

Marking juvenile sandfish, *Holothuria scabra*, with the fluorochrome oxytetracycline – a preliminary report

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Abstract

We conducted a preliminary experiment using the fluorochrome oxytetracycline (OTC) to mark the dermal spicules of juvenile *Holothuria scabra*. The marking efficiency of OTC in this preliminary experiment was much lower than tetracycline (TC) and calcein after 1, 6 and 24 h at the same water temperature (28°C), fluorochrome concentration, and duration of immersion. The highest marking efficiency achieved with OTC (18%) was similar to the 15% marking efficiency achieved with TC after 1 h, and made up only 62% of the 29% marking efficiency found with calcein after 1 h. Moreover, after 24 h, TC was found to have marked 28% of dermal spicules and calcein 39%. Although all treatment groups in this preliminary experiment suffered significant weight loss, none of the treatments resulted in mortalities. Preliminary results indicate that while OTC may be less toxic, it is not as effective as TC and calcein in marking the dermal spicules of juvenile *H. scabra*.

Introduction

Marking the dermal spicules of juvenile sandfish (*Holothuria scabra*) has proven to be a suitable technique to distinguish between hatchery-reared juveniles and natural populations. This is particularly important when evaluating the efficiencies of restocking and sea ranching programmes (Purcell et al. 2006; Purcell and Simutoga 2008; Purcell and Blockmans 2009). The preferred method for marking juveniles is immersion marking, where the fluorochrome is added to a bath solution at the desired concentration. Immersing juvenile *H. scabra* in either the fluorochrome tetracycline (TC) or calcein at a concentration of 100 mg L⁻¹ for 24 h at ≥26°C was found to be the most practical compromise in terms of marking efficiency and survival of *H. scabra* (Purcell and Blockmans 2009). An alternative TC, oxytetracycline hydrochloride (OTC), has been successfully used to mark juvenile fish (Secor et al. 1991; Kayle 1992; Brooks et al. 1994; Bumgardner and King 1996; Jenkins et al. 2002; Butcher et al. 2003; Taylor et al. 2005; Hutchings and Griffiths 2010) and abalone (Day et al. 1995). However, the effectiveness of OTC as a marker for juvenile *H. scabra* has not yet been examined.

We report on a preliminary experiment using the fluorochrome OTC to mark dermal spicules of juvenile *H. scabra*. In particular, we focused on the effect of immersion time in OTC on marking efficiency. We specifically only manipulated this one factor,

immersion time, in order to avoid time-consuming manipulation of other factors such as temperature and salinity.

Method and results

Juvenile *H. scabra* were spawned at the Darwin Aquaculture Centre in September 2010 using broodstock owned by Tasmanian Seafoods P/L, following the methods of Agudo (2006). In total, 36 juveniles (weight range 1.5–3.0 g) were randomly selected and transferred to a 600-L pre-conditioning tank. All juveniles were held in the tank for a period of eight days and every second day *Spirulina* sp. (Australian Spirulina,® TAAU Australia P/L, Darwin, Australia) was added at the rate of 5% of the total biomass of *H. scabra*. One day prior to the immersion treatment, no feed was added and the health of all juveniles was inspected according to Purcell and Eeckhaut (2005).

Each of the 36 juveniles was weighed and 3 were randomly placed into each of the 12 containers filled with 1µm-filtered seawater (34 ppt, 28.0–28.5°C). OTC was added to each container to arrive at a final concentration of 100 mg L⁻¹ (Purcell and Blockmans 2009). Groups of three containers were placed in one of four, aerated, polystyrene boxes and left undisturbed for the duration of the respective immersion period (1, 6, 24, 48 h). Each polystyrene box was specific to one immersion period. After the immersion treatment was completed, 3

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juvenile *H. scabra* from each container were placed in one of 12 labelled baskets in the 600-L tank. These individuals remained in the tank for a rest period of 14 days, during which the health of the juveniles was observed daily, *Spirulina* sp. added every second day, and faeces siphoned out twice daily.

Dermal spicule collection and processing followed the methods of Purcell and Blockmans (2009). Following that, spicules were viewed under a Leica DM 4000 Epifluorescence microscope and two sets of photos (using a Leica DFC 320 camera) were taken for each slide. Each set consisted of one photo with fluorescent light and one with natural light. The percentage of marked spicules was then calculated by dividing the number of marked spicules in the fluorescent light picture with those in the natural light picture. The number of spicules counted per photo varied from 10 to 167.

