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Effect of three tagging methods on the growth and survival of the purple sea urchin *Strongylocentrotus purpuratus*

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Abstract. We evaluated the effect of tetracycline (a fluorescent tag) and two different types of internal tags, Decimal Coded Wire Tags (CWT) and Passive Integrated Transponder tags (PIT) used alone or in combination on growth rates, survival, tag retention, and post-tagging stress of the purple sea urchin *Strongylocentrotus purpuratus* (Stimpson), under laboratory conditions (150 days). Specimens had a size range between 12.5 and 54.0 mm in diameter. Our results suggest that the tetracycline, PIT, and CWT (or their combination) do not affect growth or survival of purple urchins. PIT tags can be successfully inserted into urchins over 22.7 mm without jeopardizing growth or survival. Urchin's growth under different tagging treatments was similar to the control groups. Urchins that were tagged twice lost their tags after two days, and died in less than a month. Post-tagging stress (RAC) was significantly affected by urchin size but not by tagging methods.

Key words: Sea urchin, tagging, tetracycline, electromagnetic tags, CWT, PIT.

Resumen. Efecto de tres métodos de marcado en el crecimiento y la supervivencia del erizo de mar *Strongylocentrotus purpuratus*. Se evaluó el efecto de la tetraciclina (marcaje fluorescente) y dos tipos diferentes de marcas internas, etiquetas de alambre codificado (CWT, por sus siglas en inglés) y marcas electromagnéticas de inducción pasiva (PIT), solas o en combinación, en la tasa de crecimiento, supervivencia, retención de las marcas y el estrés post-marcaje del erizo morado *Strongylocentrotus purpuratus* (Stimpson), bajo condiciones experimentales de laboratorio (150 días). Los especímenes tuvieron un rango de tamaño entre 12.5 y 54.0 mm de diámetro. Nuestros resultados sugieren que la tetraciclina, CWT y PIT (o su combinación) no afectan el crecimiento o la supervivencia de los erizos morados. Las marcas PIT pueden ser insertadas con éxito en erizos mayores a 22.7 mm sin afectar su crecimiento o supervivencia. El crecimiento de los erizos bajo diferentes tratamientos de marcado fue similar a los grupos de control. Los erizos que fueron marcados dos veces perdieron las marcas después de dos días, y murieron en menos de un mes. El tamaño del erizo afectó significativamente al estrés post-marcado (RAC); sin embargo, el método de marcado no tuvo ningún efecto sobre el RAC.

Palabras Clave: Marcaje, erizos de mar, tetraciclina, marcas electromagnéticas, CWT, PIT.

Introduction

Harvesting purple sea urchins (*Strongylocentrotus purpuratus*) has been suggested as an alternative to reduce the harvest pressure on red urchin populations in Baja California (Andrew *et al.* 2002, Palleiro *et al.* 2008); however, little information exists regarding its life history. Since an extensive fishery has been recommended, quantifying critical parameters, such as growth becomes important to suggest adequate management strategies.

The application of mark-recapture methods in studies of growth (Pearse & Pearse 1975, Ebert 1988, Meidel & Scheibling 1998, Russell 2000, Rogers-Bennett *et al.* 2003, Pederson & Johnson 2007) and age validation (Taki 1972, Robinson &

MacIntyre 1997, Hiroyuki *et al.* 2005, Shelton *et al.* 2006) has been relatively common for Strongylocentrotids. Growth rates in purple sea urchins have been estimated with tagging techniques using the fluorochrome tags such as tetracycline and calceine (Russell 1987, Ebert 2007).

Fluorochromes that incorporate into urchin ossicles do not interfere with natural growth (Kobayashi & Taki 1969, Ebert 1988, Gage 1992, Russell & Urbaniak 2004, Ellers & Johnson 2009) and very small growth increments can be easily detected, including zero growth (Ebert 1967). A disadvantage of internal tags resides in the intensive destructive sampling required to ensure high recapture rates of tagged organisms. The development of Passive Integrated Transponders (PIT) and Decimal Wire Coded Tags (CWT) has been useful to individually identify urchins in laboratory conditions (Hagen 1996, Kalvass *et al.* 1998, Woods & James 2005), and in the field (Dugan & Miller 2001, Lauzon Guay & Scheibling 2008). Hence, the combination of internal and external tags look promise to diminish recapture rates and guarantee higher percentages of tagged individuals.

