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Assessing the reliability of two tagging techniques in the echinoid *Echinometra lucunter*

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HIGHLIGHTS

- We evaluated two types of tags in the echinoid *Echinometra lucunter*.
- We found significant differences in retention between both tags.
- There was no association between size and retention for both tags.
- We do not recommend the use of S-tag due to low retention.
- We support the use of PIT-tags for their relative high retention and survival.

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ABSTRACT

Several tagging methods have been tested to assess important aspects on ecology, such as population dynamic, movement pattern, and behavioral studies. The use of external and internal tags has been extensively used for individual identification in sea urchins. Different types of tags have been used in *Echinometra lucunter*, considered the most common sea urchin in the Caribbean. We evaluated whether PIT-tags and S-tags may affect retention, survival and growth rates under laboratory conditions, using the sea urchin *E. lucunter* as a model. The type of tag was critical in terms of retention rates, with significant differences between PIT-tag and S-tags groups (Kruskal–Wallis, $H = 5.33$, $p = 0.021$), and mortality exhibited similar rates among treatments. No significant association was detected between initial maximum test diameter and the number of weeks the individuals retained neither the PIT-tag ($r = -0.09$, $p = 0.620$), nor the S-tag ($r = -0.175$, $p = 0.413$), and no significant differences in growth rate were detected among treatments ($F = 1.66$, $df = 2$, $p = 0.401$). We do not recommend the use of S-tag due to low retention; but we support the use of PIT-tags in *E. lucunter* for their positive performance displayed in terms of retention and survival but with caution, particularly in those studies requiring 80% or higher retention.

1. Introduction

The development of suitable and effective tagging can provide insight on vital rates and currently represents a serious challenge for the design of long-term studies. Tagging devices have been extensively used in different aspects of ecology research whose experimental design rely on specific individual identification, such as evaluation of growth rates, predation, culture, movement patterns or behavioral studies (James, 2000; Tuya et al., 2003; Martínez et al., 2013; Boada et al., 2015). Early studies in echinoderms used external tags such as painted madreporite, insertion of a metallic labeled bar, anchored labels or plastic tags attached to the test; see Hagen (1996), Agatsuma et al. (2000), Duggan and Miller (2001), Clemente et al. (2007). Chemical tags (tetracycline marks, fluorochromes, and fluorescent elastomers) have been also used to track sea urchins (Ellers and Johnson, 2009; Martínez et al., 2013), but with serious limitations for uniquely identifying individuals. Recent development of Passive Integrated Transponder (PIT) tags (Gibbons and Andrews, 2004) has allowed for successful individual tracking of sea urchins using internal devices (Hazan et al., 2014; Boada et al., 2015; Rodríguez-Barreras and Sonnenholzner, 2014).

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Both PIT-tags and external plastic tags are invasive in their manipulation and artificially designed, with some important disadvantages that limit their reliability (Peterson and Black, 1994). Some authors held tagged sea urchins under laboratory conditions during short time periods to evaluate mortality and survival before using them in field experiments (Sonnenholzner et al., 2010; Fagerli et al., 2014). The evaluation of external plastic tags has been tested in echinoids with contrasting results, sometimes with the same target species (Carpenter, 1984; Rodríguez-Barreras and Sabat, 2015). The suitability of PIT-tags has exhibited variability among different species of sea urchins. Thus, some studies showed high retention and survival rates in *Strongylocentrotus* spp. (Palleiro-Nayar et al., 2009; Sonnenholzner et al., 2010) or *Tripneustes ventricosus* (Rodríguez-Barreras and Sonnenholzner, 2014), whereas low retention and survival rates were reported in *Diadema antillarum* (Rodríguez-Barreras and Sabat, 2015).

In this study, we evaluate the use of two possible tagging devices on the sea urchin *Echinometra lucunter* (Linnaeus, 1778), one of the most common Caribbean echinboid. This species inhabits in littoral shallow-water rocky areas, coral reefs and other hardgrounds (Hendler et al., 1995), and plays a crucial ecological role in the carbonate budget of reef systems for its bioeroding activity (Bak, 1994; Perry et al., 2014). Only a few tagging techniques have been tested in the genus *Echinometra*. PIT-tags were used to test the effect of different variables in growth rate and survival in *Echinometra sp. EE* from the Red Sea (Hazan et al., 2014), whereas Ebert (1988) estimated growth rates of *E. mathaei* using tetracycline marks. The only known tested technique in *E. lucunter* was the use of calcein for determining growth and survival rates (Ebert et al., 2008). The aim of this study was to determine if PIT-tags and S-tags could be used as reliable marking techniques, and how both tags may affect survival and growth rates under laboratory conditions.

