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SHORT COMMUNICATION

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First assessment of restocking efficacy of the depleted sea urchin *Paracentrotus lividus* populations in two contrasted sites

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The decrease of marine resources is widespread and concerns also marine invertebrates including sea urchins which are harvested for their prized gonads (Stefánsson et al., 2017). Sea urchin fisheries have been recognized ecologically unsustainable in many regions of the world including the Mediterranean and the Atlantic coasts of Europe (Lawrence, 2013). In these regions unregulated harvesting pressure on the common purple sea urchin *Paracentrotus lividus* (Lamarck, 1816), has caused a drastic reduction of populations that presently cannot recover with self-recruitment (Addis et al., 2012; Carboni et al., 2014; Ceccherelli et al., 2011; FAO, 2020; Gibson et al., 2003; Pais et al., 2007; Stefánsson et al., 2017). Only a few studies attribute the stock decline of *P. lividus* to global warming and scarcity of seaweed (Yeruham et al., 2015, 2019).

The application of aquaculture technologies by producing and releasing juveniles reared in 'conservation hatcheries', could be a useful tool to restock commercial species locally extinct or threatened due to overexploitation from fishing pressure (Bell et al., 2008). These authors state that the application of aquaculture through restocking, stock enhancement and sea ranching, are promising management tools to restore lost production, also allowing the recovery beyond historical levels.

Stock enhancement programmes through the releasing of hatchery-reared sea urchins in the wild, accounted for limited experimental studies in Japan (Agatsuma et al., 2003), Philippines (Juinio-Meñez et al., 2008), South Korea (National Fishery Research & Development Institute, 2000) and only one case-study in the Mediterranean Sea for *P. lividus* (Couvray et al., 2015).

Specific research projects have led the development of production methods for *P. lividus* including spawning protocols and on-growing systems (Carboni et al., 2014; Prato et al., 2018; Secci et al., 2020). Therefore, ranching and restocking of sea urchins could be a viable possibility for echinoculture allowing to reduce the cost and solve the main bottlenecks in commercial spat production. In addition, for a real cost-effectiveness assessment of a restocking programme, the overall production cycle cost should also be taken into account.

Hence, to explore the usefulness of restocking, we investigated growth and survivability of reared sea urchins released in the field in diverse environmental conditions.

We considered both small and large-scale releases and two geographical areas (Italy and Ireland) where the species has been overexploited for a long time (FAO, 2020; RAS, 2020). These areas were characterized by low abundance of *P. lividus* and in some locations the extinction of the commercial sizes (≥50 mm test diameter) has currently occurred (RAS, 2020).

In Italy (South Sardinia) two sites (Fortezza Vecchia = S1; Cava Usai = S2) have been selected for small-scale release trials in semi-rocky substrates (depth: S1 = 0.5 m; S2 = 10 m) colonized by two brown macroalgaes: *Cystoseira* Spp. and *Padina pavonica* (Figure 1). In Ireland two sites (Sligo = I1 and Achill Island = I2) have been selected for large-scale release (Figure 1). These sites were characterized by rocky substrates pools (depth = 0.5–5 m) in the inter-tidal zone and colonized by *Laminaria Spp*.

