Caribbean Journal of Science, Vol. 48, No. 2-3, 132-137, 2014

Effect of implanted PIT-tags on growth, survival, and tag retention in the sea urchin *Tripneustes ventricosus*

Ruber Rodríguez-Barreras[1,*] and Jorge Sonnenholzner[2]

[1]*Department of Biology, University of Puerto Rico, P.O. Box 23360 San Juan PR 00931-3360*
[2]*Escuela Superior Politécnicadel Litoral, ESPOL, Facultad de Ingeniería Marítima, Ciencias Biológicas, Oceánicas y Recursos Naturales. Campus Gustavo Galindo Km 30.5 Vía Perimetral, P.O. Box 09-01-5863, Guayaquil, Ecuador..*
[*]*Corresponding autor: ruber.rodriguez@outlook.com*

Abstract.– The major goal of this study was to provide valuable information about the effectiveness of tagging the whitish sea urchin *Tripneustes ventricosus* in semi-captivity conditions using one type of internal mark. A field experimental study was conducted to assess the effect of Passive Integrated Transponders (PIT) tagging on the survival and growth rate of *T. ventricosus* as a function of body size, and evaluate PIT tag loss. PIT tags were injected into the coelomic cavity of sea urchins ranging from 29 to 125 mm of test diameter (TD). Sixty sea urchins (30 tagged and 30 non-tagged) of similar sizes were monitored over 10 weeks. For all sea urchin size categories, at the end of the study, tag retention rate was 100%, and survival rate was 75.0 ± 24.0 %. Small-sized urchins exhibited the highest Gross Growth Rate during the experiment (tagged: 3.16 ± 0.34 mm TD; control: 1.78 ± 0.36 mm TD), whereas large-sized individuals displayed the lowest rates (tagged: 0.53 ± 0.0 mm TD; control: 0.70 ± 0.18 mm TD). No significant differences in survival and growth rates were found between tagged and non-tagged animals within size classes. Results suggested that urchins can be tagged using PIT tags, with negligible effects on survival and growth, and this leads to a zero tag rejection rate. Therefore, we recommend use this accurate technique to improve estimation of demographic parameters in *T. ventricosus* populations for long term tagging studies.

Keywords.–*Tripneustes ventricosus*, tagging, survivorship, PIT-tags, Puerto Rico.

Introduction

The sea urchin *Tripneustes ventricosus* (Lamarck 1816) is an important herbivore of seagrass meadows along the Western Atlantic, the Caribbean Sea, and The Gulf of México (Keller 1983, Tertschnig 1989). This echinoid is a short-lived species. Indeed, its gonads are highly appreciated in the fishery and aquaculture industry for their large size (Scheibling and Mladenov 1987, Smith and Berkes 1991, Lawrence and Bazhin 1998, Vermeer *et al*. 2005, De Beauville-Scott 2010).

The development of accurate tagging technique to estimate demographic parameters of a given species is a real necessity for fishery studies (Pradel 1996, Kalvas *et al*. 1998, Lauzon-Guay and Scheibling 2008). In this sense, sea urchins have been tagged in several ways, such as metallic labeled bars into the coelomic cavity, tetracycline marks, plastic tags, painted madreporite, anchoraged labels, polyfluorochromes, and passive integrated transponders (Olson and Newton 1979, Ebert and Russel 1992, Kalvas *et al*. 1998, Duggan and Miller 2001, Tuya *et al*. 2003, Ellers and Johnson 2009, Sonnenholzner *et al.* 2010, Rodríguez-Barreras *et al*. 2014).

Effective tags should have high retention rate and neutral effect on survival and growth rates (Ebert and Russel 1992, Williams *et al*. 2002, Amstrup *et al*. 2006). Passive Integrated Transponder (PIT) tags are one of the most recent internal devices used for accurate and individual identification on sea urchins in the wild (Mowat and Strobeck 2000, Woods and James 2005). On laboratory-controlled conditions, PIT-tags have reported promising results on retention and survivorship (Hagen 1996, Sonnenholzner *et al.* 2010). Nevertheless, field studies in echinoids remains scarce, and there is no available information about the use of PIT-tags on *T. ventricosus*. Therefore, the present study evaluates the retention of PIT tags and their effect on growth, survival and survival of *T. ventricosus* in semi-captivity conditions.

