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SHORT COMMUNICATIONS

A METHOD FOR MARKING SMALL JUVENILE GASTROPODS

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While marking and tagging methods have frequently been used to study adult marine gastropods, such methods have seldom been applied to very small juveniles. This paper presents a simple method of individually marking juvenile gastropods as small as 0·9 mm in length by applying nail polish colour codes to their shell. The method has no adverse effects on snail growth and survival, and marks persist in excess of 60 d. This marking method can be used to acquire valuable information on early juvenile gastropods or other juvenile invertebrates, for which very little information is presently available.

Methods used to identify individual organisms consistently over time have been invaluable tools in ecological studies, enabling reliable assessments of time-dependent parameters such as growth and mortality, and an accurate determination of their variance. These methods have proved to be particularly amenable to gastropods owing to the presence of an external shell on which marks or tags can be applied with little or no adverse effects on the animal. Marking and tagging techniques have enabled the study of several ecological parameters in adult marine gastropods, including growth (Frank, 1965; Hughes, 1972; Palmer, 1983; Gosselin & Bourget, 1989), mortality (Frank, 1965; Hughes, 1972), movements (Frank, 1965; Chapman, 1986), and foraging behaviour (Menge, 1974; Hughes *et al.*, 1992). Small organisms, however, can pose considerable problems for individual marking (Southwood, 1978). As a result, marking and tagging methods have seldom been applied to newly hatched or recently settled juvenile marine gastropods. Several methods have been developed for simultaneously labelling large numbers of invertebrate larvae (Levin, 1990), and some of these methods may be applicable to juvenile gastropods. The usefulness of these methods, however, is limited because all animals receive the same label and, consequently, individual animals cannot be recognized. To my knowledge, no method of individually marking very small juvenile marine gastropods has been documented. In fact, it is sometimes perceived that small juveniles cannot be individually marked due to their small size and sensitivity (Frank, 1965; Palmer, 1990). The object of this paper is to present a simple method of marking early juvenile gastropods, which consists of applying colour codes to the shells of individuals as small as 0·9 mm in length. In addition, the present study demonstrates that the method has no adverse effects on snail growth and survival, and shows that the marks are persistent over time. The method was applied to hatchlings of a marine prosobranch gastropod, *Nucella emarginata* (=*Thais emarginata*) (Deshayes). *Nucella emarginata* hatch from benthic egg capsules as juveniles capable of crawling away and measuring 0·9-1·8 mm from the apex to the tip of the siphonal canal (personal observation; Spight, 1976).

The juvenile snails were initially placed in freshwater for 30-40 s to make them retract into their shell. This decreased their susceptibility to desiccation during the following steps. Hatchling *Nucella emarginata* can be left in freshwater for several minutes without apparent effects on

subsequent behaviour or survival. Excess water was then removed by blotting snails onto a wet paper towel. Blotting must be very brief (1-2 s); if water within the shell is also absorbed the hatchling is not likely to survive the subsequent steps.

Hatchlings were then secured by gently pressing them into a thin (~2 mm) layer of non-toxic modelling clay, aperture down. Once the shell was dry, a colour code of up to three dots of nail polish were carefully applied to each hatchling under a dissecting microscope. The nail polish was applied using a small brush trimmed down to 3-5 strands. Hatchlings were returned to sea-water as soon as the nail polish was dry. These colour codes could then easily be read under a dissecting microscope. Six colours were used to mark *N. emarginata* hatchlings: blue, red, yellow, green, orange, and purple. For clarity, combinations with two consecutive dots of the same colour were not used. Thus 186 different combinations of these six colours could be generated when applying up to three dots of nail polish.

In order to determine if this method had an effect on growth or survival of juvenile *Nucella emarginata*, nine hatchlings were marked within 12 h of emerging from their egg capsules. Each individual was then measured and placed in a separate cage (size=10x10x6 cm; 610 µm mesh) in a tank with flowing sea-water. Each cage also received one unmarked hatchling from the same sample of egg capsules. Small mussels (*Mytilus* spp.) and a rock with barnacles (*Balanus glandula* Darwin and *Chthamalus dalli* Pilsbry) were added as prey. Additional prey were provided as required. The hatchlings were observed and measured regularly over a period of 365 d. An additional drop of nail polish was applied to the new shell growth of all marked hatchlings on day 71. These new marks persisted for the remainder of the experiment.

When returned to sea-water after being marked, hatchlings resumed crawling within 5 min. No difference in behaviour was apparent between marked and unmarked hatchlings. Throughout the experiment, the average size of marked and unmarked snails remained closely matched (Figure 1). Growth (shell length increment) of marked and unmarked individuals was not significantly different during the first 30 d or during the remainder of the 365 d period (Table 1). In addition, the nine pairs of snails were still alive after one year.

