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

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ABSTRACT

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

Methods. Reproductive potential was estimated by calculating Gonadosomatic Index (GSI) from June 2013 to May 2014 both for individuals of commercial size (diameter without spines, TD ≥ 50 mm) and the undersized ones with gonads (30 ≤ TD < 40 mm and 40 ≤ TD < 50 mm). Gamete Output was calculated for the commercial-size class and the undersized individuals with fertile gonads (40 ≤ TD < 50 mm) in relation to their natural density (Gamete Output per m²).

Results. The reproductive potential of populations was slightly different at the beginning of the sampling period but it progressed at different rates with an early spring spawning event in the take zone and two gamete depositions in early and late spring in the no-take zone. For each fertile size class, GSI values changed significantly during the year of our study and between the two levels of protection. Although the multiple spawning events determined a two-fold higher total Gamete Output of population (popTGO) in the no-take zone, the population Mean Gamete Output (popMGO) was similar in the two zones. In the take zone, the commercial-sized
individuals represented approximately 5% of the population, with almost all the individuals smaller than 60 mm producing an amount of gametes nearly three times lower than the undersized ones. Conversely, the high density of the undersized individuals released a similar amount of gametes to the commercial-size class in the no-take zone.

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

**INTRODUCTION**

Commercial fisheries are one of the main causes of the deterioration of marine communities (Jackson *et al.*, 2001). The systematic removal of fishery resources drastically reduces natural stocks and changes growth, production and recruitment of target species (Pinnegar *et al.*, 2000). In extreme cases, overfished populations are still present in the community but no longer interact significantly with other species (Estes, Duggins & Rathbun, 1989). This phenomenon can lead to a simplification of trophic webs with dramatic consequences for marine ecosystems (Pauly, 1995; Myers & Worm, 2003; Coll, Lotze & Romanuk, 2008a; Coll, Palomera & Tudela, 2009a; Coll *et al.*, 2009b; Lotze, Coll & Dunne, 2011; Navia *et al.*, 2012; Tunca *et al.*, 2016). One of the clearest examples reported for several temperate coastal systems is the overexploitation of target
species involved in the typical tri-trophic interaction “fish-sea urchins-macrophyte”. Trophic relationships can be altered by overfishing predatory fishes. This triggers an uncontrolled proliferation of sea urchins which then leads to an overgrazing of algal cover (Sala, Boudouresque & Harmelin-Vivien, 1998; Steneck et al., 2002; Steneck, Vavrinec & Leland, 2004). Although this is the main effect of overfishing on many Mediterranean coasts (Sala & Zabala, 1996; Sala, Boudouresque & Harmelin-Vivien, 1998), trophic imbalances have also been found to act in the opposite direction. Indeed, over the last 2-3 decades, the general decline of natural fish stocks has led to a focus on new target species, often further down the food web. In many regions sea urchin harvesting was added to higher-trophic-level fisheries (Anderson et al., 2011). This is the case of some regions in the South of Italy where the edible sea urchin Paracentrotus lividus (Lamarck, 1816) is subjected to high fishing pressure (Tortonese, 1965; Guidetti, Terlizzi & Boero, 2004; Pais et al., 2007). The most striking effect of sea urchin fishing is the rapid decrease in commercial resources in terms of total density and abundance (Andrew et al., 2002; Bertocci et al., 2014). A remarkable reduction in density and mean size of the sea urchin population would have dramatic consequences for the whole benthic community. For instance, the removal of hundreds of thousands of sea urchins from the temperate reef has coincided with the rapid development of brown algae that has led to substantial changes in the abundance of fish and benthic invertebrates (Bell et al., 2014).

Furthermore, the decline of sea urchin populations due to harvesting by humans could be even more significant because of the loss of sexually mature individuals that contribute to the local recruitment pool (Levitan & Sewell, 1998). Indeed, gonads are proportional to a sea urchin’s body-size and are more mature and developed in large sea urchins (Mita et al., 2007). The Gonadosomatic Index (GSI) is generally used to evaluate the reproductive features of echinoids.
such as fluctuations in gonad size and spawning periods (Spirlet, Grosjean & Jangoux, 1998; Shpigel et al., 2004; Gianguzza et al., 2013) and these relate to the reproductive potential of the individual (Brewin et al., 2000). Fertile size classes can produce more than one cohort of mature gametes in a single breeding season (Mita et al., 2007) and the reproductive cycle generally has one or two seasonal GSI peaks (see reviews in Boudouresque & Verlaque, 2007 and Ouréns, Fernandez & Freire, 2011). Sometimes there can be continuous spawning events of lower significance and with strong dependence on the variability of the gametogenesis (Boudouresque & Verlaque, 2007). Generally GSI shows considerable spatial and temporal variability as a result of extrinsic factors such seasonal changes in photoperiod, water temperature and phytoplankton blooms (see Ouréns, Fernandez & Freire, 2011 and references therein). Food quality and availability can also influence the reproductive cycle (Byrne, 1990; Brady & Scheibling, 2006) as well as hydrodynamic conditions (Lozano et al., 1995; Meidel & Scheibling, 1998; Guettaf, San Martin & Francour, 2000; Sellem & Guillou, 2007; Gianguzza et al., 2013).

