

1 **Hard time to be parents? Sea urchin fishery shifts potential**
2 **reproductive contribution of population onto the shoulders of the**
3 **youngest.**

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15 **ABSTRACT**

16 **Background.** In Sardinia, as in other regions of the Mediterranean Sea, sustainable fisheries of
17 the sea urchin *Paracentrotus lividus* have become a necessity. At harvesting sites, the systematic
18 removal of large individuals (diameter ≥ 50 mm) seriously compromises the biological and
19 ecological functions of sea urchin populations. Specifically, in this study, we compared the
20 reproductive potential of the populations from two Mediterranean coastal sites which have
21 different levels of protection. The sites were Su Pallosu, where fishing pressure is high (take
22 zone) and at Tavolara-Punta Coda Cavallo Marine Protected Area (no-take zone) where the
23 pressure is negligible.

24 **Methods.** Reproductive potential was estimated by calculating Gonadosomatic Index (GSI) from
25 June 2013 to May 2014 both for individuals of commercial size (diameter without spines, $TD \geq$
26 50 mm) and the undersized ones with gonads ($30 \leq TD < 40$ mm and $40 \leq TD < 50$ mm). Gamete
27 Output was calculated for the commercial-size class and the undersized individuals with fertile
28 gonads ($40 \leq TD < 50$ mm) in relation to their natural density (Gamete Output per m^2).

29 **Results.** The reproductive potential of populations was slightly different at the beginning of the
30 sampling period but it progressed at different rates with an early spring spawning event in the
31 take zone and two gamete depositions in early and late spring in the no-take zone. For each fertile
32 size class, GSI values changed significantly during the year of our study and between the two
33 levels of protection. Although the multiple spawning events determined a two-fold higher total
34 Gamete Output of population (popTGO) in the no-take zone, the population Mean Gamete
35 Output (popMGO) was similar in the two zones. In the take zone, the commercial-sized

36 individuals represented approximately 5% of the population, with almost all the individuals
37 smaller than 60 mm producing an amount of gametes nearly three times lower than the
38 undersized ones. Conversely, the high density of the undersized individuals released a similar
39 amount of gametes to the commercial-size class in the no-take zone.

40 **Discussion.** Overall, the lack of the commercial-size class in the take zone does not seem to be
41 very alarming for the self-supporting capacity of the population, and the reproductive potential
42 contribution seems to depend more on the total density of fertile sea urchins than on their size.
43 However, since population survival in the take zone is supported by the high density of
44 undersized sea urchins between 30 and 50 mm, management measures should be addressed to
45 maintain this size and to shed light on the source of the larval supply.

46

47 INTRODUCTION

48 Commercial fisheries are one of the main causes of the deterioration of marine communities
49 (Jackson *et al.*, 2001). The systematic removal of fishery resources drastically reduces natural
50 stocks and changes growth, production and recruitment of target species (Pinnegar *et al.*, 2000).
51 In extreme cases, overfished populations are still present in the community but no longer interact
52 significantly with other species (Estes, Duggins & Rathbun, 1989). This phenomenon can lead to
53 a simplification of trophic webs with dramatic consequences for marine ecosystems (Pauly, 1995;
54 Myers & Worm, 2003; Coll, Lotze & Romanuk, 2008a; Coll, Palomera & Tudela, 2009a; Coll *et*
55 *al.*, 2009b; Lotze, Coll & Dunne, 2011; Navia *et al.*, 2012; Tunca *et al.*, 2016). One of the
56 clearest examples reported for several temperate coastal systems is the overexploitation of target

57 species involved in the typical tri-trophic interaction “fish-sea urchins-macrophyte”. Trophic
58 relationships can be altered by overfishing predatory fishes. This triggers an uncontrolled
59 proliferation of sea urchins which then leads to an overgrazing of algal cover (Sala,
60 Boudouresque & Harmelin-Vivien, 1998; Steneck *et al.*, 2002; Steneck, Vavrinec & Leland,
61 2004). Although this is the main effect of overfishing on many Mediterranean coasts (Sala &
62 Zabala, 1996; Sala, Boudouresque & Harmelin-Vivien, 1998), trophic imbalances have also been
63 found to act in the opposite direction. Indeed, over the last 2-3 decades, the general decline of
64 natural fish stocks has led to a focus on new target species, often further down the food web. In
65 many regions sea urchin harvesting was added to higher-trophic-level fisheries (Anderson *et al.*,
66 2011). This is the case of some regions in the South of Italy where the edible sea urchin
67 *Paracentrotus lividus* (Lamarck, 1816) is subjected to high fishing pressure (Tortonese, 1965;
68 Guidetti, Terlizzi & Boero, 2004; Pais *et al.*, 2007). The most striking effect of sea urchin fishing
69 is the rapid decrease in commercial resources in terms of total density and abundance (Andrew *et*
70 *al.*, 2002; Bertocci *et al.*, 2014). A remarkable reduction in density and mean size of the sea
71 urchin population would have dramatic consequences for the whole benthic community. For
72 instance, the removal of hundreds of thousands of sea urchins from the temperate reef has
73 coincided with the rapid development of brown algae that has led to substantial changes in the
74 abundance of fish and benthic invertebrates (Bell *et al.*, 2014).