Mark-recapture methods have the premise that tagging does not promote any physical damage; furthermore, credibility of results from a tagging study rests on demonstrating that major assumptions and biases have been met or accounted for. The effect of different internal tagging methods has been assessed in green urchins (*Strongylocentrotus droebachiensis*) (Hagen 1996, Russell & Urbaniak 2004, Lauzon-Guay & Scheibling 2008), red urchins (*S. franciscanus*) (Kalvass *et al.* 1998), and New Zealand urchins (*Evechinus chloroticus*) (Woods & James 2005); however, no information exists about the tagging effect on purple urchins (*S. purpuratus*).

The present study evaluates the effect of three tagging methods: (1) tetracycline (free base), (2) PIT tags and (3) CWT tags, as well as their combination on purple sea urchins somatic growth, survival, post-marking-stress (RAC) and tag loss, under laboratory conditions.

Our interest in assessing the effect of the paired combination of a single-fluorochrome marking (tetracycline) with PIT or CWT tags, resides in our concern of using both tagging techniques in age determination studies to validate the periodicity of natural growth lines in urchins genital plates. Because the use of tetracycline alone requires the recapture of a high number of organisms, adding an electromagnetic tag that is easily recognized without killing the urchins, would reduce the number of samples to be collected.

Materials and Methods

A total of 128 urchins (between 12.5 mm and 54.0 mm in diameter) were collected from an intertidal site at Bajamar, Baja California, México (32°01'14" N, 116°52'52" W), and transported to the laboratories of the Universidad Autónoma de Baja California. They were acclimated in 280 l-tanks with circulating sea-water and aeration for at least one month before the experiment. Seawater temperature was maintained at 16 ± 1ºC and urchins were fed on *Egregia menziesii ad libitum*.

Tagging procedure. Three different types of internal tags were used: (1) Passive Integrated Transponders (PIT, Biosonic Corp.), (2) unique sequential Coded Wire Tags (CWT, Northwest Marine Tech. Inc.), and (3) the fluorochrome tetracycline (SIGMA). PIT tags are cylindrical glass capsules of 12 mm long x 2.1 mm in diameter. CWTs are steel wire tags 1.5 to 2 mm long x 0.25 mm diameter. Tetracycline-hydrochloride solution (1 g per 100 mL) was prepared using 0.2 μm filtered seawater. Due to the size of PIT tags these were used on medium (22–40 mm) and large (>40 mm) urchins. Small individuals (<22 mm) were tagged with CWT, and 0.1 mL of tetracycline solution was injected. Medium and large individuals received 0.2 and 0.3 mL of the solution, respectively. After five minutes of injecting tetracycline, we performed PIT and CWT tagging to avoid spill of fluids. PIT and CWT tagging was carried out by holding each urchin upside down, inserting the injector needle through the peristome membrane into the coelom. PIT tags were inserted using a 12 gauge implanter (MK7 Biomark) and CWT tags using a single-shot injector (NMT). A small gauge hypodermic needle was used for tetracycline. Tagging procedure lasted approximately 45 s per urchin. PIT tags were scanned with a Portable Transceiver System (Model 2001F-ISO, Destron Technologies), and CWTs were X-ray photographed to confirm their placement within the urchin coelom (Fig. 1).

Laboratory experimental setup. Individual urchins were distributed into experimental units consisting of PVC pipe (15 cm diameter x 16 cm height) covered with a 2 mm diagonal mesh at the bottom. Treatments were assigned randomly to experimental units that were placed on a fiberglass tray (245 cm long x 75 cm wide) into an air conditioned room (air temperature was kept at 15.0 ± 0.5ºC). An open sea water system (water flow was maintained at ca. 500 mL min^{-1}) and constant aeration was provided using airlifts. The experiment lasted 150 days from 2006/10/26–2007/03/26. The number of replicates per treatment was 9 to 11. We evaluated the effect of tagging on: 1) growth, 2) survival, 3) tag loss and 4) post-tagging stress. To diminish measurement error, test diameter (TD) was measured five times using a knife-edge vernier calipers and the mean diameter was used for the analysis.

