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# Evaluation of tagging and substrate refuges in release of juvenile sea urchins

Silvia de la Uz [a,*], José F. Carrasco [b], Carmen Rodríguez [a], Jacobo López [a]

[a] Centro de Experimentación Pesquera, Dirección General de Pesca Marítima, C/ El Muelle, s/n 33760 Castropol, Asturias, Spain
[b] Centro de Experimentación Pesquera, Dirección General de Pesca Marítima, Av. Príncipe de Asturias, s/n 33212 Gijón, Asturias, Spain

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ABSTRACT

Pilot experiments are required and helpful to identify optimal release strategies in restocking and stock enhancement programs. The selection of a suitable habitat has been identified as an important factor determining sea urchin recruitment success. Substrate complexity and adult abundance offer physical refuges and reduce mortality of juvenile sea urchins. The present study evaluated the effect of substrate shelters on the survival of *Paracentrotus lividus* juveniles released in the field. Previously, the reliability of an internal tag (coded wire tag) was assessed during four months under laboratory conditions. No significant effect of tagging was detected on survival (≥96%) and growth ($p < 0.05$) between tagged and control groups in the different size-classes. However, there were differences among size-class 1 (62%) and size-classes 2 and 3 (80%) in terms of retention rate. In the field, 100% and 80% of recaptured individuals were tagged after 6 and 14 weeks, respectively. All juveniles released on substrates with burrows did not survive the first few weeks, and only those that were released on substrates with adults successfully settled (12%). In conclusion, the present study provided a useful tag for sea urchin and identified a suitable habitat for releases of cultivated juveniles.



## 1. Introduction

Sea urchins play a key role in structuring marine ecosystems due to their grazing activity, which constitutes an important controlling factor in benthic algal communities and over other organisms (Steneck, 2013). Apart from the ecological importance, the edible species are of economic importance. Sea urchin gonads, also known as roe or uni, are considered a highly valued seafood product and a delicacy in many parts of the world.

The purple sea urchin *Paracentrotus lividus* Lamarck, 1816 is the most important commercial species in southern European regions. This echinoid is widely distributed throughout the Mediterranean Sea and in the north-eastern Atlantic, from Scotland and Ireland to Southern Morocco, including the Azores and Canary Islands (Boudouresque and Verlaque, 2013). Nowadays, the consumption of *P. lividus* is mainly limited to France and Spain and, to a lesser extent, to Italy and Greece, although harvesting occurs, or has occurred, over a much larger area (e.g. Ireland, Portugal and Croatia) for export (Boudouresque and Verlaque, 2013). In recent decades, the exploitation of this marine resource has become increasingly intensive, causing the depletion of wild stocks and the collapse of the fishery in several countries (Byrne, 1990; Andrew et al., 2002; Fernández-Boán et al., 2012; Couvray et al., 2015).

Restocking and stock enhancement can potentially mitigate these impacts and improve the natural stocks through the release of cultivated juveniles. But enhancement cannot be conducted effectively without pilot release experiments to identify optimal release strategies (Blankenship and Leber, 1995; Bell et al., 2006).

One of the key release parameters that affects the survival of juveniles released into the wild is the selection of a suitable habitat and microhabitat (Lorenzen et al., 2010). Predation is the greatest obstacle to survival of the released juveniles and smaller sizes are the most susceptible to predators. Substrate complexity and adult abundance have been recognized as important factors determining sea urchin recruitment success (Hereu et al., 2005; Clemente et al., 2013; Oliva et al., 2016). Therefore, spatial heterogeneity and availability of physical refuges reduce mortality in juvenile sea urchins.

Tagging provides the basis for distinguishing released animals from wild conspecifics and allows quantifying the release success (Blankenship and Leber, 1995). Many external and internal tags have been used for tagging sea urchins (Hagen, 1996) but it becomes more challenging for specimens of small body size (Clemente et al., 2007; Sonnenholzner et al., 2010). External tags have important advantages in field studies since they allow individual identification and are easily detectable by divers. Unfortunately, most of them are traumatic or short lived, thus limiting their use to short-term studies, within days or weeks (Tuya et al.,

* Corresponding author.
*E-mail addresses:* silviadelauz@gmail.com, sdelauz@tragsa.es (S. de la Uz).

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2003; Clemente et al., 2007). However, internal tags have higher retention and survival rates (Duggan and Miller, 2001; Rodríguez-Barreras and Sabat, 2015; Rodríguez-Barreras and Wangensteen, 2016) and no detectable effect on behavior in medium and long term (Hagen, 1996).

The aims of this study were (1) to evaluate the effect of an internal tag on the survival and growth of juvenile sea urchins in the laboratory and (2) to assess the effect of the substrate shelters, with or without adult conspecifics, on the survival and growth of urchin juveniles released into the field.

## 2. Materials and methods

### 2.1. Tagging experiment

In the tagging experiment, Coded Wire Tags (CWT, Northwest Marine Tech. Inc.) were inserted into the sea urchin using a hand-held coded wire tag injector. The injector cuts the steel wire tag (0.25 × 2.20 mm) and injects it through the peristome membrane into the coelom cavity with a 0.57 mm diameter needle. Then, the wand detector verifies the presence of the magnetic tag in the sea urchin.