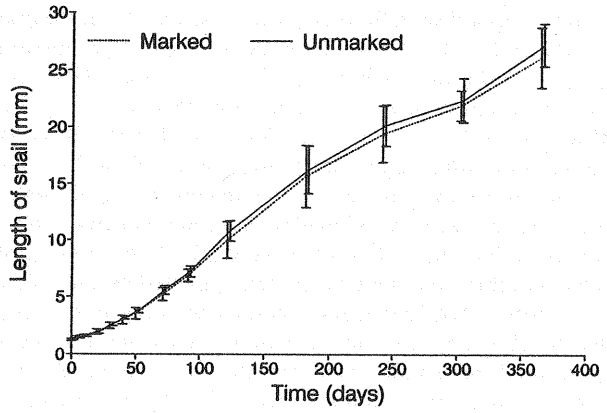

Figure 1. Growth of marked and unmarked *Nucella emarginata* hatchlings. Error bars represent standard deviations. All hatchlings were kept in flowing sea-water in the laboratory with an abundant supply of food. (N=9 marked and 9 unmarked hatchlings).

To determine the persistence time of the nail polish marks, three cages containing a total of 39 marked hatchlings were placed in flowing sea-water in the laboratory for a period of 140 d. Three other cages, containing a total of 14 hatchlings, were attached to the substratum in the intertidal zone near the Bamfield Marine Station for the same period. All cages were initially supplied with prey, and at approximately 20-d intervals the marks were examined and the supply of prey was

Table 1. *Effects of the marking method on the growth (increase in shell length) of* Nucella emarginata *hatchlings. Initial shell length, measured from the tip of siphonal canal to the apex, and shell growth were obtained from nine pairs of marked and unmarked hatchlings. The ocular micrometer of a dissecting microscope (measurement error ±0·02mm) was used for measurements on days 0 and 30. A vernier caliper (measurement error ±0·1mm) was used on day 365.*

		Marked		Unmarked		ANOVA	
		Mean	±SD	Mean	±SD	*F*	*P*
Initial length (mm)		1.23	±0.10	1.25	±0.07	0.34	0.58
Growth (mm):	Days 0-30	1.27	±0.18	1.34	±0.16	0.82	0.39
	Days 30-365	23.7	±2.8	24.6	±2.0	1.32	0.28

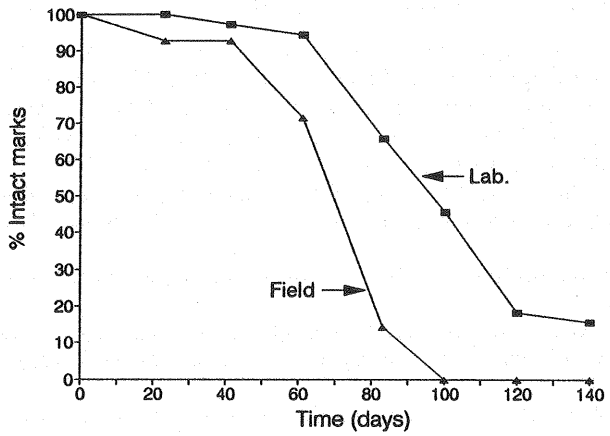

Figure 2. Persistence time of marks on *Nucella emarginata* hatchlings. One mark per hatchling, each mark consisting of up to three dots of nail polish. 'Lab.' hatchlings were placed in cages in the laboratory (N=39). 'Field' hatchlings were placed in cages attached in the intertidal zone (N=14).

replenished. Under laboratory conditions 94% of the marks were still intact after 60 d (Figure 2); 46% lasted longer than 100 d. Although the marks on hatchlings placed in field cages did not last as long as those in the laboratory, 93% were still intact after 40 d (Figure 2). Nail polish marks were lost when the larval shell eroded. However, remaining fragments of the colour marks allowed correct identification of the hatchlings well after large portions of the marks were lost.

This marking method, therefore, did not produce any detectable effect on growth, mortality, or behaviour of the hatchlings. In addition, nail polish possessed the advantageous properties of good adhesion, hardness, and rapid drying. Trials using ink and enamel paint were unsuccessful. The ink did not adhere well to the shell and flaked off within hours of being applied; the enamel paint required considerable drying time which resulted in high hatchling mortality.

Due to their small size and cryptic behaviour, early juvenile marine gastropods have mostly been studied under laboratory conditions (Largen, 1967; Rittschof *et al.*, 1983; Palmer, 1990; Gosselin & Chia, in press). However, I have successfully recovered live marked *Nucella emarginata* hatchlings after a period of 10 d in the field (unpublished data), demonstrating the feasibility of mark and recovery field experiments with juveniles with limited dispersal capabilities. This marking method has also been used to study growth and survival of *N. emarginata* hatchlings in relation to food type in the laboratory (Gosselin & Chia, in press). It is also likely to be effective for marking juveniles of certain bivalve species as well as other juvenile invertebrates bearing hard external structures. To date, most studies of the ecology of post-metamorphic marine

invertebrates have been directed at the late juvenile and adult stages. Very little information is available on the early juvenile period of these organisms. Methods such as the one described herein will facilitate the study of very young individuals and contribute to a better understanding of the ecology of juvenile marine invertebrates.

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