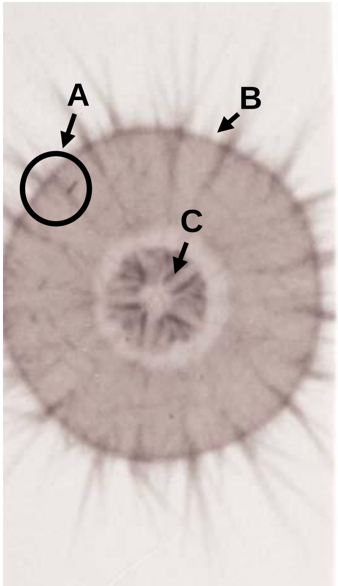

Figure 1. X-ray photograph of a purple sea urchin of 22 mm test diameter showing (A) a coded-wire tag into the coelom, (B) spines, and (C) Aristotele's lantern.

Survival, growth rate, and tag retention. Survival rate (S) was determined as the percentage of animals that survive the time interval:

$$S (\%) = [(N_t – N_0) / N_0] \times 100 \quad (1)$$

where N_0 and N_t are the number of live organisms present at time 0 and at the end of the experiment, respectively.

Gross Growth Rate (GGR) was calculated as the size increment (TD) between the beginning and the end of the experiment:

$$GGR\ (mm\ month^{-1}) = [TD_t – TD_0) / t\ (5\ months)] \quad (2)$$

where TD_0 and TD_t are the test diameters present at time 0 and at the end of the experiment (5 mo), respectively.

Daily observations were made for estimating mortality and tag retention. Some sea urchins were not correctly marked at the first time by a manipulation error. Afterward this first failed attempt, these sea urchins (*n* = 15) in a second attempt were tagged. These animals were maintained for 30 days in individual plastic containers, but in a separate tank for observational stress trials to register lost spines and death time.

Righting activity Coefficient (RAC). Sea urchins are normally oriented with the tube feet of the oral surface attached to the substratum. Overturning an individual so that the oral surface faces up and the tube feet are not in contact with the substratum elicits a righting response that can be estimated based on the time it takes an urchin to return to normal position, after being inverted onto their surface. Eight hours after tagging, all urchins (tagged and control groups) were kept in Petri dishes and righting time was measured. Mean righting times were converted to righting activity coefficients (RAC) using the formula suggested by Hagen (1994): RAC (seconds) = 1000/righting time. RAC values can indicate different levels of stress and well-being (Lawrence & Cowell, 1996). Relatively higher RAC values are associated with higher levels of stress.

Statistical analysis. We tested the effects of tagging method on growth (GGR) and post-tagging stress (RAC) using analysis of covariance (ANCOVA), using tagging methods as a fixed factor and initial urchin size as the covariate, after verifying the homogeneity of slopes among treatments (Zar, 1999).We report results as mean ± SE throughout. All statistical analyses were performed using the software STATISTICA for Windows (StatSoft, Inc. 1998).

Results

Survival and tag retention. At the end of the experiment (150 d), survival and tag retention was 100% for all test groups. We found no association between survival and urchin size or tag treatments. The tag wound healed on all urchins by day 2 when a scar was noticeable on most of the individuals. By day 5 the epidermal pigmentation appeared normal.

Severe epithelium damage was caused when urchins received two insertions. This occurred in 13% of the tagged urchins. Those urchins were placed for 4-weeks in separate experimental units until they died (Table I). In this group, mortality was exceptionally high in the first two weeks post-tagging. During this period, urchins had a low adhesive capacity to the substratum and eventually lost their spines. Tag loss occurred between one and two days after tagging. Of these, small urchins that were tagged with CWT showed a high percentage (83%) of tag loss in the first day and were all dead after one month. (Table I).

Table I. Observational trial (1-month) for the sea urchin *Strongylocentrotus purpuratus* from different treatments that were tagged twice. Tags = CWT or PIT, TC = tetracycline.

Treatments	Number of urchins	Tagging methods and number of urchins			
		(Tags lost)		(Dead urchins)	
		CWT	PIT	CWT	PIT
Tag + TC	11	4 (3)	7 (1)	4 (4)	7 (7)
Tag	4	2 (2)	2 (1)	2 (2)	2 (2)
Total	15	6 (5)	9 (2)	6	9

Growth rates. Among different treatments, mean GGR was 0.636 ± 0.042 mm mo^{-1} and ranged from 0.006 mm mo^{-1} to 1.808 mm mo^{-1}. The results of GGR for the different tagging treatments in relation to initial size are shown in Figure 2a. ANCOVA indicated an effect of size (P = <0.0001, Table II), but not of tagging methods (P = 0.806, Table II).

