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Short communication

# A novel technique for tagging the long-spined sea urchin *Diadema antillarum*

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We describe a new protocol for tagging the long-spined sea urchin *Diadema antillarum* to study the daily activity patterns of this echinoid. The technique consists of the *in situ* introduction of a fishing hook into the periproctal membrane of individual *D. antillarum* with the help of tweezers, thus allowing the individual identification of tagged urchins. Preliminary tests displayed the effectiveness of this method.

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Marking individuals is imperative when monitoring movement patterns and the behaviour of animals. Marking sea urchins is difficult because of their spines, burrowing behaviour and nocturnal activity patterns (Neill 1987). Several published techniques are available for tagging certain species of echinoid, for example *Paracentrotus lividus* (Lam.) in Europe (Shepherd & Boudouresque 1979; Dance 1987; Crook & al. 2000), *Toxopneustes roseus* (A. Agassiz) in the Gulf of California (James 2000), *Centrostephanus coronatus* (Verrill) in the Caribbean (Nelson & Vance 1979), *Tripneustes ventricosus* (Lam.) in the Virgin Islands (Tertschnig 1989), *Tripneustes gratilla* (L.) in Papua New Guinea (Nojima & Mukai 1985), *Echinus esculentus* (L.) in Europe (Lewis 1980), *Evechinus chloroticus* (Val.) in New Zealand (Dix 1970), and *Strongylocentrotus droebachiensis* (Müller) in Europe (Hagen 1996) and in North America (Duggan & Miller 2001). However, there is only one nocturnal tagging study (Carpenter 1984) of the long-spined urchin *Diadema antillarum* Philippi. No study to date has addressed the nocturnal movements of this species in the temperate Central East Atlantic Ocean using tagging procedures.

In the present study, a preliminary survey was carried out to see if any of the published tagging techniques for echinoid species could be applied to *D. antillarum* to study short-term daily movements on a population of this species at the island of Gran Canaria (27°51′N 15°23′W) where they are known to inhabit shallow rocky reefs (Garrido & al. 2000). Only those techniques which did not require the removal of the echinoid from its habitat were researched. The results of this survey showed that the techniques employed for tagging short-spined sea urchins (e.g. *Paracentrotus lividus*) by means of anchor tags, metal screw tags, etc., could not be applied to a long-spined urchin such as *D. antillarum*, because of (1) the fragility of its spines, (2) excessive spine movement and (3) the use of the pedicellariae and secondary spines to push tags up the spine. We also tested the tagging procedure described by Carpenter (1984), who used 6 mm diameter circular sections of surgical tubing in his study of *D. antillarum*. However, our observations showed that sea urchins dropped the tagged spines on a large number of occasions ($\approx$ 50% of tagged spines). Carpenter (1984) only noted spine dropping in less than 10% of tagged *D. antillarum*. The reason for this difference in behaviour is not known. Nonetheless, differences in the environmental conditions between the Virgin Islands (tropical waters) and the Canarian Archipelago (temperate waters) may provide some explanation for this different behaviour.

We therefore set about to design a new tagging technique for *D. antillarum* to study short-term movements in the population found in Gran Canaria. The technique consisted of the *in situ* introduction of a fishing hook (model HL 6020 – # 11; 20 mm long) by a SCUBA diver into the periproctal membrane of individual *D. antillarum* using tweezers. The hook was linked to a fishing line ($\phi = 0.28$ mm, $\approx 10$ cm long), which in turn was attached to a cork buoy. This marker ($\approx 2 \times 2$ cm) allowed the individual identification of tagged urchins (Fig. 1). Tags securely placed in

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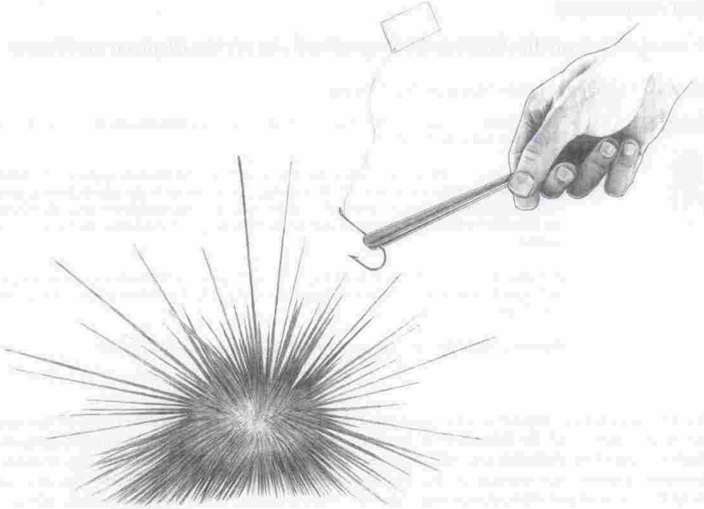

Fig. 1. *In situ* tagging of the long-spined sea urchin *Diadema antillarum* using tweezers.

an urchin were difficult to remove by simply pulling on the tag. The tagging protocol usually took less than 20 s per individual.