75 Furthermore, the decline of sea urchin populations due to harvesting by humans could be even
76 more significant because of the loss of sexually mature individuals that contribute to the local
77 recruitment pool (Levitani & Sewell, 1998). Indeed, gonads are proportional to a sea urchin’s
78 body-size and are more mature and developed in large sea urchins (Mita *et al.*, 2007). The
79 Gonadosomatic Index (GSI) is generally used to evaluate the reproductive features of echinoids

80 such as fluctuations in gonad size and spawning periods (Spirlet, Grosjean & Jangoux, 1998;
81 Shpigel *et al.*, 2004; Gianguzza *et al.*, 2013) and these relate to the reproductive potential of the
82 individual (Brewin *et al.*, 2000). Fertile size classes can produce more than one cohort of mature
83 gametes in a single breeding season (Mita *et al.*, 2007) and the reproductive cycle generally has
84 one or two seasonal GSI peaks (see reviews in Boudouresque & Verlaque, 2007 and Ouréns,
85 Fernandez & Freire, 2011). Sometimes there can be continuous spawning events of lower
86 significance and with strong dependence on the variability of the gametogenesis (Boudouresque
87 & Verlaque, 2007). Generally GSI shows considerable spatial and temporal variability as a result
88 of extrinsic factors such seasonal changes in photoperiod, water temperature and phytoplankton
89 blooms (see Ouréns, Fernandez & Freire, 2011 and references therein). Food quality and
90 availability can also influence the reproductive cycle (Byrne, 1990; Brady & Scheibling, 2006) as
91 well as hydrodynamic conditions (Lozano *et al.*, 1995; Meidel & Scheibling, 1998; Guettaf, San
92 Martin & Francour, 2000; Sellem & Guillou, 2007; Gianguzza *et al.*, 2013).

93 In Sardinia (Italy, Western Mediterranean), commercial fishing of the sea urchin *Paracentrotus*
94 *lividus* is limited by law to specimens larger than 50 mm test diameter (TD), from November to
95 April. However, despite regional decrees concerning fishing periods, minimum size and catch
96 quotas per day per fisherman, the harvesting of *P. lividus* is intensively practiced. Removal by
97 occasional recreational fishermen occurs throughout the year because of the long tourist season
98 (Pais *et al.*, 2007). The systematic removal of the largest sea urchins may decrease the number of
99 fertile individuals that release gametes into the surrounding environment. This leads to a
100 population collapse, as reported for some overfished areas (Pais *et al.*, 2007). The aim of this
101 work is to point out the differences between the reproductive potential of populations living in a
102 harvesting zone (take zone) and those in a Marine Protected Area (no-take zone). Through the

103 evaluation of the GSI over one year, we compared the Annual Gamete Output (Brewin *et al.*,
104 2000) of the commercial-size class (diameter without spines, $TD \geq 50\text{mm}$) and the fertile,
105 undersized one ($40 \leq TD < 50 \text{ mm}$) of both populations under different fishing pressures and
106 levels of protection. Our hypothesis supports that, under unchecked fishing pressure, the
107 contribution of the commercial-size class to reproductive potential drastically decreases and, as a
108 result, the risk of a population collapse increases.

109

110 **MATERIALS AND METHODS**

111 **Study sites and sea urchin sampling**

112 The reproductive potential of the *Paracentrotus lividus* populations was examined in two zones
113 of Sardinia that differ in sea urchin fishing pressure (Fig. 1). Su Pallosu (central-western
114 Sardinia, $40^{\circ} 03' \text{ N}$; $008^{\circ} 25' \text{ E}$) is subjected to very high pressure which is widespread across
115 the entire bay (take zone). In this area, harvesting is practised from November to April by 189
116 professional fishermen authorized by regional decree. Each one of them is allowed to collect up
117 to 3000 sea urchins ($TD \geq 50 \text{ mm}$) per day. On the contrary, the Marine Protected Area of
118 Tavolara – Punta Coda Cavallo (north-eastern Sardinia, $40^{\circ} 53' \text{ N}$; $009^{\circ} 40' \text{ E}$) is considered a
119 no-take zone. Sea urchin fishing is strongly restricted here and populations are well conserved
120 (Guala *et al.*, 2011). In both of these zones, sea urchin specimens were collected in two areas, at
121 a distance of 1-3 kilometers apart.

122 Sampling (approved by the Regione Autonoma Sardegna through the release of the fishing
123 license for scientific purposes n. 9727/AP SCIE/N.7 03/06/2016) involved all sea urchin size

124 classes that have gonads and contribute to the reproductive potential of the population.

125 Specifically, once a month, we collected 10 individuals of the commercial size (CS, TD \geq 50
126 mm), 5 undersized individuals (US, $40 \leq$ TD $<$ 50 mm) and 5 smaller, undersized sea urchins
127 (Small-US, $30 \leq$ TD $<$ 40 mm) for an entire year.

128 The sea urchins were collected by SCUBA diving over a rocky bottom covered in photophilic
129 algal communities at a depth of five meters. Sea urchin samples were wrapped in cloth soaked in
130 salt water, stocked in iceboxes and immediately transported to the laboratory.

131

132 **Environmental features**

133 Despite the differences in position along the Sardinian coast and in wind exposure, sampling was
134 performed in areas with similar environmental features (i.e. depth, slope, shelter from the waves),
135 on rocky bottoms.

136 Differences in food availability, potentially able to affect sea urchin gonad growth (Byrne, 1990;
137 Brady & Scheibling, 2006), were assessed as temporal changes of algal cover between zones.

138 Digital photographs were taken over three PVC quadrats of 50 x 50 cm which were randomly
139 placed on the sea bottom in each area. This was done three times (July 2013, January and May
140 2014) during the surveyed year. Image analysis was carried out using Seascape® software
141 (Segmentation and Cover Classification Analyses of Seabed Images, Teixidó *et al.*, 2011) to
142 detect the percentage cover of conspicuous algal taxa or morphological groups. A permutational
143 multivariate analyses of variance (PERMANOVA, Anderson, 2001a) was done, on the basis of a
144 Bray-Curtis dissimilarity matrix calculated from square-root transformed data (Primer-E 6
145 Permanova®), to estimate the variability of assemblages between the take and no-take zones over

146 time. A 3-way model was used with Time (random, 3 levels) and Protection (fixed, 2 levels, take
147 vs. no-take) as crossed factors, and Area (2 levels) as a random factor nested in Protection. P-
148 values were obtained through Monte Carlo random draws from the asymptotic permutation
149 distribution (Anderson, 2005) and a pairwise test was used to discriminate between various levels
150 of significant factors. A non-metric Multi-Dimensional Scaling (nMDS) ordination was used as a
151 graphical representation of data.