The righting activity coefficient (RAC). Mean RAC considering all treatments was 12.23 ± 0.25 s and ranged between 9.71 and 24.57 s. Highest and lowest RAC values were found in individuals less than 25 mm TD and larger than 30 mm TD, respectively (Fig. 2b). The highest variability of RAC values was found in those individuals of small size (< 25 mm) (Fig. 2b). ANCOVA indicated an effect of size (P = <0.0001, Table II), but not of tagging methods (P = 0.5863, Table II).

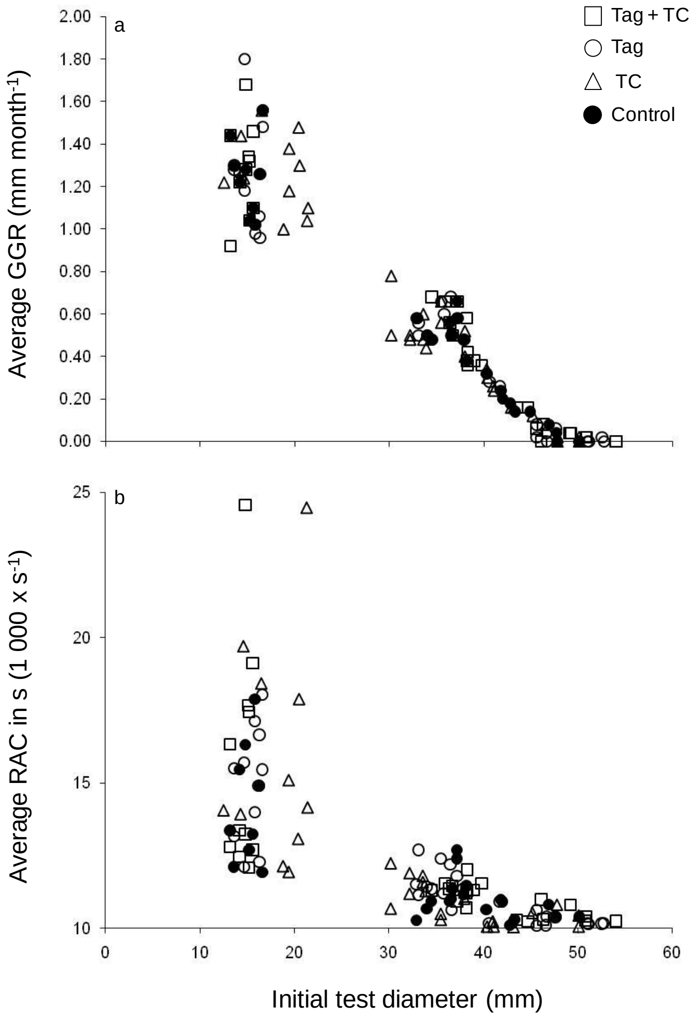

Figure 2. The relationship between the initial size and (a) the Gross Growth Rate (GGR), and (b) the Righting Activity Coefficient (RAC) of *Strongylocentrotus purpuratus* among tagging methods and control.

Table II. Analysis of Covariance (ANCOVA) to test the effects of tagging methods and size on the Gross Growth Rate (GGR), and the Righting Activity Coefficient (RAC) of *Strongylocentrotus purpuratus*.

Effect	SS	DF	MS	F-*ratio*	P-*value*
Gross Growth Rate					
Tag	0.02051	3	0.0068	0.327	0.8062
Size	29.4433	1	29.4433	1406.20	<0.0001
Error	2.5754	123	0.0209		
Righting Activity Coefficient					
Tag	7.291	3	2.430	0.647	0.5863
Size	523.353	1	523.353	139.317	<0.0001
Error	462.058	123	3.757		

Discussion

Many studies have separately evaluated the effect of fluorescent tags (tetracycline and calceine) (Ebert 1965, Russell & Urbaniak 2004), passive-induced (or integrated) transponder (PIT) and unique coded wire tags (CWT) (Hagen 1996, Kalvass *et al.* 1998, Duggan & Miller 2001, Woods & James 2005) on sea urchin growth and survival; however, the combined effect of both methods has only been assessed in the green sea urchin, *Strongylocentrotus droebachiensis* (Lauzon-Guay & Scheibling 2008, Ellers & Johnson 2009).

