
599

## 600 Tables

601 **Table 1:** Analyses of Deviance Table of the multivariate and univariate Generalized Linear  
602 Model fits on the estimated rates of tethered fishes to be completely consumed 120 minutes  
603 after the experimental unit deployment. The multivariate GLM fit considers the 3 tethered  
604 fish species. Univariate fits consider one species at a time. Upper panel: full tests. Lower  
605 panel: pairwise comparisons among pairs of levels of the Location factors. CAB: Cabo de  
606 Palos, TAV: Tavolara, KAS: Kaş; BOO.BOO.: *Boops boops*, SAR.PIL.: *Sardina pilchardus*,  
607 SIG.RIV.: *Siganus rivulatus*.

	Res. Df	Df. diff	Multivariate		BOO.BOO.		SAR.PIL.		SIG.RIV.	
			Dev	p	Dev	p	Dev	p	Dev	p
LOCATION	105	2	18.113	<b>0.021</b>	5.917	0.116	11.628	<b>0.014</b>	0.568	0.729
PROTECTION	104	1	2.194	0.541	1.129	0.642	0.687	0.671	0.379	0.671
LOCATION X PROTECTION	102	2	13.009	0.091	3.549	0.411	1.203	0.586	8.256	0.080
CAB vs TAV	70	2	15.049	<b>0.003</b>	3.339	0.143	11.551	<b>0.002</b>	0.159	0.704
CAB vs KAS	70	2	8.129	0.075	5.623	0.064	2.379	0.230	0.127	0.782
TAV vs KAS	70	2	4.475	0.243	0.322	0.688	3.585	0.180	0.568	0.688

608

609 **Table 2:** Analyses of Deviance Table of the multivariate and univariate Generalized Linear  
610 Model fits on the estimated rates of tethered fishes to be bitten (partially or completely  
611 consumed) within 30 minutes (upper panel), 60 minutes (central panel) and 90 minutes (lower  
612 panel). The multivariate GLM fit considers the 3 tethered fish species. Univariate fits consider  
613 one species at a time. Full tests and pairwise comparisons among pairs of levels of the  
614 Location factors. Refer to Table 1 for location and species abbreviations.

	Res. Df	Df. diff	Multivariate		BOO.BOO.		SAR.PIL.		SIG.RIV.	
			Dev	p	Dev	p	Dev	p	Dev	p
LOCATION	33	2	26.456	<b>0.001</b>	16.920	<b>0.001</b>	4.834	0.194	4.702	0.194
PROTECTIO N	32	1	5.022	0.240	0.000	0.914	4.765	0.106	0.257	0.909
LOCATION X PROTECTIO N	30	2	2.616	0.779	1.928	0.743	0.494	0.920	0.194	0.920
CAB vs TAV	22	2	4.379	0.304	1.247	0.561	0.178	0.706	2.955	0.319
CAB vs KAS	22	2	24.327	<b>0.003</b>	15.407	<b>0.001</b>	4.332	0.089	4.589	0.089
TAV vs KAS	22	2	11.853	<b>0.028</b>	8.795	<b>0.020</b>	2.805	0.221	0.254	0.635
LOCATION	33	2	29.303	<b>0.001</b>	16.920	<b>0.001</b>	4.029	0.147	8.353	<b>0.046</b>
PROTECTIO N	32	1	5.083	0.232	0.000	0.921	4.864	0.119	0.219	0.921
LOCATION X PROTECTIO N	30	2	3.084	0.675	1.928	0.699	1.030	0.776	0.126	0.776
CAB vs TAV	22	2	4.201	0.323	1.247	0.563	0.000	0.904	2.955	0.330
CAB vs KAS	22	2	26.773	<b>0.001</b>	15.407	<b>0.002</b>	3.104	0.084	8.263	<b>0.015</b>
TAV vs KAS	22	2	13.759	<b>0.009</b>	8.795	<b>0.015</b>	3.104	0.190	1.860	0.190
LOCATION	33	2	25.425	<b>0.001</b>	15.928	<b>0.001</b>	3.342	0.229	6.155	0.132
PROTECTIO N	32	1	3.752	0.353	0.191	0.923	3.381	0.244	0.180	0.923
LOCATION X PROTECTIO	30	2	5.441	0.500	1.286	0.751	1.498	0.751	2.657	0.637