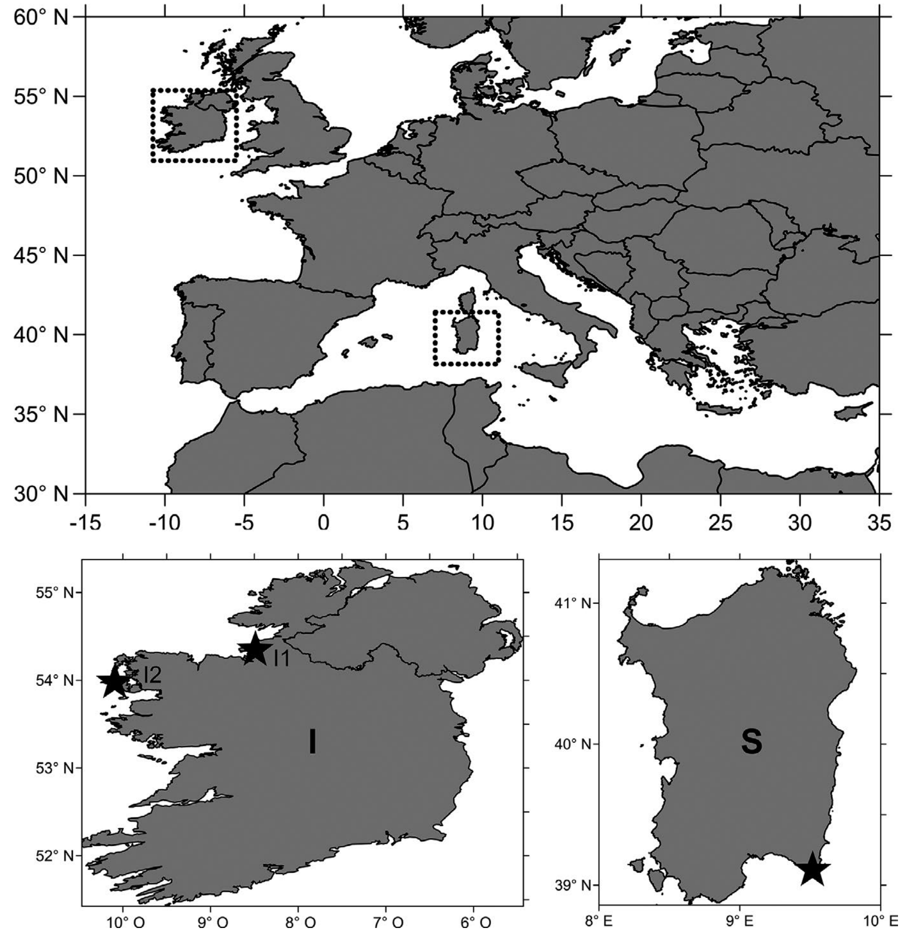

FIGURE 1 Sites (stars) considered in the restocking experiment of *Paracentrotus lividus* in Ireland (I) and Sardinia (S)

Juveniles *P. lividus* were produced using the same spawning protocol (Carboni et al., 2014; Hannon et al., 2015) at the experimental hatchery of the University of Cagliari (Italy) and at the commercial hatchery of Dunmanus Seafoods Ltd (Ireland). Specimens were grown as far as the size of ~20 mm of test diameter (TD) (~25 months old). Juveniles were transported to the release sites stocked in cooling boxes in overlapping layers separated by fresh macroalgae and with gel ice blocks. Delivery time to sites was within 2 h in Sardinia and within 7 h in Ireland.

In S1 a total of 66 specimens (TD = 21.2 ± 0.29 mm; mean ± *SE*) were enclosed in 6 plastic mesh fences (Mesh = 1 × 1 cm, Height = 1.2 m, Diameter = 0.55 m, surface = ~5 m^2) in November 2015 (Month = 0). The fences prevented the entry of predators for sea urchins (eg. sparids and labrids). Moreover, a biological vacuum was carried out in each fence to remove macrobenthic invertebrates (crabs, murex shells) and wild sea urchins. In S2 a total of 200 specimens (TD = 23.0 ± 0.36 mm) were released in two rocky bottom sites without protection fences in July 2016 (Month = 0). The removal of wild sea urchins from S2 (*N* ~ 150) was carried out before the beginning of the trial to avoid a mixing effect with the reared urchins. S2 was surrounded by a wide, sandy bottom and it is difficult to be recruited by adult wild sea urchins. Survival rate (by counting the number of survivors) and the growth rate (TD; measuring test diameter on the whole collected specimens) were checked every 30 days until 120 days after the release (Month = 3) in both trials (S1–S2).

In I1 and I2 a total of 20,000 urchins (TD = 18.7 ± 0.69 mm) were released (10,000 in each site) in July 2016 (Month = 0). I1 and I2 were fully overharvested sites. Survival rate and growth rate were checked after 11 and 21 months the release.