152 In order to investigate any potential thermic anomalies, seasonal variations of the coastal water
153 temperature were monitored with the “Mediterranean Sea – High Resolution and Ultra High
154 Resolution L3S Sea Surface Temperature” product ([http://marine.copernicus.eu/web/69-
155 myocyan-interactive-catalogue.php](http://marine.copernicus.eu/web/69-myocyan-interactive-catalogue.php)). Daily sea surface temperatures (SST) were extrapolated
156 from the catalogue after choosing an intermediate point between the two sampling areas within
157 the zones, then mean monthly temperatures were obtained and used to represent the annual trend.

158

159 **Gonadosomatic Index and fertility**

160 The Gonadosomatic Index (GSI) was examined every month from June 2013 to May 2014 (with
161 the exception of November due to adverse weather conditions) for all of the three sampled size
162 classes. Sea urchins were allowed to drip dry for some minutes and then weighed. The test
163 diameter (TD) without spines was measured and the gonads were successively extracted and
164 weighed as well.

165 GSI was calculated by the formula: $GSI = [\text{gonad wet weight} / \text{total wet weight}] \times 100$ (Sánchez-
166 España, Martínez-Pita & García, 2004).

167 The fertility of the US individuals was tested according Brundu *et al.* (2016) during the months
168 of maximum gonadal development (i.e. from December to April). Gonads from females were
169 extracted and gently shaken in filtered seawater to allow the mature ova to come out. Sperm was
170 then added and finally, the fertility of the individuals was assessed as a percentage of the
171 effectively fertilized eggs after the appearance of the fertilization membrane (fertilization was
172 considered successful if it took place in at least 80% of the eggs according Falugi & Angelini,
173 2002). The percentage of achievement of the first larval stage (development of four-arm
174 echinopluteus) was also measured. A fertility test was not carried out on individuals belonging to
175 the Small-US class because of the paucity of their gonads (see “Results”). Nor was it done on the
176 commercial-sized individuals because they were assumed to be fertile (i.e. Ouréns, Fernández &
177 Freire, 2011).

178 Monthly mean values of GSI were calculated for the two zones and areas of sampling. A four-
179 way ANOVA (using Statistica 6.0, Statsoft Inc.) was performed to highlight the differences in
180 GSI values in different months (fixed and orthogonal factor, 11 levels), protection levels (fixed
181 and orthogonal factor, 2 levels), areas (random and nested in protection, 2 levels), and fertile size
182 classes (fixed and orthogonal factor, 2 levels, CS and US). Eight replicates for each size class
183 were haphazardly selected from among those available in order to get a balanced design.
184 Cochran’s C test was used to check for the assumption of homogeneity of variances and a
185 posteriori SNK tests were performed to find alternative hypotheses (Underwood, 1997).

186

187 **Population structure and potential reproductive contribution**

188 Abundance and size-frequency distribution of the populations were estimated for both take and
189 no-take zones by counting all sea urchins found in the PVC plots and measuring them with
190 calipers. The plots were 50 x 50 cm and were placed randomly as many times as necessary to
191 cover two replicates of 25 m² in each area (100 m² for each zone).

192 Sea urchin abundance was estimated as the total density (individuals m⁻²) and the density of each
193 10 mm size class: TD < 10 mm, 10 ≤ TD < 20 mm, 20 ≤ TD < 30 mm, 30 ≤ TD < 40 mm, 40 ≤
194 TD < 50 mm and 50 ≤ TD < 60 mm, TD ≥ 60 mm. Size-class densities were then translated into
195 frequency percentages and used to compose the population structure for each level of protection
196 (take and no-take zones). The Gamete Output and the Spawning Magnitude of each spawning
197 event were calculated for the fertile CS and US classes according to Brewin *et al.* (2000). The
198 highest and the lowest mean monthly GSI recorded during the year of sampling corresponded to
199 the period just before the beginning (pre-spawning) and after the end (post-spawning) of the
200 spawning events. We defined the mean Individual Gamete Output (IGO), in units of gamete wet
201 weight per urchin per spawning event (g g⁻¹ se⁻¹), as the difference of the mean monthly pre-
202 spawning GSI and the mean monthly post-spawning GSI. The Spawning Magnitude was defined
203 as the percentage ratio of the mean Individual Gamete Output and the mean monthly pre-
204 spawning GSI.

205 For each spawning event, we calculated the Gamete Output per m² (GO, g g⁻¹ m⁻² se⁻¹), released by
206 fertile size classes in take and no-take zones, as their IGO multiplied for the respective natural
207 density. The Total Gamete Output (TGO) and the Mean Gamete Output (MGO), used to estimate
208 the reproductive contribution of each size class per m² per year, were defined respectively as the
209 sum and the average of GO (g g⁻¹ m⁻² yr⁻¹). Finally, the potential reproductive contribution of the

210 whole population in both take and no-take zones (popTGO and popMGO, respectively) were
211 calculated for the investigated year as the sum of the contributions of both fertile size classes.

212

213 **RESULTS**

214 **Environmental features**

215 Multivariate analysis on algal cover has detected a significant interaction between Time and
216 Protection. More specifically, pairwise tests highlighted that algae where sea urchins live
217 changed significantly over time but not between take and no-take zones (Table S1). The nMDS
218 showed that plots of the take zone were interspersed on the graph with those of the no-take zone
219 (Fig. S1). Also, the water temperature changed over time but no difference was observed in the
220 annual trend between the two zones studied (Fig. S2).

221

222 **Gonadosomatic Index and fertility**

223 At each area, 220 specimens of the commercial-size class (CS) and 110 undersized and smaller
224 undersized individuals (US and Small-US) were randomly collected over a year to compare
225 reproductive potential between populations. Regarding CS individuals, sea urchins with $TD \geq 60$
226 mm were 24 of 440 (5.5%) in the take zone and 219 of 417 (53%) in the no-take zone.
227 Fertility tests showed that 100% of the undersized individuals (US) checked were fertile and
228 contributed to the reproductive potential of the populations, with high percentages of fertilized
229 eggs (ranging from 87 to 96%) and developing larvae (ranging from 95 to 100%) (Table S2).