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

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evaluation of the GSI over one year, we compared the Annual Gamete Output (Brewin et al., 2000) of the commercial-size class (diameter without spines, TD ≥ 50 mm) and the fertile, undersized one (40 ≤ TD < 50 mm) of both populations under different fishing pressures and levels of protection. Our hypothesis supports that, under unchecked fishing pressure, the contribution of the commercial-size class to reproductive potential drastically decreases and, as a result, the risk of a population collapse increases.

MATERIALS AND METHODS

**Study sites and sea urchin sampling**

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

Sampling (approved by the Regione Autonoma Sardegna through the release of the fishing license for scientific purposes n. 9727/AP SCIE/N.7 03/06/2016) involved all sea urchin size
classes that have gonads and contribute to the reproductive potential of the population.

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

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

Environmental features

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

Differences in food availability, potentially able to affect sea urchin gonad growth (Byrne, 1990; Brady & Scheibling, 2006), were assessed as temporal changes of algal cover between zones. Digital photographs were taken over three PVC quadrats of 50 x 50 cm which were randomly placed on the sea bottom in each area. This was done three times (July 2013, January and May 2014) during the surveyed year. Image analysis was carried out using Seascape® software (Segmentation and Cover Classification Analyses of Seabed Images, Teixidó et al., 2011) to detect the percentage cover of conspicuous algal taxa or morphological groups. A permutational multivariate analyses of variance (PERMANOVA, Anderson, 2001a) was done, on the basis of a Bray-Curtis dissimilarity matrix calculated from square-root transformed data (Primer-E 6 Permanova®), to estimate the variability of assemblages between the take and no-take zones over
time. A 3-way model was used with Time (random, 3 levels) and Protection (fixed, 2 levels, take vs. no-take) as crossed factors, and Area (2 levels) as a random factor nested in Protection. P-values were obtained through Monte Carlo random draws from the asymptotic permutation distribution (Anderson, 2005) and a pairwise test was used to discriminate between various levels of significant factors. A non-metric Multi-Dimensional Scaling (nMDS) ordination was used as a graphical representation of data.

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

**Gonadosomatic Index and fertility**

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

GSI was calculated by the formula: $GSI = \frac{\text{gonad wet weight}}{\text{total wet weight}} \times 100$ (Sánchez-España, Martinez-Pita & Garcia, 2004).
The fertility of the US individuals was tested according Brundu et al. (2016) during the months of maximum gonadal development (i.e. from December to April). Gonads from females were extracted and gently shaken in filtered seawater to allow the mature ova to come out. Sperm was then added and finally, the fertility of the individuals was assessed as a percentage of the effectively fertilized eggs after the appearance of the fertilization membrane (fertilization was considered successful if it took place in at least 80% of the eggs according Falugi & Angelini, 2002). The percentage of achievement of the first larval stage (development of four-arm echinopluteus) was also measured. A fertility test was not carried out on individuals belonging to the Small-US class because of the paucity of their gonads (see “Results”). Nor was it done on the commercial-sized individuals because they were assumed to be fertile (i.e. Ouréns, Fernández & Freire, 2011).

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

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

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

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

RESULTS

Environmental features

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

Gonadosomatic Index and fertility

At each area, 220 specimens of the commercial-size class (CS) and 110 undersized and smaller undersized individuals (US and Small-US) were randomly collected over a year to compare reproductive potential between populations. Regarding CS individuals, sea urchins with TD ≥ 60 mm were 24 of 440 (5.5%) in the take zone and 219 of 417 (53%) in the no-take zone. Fertility tests showed that 100% of the undersized individuals (US) checked were fertile and contributed to the reproductive potential of the populations, with high percentages of fertilized eggs (ranging from 87 to 96%) and developing larvae (ranging from 95 to 100%) (Table S2).
Conversely, the Small-US individuals had reduced gonads (Fig. 2), and their contribution to the reproductive potential of the population can be considered negligible.

The GSI trend over the year was generally higher for CS individuals than US ones for both levels of protection (Fig. 2). At the take zone, we recorded a single large spawning period from March to May for both fertile classes (see Fig. 2a). In pre-spawning time, GSI values reached 6.6 ± 0.3\% (mean ± standard error) and 4.4 ± 0.4\%, while in post-spawning time values were 1.3 ± 0.2\% and 1.6 ± 0.1\% for the CS and US classes respectively (Fig. 2a).

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

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

**Population structure and potential reproductive contribution**
Size class distribution was consistently different between take and no-take zones (Fig. 3). In the take zone, sea urchin density was almost two-fold higher than in the no-take zone: 10 ± 1.4 and 5.4 ± 0.5 individuals per m² respectively. Sea urchins with TD ranging from 0 to 20 mm were 1.7 ± 0.1 per m² and 1.1 ± 0.6 per m² in the take and no-take zones respectively, and they represent 17% and 21% of their populations. The most abundant size classes were those ranging from 20 to 50 mm diameter (77%) with a density of 3 ± 0.6 and 3 ± 0.3 individuals per m² for the Small-US and US classes respectively (30 ≤ TD < 40 mm and 40 ≤ TD < 50 mm). The proportion of individuals of the CS class with respect to the entire population was 6% (0.6 ± 0.2 individuals per m²) and all the individuals were included in the range of 50 ≤ TD < 60 mm (Fig.3). In the no-take zone, the individuals between 20 and 50 mm represented 28% of the population while the CS class was 52% with 1 ± 0.4 and 2 ± 0.2 individuals per m² for 50 ≤ TD < 60 mm and TD ≥ 60 mm respectively (Fig. 3).