In the present study we tested the combination of tetracycline with two tagging techniques (PIT and CWT) and our results suggest that these do not significantly interfere with growth and survival in *S. purpuratus*. Although Kalvass *et al.* (1998) performed a similar study in *S. franciscanus*; their experiments were not designed to analyze the combined effect of tetracycline, PIT and CWT tags. Because the authors were interested in comparing growth rates calculated from direct measurements (PIT and CWT tags) and from indirectly-measured growth (tetracycline back-calculation method), they did not use true controls in their experiments, so their results cannot be conclusive.

Our results also suggest that the separate effect of the three tested methods (tetracycline, PIT and CWT tags) was not evident on the growth or survival of purple sea urchins. These results are in agreement with what has been found in other urchin species. Hagen (1996), Duggan & Miller (2001), Russell & Urbaniak (2004), Ellers & Johnson (2009) found that growth and survival of the green sea urchin, *S. droebachiensis* did not differ between tagged individuals and controls. In a similar study, Woods and James (2005) studied the effect of PIT tags in the sea urchin *Evechinus chloroticus*. They found 100% survival after five months, and no significant differences in test diameter, wet weight, and gonad index between tagged and untagged sea urchins.

A recent study (Lauzon-Guay & Scheibling 2008) shows contradictory evidences when tagging the green sea urchin *S. droebachiensis,* suggesting that PIT- tags can have an adverse effect in growth, gonad index, survival, and feeding rate in tagged urchins under field conditions; however, this effect was not evident under laboratory conditions. A shortcoming in their study was that short term movement and feeding rate experiments were conducted right after tagging, during the period when urchins were recovering. Although the authors do not recommend the use of PIT tags in the field for growth studies, it would be desirable to conduct a longer experiment, since it has been suggested that the effect of tagging could be noticed at the beginning of the experiment, but differences in growth between tagged and untagged urchins decrease through time (Hagen 1996, Russell & Urbaniak 2004, Ellers & Johnson 2009).

Previous studies have not tested the effect of tagging in different sea urchin sizes. In this study, although growth rates differed among different size categories, none of the tagging methods showed an effect on growth or survival. This result also applies to sea urchins less than 22 mm in test diameter and the authors are unaware of previous tagging studies on sea urchins of this size. Although Hagen (1996) included an ample size range (25–55 mm), it was difficult to assess a size effect due to small sample sizes (2–4 urchins/size interval) and a lack of replicates to perform statistical inferences. Dugan & Miller (2001) tested two size ranges; however, urchin sizes were large (40–50 and 55–62 mm), and no growth differences were found between sizes, since growth rate is a decreasing function of diameter (Ellers & Johnson 2009).

The inclusion of size categories allowed realizing that post-tagging stress (RAC) can differ among sizes, being small urchins more susceptible to the stress of tagging. This response becomes important when tagging urchins in the field, where a wave swept environment is typical of tidal pools in a high-energy zone. Survival of tagged sea urchins under such harsh conditions might depend on its ability to quickly attach to the substratum and seek out protection. According to Santos *et al.* (2005) y Santos & Flammang (2007), transplanted sea urchins respond to the new hydrodynamic conditions and can be completely attached to the substrate in few minutes.

To assure an accurate estimate of the seasonal growth and survival, tagged organisms must be easily found and identified. This goal can be achieved when PIT and CWT tags have good retention characteristics. We found high retention rates in all sizes and tag losses occurred only during the first two days after tagging, only in specimens that were tagged twice.

CWT tags were useful to tag small sea urchins in the laboratory; however, under field conditions the recapture of tagged organisms is only possible if a wand or metal detector is available. There is still another limitation when using CWTs. Seasonal growth increments cannot be obtained from tagged organisms because individual codes cannot be detected without killing the animal. In spite of this disadvantage, CWTs are the only coded tags for individual recognition of small sea urchins.

Results from this study suggest that the combination of internal tags: (1) tetracycline + PIT or (2) tetracycline + CWT, can be applied in age determination studies of sea urchins, where the fluorescent tag, tetracycline can be used to validate the periodicity of natural growth lines in the genital plates, and PIT or CWT tags guarantee the recapture of individually tagged organisms, reducing the number of samples to be collected.

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