230 Conversely, the Small-US individuals had reduced gonads (Fig. 2), and their contribution to the
231 reproductive potential of the population can be considered negligible.
232 The GSI trend over the year was generally higher for CS individuals than US ones for both levels
233 of protection (Fig. 2). At the take zone, we recorded a single large spawning period from March
234 to May for both fertile classes (see Fig. 2a). In pre-spawning time, GSI values reached $6.6 \pm 0.3\%$
235 (mean \pm standard error) and $4.4 \pm 0.4\%$, while in post-spawning time values were $1.3 \pm 0.2\%$ and
236 $1.6 \pm 0.1\%$ for the CS and US classes respectively (Fig. 2a).

237 At the no-take zone, a spawning event was observed twice (Fig. 2b). The first one was recorded
238 from June to December with a pre-spawning GSI of $6.7 \pm 0.3\%$ and $5.2 \pm 0.5\%$, and a post-
239 spawning GSI of $2.5 \pm 0.3\%$ and $1 \pm 0.2\%$ for the CS and US classes respectively. The second
240 spawning event occurred from February to April with a pre-spawning GSI of $5.4 \pm 0.4\%$ and a
241 post-spawning GSI of $2.5 \pm 0.2\%$ for the CS class. From February to May we observed a pre-
242 spawning GSI of $4 \pm 0.6\%$ and a post-spawning GSI of $1.2 \pm 0.2\%$ for the US class (Fig. 2b).

243 ANOVA highlighted significant differences during the year and between the different size
244 classes, while there were no major statistical differences found between sampling areas. A
245 significant interaction between Protection Level and Size Class was detected, and the GSI of the
246 CS class was significantly higher than that of the US individuals for both zones. Between
247 protection levels, the CS class had higher GSI values in the no-take zone while no differences
248 were found for the US individuals (Table 1). A significant interaction was also found between
249 Month and Size Class with SNK pointing out significantly higher GSI values for the CS
250 individuals during the whole sampling year excluding April (Table 1).

251

252 **Population structure and potential reproductive contribution**

253 Size class distribution was consistently different between take and no-take zones (Fig. 3). In the
254 take zone, sea urchin density was almost two-fold higher than in the no-take zone: 10 ± 1.4 and
255 5.4 ± 0.5 individuals per m^2 respectively. Sea urchins with TD ranging from 0 to 20 mm were 1.7
256 ± 0.1 per m^2 and 1.1 ± 0.6 per m^2 in the take and no-take zones respectively, and they represent
257 17% and 21% of their populations. The most abundant size classes were those ranging from 20 to
258 50 mm diameter (77%) with a density of 3 ± 0.6 and 3 ± 0.3 individuals per m^2 for the Small-US
259 and US classes respectively ($30 \leq \text{TD} < 40$ mm and $40 \leq \text{TD} < 50$ mm). The proportion of
260 individuals of the CS class with respect to the entire population was 6% (0.6 ± 0.2 individuals per
261 m^2) and all the individuals were included in the range of $50 \leq \text{TD} < 60$ mm (Fig.3). In the no-take
262 zone, the individuals between 20 and 50 mm represented 28% of the population while the CS
263 class was 52% with 1 ± 0.4 and 2 ± 0.2 individuals per m^2 for $50 \leq \text{TD} < 60$ mm and $\text{TD} \geq 60$
264 mm respectively (Fig. 3).

265 In relation to the population structure, the reproductive contribution was compared between the
266 take and no-take zones, but no comparisons were made between the sampling areas because no
267 differences were found (see Table 1). The potential reproductive contribution was calculated
268 according to the number of spawning events during the surveyed year and the natural density of
269 the fertile size classes (US and CS). In the take zone, a single spawning event occurred and the
270 Spawning Magnitude for the year was 73% on average (Table 2) with an Individual Gamete
271 Output (IGO) of 0.03 and $0.05 \text{ g g}^{-1} \text{ se}^{-1}$ for the US and CS individuals, respectively (Table 2).
272 The Gamete Output (GO), calculated in relation to the natural density of this zone, was 0.08 g g^{-1}
273 $\text{m}^{-2} \text{ se}^{-1}$ for the US sea urchins (2.7 ± 0.3 individuals per m^2) and $0.03 \text{ g g}^{-1} \text{ m}^{-2} \text{ se}^{-1}$ for the CS
274 class (0.6 ± 0.2 individuals per m^2). Because of the single spawning event, the Total Gamete
275 Output per m^2 (TGO) overlaps the Mean Gamete Output per m^2 (MGO) (Table 2). Accordingly,

276 the Total Gamete Output of the whole population (i.e. sum of TGO of the two fertile size classes)
277 corresponded to the Mean Gamete Output for m^2 (popMGO) with a value of $0.11 \text{ g g}^{-1} \text{ m}^{-2} \text{ yr}^{-1}$
278 (Table 2).