	Res. Df	Df. diff	Dev	p	Dev	p	Dev	p	Dev	p
CAB vs TAV	22	2	2.959	0.481	2.403	0.453	0.168	0.816	0.387	0.816
CAB vs KAS	22	2	23.965	<b>0.002</b>	15.407	<b>0.001</b>	3.104	0.075	5.455	0.057
TAV vs KAS	22	2	11.476	<b>0.014</b>	6.511	<b>0.041</b>	1.860	0.175	3.104	0.175

615

616 **Table 3:** Analyses of Deviance Table of the multivariate and univariate Generalized Linear  
617 Model fits on the estimated rates of tethered fish to be partially or completely consumed after  
618 90 min. The multivariate GLM fit considers the 3 tethered fish species. Univariate fits  
619 consider one species at a time. Full tests and pairwise comparisons among pairs of levels of  
620 the Location factors. Refer to Table 1 for location and species abbreviations.

		Res. Df	Df. diff	Multivariate Dev	Multivariate p	BOO.BOO. Dev	BOO.BOO. p	SAR.PIL. Dev	SAR.PIL. p	SIG.RIV. Dev	SIG.RIV. p
	LOCATION	33	2	79.259	<b>0.001</b>	31.727	<b>0.001</b>	31.727	<b>0.001</b>	15.805	<b>0.001</b>
Alien	CAB vs TAV	22	2	<0.001	1.000	<0.001	1.000	<0.001	1.000	<0.001	1.000
	CAB vs KAS	22	2	53.932	<b>0.001</b>	21.788	<b>0.001</b>	21.788	<b>0.001</b>	10.357	<b>0.005</b>
	TAV vs KAS	22	2	53.932	<b>0.001</b>	21.788	<b>0.001</b>	21.788	<b>0.001</b>	10.357	<b>0.006</b>
	LOCATION	33	2	14.958	<b>0.034</b>	6.851	0.114	5.152	0.132	2.955	0.394
Native	CAB vs TAV	22	2	2.959	0.478	2.403	0.436	0.168	0.797	0.387	0.797
	CAB vs KAS	22	2	5.963	0.096	1.430	0.304	3.104	0.127	1.430	0.304
	TAV vs KAS	22	2	13.946	<b>0.005</b>	6.351	<b>0.044</b>	4.641	0.052	2.955	0.112
Alien High Trophic level	LOCATION	33	2	6.890	0.154	0.000	1.000	4.635	0.093	2.255	0.191
Alien	LOCATION	33	2	71.430	<b>0.001</b>	31.727	0.001	26.992	0.001	12.711	<b>0.002</b>
Low-Medium Trophic level	CAB vs TAV	22	2	<0.001	1.000	<0.001	1.000	<0.001	1.000	<0.001	1.000
	CAB vs KAS	22	2	48.310	<b>0.001</b>	21.788	<b>0.001</b>	18.259	0.001	8.263	<b>0.010</b>
	TAV vs KAS	22	2	48.310	<b>0.001</b>	21.788	<b>0.001</b>	18.259	0.001	8.263	<b>0.013</b>
Native High Trophic level	LOCATION	33	2	9.269	0.226	4.635	0.340	2.955	0.410	1.680	0.739
Native	LOCATION	33	2	14.597	<b>0.021</b>	4.736	0.127	7.607	<b>0.042</b>	2.255	0.422
Low-Medium Trophic level	CAB vs TAV	22	2	3.346	0.397	1.247	0.758	0.670	0.758	1.430	0.758
	CAB vs KAS	22	2	5.237	0.065	1.430	0.156	3.807	0.076	-0.000	1.000
	TAV vs KAS	22	2	13.387	<b>0.009</b>	4.589	0.074	7.368	<b>0.025</b>	1.430	0.307

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**Table 4:** List of species that interacted with the tethered fish per site. Species are distinguished by their behavior i.e. whether they were interested in the tethered fish or were consumers (including both partial and full consumers), their trophic level (low & medium vs high), and their origin (N = native, A = alien).