One-way ANOVA (α = 0.05) was used to evaluate the survival and growth of the sea urchin released over time in each site (factor: Month: 0, 1, 2, 3 in S1 and S2; Month: 0, 11, 21 in I1 and I2). The homogeneity of the variance was evaluated by the Cochran test (α = 0.05) and, whenever necessary, data were appropriately transformed. Where data transformation did not correct violations in the assumption of homogeneous variances (i.e. growth in S1), an alpha-level adjustment to 0.01 was used to compensate for increased type I errors (Underwood, 1997). The statistical analysis was carried out by the software Statgraphics centurion XVI (Statpoint technologies Inc, United States).

At the end of the experiment sea urchins in S1 showed a significant growth of TD (p = 0.0004). The average size (± *SE*) at the end of the experiment was 23.6 ± 0.47 mm (Figure 2). In S2 non-significant growth was observed in both rocky bottom sites (p = 0.0342) (Figure 2). The survival rate in S1 showed significant differences between the Month two and Month zero (p = 0.0385); however

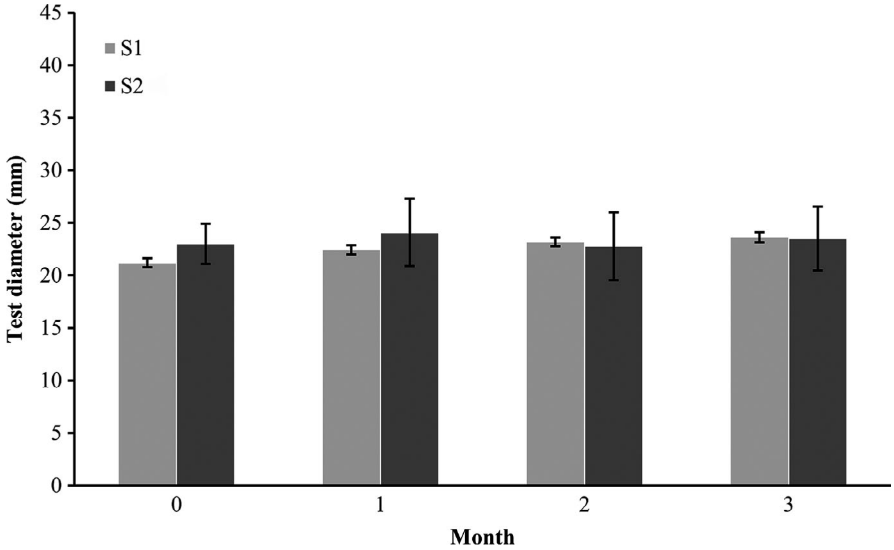

FIGURE 2 Average size (± standard error) of the sea urchin released inside the fences (S1) and sea urchin released at the open water site (S2) during the 120 days of experimentation

non-significant differences were observed between the final values (72.12 ± 1.01%) and the initial value (Month zero). The final survival rate in S2 was 30 ± 10.6% and non-significant differences were observed among Months ($p = 0.2487$).

Sea urchins seeded in Ireland showed a significant growth in both sites (I1: $p = 0.02$; I2: $p = 0.018$) (Figure 3). The final mean TD was 43.8 ± 0.88 mm in I1. The final measure in I2 was 41.3 ± 1.1 mm. The final survival rate was 60% and the 65% for I1 ($p = 0.248$) and I2 ($p = 0.257$), respectively.

Sea urchins used in these trials were about 2-year-old. When released in the natural environment they grew up with an average 0.8 mm/month (Sardinia) and 11 mm/year (Ireland). These growth rates were similar to those indicated in literature for wild specimens which grow with an average 10–12 mm per year (Lawrence, 2013). These results indicate that reared sea urchins were able to forage once seeded into the wild confirming that sea urchins used the available seaweeds and animal proteins as source of food (Prato et al., 2018).