Methods

Sampling site

Sea urchins of the species *Tripneustes ventricosus* were collected randomly from a shallow-water reef lagoon at Mar Azul beach, municipality of Luquillo, in the northeastern coast of Puerto Rico (18°23'18.46"N, 65°43'5.52"W). The area is a well-developed seagrass meadow dominated by the flowering plants *Thalassia testudinum* and *Syringodium filiforme*, with an average of 1.5 m depth. Substrate consists mainly in a mix of sand and rubbles from remains of the coral *Porites porites*, the most abundant coral in the area.

Tagging procedure

One Passive Integrated Transponder (cylindrical glass capsule of 8.0 mm long and 1.4 mm in test diameter, PIT-tags, Oregon RFID Corp.) was used on small (< 50.0 mm, mean ± SE= 40.4 ± 1.1 mm test diameter, TD), medium (50.1- 80.0 mm, 62.0 ± 1.4 mm TD), and large specimens (> 80.1 mm, 108.0 ± 2.6 mm TD). The PIT-tags were carried out by holding each urchin upside down, inserting the needle injector through the peristome membrane into the coelom (1.75 mm gauge implanter). Tagging procedure lasted approximately 45 s per urchin. PIT tags were scanned with a Portable Transceiver System (Pocket Reader, EX-model, Biomark Inc.). Individuals were measured and scanned weekly for 10 weeks. All sea urchins were not handled incorrectly during the tagging procedure. This was established to avoid trials for manipulation errors.

Field experimental setup

Sixty sea urchins (sizes range were 29 to 125 mm test diameter, TD) between classified by size into three range categories: small (29.0-47.0 mm TD, mean= 40.4 ± 1.1 mm test diameter, TD), medium (52.0-75.0 mm TD, mean= 62.0 ± 1.4 mm TD), and large (82.0-125.0 mm TD, mean= 108.1 ± 2.6 mm). Of them, twenty individuals of each size class (30 urchins per cage, 10 individuals of each size category) were randomly placed in two bottomless metallic cages (3.0 m length x 1.5 m width x 1.5 m height) covered with 1.4 cm of mesh size were anchoraged to the substrate with iron bars. Treatments (tagged and non-tagged: control) were assigned randomly to experimental units. Urchins were fed *ad libitum* for 10 weeks (3.8 kg wk^{-1}) with the seagrass *Thalassia testudinum* and mix of algae present in the area where sea urchins were collected. The experiment lasted 70 days from 2013/02/09–2013/04/19 (ten weeks).

Survival and growth rate

Survival rate (S) was determined as the percentage of animals that survived the time interval (Equation 1), where N_0 and N_t are the number of live organisms present at time 0 and at the end of the experiment, respectively.

$$S\,(\%) = \left[\frac{(Nt - No)}{No}\right] \times 100 \qquad (\text{Eq. } \mathbf{1})$$

Gross Growth Rate (GGR) was calculated as the size increment (TD) between the beginning and the end of the experiment (Equation 2), where TD_0 and TD_t are the test diameters present at time 0 and at the end of the experiment (2.5 month), respectively.

$$GGR\,(\text{mm. month}^{-1}) = \left[\frac{(TDt - TDo)}{t}\right] \qquad (\text{Eq. } \mathbf{2})$$

Analysis of data

To determine the effect of tagging on survival, a paired-t test was performed between tagged and control treatments, considering the three size groups as the pairing criterion. Percent survival data were previously subjected to the arcsine transformation. To test the effect of tagging on the GGR, separate t-tests were performed for each size group between tagged and control urchins. We report results as mean ± SE throughout. All statistical analyses were performed at a significance level 0.05 using the program Statistica, version 6.0 (StataCorp.

2003).

Results

Survival

An overall survival rate was 75.0 ± 23.5 % for all size categories at the end of the study; where tagged and control treatments were 73.3 ± 30.6 % and 76.7 ± 20.8 %, respectively. The lowest survival rate was found in small individuals with an overall survivorship of 40-60%. The medium sized urchins had a survivorship between 80 % for tagged and 70 % for control treatments. No mortality occurred in large sized individuals (Fig. 1); and there was not a significant effect of tagging on growth gross rate ($t = 0.307$; $p = 0.7879$).

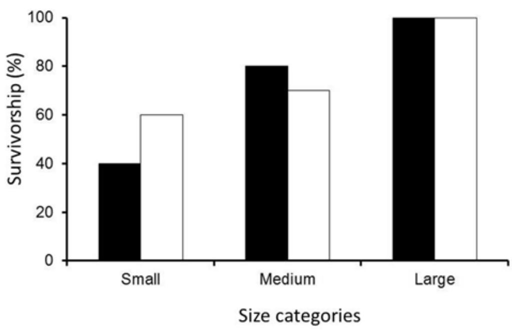

Fig. 1. Survival of sea urchins *Tripneustes ventricosus* in the experiment. Dark bars represent the PIT-tag treatment and white bars represent the Control treatment.

