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## METHODS & TECHNIQUES

### A Method for Marking Nudibranchs

BY

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## INTRODUCTION

MANY OF THE PUBLISHED METHODS for marking invertebrates depend on the presence of a hard exoskeleton or shell (SOUTHWOOD, 1966). These methods are not practical for use on nudibranchs which are covered externally with a fleshy mantle. SOUTH (1965) was able to mark the slug *Agriolimax reticulatus* by feeding the animals on agar jelly with 0.2% neutral red. The darkly stained digestive glands of these slugs were visible through their feet. Although this technique is probably applicable for the group marking of some nudibranchs, the intense pigmentation of other species, such as *Rostanga pulchra* MacFarland, 1905, may render it impractical.

During a study of the behavior and ecology of the dorid nudibranch, *Rostanga pulchra* (ANDERSON, 1971), several methods were tried to mark individual animals so that they could be followed from day to day in the field. This paper will report on the varied success of the 3 marking methods used.

## MATERIALS AND METHODS

The field experiments were done at the Great Tide Pool near Point Pinos, Pacific Grove, California, during periods of low tides. A detailed map of the study area, showing the

exact locations at which the nudibranchs were found, was made to facilitate recovery. "Recovery" involved noting the location and behavior of the animals of interest and immediately returning them to the substrate on which they were found.

Three marking methods were tested: Some nudibranchs were marked by putting small loops of variously colored thread through holes made near the edge of the mantle. In other animals, following the suggestion of G. Robilliard (personal communication) the dorsum was gently scraped to remove mucus, and spots of vital stains (*e. g.*, methylene blue) were applied. Finally, a method proposed by Dr. John Pearse was used. Using a fine scissors, a small V-shaped notch of tissue, approximately 1 mm across, was cut from the edge of the mantle. Notches could be made at 7 different locations: anteriorly, posteriorly, and centrally along both the left and right sides of the body and at the posterior end of the body. Because of the proximity of the oral tentacles, no notches were made at the anterior end of the body. Using combinations of one and two notches, many nudibranchs could be marked distinctively. The notches were detectable by careful examination of the edge of the mantle.

Unmarked animals were used as controls and were carefully chosen to be distinctive with respect to their coloration, size, and the pattern of the dark spots on the dorsal surface.

## RESULTS AND DISCUSSION

The first two marking techniques were not successful. Inserting a loop of thread into the mantle of *Rostanga pulchra* frequently tears the mantle. Once in place, the loops commonly snag and are torn out. In the case of marking with vital stains, some of the marks made were not easily visible and there was a high mortality rate among marked nudibranchs.

The reasons for this high mortality rate were not investigated. SOUTH's (1965) results suggest that vital stains are not toxic. However, scraping the dorsum may have caused injury and infection of the epithelium.

The notching technique was successfully used. The survival of notched *Rostanga pulchra* was first observed in the laboratory. Five marked and 5 control animals were kept in an aquarium with the sponge, *Ophlitaspongia pennata* Lambe, for food. The behavior of the nudibranchs and the condition of their wounds were observed daily. The observations were discontinued after 24 days due to the degeneration of the food sponge. The loco-



motory behavior of the notched animals did not differ from that of the controls. Both groups exhibited normal feeding and reproductive behavior. At the end of the observation period, 3 of the marked and 3 of the control animals were still alive. Two marked animals and one of the controls died from injuries received from the water pump of the aquarium. The other control animal died of unknown causes. Thus, under these aquarium conditions it appeared that the marking procedure did not affect the ability of the nudibranchs to survive. The notches were still easily detectable at the end of these observations.

A field experiment was then conducted with 8 pairs of nudibranchs. An attempt was made to choose as one member of each pair an animal that was sufficiently distinctive to be identified without being marked. This was not entirely successful. The other member of each pair was marked. As is apparent from the recovery data (Table 1), it is not always possible to identify unmarked animals

Table 1

## Results of a Marking Experiment

Four sites were chosen at each of which one or more pairs of *Rostanga pulchra* were found. One member of each pair, which was distinctive in appearance, was not marked. The other member of each pair was marked.

Site		Number of animals	Number of animals recovered on - days after marking			
			Day 1	Day 2	Day 3	Day 4
1	Marked	4	4	3	4	3
	Unmarked	4	5	4	4	3
2	Marked	1	1	1	1	1
	Unmarked	1	1	1	1	1
3	Marked	2	2	2	2	2
	Unmarked	2	2 (2) <sup>1</sup>	3 (1)	4	4 (3)
4	Marked	1	1	1	1	1
	Unmarked	1	1	1	0	1

<sup>1</sup> The animals listed in parentheses are unmarked animals, not fitting the descriptions of the original unmarked animals that were observed at the recovery sites.

with certainty. However, the recovery of marked *Rostanga pulchra* was similar to that of unmarked animals indicating that the marking method does not bias recovery.

According to SOUTHWOOD (1966) an effective marking technique should be durable, inconspicuous, and not affect the behavior or the longevity of the animals. Notching the edge of the mantle of *Rostanga pulchra* satisfies all of these criteria. Furthermore, nudibranchs can be notched quickly either in the field or in the laboratory. The technique is suitable for both group marking, in which large numbers of nudibranchs are marked identically, and for individually marking animals.

This marking method was used in extensive field observations (ANDERSON, 1972) to study the day to day movements of *Rostanga pulchra* within the intertidal zone. Nudibranchs ranging in length from 6 to 18 mm were successfully marked. Notched nudibranchs were recovered from the substrates on which they were initially found, as well as up to 60 cm away from their original locations. Twenty-nine animals were marked in one experiment; 24 of these animals (83%) were recovered on the first day after marking, 15 animals (52%) on the second and third days, and 11 animals (38%) on the fourth day. In one case a nudibranch was recovered 37 days after marking although this is not regarded as an upper limit. These experiments suggest that notching the edge of the mantle is an effective technique for marking dorid nudibranchs. It may also be useful for marking other mollusks.

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