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FIELD METHODS NOTE

IDENTIFICATION OF INDIVIDUAL *APLYSIA* SPP. BY HEAD PIGMENT PATTERNS

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To study social, reproductive and migratory behavior, in *Aplysia* spp. individual identification is important (Switzer-Dunlap, Meyers-Schulte & Gardner, 1984; Zaferes, Skolnick & Tobach, 1988). Identification has been implemented by tagging techniques using floy tags (Audesirk, 1975), DYMO plastic tape (Nishiwaki, Veda & Makioka, 1975), safety pins with colored tape codes (Lederhendler & Tobach, 1977; Zaferes, et al., 1988), and nylon line (Migenis-Lopez, personal communication, 1987). Tags have been attached to most parts of the *Aplysia* body, and have been reported not to remain attached to the animal for prolonged periods, especially in the field. Nishiwaki et al. (1975) tagged and surveyed 1,427 *A. kurodai* in Nabeta Bay, Japan, over a period of 5 months. By the fifth month only one tagged animal was recaptured, and 121 loose tags were found in the waters where the *Aplysia* had been tagged. Other studies using tags in field and laboratory conditions reported similar results of lost tags (Lederhendler, Bell & Tobach, 1975; Migenis-Lopez, personal communication, 1987).

Aplysia are covered with spots and lines of pigment of different colors: purple, green, aqua, black, brown or very light tan. These colors reflect species differences and the algae eaten (Winkler, 1959). In the course of a study of *A. brasiliana* in waters off Guayama, Puerto Rico (P.R.), it was found that no two individuals had the same pattern of pigment spots and lines on the head in the area between the buccal tentacles and the rhinophores. To verify this observation, *A. brasiliana* were studied in the field and laboratory and *A. dactylomela* were observed in the laboratory.

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THE HEAD PATTERN IDENTIFICATION TECHNIQUE

1. Handling Technique

Animals were obtained while walking, snorkeling, and scuba diving, and were removed by hand from the substrate by sliding one's fingers under an animal's foot and gently scooping it off the substrate. Animals were picked up without touching the visceral mass or head area, to prevent the animals from contracting into a balled position which hides much of the dorsal surface of the head from the observer's view. When held with minimal pressure with its head slightly lower than its tail, the *Aplysia* generally stretched its neck and head, providing a full view of the head pattern (Figure 1). An *Aplysia* usually remains inactive in this position for at least 20 sec.

2. Characteristics

Each time an animal was found in the field, the pattern was drawn onto a waterproof writing slate, and notes were made of where and where the animal was found, its volume and the characteristics of the animal's markings. The characteristics recorded in addition to the head pattern were as follows:

1. rhinophores:
 - a. symmetrical or asymmetrical
 - b. striped or not striped



FIGURE 1. Procedure used to hold an *Aplysia* while recording head pattern.

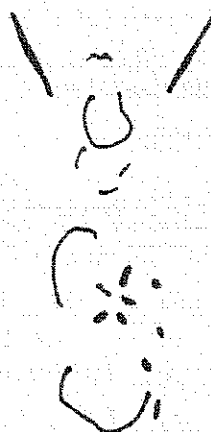


FIGURE 2. A. Photograph of the head area of an *Aplysia* taken in the field May 14, 1987. The two rhinophores on top and the buccal tentacles on the bottom of the picture are partially contracted. B. The head pattern drawing recorded when photograph "A" was made. The two dark lines on top represent the rhinophores. Other characteristics recorded on the slate: 1. rhinophores symmetrical and unmarked 2. unmarked foot 3. none (scars, identifiable patterns) 4. a. broken b. large c. thin 5. 72 ml.

2. foot: spotted or unmarked
3. body/head: scars, distinguishable markings such as geometric patterns or lumps on the body
4. pigmented circles on body wall:
 - a. continuous or broken;
 - b. large, intermediate or small;
 - c. thick or thin
5. Each animal's volume was measured by placing it in a 1000 ml graduated cylinder filled partially with sea water. Otsuka, Rouger, & Tobach (1972) found that the amount of sea water an *Aplysia* displaced was equal to its dry weight.

The entire process of recording a head pattern, volume and physical description took approximately 1-3 min depending on the animal and water conditions (Figure 2). At the end of each survey, the information from the slate was copied onto index cards. Photographs were

taken of the head area with a Nikonos V camera from time to time when the *Aplysia* was recaptured to check for drawing accuracy and to record changes that may have taken place.

LOCATION IN WHICH ANIMALS WERE FOUND AND DESCRIBED

1. Field sites (Table 1)

a) Guayama, Playa de Jobos (Southern P.R.)

Daily surveys were made hourly during daylight in an area approximately 15 m wide and 30 m long. Animals were individually identified by using both head pattern and tagging with a safety pin with colored tape technique (Lederhendler & Tobach, 1977). Several animals lost their tags on one or more occasions, but the head pattern technique proved helpful in the identification of these animals. A total of 17 *A. brasiliana* were found, of which 14 animals were seen more than one day (Table 1). At the end of four or five days most animals' head patterns could be recognized easily on sight.

b) Fajardo, Seven Seas Beach (Northeastern P.R.)