GGR

Overall GGR was 1.59 ± 0.17 mm mo^{-1}, and ranged from 0.53 mm mo^{-1} to 4.21 mm mo^{-1} among treatments. The growth rate between tagged individuals of the same size-class was not different to the control group (Table 1). Small-sized urchins had the highest GGR during the experiment (tagged: 2.63±1.70 mm TD; control: 2.01 ± 2.3 mm TD), whereas large-sized individuals displayed the lowest rates (tagged: 0.02 ± 0.98 mm TD; control: 0.51 ± 0.35 mm TD) (Fig. 2).

Table 1. A t-test performed to compare Gross Growth Rate for each size group between tagged and control treatments for the sea urchin *Tripneustes ventricosus*.

Urchin size group	**df**	**t**	**p**
Small	9	1.5126	0.1647
Medium	9	1.6500	0.1333
Large	9	1.4638	0.0886

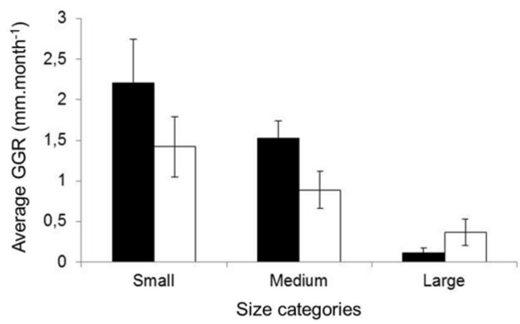

Fig. 2. Average Gross growth rates (GGR) of the sea urchin *Tripneustes ventricosus* for three size classes. Dark bars mean PIT-tag treatment and white bars represent the Control treatment.

Pit-tag retention

In living sea urchins, tag retention was 100% for all size classes at the end of the study (Fig. 3). Indeed, some PIT-tags were recovered from recently dead animals. Mortality did not occur until the fourth week for tagged individuals, and in the seventh week for those non-tagged (control) (Fig. 3).

Discussion

The major goal of this study was to provide valuable information about the effectiveness of tagging the sea urchin *Tripneustes ventricosus* in semi-captivity conditions using PIT-tags. Several studies have emphasized that high retention and none mortality effect are essential for successful long-term ecological studies (McPherson 1965, Olson and Newton 1979,

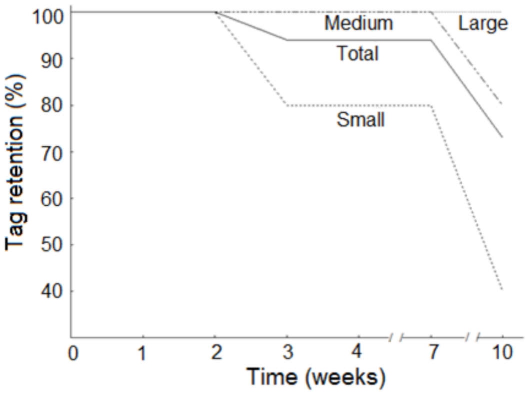

FIG. 3. Tag retention of the sea urchin Tripneustes ventricosus by size-classes.

Hagen 1996, Duggan and Miller 2001, Williams *et al*. 2002, Amstrup *et al*. 2006). The fact that 100% of living *T. ventricosus* retained the PIT-tag meets this requirement.

This result agrees with other studies where sea urchins displayed a tag loss of 10% or lower (Hagen 1996, Kalvas *et al*. 1998, Woods and James 2005, Palleiro-Nayar *et al.* 2009, Sonnenholzner *et al.* 2010). Although those previous studies were conducted under controlled conditions reported higher survival rates than in our study, here we demonstrated that is possible tagging the whitish sea urchin *T. ventricosus* for obtaining a successfully individual response, based on their survivorship, growth rates and retention of marks on wild and semi-captivity conditions. Although, our data did not include the release of those tagged sea urchins in the sea; of them, twenty-two pit-tagged sea urchins were released at the end of the experiment, and after one month were recaptured 13 (59 %) individuals with their tagging marks *(unpublished data)*.

Therefore, mortality here reported for tagged and non-tagged small and medium size urchins was due to the experimental setup design here used that allowed an increased intra-especific competition between animals by the crowding condition (a high urchin density to limited number of cages), but no the tagging procedure. Some large urchins where observed mounted (in four of the seven visits done) on small and medium size ones. Small and medium urchins had peeled spines and sanded test epithelium. In fact, these two size categories reported lower survival while large urchins were alive throughout the experiment.