In relation to the population structure, the reproductive contribution was compared between the take and no-take zones, but no comparisons were made between the sampling areas because no differences were found (see Table 1). The potential reproductive contribution was calculated according to the number of spawning events during the surveyed year and the natural density of the fertile size classes (US and CS). In the take zone, a single spawning event occurred and the Spawning Magnitude for the year was 73% on average (Table 2) with an Individual Gamete Output (IGO) of 0.03 and 0.05 g g⁻¹ se⁻¹ for the US and CS individuals, respectively (Table 2). The Gamete Output (GO), calculated in relation to the natural density of this zone, was 0.08 g g⁻¹ m⁻² se⁻¹ for the US sea urchins (2.7 ± 0.3 individuals per m²) and 0.03 g g⁻¹ m⁻² se⁻¹ for the CS class (0.6 ± 0.2 individuals per m²). Because of the single spawning event, the Total Gamete Output per m² (TGO) overlaps the Mean Gamete Output per m² (MGO) (Table 2). Accordingly,
the Total Gamete Output of the whole population (i.e. sum of TGO of the two fertile size classes) corresponded to the Mean Gamete Output for m² (popMGO) with a value of 0.11 g g⁻¹ m⁻² yr⁻¹ (Table 2).

Conversely, in the no-take zone, two spawning events were observed (Fig. 2b). Spawning Magnitude varied from 54 to 81% with higher values for US individuals (Table 2). IGO was similar for both size classes with values ranging from 0.03 to 0.04 g g⁻¹ sec⁻¹ according to the spawning period. The GO of the US individuals was 0.02 g g⁻¹ m⁻² sec⁻¹ during the first spawning event and 0.01 g g⁻¹ m⁻² sec⁻¹ during the second one, while it was 0.11 and 0.08 g g⁻¹ m⁻² sec⁻¹ for the CS class. Total Gamete Output (TGO) and Mean Gamete Output (MGO) of the US individuals, whose density was 0.4 ± 0.1 individuals per m², were 0.03 and 0.01 g g⁻¹ m⁻² yr⁻¹ respectively. Meanwhile they were 0.19 and 0.10 g g⁻¹ m⁻² yr⁻¹ for the CS class, whose natural density was 2.7 ± 0.3 individuals per m². Consequently, the Total Gamete Output of the whole population was estimated to be 0.22 g g⁻¹ m⁻² yr⁻¹ and the total Mean Gamete Output was 0.11 g g⁻¹ m⁻² yr⁻¹ (Table 2).

**DISCUSSION**

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

While recruits (TD < 20 mm) had the same proportion of population structure, the density of the fertile undersized individuals (US) was 4.5-fold higher at the take zone than at the no-take one, and the density of the commercial size (CS) was 6.8-fold lower. Furthermore, within the
commercial size, a large percentage was composed of by individuals ≥ 60 mm at the no-take zone, while they were nearly absent at the take zone (none have been detected during the sampling for the estimation of the population structure, only 24 throughout the annual sampling for the GSI assessment).

Both populations showed a spawning event at the end of winter or at the beginning of spring, which lasted until April-May for the two fertile size classes examined (US and CS). Moreover, a second relevant peak of GSI was registered in the no-take zone which was demonstrated to be concomitant with a spawning event from June 2013 (Siliani et al., 2016) by histological analysis. This is consistent with observations from other areas of the Mediterranean where one or two annual spawning periods were commonly identified, regardless of the proportion of size classes shaping the population structure (Fenaux, 1968; Semroud & Kada, 1987; Pedrotti & Fenaux, 1992; Pedrotti, 1993; Semroud, 1993; Lozano et al., 1995; Fernandez & Boudouresque, 1997; Lópe et al., 1998; Guetaf, San Martin & Francour, 2000; Leoni et al., 2003; Martínez et al., 2003; Sánchez-Españo, Martinez-Pita & Garcia, 2004; Tomas, Romero & Turon, 2004; Sellem & Guillou, 2007). The differences identified in reproductive potential between the populations were not ascribed to different environmental features since the sampling zones were similar in terms of sheltering from waves and there were no differences found for either water temperature or algal assemblages. At both zones, GSI of the Small US individuals was lower than 1% throughout the year and its contribution to the reproductive potential of the population was considered negligible. On the contrary, GSI of the two fertile size classes (US and CS) changed significantly over the year and between the different levels of protection. Specifically, GSI of the CS class was significantly higher than those of the US individuals and it was significantly higher in the no-take zone than in the take zone.
As widely described in many other locations of the Mediterranean (Guidetti, Terlizzi & Boero, 2004; Gianguzza et al., 2006; Pais et al., 2007; Ceccherelli, Pinna & Sechi, 2009; Bertocci et al., 2014), our study clearly points out how human predation adversely affects the population structure in the take zone, truncating the adult cohorts with TD ≥ 50 mm. The high presence of commercial-size adults (TD ≥ 50 mm) at the no-take zone, and in particular the high number of individuals larger than 60 mm, confirmed this evidence. More interestingly, our results demonstrate the existence of a strong connection between the contribution to the reproductive potential of the fertile size classes and the level of pressure of commercial harvesting.