	Cabo de Palos		Tavolara		Kaş	
	Interested	Consumer	Interested	Consumer	Interested	Consumer
<b>Low &amp; medium TL</b>						
<i>Diplodus annularis</i> (N)	x	x	x	x		
<i>Diplodus sargus</i> (N)	x		x	x	x	
<i>Diplodus vulgaris</i> (N)	x		x	x	x	
<i>Sargocentron rubrum</i> (A)					x	x
<i>Serranus cabrilla</i> (N)	x		x	x	x	x
<i>Serranus scriba</i> (N)	x	x	x	x	x	
<i>Sparus aurata</i> (N)			x		x	
<i>Spondyliosoma cantharus</i> (N)	x		x	x		
<i>Torquigener flavimaculatus</i> (A)					x	x
<b>High TL</b>						
<i>Dentex dentex</i> (N)	x	x	x			
<i>Epinephelus costae</i> (N)	x				x	
<i>Epinephelus marginatus</i> (N)	x		x		x	
<i>Fistularia commersonii</i> (A)					x	
<i>Lagocephalus sceleratus</i> (A)					x	x
<i>Muraena helena</i> (N)	x	x	x	x	x	x
<i>Mycteroperca rubra</i> (N)	x					
<i>Pomatomus saltatrix</i> (N)			x			
<i>Seriola dumerili</i> (N)	x		x		x	
<i>Sphyraena sphyraena</i> (N)	x		x		x	

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**Figure Captions**

632 **Fig. 1** Study locations. Two locations are situated in the Western Mediterranean, including  
633 the Cabo de Palos MPA in Spain and the Tavolara MPA in Italy. The third location is  
634 situated in the Eastern Mediterranean, including the Kaş - Kekova MPA in Turkey.

635 **Fig. 2** Mean rate (and 95% confidence interval) of consumption of tethered fish 120 min after  
636 the experimental unit was deployed. The estimation of the consumption rate was based  
637 on *in situ* visual observations from the tethering experiment. The red line corresponds  
638 to values in the no-take zone (NTZ) of the MPAs and the turquoise line to values in  
639 unprotected areas (OUT). Refer to Table 1 for location and species abbreviations.

640 **Fig. 3** Mean estimated rate (and 95% confidence interval) of a tethered fish being bitten  
641 within a) 30 minutes, b) 60 minutes, and c) 90 minutes after the experimental unit was  
642 deployed. The estimated rates were assessed based on video recording data. The red  
643 line corresponds to values in the no-take zone (NTZ) of the MPAs and the turquoise  
644 line to values in unprotected areas (OUT). Refer to Table 1 for location and species  
645 abbreviations.

646 **Fig. 4** Mean probability (and 95% confidence interval) of a tethered fish being bitten within  
647 90 minutes after the experimental unit was deployed a) distinguishing predators based  
648 on origin (alien *vs* native fish), and b) distinguishing predators based on origin and  
649 trophic level (high *vs* low-medium). The red line corresponds to alien species and the  
650 turquoise line to native species. Refer to Table 1 for location and species  
651 abbreviations.