279 Conversely, in the no-take zone, two spawning events were observed (Fig. 2b). Spawning
280 Magnitude varied from 54 to 81% with higher values for US individuals (Table 2). IGO was
281 similar for both size classes with values ranging from 0.03 to $0.04 \text{ g g}^{-1} \text{ se}^{-1}$ according to the
282 spawning period. The GO of the US individuals was $0.02 \text{ g g}^{-1} \text{ m}^{-2} \text{ se}^{-1}$ during the first spawning
283 event and $0.01 \text{ g g}^{-1} \text{ m}^{-2} \text{ se}^{-1}$ during the second one, while it was 0.11 and $0.08 \text{ g g}^{-1} \text{ m}^{-2} \text{ se}^{-1}$ for the
284 CS class. Total Gamete Output (TGO) and Mean Gamete Output (MGO) of the US individuals,
285 whose density was 0.4 ± 0.1 individuals per m^2 , were 0.03 and $0.01 \text{ g g}^{-1} \text{ m}^{-2} \text{ yr}^{-1}$ respectively.
286 Meanwhile they were 0.19 and $0.10 \text{ g g}^{-1} \text{ m}^{-2} \text{ yr}^{-1}$ for the CS class, whose natural density was 2.7
287 ± 0.3 individuals per m^2 . Consequently, the Total Gamete Output of the whole population was
288 estimated to be $0.22 \text{ g g}^{-1} \text{ m}^{-2} \text{ yr}^{-1}$ and the total Mean Gamete Output was $0.11 \text{ g g}^{-1} \text{ m}^{-2} \text{ yr}^{-1}$
289 (Table 2).

290 DISCUSSION

291 The results showed a considerable difference between the take and no-take zones in population
292 structure and in abundance of sea urchins, as well as in the Gonadosomatic Index trend
293 throughout the surveyed year. As consequence, even the potential reproductive contribution
294 differed between the populations subjected to different levels of sea urchin harvesting.

295 While recruits ($TD < 20 \text{ mm}$) had the same proportion of population structure, the density of the
296 fertile undersized individuals (US) was 4.5-fold higher at the take zone than at the no-take one,
297 and the density of the commercial size (CS) was 6.8-fold lower. Furthermore, within the

298 commercial size, a large percentage was composed of by individuals ≥ 60 mm at the no-take
299 zone, while they were nearly absent at the take zone (none have been detected during the
300 sampling for the estimation of the population structure, only 24 throughout the annual sampling
301 for the GSI assessment).

302 Both populations showed a spawning event at the end of winter or at the beginning of spring,
303 which lasted until April-May for the two fertile size classes examined (US and CS). Moreover, a
304 second relevant peak of GSI was registered in the no-take zone which was demonstrated to be
305 concomitant with a spawning event from June 2013 (Siliani *et al.*, 2016) by histological analysis.
306 This is consistent with observations from other areas of the Mediterranean where one or two
307 annual spawning periods were commonly identified, regardless of the proportion of size classes
308 shaping the population structure (Fenaux, 1968; Semroud & Kada, 1987; Pedrotti & Fenaux,
309 1992; Pedrotti, 1993; Semroud, 1993; Lozano *et al.*, 1995; Fernandez & Boudouresque, 1997;
310 López *et al.*, 1998; Guettaf, San Martin & Francour, 2000; Leoni *et al.*, 2003; Martínez *et al.*,
311 2003; Sánchez-España, Martinez-Pita & Garcia, 2004; Tomas, Romero & Turon, 2004; Sellem &
312 Guillou, 2007). The differences identified in reproductive potential between the populations were
313 not ascribed to different environmental features since the sampling zones were similar in terms of
314 sheltering from waves and there were no differences found for either water temperature or algal
315 assemblages. At both zones, GSI of the Small US individuals was lower than 1% throughout the
316 year and its contribution to the reproductive potential of the population was considered
317 negligible. On the contrary, GSI of the two fertile size classes (US and CS) changed significantly
318 over the year and between the different levels of protection. Specifically, GSI of the CS class was
319 significantly higher than those of the US individuals and it was significantly higher in the no-take
320 zone than in the take zone.

321 As widely described in many other locations of the Mediterranean (Guidetti, Terlizzi & Boero,
322 2004; Gianguzza *et al.*, 2006; Pais *et al.*, 2007; Ceccherelli, Pinna & Sechi, 2009; Bertocci *et al.*,
323 2014), our study clearly points out how human predation adversely affects the population
324 structure in the take zone, truncating the adult cohorts with $TD \geq 50$ mm. The high presence of
325 commercial-size adults ($TD \geq 50$ mm) at the no-take zone, and in particular the high number of
326 individuals larger than 60 mm, confirmed this evidence. More interestingly, our results
327 demonstrate the existence of a strong connection between the contribution to the reproductive
328 potential of the fertile size classes and the level of pressure of commercial harvesting.

329 Since the mean density of the US individuals at the take zone was similar to the mean density of
330 the CS sea urchins at the no-take zone, and vice versa, the Mean Gamete Output of the population
331 (popMGO) was similar at the two zones but the contribution of the two fertile size classes was
332 specular. The US individuals were therefore the main producers of gametes at the take zone while
333 the CS ones were annihilated by harvesting. On the other hand, at the no-take zone, the main
334 producer was the CS class probably because the US individuals were strongly reduced by fish
335 predation as a response to protection measures (Sala, 1997; Hereu *et al.*, 2005; Parravicini *et al.*,
336 2010).

337 In contrast, the Total Gamete Output of the population (popTGO), which represented the annual
338 reproductive contribution of populations throughout the year, was two-fold higher at the no-take
339 zone. Mita *et al.* (2007) suggested that gonad size increases volumetrically with sea urchin test
340 diameter, with the largest body size implying the most mature and developed gonads (and the
341 consequent possibility to produce more than one cohort of gametes). Effectively, in marine
342 reserves, more fertile gametes are produced by the largest sea urchins compared to sites where

343 large sea urchins are lacking (Lundquist, 2000). Moreover, the very important effects related to
344 the individual's age and size are proven by studies conducted on different fish species that have
345 demonstrated how a greater age diversity in a spawning stock may extend spawning both
346 temporally and spatially. This, in consequence, increases the chances that more offspring
347 encounter favourable conditions for development (Secor, 2000; Berkeley *et al.*, 2004b; Fiorentino
348 *et al.*, 2008).