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

In contrast, the Total Gamete Output of the population (popTGO), which represented the annual reproductive contribution of populations throughout the year, was two-fold higher at the no-take zone. Mita et al. (2007) suggested that gonad size increases volumetrically with sea urchin test diameter, with the largest body size implying the most mature and developed gonads (and the consequent possibility to produce more than one cohort of gametes). Effectively, in marine reserves, more fertile gametes are produced by the largest sea urchins compared to sites where...
large sea urchins are lacking (Lundquist, 2000). Moreover, the very important effects related to the individual’s age and size are proven by studies conducted on different fish species that have demonstrated how a greater age diversity in a spawning stock may extend spawning both temporally and spatially. This, in consequence, increases the chances that more offspring encounter favourable conditions for development (Secor, 2000; Berkeley et al., 2004b; Fiorentino et al., 2008).

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

In general, sea urchin population dynamics is driven by various ecological processes that operate on different spatial and temporal scales. Larval supply fluctuates widely from region to region as it is associated with oceanic currents (Fenaux, Cellario & Rassoulzadegan, 1988; Prado et al., 2012). The success of settlements is influenced by local constraints linked to habitat (e.g. adult abundance, presence of crustose algae, substrate rugosity) (Boudouresque & Verlaque, 2007, Oliva et al., 2016). Finally, predation becomes the prevalent mechanism of sea urchin population
control after settlement and serves as a critical bottleneck for urchin populations (Guidetti, 2004; Hereu et al., 2005; Farina et al., 2009; Farina et al., 2014; Boada et al., 2015). In our case, the lack of predatory fish in the take zone (Marra et al., 2016; Oliva et al., 2016) and the high pressure of harvesting shifted the whole potential reproductive contribution onto the young, fertile undersized sea urchins. On the contrary, in the no-take zone, it mostly depended on commercial-sized individuals since predation in marine reserves significantly affects sea urchins until they reach the size of 50 mm TD (Guidetti, 2004).

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

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

In a marine reserve context, where sea urchin populations have a normal bimodal structure (Brundu et al., 2013), the opening of commercial harvesting should be avoided (despite the
insistence of local fishermen) to prevent the depletion of fertile individuals, as a result of the synergistic action with the natural predation, and the breakdown of the population. On the contrary, in areas affected by fishing pressure, larger individuals are lacking (Baroli et al., 2006), but the survival of the fertile, intermediate-size classes, which therefore support the mean gamete output of the whole population, is guaranteed by laws (as harvesting is authorized only for individuals ≥ 50 mm) and by the absence of predatory fishes, which in turn are targeted by commercial fishing. Our present findings suggest that the reproductive potential contribution of the population does not depend on the size of fertile individuals but on their density. For this reason, the harvesting of individuals between 40 and 50 mm should be avoided particularly in over-fished locations, since they are the only ones capable of generating new life, as far as we know without more information on the origin of the larvae present.

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REFERENCES

Anderson MJ. 2001a. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26: 32-46.

Anderson MJ. 2005. PERMANOVA: a FORTRAN computer program for permutational multivariate analysis of variance. Department of Statistics, University of Auckland, New Zealand.

Anderson SC, Mills Flemming J, Watson R, Lotze HK. 2011. Rapid Global Expansion of Invertebrate Fisheries: Trends, Drivers, and Ecosystem Effects. *PLoS ONE* 6: e14735.

Andrew NL, Agatsuma Y, Ballesteros E, Bazhin AG, Creaser EP, Barnes DKA, Botsford LW, Bradbury A, Campbell A, Dixon JD, Einarsson S, Gerring PK, Hebert K, Hunter M, Hur SB, Johnson CR, Juinio-Menez MA, Kalvass P, Miller RJ, Moreno CA, Palleiro JS, Rivas D, Robinson SML, Schroeter SC, Steneck RS, Vadas RL, Woodby DA, Xiaoqi Z. 2002. Status and management of world sea urchin fisheries. *Oceanography And Marine Biology An Annual Review* 40: 343–425.

Baroli M, De Falco G, Sanna O, Paliaga B. 2006. Monitoraggio della popolazione del riccio di mare *Paracentrotus lividus* nell’Area Marina Protetta Penisola del Sinis – Isola di Mal di Ventre. Technical report Fondazione IMC, 35 pp.

Bell JE, Bishop MJ, Taylor RB, Williamson JE. 2014. Facilitation cascade maintains a kelp community. *Marine Ecology Progress Series* 501: 1–10.
Berkeley SA, Hixon MA, Larson RJ, Love MS. 2004b. Fisheries sustainability via protection of age structure and spatial distribution of fish populations. *Fisheries* 29: 23–32.