652 **Fig. 5** Fish arrival time in the camera field of view represented in a) species accumulation  
653 curves through time, and b) box plots of number of species after 90 min of video  
654 deployment. In both panel figures the predators are distinguished by trophic level  
655 (High *vs* Low-Medium trophic level predators), location (CAB, TAV, KAS) and  
656 protection level (no-take zone: NTZ, unprotected areas: OUT). Solid lines in (a) are  
657 smoothed conditional means (method LOESS), and grey envelopes are the 95%  
658 Confidence Intervals. Dashed lines are site-specific curves (see also Table 1). The red  
659 colour corresponds to values in the no-take zone (NTZ) of the MPAs and the turquoise  
660 colour to values in unprotected areas (OUT). Refer to Table 1 for location and species  
661 abbreviations.

662 **Fig. 6** Time of first predator - tethered fish interaction (predator approaching the tethered fish  
663 with eventual partial or complete consumption) represented by a) species  
664 accumulation curves through time, and b) Box Plots of number of species after 90 min  
665 of video deployment. In both panel figures the predators are distinguished by trophic

666 level (High vs Low-Medium), location (CAB, TAV, KAS) and protection level (no-  
667 take zone: NTZ, unprotected areas: OUT). Solid lines in (a) are smoothed conditional  
668 means (method LOESS), and grey envelopes are 95% Confidence Intervals. Dashed  
669 lines are site-specific curves (see also Table 2). The red colour corresponds to values  
670 in the no-take zone (NTZ) of the MPAs and the turquoise colour to values in  
671 unprotected areas (OUT). Refer to Table 1 for location and species abbreviations.





Cabo De Palos

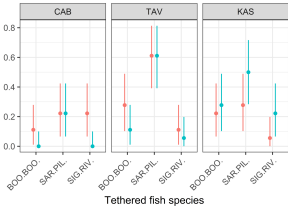
Tavolara

Kas-Kekova

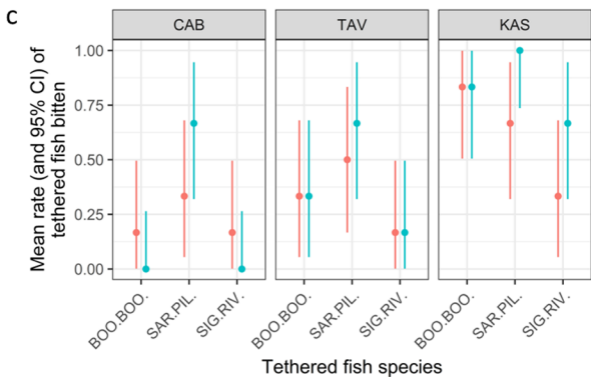
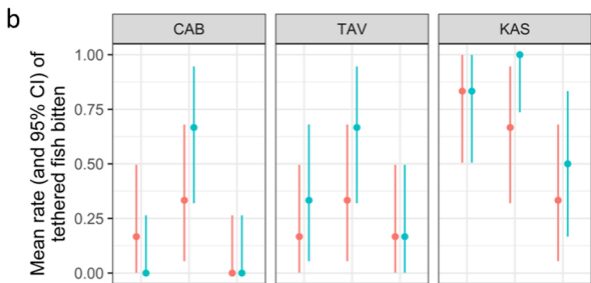
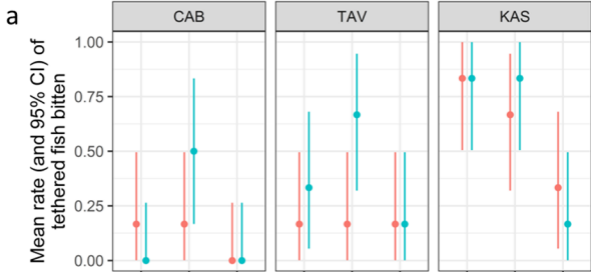
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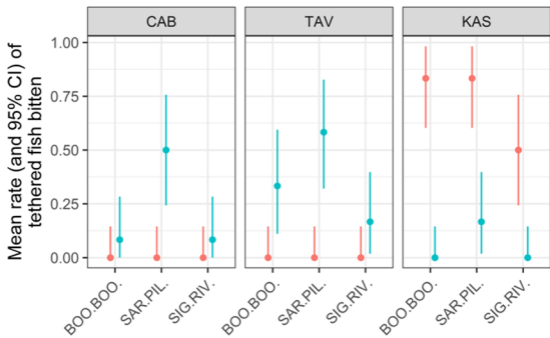
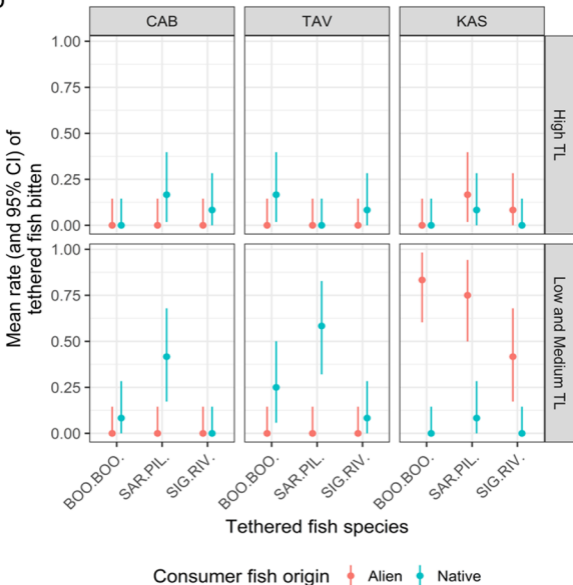
Mean rate (and 95% CI) of tethered fish consumed