The overall survivability ranged from 30% to 72%. These values were similar to those obtained for the restocking of *Strongylocentrotus intermedius* (TD = 15–20 mm) in Japan (Agatsuma et al., 2003) and they were much higher than those obtained during the experimental release of post-larvae of *P. lividus* in the Mediterranean French coast (Couvray et al., 2015). Our results highlighted the importance of releasing well developed young sea urchins considering that size is one of the main

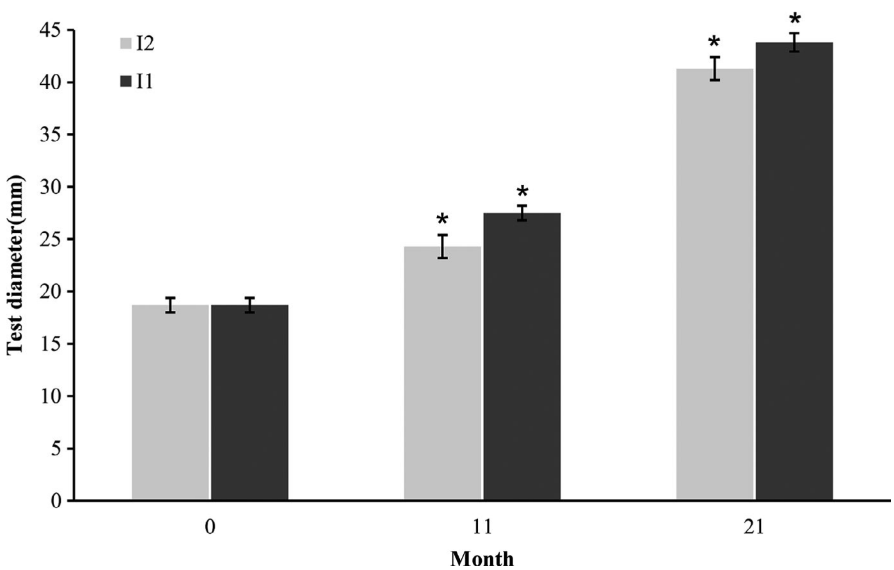

FIGURE 3 Survival average percentage (± standard error) of the sea urchin released in the Experiment I1 and I2 during the 120 days of experimentation. * shows significative differences ($p < 0.05$)

constraints affecting survival (Agatsuma et al., 2003). Smaller sea urchins (TD < 20 mm) are indeed susceptible to predation from crabs, fish and gastropods (Guidetti, 2006).

The count of specimens released on each sampling site was another issue of this study. As a matter of fact, once sea urchins were seeded out, they began to move into new habitat occupying crevices and the underneath of boulders in the bottom. This cryptic behaviour occurred both in Sardinian and Irish sites causing the lack in retrieving released specimens and thus the estimation of survival rate within some months.

As to the evaluation of the overall production costs in Sardinia, it was estimated an expense of 0.36 €/specimen (referred to a 2-year-old specimen or 20 mm TD) for a production cycle of about 200,000 individuals (RAS, 2015). This production cost coupled with the slow growth of juvenile sea urchins is still an industry barrier for echinoculture in Europe and internationally which requires further investigation (Hannon et al., 2015; Stefánsson et al., 2017).

Moreover, there is a lack of legislation around echinoderms in Europe which includes also reseeding activities (Hannon et al., 2015; Secci et al., 2020; Stefánsson et al., 2017). Taking into account the underdeveloped invertebrate aquaculture industry and a lack of legislation, this has a knock-on effect which was one of the causes of the lack for sea urchin hatchery across Europe (Hannon et al., 2015; Secci et al., 2020; Stefánsson et al., 2017).

In conclusion, as far as we know this is the first study which has evaluated the growth and survival of reared *P. lividus* in natural environments. The results of these restocking trials are promising to support overexploited populations and probably help the development of echinoculture. However, we clearly show an influence upon the selected site on growth and survival of released sea urchins. Hence, the reseeding in overexploited areas showed encouraging results but the effects of stock enhancement should be investigated on a longer timescale to validate the efficacy, production cost and ecological relevance.

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CONFLICT OF INTEREST

The Authors have not conflict of interest to declare

AUTHOR CONTRIBUTIONS

Ambra Angelica Giglioli wrote the manuscript and collected the data. Viviana Pasquini analyzed the data. Marco Secci edited the manuscript and collected the data. Colin Hannon and Pierantonio Addis conceived and design the research.

DATA AVAILABILITY STATEMENT

Data are available upon request to the corresponding author.

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