Bertocci I, Dominguez R, Machado I, Freitas C, Domínguez Godino J, Sousa-Pinto I, Gonçalves M, Gaspar MB. 2014. Multiple effects of harvesting on populations of the purple sea urchin *Paracentrotus lividus* in north Portugal. *Fisheries Research* 150: 60–65.

Boada J, Rohan A, Farina S, Santana Y, Mascaró O, Romero J, Alcoverro T. 2015. Hotspots of predation persist outside marine reserves in the historically fished Mediterranean Sea. *Biological Conservation* 191: 67-74.

Boudouresque CF, Verlaque M. 2007. Ecology of *Paracentrotus lividus*. In: Lawrence JM, ed. *Edible sea urchins: biology and ecology*. Amsterdam: Elsevier, 243-285.

Brady SM, Scheibling RE. 2006. Changes in growth and reproduction of green sea urchins, *Strongylocentrotus droebachiensis* (Müller), during repopulation of the shallow subtidal zone after mass mortality. *Journal of Experimental Marine Biology and Ecology* 335: 277–291.

Brewin PE, Lamare MD, Keogh JA, Mladenov PV. 2000. Reproductive variability over a four-year period in the sea urchin *Evechinus chloroticus* (Echinoidea: Echinodermata) from differing habitats in New Zealand. *Marine Biology* 137: 543–557.

Brundu G, Loi B, Baroli M, Frau F, Panzalis P, Bernardi G, Navone A, Guala I. 2013. Change of density and structure of sea urchin population at the MPA Tavolara – Punta Coda Cavallo). *Biologia Marina Mediterranea* 20: 122-123.

Brundu G, Vallainc D, Baroli M, Figus MA, Pinna A, Carboni S. 2016. Effects of on-demand
feeding on sea urchin larvae (*Paracentrotus lividus*; Lamarck, 1816), development, survival and microalgae utilization. *Aquaculture Research* 1–11.

Byrne M. 1990. Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. *Marine Biology* 104: 275–289.

Ceccherelli G, Pinna S, Sechi N. 2009. Evaluating the effects of protection on *Paracentrotus lividus* distribution in two contrasting habitats. *Estuarine, Coastal and Shelf Science* 81: 59–64.

Coll M, Lotze HK, Romanuk TN. 2008a. Structural Degradation in Mediterranean Sea Food Webs: Testing Ecological Hypothesis Using Stochastic and Mass-Balance Modeling. *Ecosystems* 11: 939-960.

Coll M, Palomera I, Tudela S. 2009a. Decadal Changes in a NW Mediterranean Sea Food Web in Relation to Fishing Exploitation. *Ecological Modelling* 220: 2088-2102.

Coll M, Santojanni A, Palomera I, Arneri E. 2009b. Food Web Changes in the Adriatic Sea Over Last Three Decades. *Marine Ecology Progress Series* 381: 17-37.

Estes JA, Duggins DO, Rathbun GB. 1989. The ecology of extinctions in kelp forest communities. *Conservation Biology* 3: 252–264.

Falugi C, Angelini C. 2002. Sea urchin development from the egg to metamorphosis: an integrated model for cell-to-cell and environment interaction. In: Yokota Y, Matranga V, Smolenicka Z, eds. *The sea urchin: from basic biology to aquaculture*. Lisse: AA Balkema, 73-93.
Farina S, Tomas F, Prado P, Romero J, Alcoverro T. 2009. Seagrass meadow structure alters interactions between the sea urchin Paracentrotus lividus and its predators. *Marine Ecology Progress Series* 377: 131-137.

Farina S, Arthur R, Pagès JF, Prado P, Romero J, Vergés A, Hyndes G, Heck KL, Glenos S, Alcoverro, T. 2014. Differences in predator composition alter the direction of structure-mediated predation risk in macrophyte communities. *Oikos* 123: 1311–1322.

Fenaux L. 1968. Maturation des gonades et cycle saisonnier des larves chez *A. lixula*, *P.lividus* et *P.microtuberculatus* à Villefranche-Sur-Mer. *Vie Milieu Serie A* 19: 1–52.

Fenaux L, Cellario C, Rassoulzadegan F. 1988. Sensitivity of different morphological stages of the larva of *Paracentrotus lividus* (Lamarck) to quantity and quality of food. In: Burke D, Mladenov PV, Lambert P, Parsley RL, eds. Echinoderm biology. Rotterdam: A.A. Balkema, 259–266.

Fernandez C, Boudouresque CF. 1997. Phenotypic plasticity of *Paracentrotus lividus* (Echinodermata: Echinoidea) in a lagoonal environment. *Marine Ecology Progress Series* 152: 145–154.

Fiorentino F, Badalamenti F, Anna GD, Garofalo G, Gianguzza P, Gristina M, Pipitone C, Rizzo P, Fortibuoni T, Anna D, Changes T. 2008. Changes in spawning-stock structure and recruitment pattern of red mullet, *Mullus barbatus*, after a trawl ban in the Gulf of Castellammare (central Mediterranean Sea). *ICES Journal of Marine Science* 65: 1175–1183.

Gianguzza P, Chiantore M, Bonaviri C, Cattaneo-Vietti R, Vielmini I, Riggio S. 2006. The effects of recreational *Paracentrotus lividus* fishing on distribution patterns of sea urchins at
Ustica Island MPA (Western Mediterranean, Italy). *Fisheries Research* 81: 37–44.