2. Methods

Adult individuals of *Echinometra lucunter* (29.0–59.5 mm of maximum horizontal test diameter) were collected from an intertidal area in Cataño municipality (18° 28′5.39″N & 66° 8′12.97″W). Sea urchins were transported to the Laboratory of Population Ecology at the University of Puerto Rico, and placed in 37-L glass tanks provided with water filtering systems and airlift pumps for constant aeration. Salinity fluctuated between 35 and 37‰, and temperature was maintained between 22 and 24 °C. The experiment lasted 10 weeks, from March to May of 2015. Sea urchins were released at the same locality where they were previously collected once the experiment was finished. The species used in this study is not listed in any threatened or endangered category of the International Union for the Conservation of Nature (http://www.iucnredlist.org/). The necessary permit for sampling at sea was previously obtained from the corresponding authority.

2.1. Experimental setup

Sea urchins were left three days in acclimation before starting the experiment. The size range of the specimens was evenly distributed among fish tanks to minimize a possible bias due to differences in size. Treatments were PIT-tag, the Snap lock pin (S)-tag, and the control group (no manipulation). The number of replicates per treatment was four, and each experimental unit had 12 individuals. Sea urchins were tagged using 8 × 1.20 mm PIT-tag (Fig. 1(A)). The PIT-tag was injected into the celom through the aboral membrane using a plastic injector with a 1.25 mm diameter needle. PIT-tags were read with a handheld portable reader model EX (HDX/FDX-134.2 kHz). Rejected tags were recovered with a magnet bar immediately after detecting a new "no-signal individual" and were removed from the bottom of the tank to avoid any accidental ingestion.

The Snap lock pin (S-tag) has a structure with three teeth and a cavity in the proximal end and a 25-mm tail (Fig. 1(A)). An identification number was attached to the distal end of the tag. We attached the S-tag to a spine close to the aboral membrane to reach maximum visibility (Fig. 1(B)). The proximal side of the tag was pushed through the spine down to the spine base using a plastic pipette. Once the tag was in the correct position, the pipette was withdrawn, and the proximal side of the S-tag was fixed to the spine by adding a droplet of non-toxic glue (www.ecotechmarine.com).

Both tagging procedures took 40–60 s. Individuals were measured at the beginning and the end of the experiment with a Vernier caliper (reading error ±0.5 mm) to determine Gross Growth Rate (GGR) by subtracting the final maximum test diameter to the initial one. The Retention (R) and Survival (S) rates were determined and expressed as the percentage of individuals that retained or survived the experimental length, where N_0 and N_t were the number of tagged/live animals at time 0, and at the end of the experiment respectively:

$$R \text{ or } S = \left[\frac{(N_t - N_o)}{N_o}\right] \times 100.$$

2.2. Statistical analyses

We tested the relationship between initial size (maximum diameter) of living individuals at the beginning of the experiment, and the amount of time (weeks) they retained the tag using a non-parametric Spearman test. We tested the effect of tagging in GGR using one-way ANOVA. Differences in retention rates between both types of tags were tested using a Kruskal–Wallis test, and differences in sea urchin mortality rates among treatments (Control, PIT-tag, and S-tag) were assessed using a two-way ANOVA (with treatment and time as fixed factors) and a posteriori Tukey-HSD tests. Normality of residuals and homogeneity of variances were tested using Shapiro–Wilk and Bartlett tests respectively (Zar, 2010). All analyses were performed using the free licensed statistical software R version 3.2.3 (R Development Core Team, 2014).

3. Results

No significant differences in mortality rates were detected among treatments; but we found temporal differences within each group (Table 1). The first week was critical in terms of mortality rates. We experimented a significant mortality during the first week, but later it stabilized in the second week and maintained with no changes throughout the experiment. No mortality was reported for any treatment after the first week. The S-tag exhibited the highest mortality rate with 21.02 ± 10.45% (mean ± SD), followed by the control with 16.75 ± 11.78%, and lastly the PIT-tag with 12.50 ± 10.35%. In the majority of S-tag groups, the presence of the mark caused the loss of the entire spine, and less than 10% exhibited necrotic symptoms in the exposed tubercle for a short period of time (Fig. 1(B) and (C)). A posteriori test found that mortality between the day one and the first week increased significantly in the S-tag group, the PIT-tags, and the control group ($p < 0.05$).

Type of tag was critical in terms of retention rates, with significant differences between PIT-tag and S-tags groups (Kruskal–Wallis, $H = 5.33$, $p = 0.021$), mainly after the fifth day of the experiment. The first week was critical and delineated the