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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 1st minute until the 90th 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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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	0.021	5.917	0.116	11.628	0.014	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	0.003	3.339	0.143	11.551	0.002	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	0.001	16.920	0.001	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	0.003	15.407	0.001	4.332	0.089	4.589	0.089
TAV vs KAS	22	2	11.853	0.028	8.795	0.020	2.805	0.221	0.254	0.635
LOCATION	33	2	29.303	0.001	16.920	0.001	4.029	0.147	8.353	0.046
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	0.001	15.407	0.002	3.104	0.084	8.263	0.015
TAV vs KAS	22	2	13.759	0.009	8.795	0.015	3.104	0.190	1.860	0.190
LOCATION	33	2	25.425	0.001	15.928	0.001	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	0.002	15.407	0.001	3.104	0.075	5.455	0.057
TAV vs KAS	22	2	11.476	0.014	6.511	0.041	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	Dev	p	Dev	p	Dev	p	Dev	p
	LOCATION	33	2	79.259	0.001	31.727	0.001	31.727	0.001	15.805	0.001
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	0.001	21.788	0.001	21.788	0.001	10.357	0.005
	TAV vs KAS	22	2	53.932	0.001	21.788	0.001	21.788	0.001	10.357	0.006
	LOCATION	33	2	14.958	0.034	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	0.005	6.351	0.044	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	0.001	31.727	0.001	26.992	0.001	12.711	0.002
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	0.001	21.788	0.001	18.259	0.001	8.263	0.010
	TAV vs KAS	22	2	48.310	0.001	21.788	0.001	18.259	0.001	8.263	0.013
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	0.021	4.736	0.127	7.607	0.042	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	0.009	4.589	0.074	7.368	0.025	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
Low & medium TL						
<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
High TL						
<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.