Gianguzza P, Bonaviri C, Prato E, Fanelli G, Chiantore M, Privitera D, Luzzu F, Agnetta D. 2013. Hydrodynamism and its influence on the reproductive condition of the edible sea urchin *Paracentrotus lividus*. *Marine Environmental Research* 85: 29–33.

Guala I, Simeone S, Baroli M. 2009. Abbondanza e struttura della popolazione di *Paracentrotus lividus* nell’Area Marina Protetta Tavolara – Punta Coda Cavallo. Technical report Fondazione IMC, 34 pp.

Guala I, Simeone S, Navone A, Panzalis P, Baroli M. 2011. Valutazione della sostenibilità del prelievo del riccio di mare *Paracentrotus lividus* nell’Area Marina Protetta Tavolara – Punta Coda Cavallo. *Studi Trentini di Scienze Naturali* 89: 119-121.

Guettaf M, San Martin GA, Francour P. 2000. Interpopulation variability of the reproductive cycle of *Paracentrotus lividus* (Echinodermata: Echinoidea) in the south-western Mediterranean. *Journal of the Marine Biological Association of the UK* 80: 899–907.

Guidetti P. 2004. Consumers of sea urchins, *Paracentrotus lividus* and *Arbacia lixula*, in shallow Mediterranean rocky reefs. *Helgoland Marine Research* 58: 110–116.

Guidetti P, Terlizzi A, Boero F. 2004. Effects of the edible sea urchin, *Paracentrotus lividus*, fishery along the Apulian rocky coast (SE Italy, Mediterranean Sea). *Fisheries Research* 66: 287–297.

Hereu B, Zabala M, Linares C, Sala E. 2005. The effects of predator abundance and habitat structural complexity on survival of juvenile sea urchins. *Marine Biology* 146: 293–299.
Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Estes JA, Hughes TP, Kidwell S, Lange CB, Lenihan HS, Pondolfi JM, Peterson CH, Steneck RS, Tegner MJ, Warner RR. 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science 293: 629–638.

Leoni V, Fernandez C, Johnson M, Ferrat L, Pergent-Martini C. 2003. Preliminary study on spawning periods in the sea urchin Paracentrotus lividus from lagoon and marine environments. In: Féra JP, David B, eds. Echinoderm research 2001. Lisse: AA Balkema, Lisse, 277–280.

Levitan DR, Sewell MA. 1998. Fertilization success in free-spawning marine invertebrates: review of the evidence and fisheries implications. In: G.S. Jamieson and Campbell A., eds. Canadian Special Publication of Fisheries and Aquatic Sciences 125: 159-164.

López S, Turon X, Montero E, Palacín C, Duarte CM, Tarjuelo I. 1998. Larval abundance, recruitment and early mortality in Paracentrotus lividus (Echinoidea). Interannual variability and plankton-benthos coupling. Marine Ecology Progress Series 172: 239–251.

Lotze HK, Coll M, Dunne JA. 2011. Historical Changes in Marine Resources, Food Web Structure and Ecosystem Functioning in the Adriatic Sea, Mediterranean. Ecosystems 14: 198-222.

Lozano J, Galera J, Lopez S, Turon X, Palacin C, Morera G. 1995. Biological cycles and recruitment of Paracentrotus lividus (Echinodermata: Echinoidea) in two contrasting habitats. Marine Ecology Progress Series 122: 179–192.

Lundquist CJ. 2000. Effects of density dependence and environment on recruitment of coastal invertebrates. D. Phil. Thesis, University of California.
Marra S, Coppa S, Camedda A, Mazzoldi C, Wrachien F, Massaro G, De Lucia GA. 2016. Recovery trends of commercial fish: The case of an underperforming mediterranean marine protected area. *PLoS ONE* 11: 1–22.

Martínez I, García FJ, Sánchez AI, Daza JL, Del Castillo F. 2003. Biometric parameters and reproductive cycle of *Paracentrotus lividus* (Lamarck) in three habitats of Southern Spain. In: Féral JP, David B, eds. *Echinoderm Research 2001*. Lisse: AA Balkema, 281–287.

Meidel SK, Scheibling RE. 1998. Annual reproductive cycle of the green sea urchin, *Strongylocentrotus droebachiensis*, in differing habitats in Nova Scotia, Canada. *Marine Biology* 131: 461–478.

Mita M, Sato J, Hirosawa Y, Nakamura M. 2007. Gonadal maturation is dependent on body size in the sea urchin, *Echinometra tsumajiroi*. *Invertebrate Reproduction & Development* 50: 187–190.

Myers RA, Worm B. 2003. Rapid worldwide depletion of predatory fish communities. *Nature* 423: 280–283.

Navia AF, Cortés E, Jordán F, Cruz-Escalona VH, Mejía-Falla PA. 2012. Changes to marine trophic networks caused by fishing. In: Mahamane A, ed. *Diversity of Ecosystems*. Croatia: InTech Press, 418-452.