Three hundred juvenile sea urchins *Paracentrotus lividus* (Lamarck, 1816) reared at the hatchery of the Centro de Experimentación Pesquera, Castropol (Spain) were used in the experiment. Fifty tagged and fifty untagged (controls) sea urchins were used in each of the following size-classes: class 1 (10 > 15 mm), class 2 (15 > 20 mm) and class 3 (20 > 25 mm). Horizontal test diameter (without spines) was measured using a digital calliper (±0.01 mm) at the beginning of the experiment and at monthly intervals during a four-month period. Also, tag retention and survival rate were recorded.

During the experimental period (March 27th—July 29th, 2009), mean water temperature increased from 13.6 ± 1.2 to 19.7 ± 0.7 °C and sea urchins were fed *ad libitum* with the intertidal brown algae *Ascophyllum nodosum*, present near the hatchery.

### 2.2. Release experiment

The release experiment was conducted on rocky bottoms in a shallow area (1.5 to 2.5 m depth) located next to a group of small islands, Las Pantorgas (43°33′40″ N, 6°59′04″ W), on the northwest coast of Asturias, northern Spain. This area is characterized mainly by large rocks alternating with vertical walls, oriented in different directions. Small boulders and crevices are present, which represent suitable refuges for small sea urchins. The rocky substrate is covered by the calcareous algae *Lithophyllum incrustans* and *Mesophyllum lichenoides* (especially in vertical walls), and upper surfaces of rocks have an abundant algal cover dominated by *Codium* spp., *Gelidium corneum*, *Asparagopsis armata* and *Cystoseira baccata*.

In order to evaluate the effect of habitat on survival of released sea urchin juveniles, two types of substrates offering different available shelter were selected: substrata with adult presence (A) and substrata with empty sea urchin burrows in the bedrock (B), indicating that sea urchins were once present. Hence, within each substrate, two sites of approximately 2 $m^2$ were established. To quantify the initial density of sea urchins in substrates with adults, a quadrat (0.32 $m^2$) was randomly placed in an area adjacent to A sites and all sea urchins were counted and measured.

Tagged sea urchins with CWT were used in A sites, in order to differentiate the released juveniles from the wild ones, and untagged juveniles were used in B sites. Net bags containing 1200 juveniles (10–30 mm) were transferred from the hatchery to the experimental sites and 300 sea urchins per site were released by a scuba diver close to the bottom. The release experiment started in June and lasted until September (summer season).

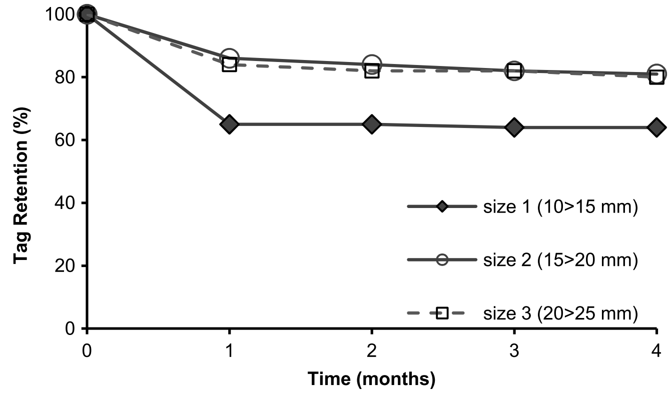


**Fig. 1.** Tag retention (%) of *P. lividus* juveniles for the three size-classes during the 4-month experiment.

During the first days after the release, visual inspections of the four sites were conducted. Six and fourteen weeks after the beginning of the experiment, a site of each substrate was sampled. On each sampling date, all sea urchins of juvenile size at each site and 1.5 m around the site (Hereu, 2005) were removed and tag retention and test diameter were recorded.

### 2.3. Statistical analysis

Differences in survival between tagged and control groups, and differences in tag retention between classes of size, were tested in a contingency table analysis comparing the number of alive or tagged sea urchins in the groups. To test the effect of tagging on the growth, separate $t$-tests were performed for each size-class between tagged and control urchins. Normality and homogeneity of variances were tested using Kolmogorov Smirnoff and Levene tests, respectively.

In the release experiment, Mann–Whitney $U$ tests were used to compare differences in juvenile diameter between the replicates before release and between the different sampling dates. The non-parametric test was performed due to non-compliance of normality.

## 3. Results

### 3.1. Tagging experiment

Over the 4-month experiment, survival rate was not significantly different ($p > 0.05$) between tagged and control urchins of the three sizes. In size-class 1 (10 > 15 mm) and 2 (15 > 20 mm) the tagged sea urchins obtained a final survival rate of 96 and 98%, respectively. The same survival rate as their respective control groups (only 2 and 1 juveniles died). In size-class 3 (20 > 25 mm), none of the 50 tagged and 50 untagged urchins died ($\chi^2 = 0$, $p > 0.05$) during the experiment.

Tag retention rate was 62% in size-class 1 and 80% in size-classes 2 and 3, at the end of the experimental period. Most tags ($n = 33$) were lost during the first month after tagging. The first month was critical in terms of retention rate, but on the second month, it stabilized and maintained with almost no changes throughout the experiment (Fig. 1). During the following months, only 7 sea urchins lost their tags.

The growth between tagged individuals of the same size-class was not significantly different to the control group ($p > 0.05$) at the end of the experiment (Table 1). Largest urchins had the lowest growth rate (1.17 mm $month^{-1}$), whereas size-class 1 and 2 reached higher rates (2.00 and 1.91 mm $month^{-1}$, respectively).

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