Weight data for juvenile *H. scabra* weighed just prior to immersion and after the 14-day resting period are shown in Table 1. Differences in initial weight of juveniles in all treatment groups were not significant. However, the difference between initial and final weight of juveniles within a treatment was significant for all exposure times. Weight loss ranged from 0.60 ± 0.05 g in juveniles immersed for 6 h, to 0.73 ± 0.15 g in juveniles immersed for 24 h. There was no significant difference in weight loss among treatment groups, and no mortalities were

recorded during the duration of the experiment. The observed weight loss may have been caused by subtle, toxic effects of OTC and/or handling stress, which will be examined in future studies.

Juvenile *H. scabra* immersed in 100 mg L^{-1} OTC for 24 h had a significantly higher percentage of marked spicules ($18.0 \pm 2.2\%$) compared with other treatments (Fig. 1). Hence, percentage marking with OTC increased with time up to 24 h as was shown for TC and calcein (Purcell and Blockmans 2009). Immersing juvenile *H. scabra* in OTC for 48 h resulted in a significantly lower percent marking as compared with 24 h (Fig. 1). Although all treatment groups suffered a significant loss of weight after the 14-day resting period (Table 1), percentage marking was only affected after 24 h. In contrast, immersion in 100 mg L^{-1} TC or calcein had no effect on the growth of juvenile *H. scabra* as compared with an unmarked control group (Purcell and Blockmans 2009). However, while none of the 36 juveniles used in the present study died, immersion in either TC or calcein (concentrations not stated) resulted in the death of 6 (from TC) and 1 (calcein) juveniles out of 108 (Purcell and Blockmans 2009). Percentage marking in *H. scabra* immersed in OTC for 1 h was significantly lower than in all other treatments ($2.3 \pm 0.4\%$). There was no significant difference between percentage marking in juveniles immersed for either 6 h ($10.3 \pm 1.4\%$) or 48 h ($12.5 \pm 3.2\%$).

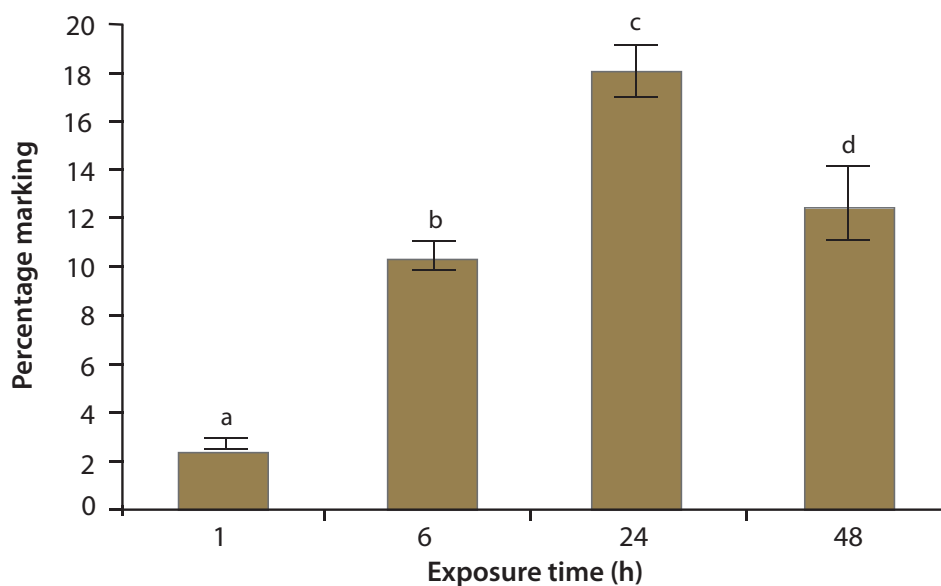


Figure 1. Percentage marking of spicules of juvenile *Holothuria scabra* immersed in the fluorochrome oxytetracycline for up to 48 h. Values are the mean \pm SE ($n = 3$). Significant differences are indicated by different letters ($p \leq 0.05$).

Table 1. Initial and final weights of juvenile sandfish, *Holothuria scabra*, marked with the fluorochrome oxytetracycline for up to 48 h. Values are the mean \pm SE (n = 3). Significant differences within rows are shown by * ($p \leq 0.05$).

Exposure time (h)	Initial weight (g)	Final weight (g)
1	2.30 \pm 0.06	1.63 \pm 0.14*
6	2.23 \pm 0.09	1.61 \pm 0.03*
24	2.23 \pm 0.03	1.53 \pm 0.14*
48	2.00 \pm 0.10	1.36 \pm 0.07*

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