Oliva S, Farina S, Pinna S, Guala I, Agnetta D, Ariotti PA, Mura F, Ceccherelli G. 2016. Determinants of *Paracentrotus lividus* sea urchin recruitment under oligotrophic conditions: implications for conservation management. *Marine Environmental Research* 117: 13–20.
Ouréns R, Fernández L, Freire J. 2011. Geographic, population, and seasonal patterns in the reproductive parameters of the sea urchin Paracentrotus lividus. Marine Biology 158: 793–804.

Pais A, Chessa LA, Serra S, Ruiu A, Meloni G, Donno Y. 2007. The impact of commercial and recreational harvesting for Paracentrotus lividus on shallow rocky reef sea urchin communities in North-western Sardinia, Italy. Estuarine, Coastal and Shelf Science 73: 589–597.

Parravicini V, Guidetti P, Morri C, Montefalcone M, Donato M, Bianchi CN. 2010. Consequences of sea water temperature anomalies on a Mediterranean submarine cave ecosystem. Estuarine, Coastal and Shelf Science 86: 276–282.

Pauly D. 1995. Anecdotes and the shifting baseline syndrome of fisheries. Trends in Ecology & Evolution 10: 430.

Pedrotti ML, Fenaux L. 1992. Dispersal of echinoderm larvae in a geographical area marked by upwelling (Ligurian Sea, NW Mediterranean). Marine Ecology Progress Series 86: 217–227.

Pedrotti ML. 1993. Spatial and temporal distribution and recruitment of echinoderm larvae in the Ligurian Sea. Journal of the Marine Biological Association UK 73: 513–530.

Pinnegar JK, Polunin NVC, Francour P, Badalament F, Chemello R, Harmelin-Vivien ML, Hereu B, Milazzo M, Zabala M, D’Anna G, Pipitone C. 2000. Trophic cascades in benthic marine ecosystems: lessons for fisheries and protected-area management. Environmental Conservation 27: 179–200.
Prado P, Tomas F, Pinna S, Farina S, Roca G, Ceccherelli G, Romero J, Alcoverro T. 2012. Habitat and Scale Shape the Demographic Fate of the Keystone Sea Urchin *Paracentrotus lividus* in Mediterranean Macrophyte Communities. *PLoS ONE* 7: e35170.

Sala E, Zabala M. 1996. Fish predation and the structure of the sea urchin *Paracentrotus lividus* populations in the NW Mediterranean. *Marine Ecology Progress Series* 140: 71-81.

Sala E. 1997. Fish predators and scavengers of the sea urchin *Paracentrotus lividus* in protected areas of the north-west Mediterranean Sea. *Marine Biology* 129: 531–539.

Sala E, Boudouresque CF and Harmelin-Vivien M. 1998. Fishing, trophic cascades, and the structure of algal assemblages: evaluation of an old but untested paradigm. *Oikos* 82: 425–439.

Sala E, Ribes M, Hereu B, Zabala M, Alvà V, Coma R, Garrabou J. 1998. Temporal variability in abundance of the sea urchins *Paracentrotus lividus* and *Arbacia lixula* in the northwestern Mediterranean: Comparison between a marine reserve and an unprotected area. *Marine Ecology Progress Series* 168: 135–145.

Sánchez-España AI, Martínez-Pita I, García FJ. 2004. Gonadal growth and reproduction in the commercial sea urchin *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata: Echinoidea) from southern Spain. *Hydrobiologia* 519: 61–72.

Secor DH. 2000. Spawning in the nick of time? Effect of adult demographics on spawning behaviour and recruitment in Chesapeake Bay striped bass. *ICES Journal of Marine Science* 57: 403–411.

Sellem F, Guillou M. 2007. Reproductive biology of *Paracentrotus lividus* (Echinodermata:...
Echinoidea) in two contrasting habitats of northern Tunisia (south-east Mediterranean). *Journal of the Marine Biological Association of the UK* 87: 763-767.

Semroud R, Kada H. 1987. Contribution a l’etude de l’oursin *Paracentrotus lividus* dans la region d’Alger: indice de repletion et indice gonadique. In: Boudouresque CF, ed. *Colloque international sur Paracentrotus lividus et les oursins comestibles*. Marseilles: GIS Posidonie, 117-124.

Semroud R. 1993. Contribution à la connaissance de l’écosystème à *Posidonia oceanica* (L.) Delile dans la région d’Alger (Algérie): etude de quelues compartiments. D. Phil. Thesis, Université des Sciences et de la Technologie Houari Boumediene, Algeria.

Shpigel M, McBride SC, Marciano S, Lupatsch I. 2004. The effect of photoperiod and temperature on the reproduction of the European sea urchin *Paracentrotus lividus*. *Aquaculture* 232: 343–355.

Siliani S, Melis R, Loi B, Guala I, Baroli M, Sanna R, Uzzau S, Roggio T, Filippa M, Anedda R. 2016. Influence of seasonal and environmental patterns on the lipid content and fatty acid profiles in gonads of the edible sea urchin *Paracentrotus lividus* from Sardinia. *Marine Environmental Research* 113: 124–133.