349 Despite the fact that a single year of study is not enough to unambiguously establish the
350 regularity of reproductive episodes throughout time, the number of spawning events we observed
351 at no-take and take zones are consistent, respectively, with previous GSI studies conducted at
352 Tavolara MPA (Guala, Simeone & Baroli, 2009) and in a location contiguous to Su Pallosu
353 (Baroli *et al.*, 2006) that is intensely affected by fishing (Marra *et al.*, 2016). Thus, since the
354 popMGO strongly depended on the density per m² of fertile individuals regardless of their size
355 class, it would be plausible that the continuous catch of commercial-sized sea urchins has led to a
356 reduction of spawning events due to a considerable decrease of the body size of the fertile
357 individuals. Hence, it might be reasonable to suppose that spawning events were favoured by the
358 well-structured spawning stock and larger sea urchins which are typical of protected populations.

359 In general, sea urchin population dynamics is driven by various ecological processes that operate
360 on different spatial and temporal scales. Larval supply fluctuates widely from region to region as
361 it is associated with oceanic currents (Fenaux, Cellario & Rassoulzadegan, 1988; Prado *et al.*,
362 2012). The success of settlements is influenced by local constraints linked to habitat (e.g. adult
363 abundance, presence of crustose algae, substrate rugosity) (Boudouresque & Verlaque, 2007,
364 Oliva *et al.*, 2016). Finally, predation becomes the prevalent mechanism of sea urchin population

365 control after settlement and serves as a critical bottleneck for urchin populations (Guidetti, 2004;
366 Hereu *et al.*, 2005; Farina *et al.*, 2009; Farina *et al.*, 2014; Boada *et al.*, 2015). In our case, the
367 lack of predatory fish in the take zone (Marra *et al.*, 2016; Oliva *et al.*, 2016) and the high
368 pressure of harvesting shifted the whole potential reproductive contribution onto the young,
369 fertile undersized sea urchins. On the contrary, in the no-take zone, it mostly depended on
370 commercial-sized individuals since predation in marine reserves significantly affects sea urchins
371 until they reach the size of 50 mm TD (Guidetti, 2004).

372 Therefore, the self-sustenance of the sea urchin populations did not change between zones with
373 different levels of fishing pressure. The high number of recruits at the take zone ensured an
374 availability of new juveniles for the immediate future and suggested an important larval input of
375 which the origin still has to be investigated. However, the two spawning events at the no-take
376 zone suggested a more abundant and successful gamete production than in the population which
377 was under strong fishing pressure. This is probably due to a well-structured spawning stock
378 which enhanced the resilience of the pristine population. Hence, in equal recruitment conditions,
379 when the density of fertile individuals decreased (e.g. as a result of intense commercial
380 harvesting or natural predation) the mean reproductive contribution consequently diminished.

381 Because of over-exploitation of *P. lividus* populations in several Mediterranean areas and their
382 ecological and economic relevance, sea urchin fisheries need urgent and effective regulation. Our
383 findings may have important implications for creating a management of sea urchin fisheries that
384 is effective in terms of the sustainable conservation of resources.

385 In a marine reserve context, where sea urchin populations have a normal bimodal structure
386 (Brundu *et al.*, 2013), the opening of commercial harvesting should be avoided (despite the

387 insistence of local fishermen) to prevent the depletion of fertile individuals, as a result of the
388 synergistic action with the natural predation, and the breakdown of the population. On the
389 contrary, in areas affected by fishing pressure, larger individuals are lacking (Baroli *et al.*, 2006),
390 but the survival of the fertile, intermediate-size classes, which therefore support the mean gamete
391 output of the whole population, is guaranteed by laws (as harvesting is authorized only for
392 individuals ≥ 50 mm) and by the absence of predatory fishes, which in turn are targeted by
393 commercial fishing. Our present findings suggest that the reproductive potential contribution of
394 the population does not depend on the size of fertile individuals but on their density. For this
395 reason, the harvesting of individuals between 40 and 50 mm should be avoided particularly in
396 over-fished locations, since they are the only ones capable of generating new life, as far as we
397 know without more information on the origin of the larvae present.