In the wild, echinoderms are vulnerable to predators and other echinoderms (Berstein *et al.* 1981, Clemente et al., 2007). Thus, large sea urchins tend to compete for space and available food resources with their own congeners (Sonnenholzner *et al.* 2011). Our semi-captivity conditions where the mesh cage diameter was 7.0 mm, did not allow the entrance of large predators. Therefore, we did not consider predation as a main source of mortality. Indeed, density of *T. ventricosus* within cages was about 15 urchin m^{-2}, when natural density of *T. ventricosus* usually fluctuates between 0.4-3.8 urchin m^{-2} (Scheibling and Mladenov 1987).

No differences in growth between treatments on *T. ventricosus* agrees with other studies that found no differences between tagged and non-tagged sea urchins on growth using PIT-tags (McPherson 1965, Hagen 1996, Sonnenholzner *et al*. 2010), as well as in other types of internal devices (Russell and Urbaniak 2004, Ellers and Johnson 2009). For instance, McPherson (1965) tagged small *T. ventricosus* with a plastic disk fastened by a stainless steel wire, but he did not find differences on growth between tagged and non-tagged individuals. Despite of both studies used a different type of tags; results suggest a neutral effect of tagging in GGR.

Growth in sea urchins may also vary according to experimental conditions (Kalvas *et al.* 1998, Maciá and Robinson 2008). Thus, Palleiro-Nayar *et al.* (2009) found that the tagged echinoid *Strongylocentrotus franciscanus* grew in the laboratory more than in the wild, because echinoids spend less energy under controlled conditions. These results evidence that experimental setup is determinant in GGR estimations, sometimes more than tagging *per se*, and emphasize the importance to test tagging devices under control and wild conditions.

The development of suitable and accurate tagging techniques constitutes a real necessity for long-term studies. We found that PIT-tags displayed positive features for long-term studies:

non-destructive identification of individual, low mortality and no remarkable negative effects on growth. Therefore, we recommend the use of PIT-tags on *T. ventricosus* for their positive performance displayed in terms of retention and survival. Nevertheless, further studies would need to clarify other important aspects of PIT-tags in the species biology, such as the effect of tagging on behavior, reproduction, and susceptibility to predation.

Acknowledgements.–This study was funded by the Sea Grant projects PD-295 and R92-110. We want to thanks Ricardo Searcy assisted with the statistical analyses and Julián López Morell for supporting field works.

Literature Cited

Amstrup, S., L. MacDonald and B. Manly. 2006. *Handbook of Capture-Recapture Analysis*. Princeton University Press, 296 pp.

Bernstein, B. B., B. E. Williams and K. H. Mann. 1981. The role of behavioral responses to predators in modifying urchin's (*Strongylocentrotus droebachiensis*) destructive grazing and seasonal foraging patterns. *Marine Biology* 63:39-49.

Clemente, S., J. C. Hernández, and A. Brito. 2007. An external tagging technique for the long-spined sea urchin *Diadema antillarum*. *Journal of the Marine Biological Association of the United Kingdom* 87:777-779.

De Beauville-Scott, S. 2010. Saint Lucia national sea egg country report. In FAO/SLC, eds. *Report of the special workshop on the white sea urchin (Tripneustes ventricosus) fisheries in the eastern Caribbean.* 61st Gulf and Caribbean Fisheries Institute (GCFI), Le Gosier, Guadeloupe, 14 November 2008, 15-23. FAO Fisheries and Aquaculture Report. No. 933. Rome, FAO. 2010. 80 pp.

Duggan, R. E. and R. J . Miller. 2001. External and internal tags for the green sea urchin, *Journal of Experimental Marine Biology and Ecology* 258(1):115-122.

Ebert, T. A. and M. P. Russel. 1992. Growth and mortality estimates for red sea urchin *Strongylocentrotus franciscanus* from San Nicolas Island, California. *Marine Ecology Progress Series* 81:31-41.

Ellers, O. and A. S. Johnson. 2009. Polyfluorochrome marking slows growth only during the marking month in the green sea urchin *Strongylocentrotus droebachiensis*. *Invertebrate Biology* 128:126-144.

Hagen, N.T. 1996. Tagging sea urchins: a new technique for individual identification. *Aquaculture* 139(3/4):271-284.