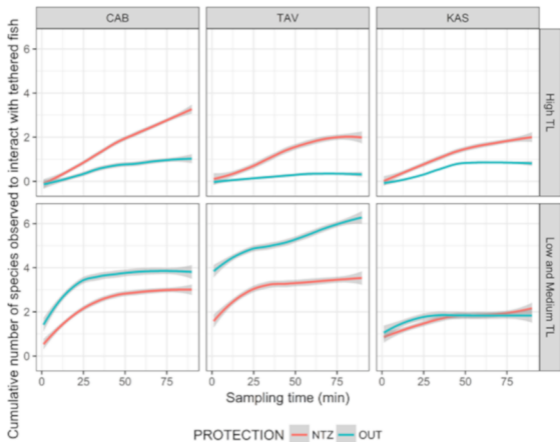
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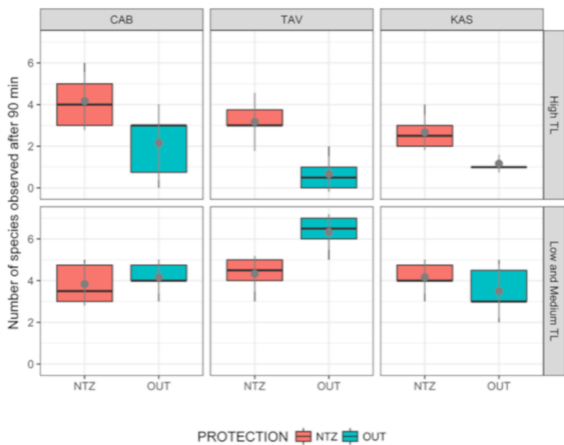
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**a****b**

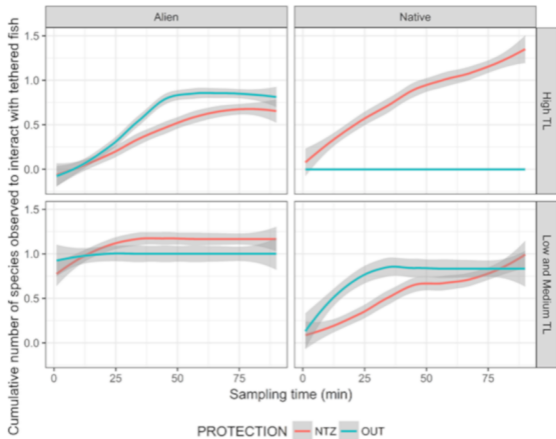
a



b



a



b