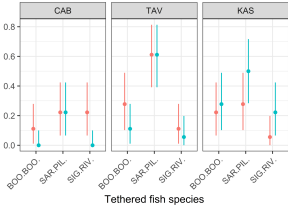
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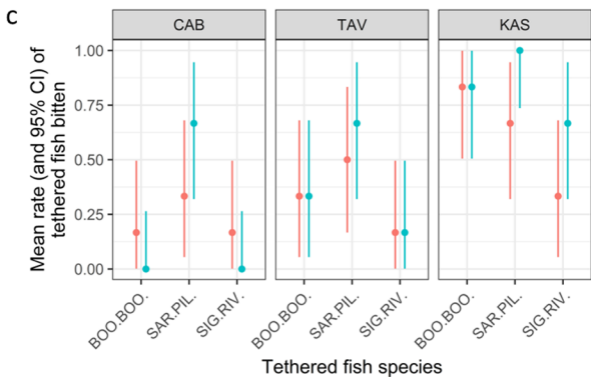
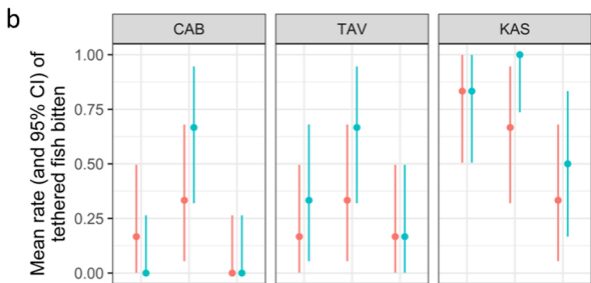
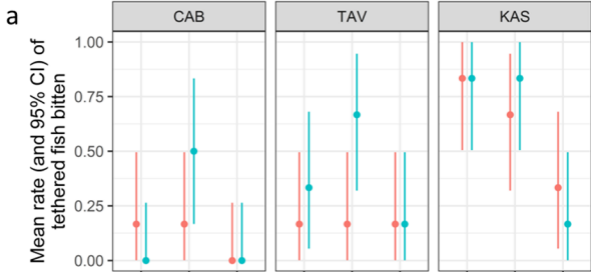
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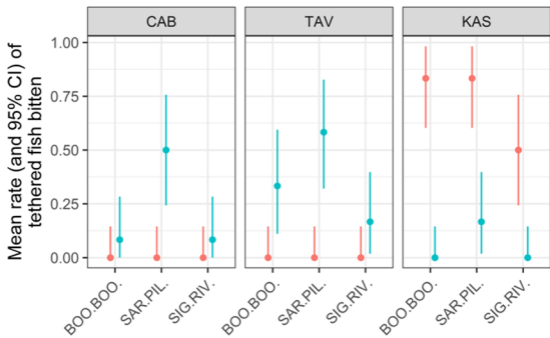
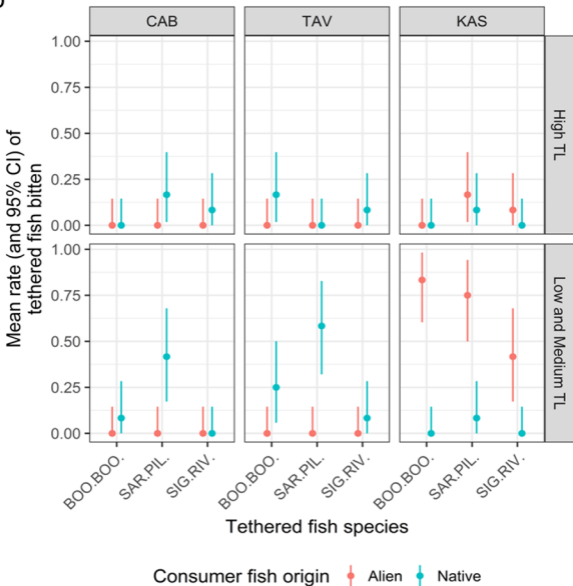
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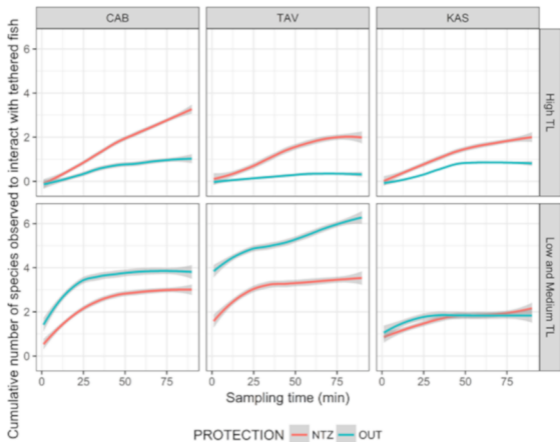
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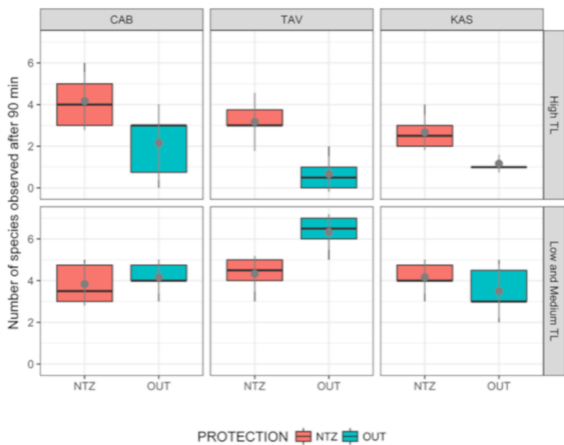
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a**b**

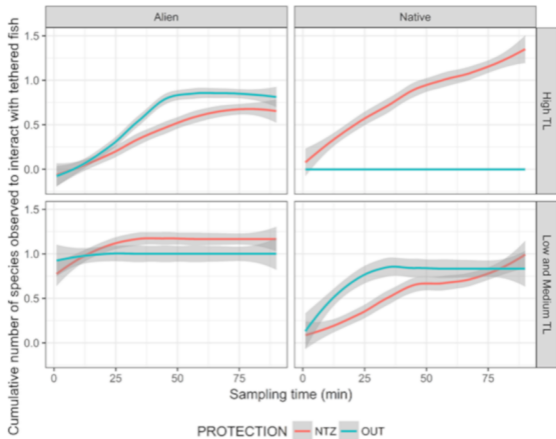
a



b



a



b