Kalvas, P. E., J. M. Henrix and P. M. Law. 1998. Experimental analysis of 3 internal marking methods for red sea urchins. *California Fish and Game* 84:88-99.

Keller, B. D. 1983. Coexistence of sea urchins in seagrass meadows: an experimental analysis of competition and predation. *Ecology* 64:1581-1598.

Lauzon-Guay, J. S. and R. E. Scheibling. 2008. Evaluation of passive integrated transponder (PIT) tags in studies of sea urchins: caution advised. *Aquatic Biology* 2:105-112.

Lawrence, J. M. and A. Bazhin. 1998. Life-history strategies and the potential of sea urchins for aquaculture. *Journal of Shellfish Research* 17(5):1515-1522.

Maciá, S. and M. P. Robinson. 2008. Habitat-dependent growth in a Caribbean sea urchin *Tripneustes ventricosus*: the importance of food type. *Hegoland Marine Research* 62:303-308.

McPherson, B. F. 1965. Contribution to the biology of the sea urchin *Tripneustes ventricosus*. *Bulletin of Marine Science* 15:228-244.

Mowat, G. and C. Strobeck. 2000. Estimating population size of grizzly bears using hair capture, DNA profiling, and mark-recapture analysis. *The Journal of Wildlife Management* 64:183-193.

Olson, M. and G. Newton. 1979. A simple, rapid method for marking individual sea urchins. *California Fish and Game* 65:58-62.

Palleiro-Nayar, J., O. Sosa-Nishizaki and G. Montaño-Moctezuma. 2009. Estimación de la tasa de crecimiento corporal del erizo rojo *Strongylocentrotus franciscanus* en cautiverio y en el Arrecife Sacramento en la Bahía El Rosario, Baja California, México. *Ciencia Pesquera* 17:21-28.

Pradel, R. 1996. Utilization of capture-mark-recapture for the study of recruitment and population growth rate. *Biometrics* 52:703-709.

Rodríguez-Barreras, R., S. Serrano-Torres and D. Macías-Reyes. 2014. A study of two tagging methods in the sea cucumber *Holothuria mexicana*. *Marine Biodiversity Records* 7:1-4.

Russell, M. P. and L. M. Urbaniak. 2004. Does calcein affect estimates of growth rates in sea urchins? In *Echinoderms: München,* eds. T. Heinzeller, and J. H. Nebelsick, 53-57. London: Taylor and Francis Group.

Scheibling, R. E. and P. V. Mladenov. 1987. The decline of the sea urchin*, Tripneustes ventricosus*, fishery of Barbados: A survey of fishermen and consumers. *Marine Fisheries Review* 49(3):62-69.

Smith, A. H. and F. Berkes. 1991. Solutions to the 'Tragedy of the Commons': sea-urchin management in St Lucia, West Indies. *Environmental Conservation* 18:131-136.

Sonnernholzner, J. I., G. Montaño-Moctezuma and R. Searcy-Bernal. 2010. Effect of three tagging methods on the growth and survival of the purple sea urchin *Strongylocentrotus purpuratus*. *Pan-American Journal of Aquatic Sciences* 5(3):414-420.

Sonnenholzner, J., G. Montaño-Moctezuma, R. Searcy-Bernal and A. Salas-Garza. 2011. Effect of macrophyte diet and initial size on the survival and somatic growth of juvenile *Strongylocentrotus purpuratus*: a laboratory experimental approach. *Journal of Applied Phycology* 23:505-513.

StataCorp. 2003. Stata Statistical Software: Release 6. College Station, TX: StataCorp LP.

Tuya, F., J. A. Martin and A. Luque. 2003. A novel technique for tagging the long-spined sea urchin *Diadema antillarum*. *Sarsia* 88:365-368.

Tertschnig, W. P. 1989. Diel activity patterns and foraging dynamics of the sea urchin *Tripneustes ventricosus* in a tropical seagrass community and a reef environment (Virgin Islands). *Marine Ecology* 10:3-21.

Vermeer, L. A., W. Hunte and H. A. Oxenford. 2005. An assessment of the potential for community level management of the sea urchin fishery in Barbados. *Proceedings of the Gulf and Caribbean Fisheries Institute* 47:79-103.

Williams, K., J. Nichols and M. Conroy. 2002. *Analysis and Management of Animal Populations*. San Diego: Academic Press.

Woods, C. M. C. and P. J. James. 2005. Evaluation of passive integrated transponder tags for individually identifying the sea urchin *Evechinus chloroticus* (Valenciennes). *Aquaculture Research* 36:730-732.