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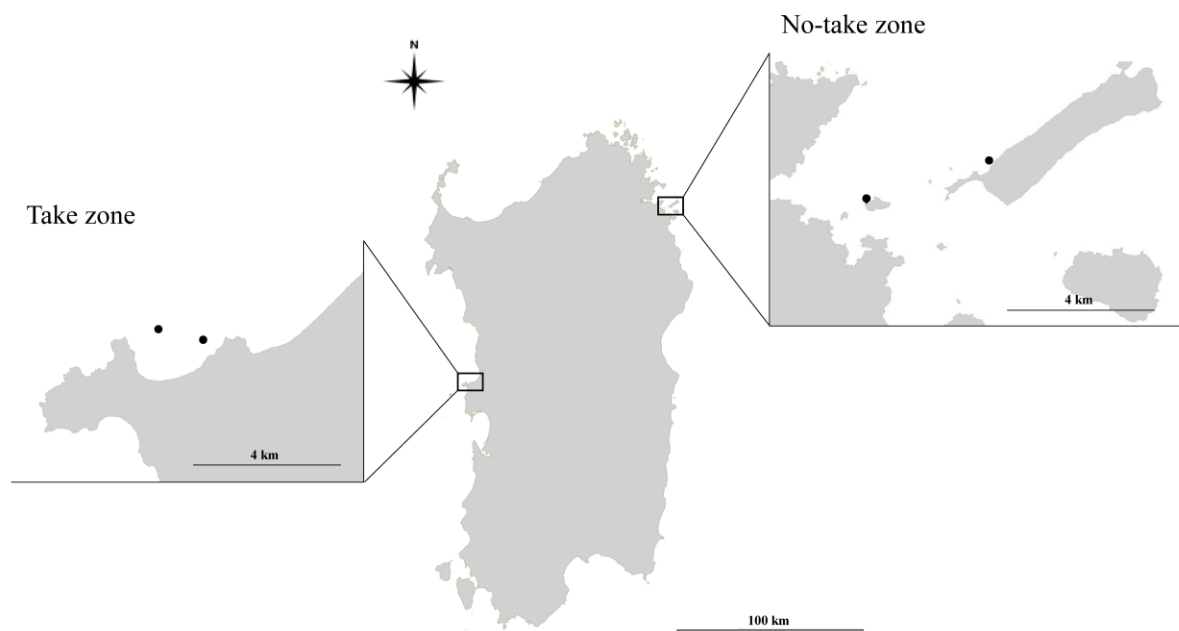
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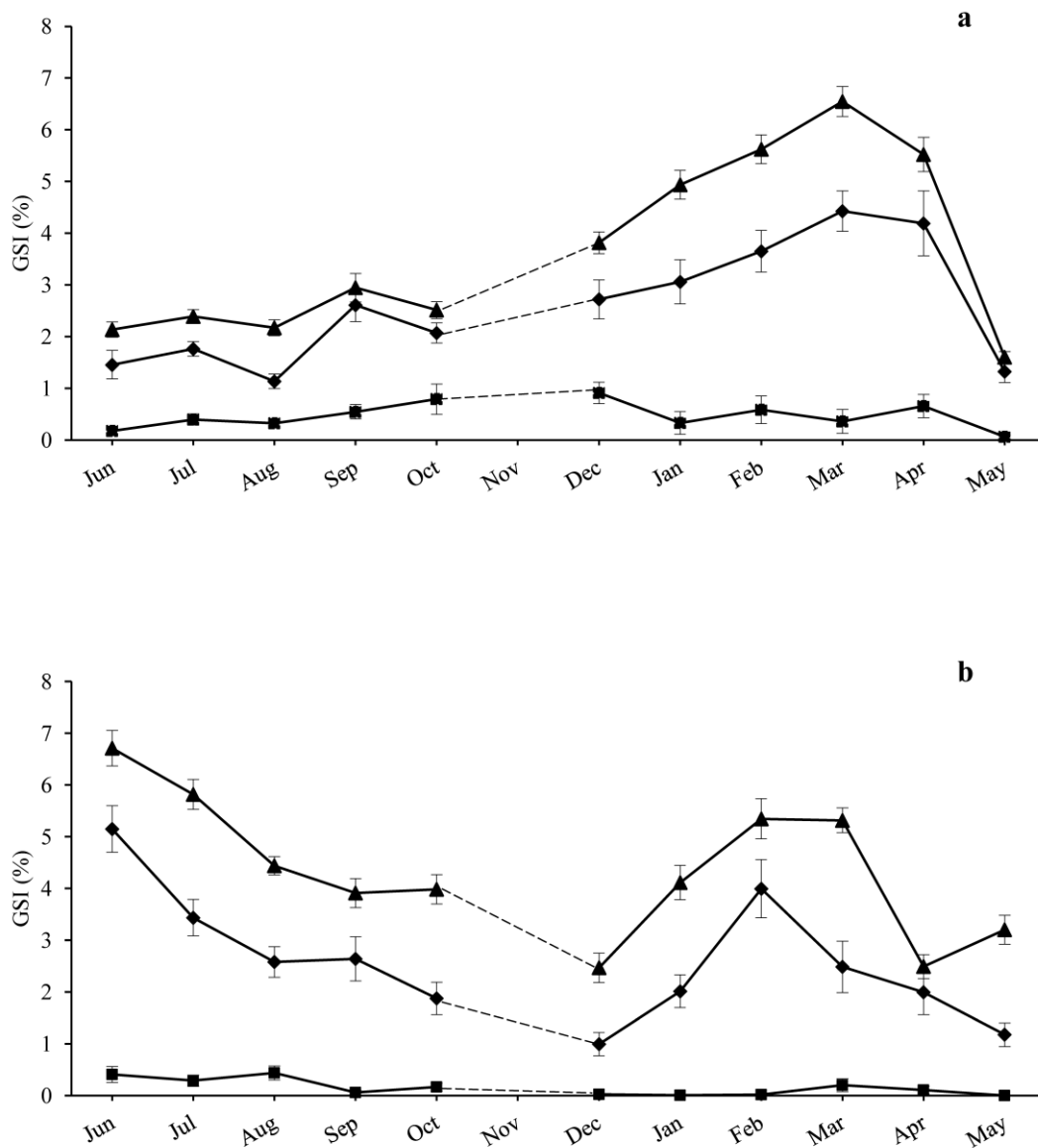
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626 **FIGURES**

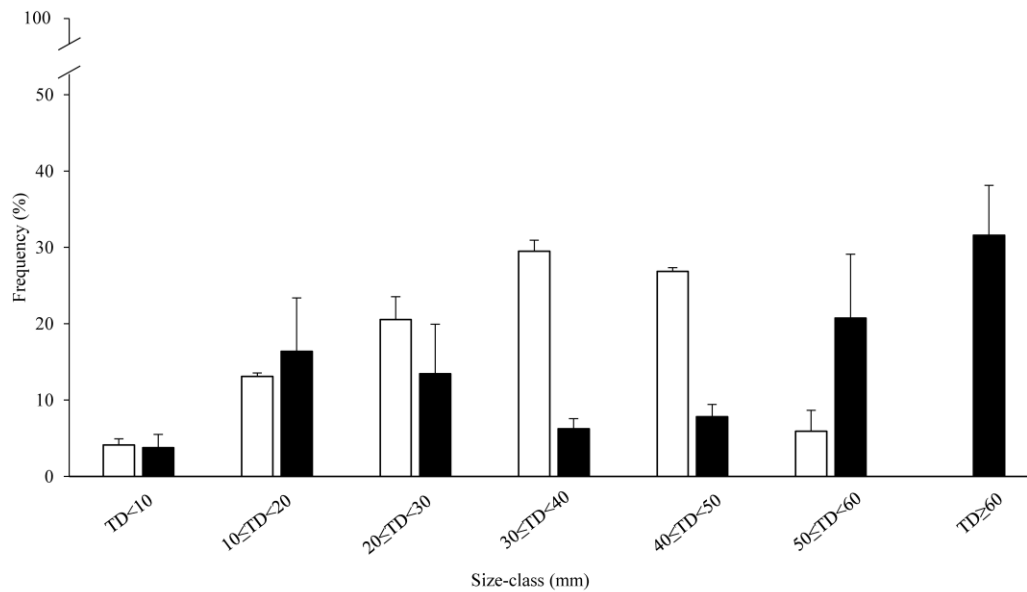
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628 Figure 1. Map of the study zones with different level of fishing pressure: take zone (Su Pallosu, central-
629 western Sardinia) and no-take zone (Tavolara-Punta Coda Cavallo Marine Protected Area, north-eastern
630 Sardinia). Sampling areas are indicated with black dots.