Preliminary tests were carried out at shallow rocky reefs to check the efficacy of the technique, as well as to assess the possible damage incurred by the tagging procedure. The distances travelled by tagged individuals were monitored and compared with non-tagged individuals by observers equipped with free-diving equipment, waterproof paper and metric tapes, for about 4–5 h per day. Non-tagged urchins were identified by their test diameters using callipers (James 2000). These comparisons were repeated three times (days) in an intertidal pool at Arinaga (Gran Canaria Island). Adult sea urchins (size class III 3.5–5.5 cm test diameter and size class IV > 5.5 cm; according to Casañas & al. 1998) were randomly selected each time, to avoid problems of non-independence and pseudoreplication of data (*sensu* Hurlbert 1984). The significance of the difference between mean individual distances moved per day between tagged and non-tagged sea urchins was calculated by means of the non-parametric Mann–Whitney U statistic (James 2000).

The percentage of tag retention per test ranged between 80 and 90% (Table 1). No significant differences in terms of movement of tagged and non-tagged individuals were obtained for any of the 3 days (Table 1). We therefore pooled the data from the three tests and calculated an overall pooled data Mann–Whitney U statistic. The probability level (p value, Table 1) of this test was not low enough to reject the null hypothesis, indicating therefore the non-significance of the comparison between the movement of tagged and non-tagged sea urchins. We calculated the power of this test using the Pass 6.0 package (Hintze 1996), taking into account that the power analysis for a non-parametric test (Mann–Whitney U) has to be conducted by adjusting the result obtained for the corresponding parametric test (t-test) (Hintze 1996). The low power detected (0.31 at $\alpha = 0.05$) was probably a function of (1) the low number of non-tagged sea urchins that could be followed, as it is logistically difficult to follow non-tagged urchins in the field (Dance 1987) and (2) the high variability associated with the distances travelled by sea urchins (Dance 1987; James 2000). In addition, we determined how many

Table 1. Percentage of tag retention and mean (± standard deviation) travelled distances (cm) by tagged and non-tagged *Diadema antillarum* for each test (day) and for pooled untransformed data. The Mann–Whitney U statistic and the associated p value indicate no significant differences between tagged and non-tagged individuals.

| | Test 1 | | Test 2 | | Test 3 | | Pooled data | |
|---|---|---|---|---|---|---|---|---|
| | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD |
| Tagged | 10 | 97.3 ± 26.09 | 10 | 88.65 ± 39.68 | 8 | 81.18 ± 26.31 | 28 | 89.60 ± 30.96 |
| Non-tagged | 5 | 111.81 ± 23.88 | 5 | 96.1 ± 25.65 | 4 | 93.00 ± 41.13 | 14 | 100.82 ± 28.89 |
| Mann–Whitney U statistic | 18.00 | | 17.50 | | 14.00 | | 146.5 | |
| p value | 0.39 NS | | 0.35 NS | | 0.73 NS | | 0.18 NS | |
| % tag retention | 80 | | 90 | | 87.5 | | 85.83 | |

SD – Standard deviation; NS – Not significant.

tagged sea urchins would have been required for the null hypothesis to be rejected at the $\alpha = 0.05$ level. A total of 662,296 tagged sea urchins would have been necessary to reject the null hypothesis; with an increase in power from 0.31 to 0.56. Consequently, the results from these statistical tests displayed evidence of an absence of alteration in movement due to tagging. Therefore, we assumed that the observations of tagged individuals were not confounded by the tagging protocol.

As Dance (1987) indicated, the effect of tagging on short-term echinoid activity is difficult to determine, as non-tagged individuals cannot easily be followed in the field during night hours. Although no flight reaction or podia movement was observed immediately after tagging, it is difficult to provide evidence of the possible damage caused by tagging on the behaviour of sea urchins, as considered in many tagging studies (Sinclair 1959; Gamble 1965; Shepherd & Boudouresque 1979; Lewis 1980; Dance 1987).

The technique used in this paper is cheap, quick and easy for experienced SCUBA divers to perform. The procedure is carried out *in situ* and does not require the removal of sea urchins from their habitat, which is an important parameter when working with species of sea urchins that burrow (Neill 1987). It can, therefore, be effectively applied for marking multiple individuals to study short-term (daily) migrations of sea urchins. However, a potential problem that we have not yet evaluated, and which should be dealt with in future tagging experiments, is the possibility that inserted tags may act as an attractant to sea urchin predators such as fish.

At present there is considerable research activity regarding the ecology of *D. antillarum* populations along the Canarian Archipelago. The tagging technique presented in this paper is enabling us to carry out experiments to study the daily movements and the homing behaviour of this invertebrate species along the Canary Islands, in the framework of a research project concerning the rapid increase in urchin-grazed barrens along the Canaries (Casañas & al. 1998; Garrido & al. 2000).


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