This site was studied from May 12 to June 8, 1987 during daylight hours, and during two night observations from sunset to sunrise. Individuals were identified by the head pattern technique. A total of 70 animals were found, of which 42 animals were seen on more than one day (Table 1). There were no observable changes in the head pigment patterns of these animals found more than once. Animal volume was used to group the animals into three categories, small (30 ml - 45 ml), medium (50 ml - 75 ml), and large (80 ml - 105 ml), to make the process of locating specific head patterns easier.

c) Palmas del Mar (Southeastern P.R.)

A total of 51 animals were found and identified with the head pattern technique, of which 17 were seen on more than one day (Table 1).

2. Laboratory

As the animals were observed in groups, individual identification was necessary. The head pattern technique was used as the identification method.

a) Nine *A. dactylomela* and 20 *A. brasiliana* were brought to Comparative Psychology Research Group Laboratory in the American Museum of Natural History for group observation in several social behavior experiments from July 7 through August 25, 1987 (Zafe & Tobach, in preparation). Animals were maintained in individual

TABLE 1
Number of *A. brasiliensis* Identified by Head Pattern

Number of Animals	Total Number of Days Seen	Median Number of Days between First and Last Sightings	Number of Days between First and Last Sightings	
			Least	Most
<i>A. Playa de Jobos, March 14, to 23, 1987</i>				
3	1	—	—	—
4	2*	—	—	—
1	3*	—	—	—
2	4*	—	—	—
4	5	6	5	6
3	6*	—	—	—
<i>B. Seven Seas Beach, May 12 to June 8, 1987</i>				
28	1	—	—	—
14	2	9	2	26
7	3	20	10	23
7	4	13	10	26
6	5	20	5	28
4	6	22.5	11	28
1	7	—	22	—
2	9#	—	27	—
1	10	—	27	—
<i>C. Palmas del Mar, March 23 to April 1, 1987</i>				
34	1	—	—	—
13	2*	—	—	—
4	3*	—	—	—

* Found on consecutive days.

Both animals seen during an interval of 27 days.

mesh cages when their social behavior was not being observed and recorded.

b) Identification reliability check.

During the first week, before the observations began, two observers studied photographs and both drew head patterns of each animal. Each observer was then presented with a drawing of each animal without any code name identification, and was able to then pick out the individual animal in a group situation with 100% accuracy without consultation between the observers. There were not differ-

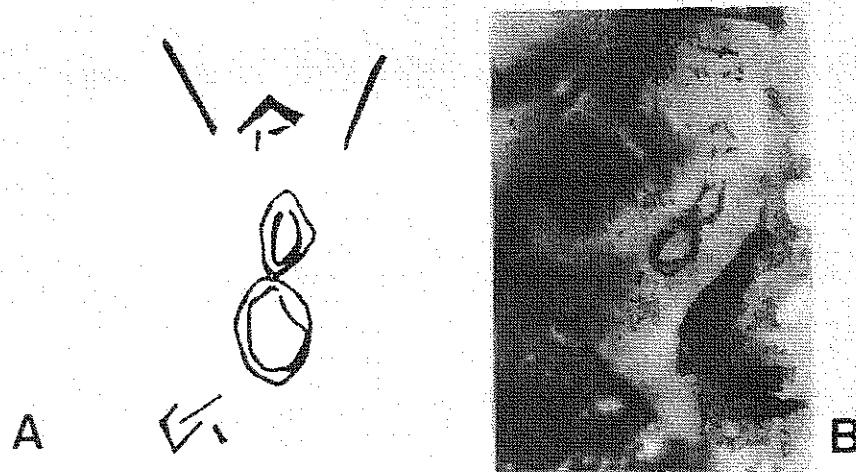


FIGURE 3. Drawing and photograph of an *A. brasiliiana*. A. The head pattern of an *Aplysia* recorded on a slate during a field observation on June 2, 1987. B. The head of that same *Aplysia* photographed in the laboratory on August 16, 1987.

ences when their own or the other observers' drawings were used. Head patterns were recorded for all animals every 10 to 14 days by drawings and/or photographs. Figure 3 shows the pattern of an animal drawn in the field and photographed in the laboratory. No changes in the head pattern of any animal were ever observed.

DISCUSSION

The method has proved useful in field and laboratory with populations of limited size (under 70), or where photographs can be made easily. Head pattern identification may not be necessarily the method of choice in such extensive studies as that by Nishiwaki et al. (1975) where several hundred animals were surveyed, but it can be an ancillary tool.

It is also possible that head patterns may be useful for species as well as for individual identification. The population of *A. dactylorella* in the laboratory were easily distinguished from the *A. brasiliiana* by their head patterns with the head pattern of *A. dactylorella* having many fine cracks and lines, as well as more complicated spot and circle patterns than the head patterns found on *A. brasiliiana*.

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