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 642 Figure 2. Annual trend of Gonadosomatic Index as mean (\pm standard error) of the three size classes
 643 examined (CS, US, Small-US) at a) take zone and b) no-take zone. Observation began in June 2013 and
 644 ended in May 2014. Dotted lines represent an approximation of the expected values of GSI for November
 645 2013 (no sampling was done). Triangles = CS; rhombuses = US; squares = Small-US.

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648 Figure 3. Size-frequency distribution (%) of sea urchin population at the take and no-take zones. The range
649 of the size classes is 10 mm of test diameter without spines (TD). Commercial size classes under fishing
650 pressure are those larger than 50 mm test diameter. White bars = take zone; black bars = no-take zone.

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661 **TABLES**

662 Table 1. Results of four-way ANOVA to test the effects on Gonadosomatic Index of Month, Protection and
 663 Size class (orthogonal fixed factors) and Area (random nested factor in Protection).

664 Bold value is statistically significant at $p < 0.05$. SNK tests for comparisons of significant interactions.

| Source of variation | <i>df</i> | MS | F | p |
|----------------------------------|-----------|-----------|--------|---------------|
| <i>Month = Mo</i> | 10 | 933.74 | 2.94 | 0.0193 |
| <i>Protection = Pr</i> | 1 | 11.883.74 | 14.26 | 0.0635 |
| <i>Area(Protection) = Ar(Pr)</i> | 2 | 833.46 | 2.27 | 0.1039 |
| <i>Size class = Sc</i> | 1 | 117763.64 | 362.25 | 0.0027 |
| <i>Mo x Pr</i> | 10 | 597.33 | 1.88 | 0.1104 |
| <i>Mo x Ar(Pr)</i> | 20 | 317.95 | 0.87 | 0.6301 |
| <i>Mo x Sc</i> | 10 | 2000.32 | 7.12 | 0.0001 |
| <i>Pr x Sc</i> | 1 | 13457.07 | 41.39 | 0.0233 |
| <i>Sc x Ar(Pr)</i> | 2 | 325.10 | 0.89 | 0.4126 |
| <i>Mo x Pr x Sc</i> | 10 | 648.71 | 2.31 | 0.0535 |
| <i>Sc x Mo x Ar(Pr)</i> | 20 | 281.10 | 0.77 | 0.7552 |
| <i>Residual</i> | 616 | 366.70 | | |

Transformation: none; Cochran's test $C = 0.0664$, $p < 0.05$

SNK
Pr x Sc: Take zone (US < CS)
 No-take zone (US < CS)
 US (Take zone = No-take zone)
 CS (Take zone < No-take zone)
Mo x Sc: April (US = CS)
 Other months (US < CS)

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668 Table 2. Timing and extent of mean monthly Gonadosomatic Index, Spawning Magnitude, and Gamete
 669 Output for US ($40 \leq TD < 50$ mm) and CS ($TD \geq 50$ mm) class, for spawning event and in relation with
 670 their natural density, and for the whole populations at take and no-take zone.

| | | Take zone | | No-take zone | | | |
|---|---------------|-----------|---------|--------------|---------|---------|---------|
| Size class | | US | CS | US | CS | US | CS |
| Spawning period | Start | Mar '14 | Mar '14 | Jun '13 | Feb '14 | Jun '13 | Feb '14 |
| | End | May '14 | May '14 | Dec '13 | May '14 | Dec '13 | Apr '14 |
| Mean monthly GSI (%) | Pre-spawning | 4.4 | 6.6 | 5.2 | 4.0 | 6.7 | 5.4 |
| | Post-spawning | 1.3 | 1.6 | 1.0 | 1.2 | 2.5 | 2.5 |
| IGO ($\text{g g}^{-1} \text{se}^{-1}$) | | 0.03 | 0.05 | 0.04 | 0.03 | 0.04 | 0.03 |
| Spawning magnitude (%) | | 70.5 | 75.8 | 80.8 | 70.0 | 62.7 | 53.7 |
| Natural density (ind m^{-2}) | | 2.7 | 0.6 | 0.4 | | 2.7 | |
| GO ($\text{g g}^{-1} \text{m}^{-2} \text{se}^{-1}$) | | 0.08 | 0.03 | 0.02 | 0.01 | 0.11 | 0.08 |
| TGO ($\text{g g}^{-1} \text{m}^{-2} \text{yr}^{-1}$) | | 0.08 | 0.03 | 0.03 | | 0.19 | |
| popTGO ($\text{g g}^{-1} \text{m}^{-2} \text{yr}^{-1}$) | | | 0.11 | | 0.22 | | |
| MGO ($\text{g g}^{-1} \text{m}^{-2} \text{yr}^{-1}$) | | 0.08 | 0.03 | 0.01 | | 0.10 | |
| popMGO ($\text{g g}^{-1} \text{m}^{-2} \text{yr}^{-1}$) | | | 0.11 | | 0.11 | | |

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 672 GO = Gamete Output per m^2 ; TGO = Total Gamete Output per m^2 ; popTGO = Total Gamete Output of the
 673 whole population per m^2 ; MGO = Mean Gamete Output per m^2 ; popMGO = Mean Gamete Output of the
 674 whole population per m^2 .