Spirlet C, Grosjean P, Jangoux M. 1998. Reproductive cycle of the echinoid *Paracentrotus lividus*: analysis by means of the maturity index. *Invertebrate Reproduction & Development* 34: 69–81.
Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ. 2002. Kelp Forest Ecosystem: Biodiversity, Stability, Resilience and their Future. *Environmental Conservation* 29: 436–459.

Steneck RS, Vavrinec J, Leland AV. 2004. Accelerating Trophic Level Dysfunction in Kelp Forest Ecosystems of the Western North Atlantic. *Ecosystems* 7: 323–332.

Teixidó N, Albajes-Eizagirre A, Bolbo D, Le Hir E, Demestre M, Garrabou J, Guigues L, Gili JM, Piera J, Prelot T, Soria-Frisch A. 2011. Hierarchical Segmentation based software for Cover Classification Analyses of Seabed Images (Seascape). *Marine Ecology Progress Series* 431: 45-53.

Tomas F, Romero J, Turon X. 2004. Settlement and recruitment of the sea urchin *Paracentrotus lividus* in two contrasting habitats in the Mediterranean. *Marine Ecology Progress Series* 282: 173–184.

Tortonese E. 1965. *Echinodermata (Vol. VI)*. *Fauna d’Italia*. Bologna: Calderini.

Trippel EA. 1995. Age at maturity as a stress indicator in fisheries. *BioScience*, 45: 759–771.

Tunca S, Ünal V, Miran B, Güçlüsoy H, Gordoa A. 2016. Biosocioeconomic analysis of marine recreational fisheries: A comparative case study from the Eastern Mediterranean, Turkey. *Fisheries Research* 174: 270-279.

Underwood AJ. 1997. *Experiments in Ecology. Their logic design and interpretation using analysis of variance*. Cambridge: Cambridge University Press.
Figure 1. Map of the study zones with different level of fishing pressure: take zone (Su Pallosu, central-western Sardinia) and no-take zone (Tavolara-Punta Coda Cavallo Marine Protected Area, north-eastern Sardinia). Sampling areas are indicated with black dots.
Figure 2. Annual trend of Gonadosomatic Index as mean (± standard error) of the three size classes examined (CS, US, Small-US) at a) take zone and b) no-take zone. Observation began in June 2013 and ended in May 2014. Dotted lines represent an approximation of the expected values of GSI for November 2013 (no sampling was done). Triangles = CS; rhombuses = US; squares = Small-US.
Figure 3. Size-frequency distribution (%) of sea urchin population at the take and no-take zones. The range of the size classes is 10 mm of test diameter without spines (TD). Commercial size classes under fishing pressure are those larger than 50 mm test diameter. White bars = take zone; black bars = no-take zone.
Table 1. Results of four-way ANOVA to test the effects on Gonadosomatic Index of Month, Protection and Size class (orthogonal fixed factors) and Area (random nested factor in Protection).

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

| Source of variation                          | df  | MS     | F     | p   |
|---------------------------------------------|-----|--------|-------|-----|
| Month = Mo                                  | 10  | 933.74 | 2.94  | 0.0193 |
| Protection = Pr                             | 1   | 11.883.74 | 14.26 | 0.0635 |
| Area(Protection) = Ar(Pr)                   | 2   | 833.46 | 2.27  | 0.1039 |
| Size class = Sc                             | 1   | 117763.64 | 362.25 | 0.0027 |
| Mo x Pr                                     | 10  | 597.33 | 1.88  | 0.1104 |
| Mo x Ar(Pr)                                 | 20  | 317.95 | 0.87  | 0.6301 |
| Mo x Sc                                     | 10  | 2000.32 | 7.12  | 0.0001 |
| Pr x Sc                                     | 1   | 13457.07 | 41.39 | 0.0233 |
| Sc x Ar(Pr)                                 | 2   | 325.10 | 0.89  | 0.4126 |
| Mo x Pr x Sc                               | 10  | 648.71 | 2.31  | 0.0535 |
| Sc x Mo x Ar(Pr)                            | 20  | 281.10 | 0.77  | 0.7552 |
| Residual                                    | 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)
Table 2. Timing and extent of mean monthly Gonadosomatic Index, Spawning Magnitude, and Gamete Output for US (40 ≤ TD < 50 mm) and CS (TD ≥ 50 mm) class, for spawning event and in relation with their natural density, and for the whole populations at take and no-take zone.

| Size class | Take zone | No-take zone |
|------------|-----------|--------------|
|            | 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 (g g⁻¹ 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 (g g⁻¹ m⁻² se⁻¹) | 0.08 | 0.03 | 0.02 | 0.01 | 0.11 | 0.08 |
| TGO (g g⁻¹ m⁻² yr⁻¹) | 0.08 | 0.03 | 0.03 | 0.19 |
| popTGO (g g⁻¹ m⁻² yr⁻¹) | 0.11 | 0.22 |
| MGO (g g⁻¹ m⁻² yr⁻¹) | 0.08 | 0.03 | 0.01 | 0.10 |
| popMGO (g g⁻¹ m⁻² yr⁻¹) | 0.11 | 0.11 |

GO = Gamete Output per m²; TGO = Total Gamete Output per m²; popTGO = Total Gamete Output of the whole population per m²; MGO = Mean Gamete Output per m²; popMGO = Mean Gamete Output of the whole population per m².