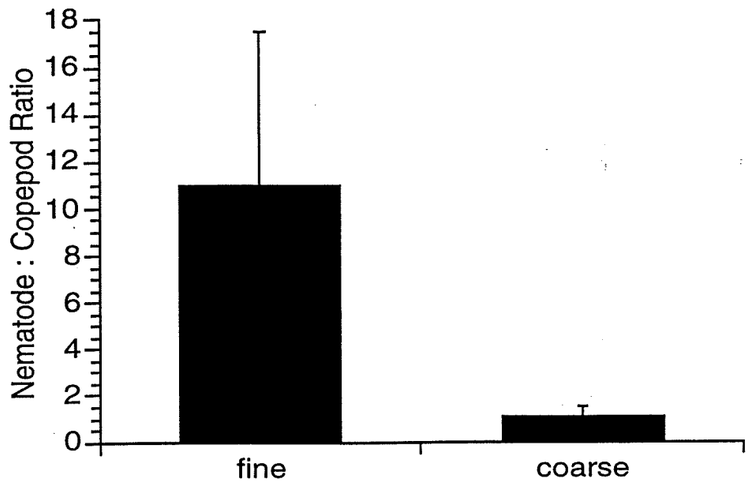

FIG. 5. Mean (± 1 S.E.) of nematode to copepod ratio found in fine sediment and coarse sediment samples from a depth of 10 m in Discovery Bay, Jamaica (t-test, t=1.53, df=10, P=0.16).

TABLE 1. Mean abundances of meiofauna in fine and course sediments (N=12) collected from a depth of 10 m Discovery Bay, Jamaica.

Meiofauna type	Fine Sediment Mean ± 1. S.E.	Coarse Sediment Mean ± 1 S.E.	t	df	P
Nematode	59.2 ± 7.3	16.2 ± 6.7	4.34	10	0.0015
Copepod	13.8 ± 3.9	21.8 ± 8.9	0.82	10	0.43
Ostracod	8.8 ± 2.6	15.8 ± 6.4	1.02	10	0.33
Gastropod	8.8 ± 1.4	8.7 ± 2.9	0.05	10	0.96
Polychaete	6.3 ± 2.2	8.8 ± 2.2	0.80	10	0.44
Isopod	2.7 ± 1.1	0.7 ± 1.1	1.24	10	0.25
Cladoceran	1.0 ± 0.4	3.2 ± 1.4	1.55	10	0.15
Other	6.5 ± 3.4	5.3 ± 1.5	0.31	10	0.76
TOTAL	107.2 ± 8.3	80.5 ± 21.1	1.19	10	0.27

INKING AS A POTENTIAL SOCIAL BEHAVIOR IN THE SEA HARE *APLYSIA DACTYLOMELA*

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Abstract. In octopus and squid, inking has been determined to function as a defensive mechanism. Sea hares (Mollusca) also release ink when disturbed and exhibit other behavioral responses that may deter predators. We examined sea hare ink as a possible mechanism for conspecific communication whereby hares near inking conspecifics would learn of the predation risk and respond with predator deterrant behaviors. Based on the finding of a past study, we predicted that the sea hare (*Aplysia dactylomela*) would demonstrate defensive behaviors when exposed to conspecific ink in both laboratory and field conditions. We found that the hares reacted to fresh conspecific ink in both the laboratory and field experiments. We also found a large majority of sea hares in groups of two to three hares in the field, suggesting that hares may prefer to congregate, making it possible for them to utilize this inking mechanism as a way to warn other individuals of a potential threat. However, further studies need to examine the inking behavior of sea hares in the field to truly determine if inking is functioning in social communication.

Key Words: Aplysia Dactylomela, sea hare, Mollusca, inking

INTRODUCTION

The release of ink by squid and octopus when disturbed has been determined to be a defensive mechanism in these cephalopods (Humann 1992). Similar to these molluscs, many sea hares (genus Aplysia, Mollusca, Gastropoda, Opisthobranchia) release a purple ink when disturbed (Barnes 1987). Past studies have examined inking of sea hares to act as a potential defensive mechanism as well. These studies have observed that predators avoid palatable food after it has been exposed to sea hare ink (DiMatteo 1982; Tobach 1989). Chemical analysis of the ink has revealed that the purplish pigments (Barnes 1987) as well as the predator-deterring compounds (Pennings 1990; Paul 1991) are both sequestered from red algae, the primary food of sea hares.

Although the chemicals contained in sea hare ink have been shown to deter predators, a second possible role for the ink has been proposed. Fiorito et al. (1990) have shown through laboratory manipulations that the ink of *Aplysia fasciata* causes behaviorial changes, from generalized to defensive, in conspecifics. Using a similar methodology as presented by Fiorito et al., we conducted our experiment on the congeneric, *Aplysia dactylomela*. We hypothesized that *A. dactylomela* would show an increase in defensive behaviors when exposed to the ink of a conspecific. We sought to examine their response in both the lab and the field to determine whether or not their responses to the ink would vary under different experimental conditions. In addition, we predicted that we would find individuals in groups more often than in a solitary state. For inking to have a function in conspecific communication of predation risk, then individual sea hares would need to be within range of the ink in order for this social function to be satisfied.

METHODS

On the evening of 6 March, 1997 we collected 14 Sea Hares (*Aplysia dactylomela*) from Discovery Bay, Jamaica and placed them into tanks in the Discovery Bay Marine Laboratory. Sea hares were exposed to three types of ink treatments in the field and in the lab-- old ink, new ink, and red dye. Ink was immediately collected from one hare by placing it into a small tank with approximately 50 ml of sea water and agitating the hare until it began inking. This ink was labeled "old ink" and set aside until the following day. The inking procedure was repeated the next evening with a different hare to obtain "new ink". Using red food coloring and sea water, a solution of "red dye" was also prepared. We decided to use artificial red-dye as one of the two control treatments since it is similar to the color of the ink secreted by *A. dactylomela*. This allowed us to examine whether the color or the chemical content of the ink was the primary cue inducing behavioral changes in conspecifics. The one-day old ink treatment was used because of its closer resemblance to the fresh ink released by *A. dactylomela*. By using one-day old ink, we hoped to

determine whether or not sea hare ink loses its ability over time to elicit a response in a conspecific.

Hare behavior was characterized into general and defensive behaviors. General behaviors were defined as the following: crawling, eating, head moving, parapoidia erect, rhinophores erect, and siphon extended. Defensive behaviors were defined as the following: balled up, head withdrawn, parapoidia closed, rhinophores crossed, and siphon withdrawn. All of these behaviors were considered "defensive" since these actions afforded the hare physical protection from a potential predator. Because many of the general behaviors are binary opposites of the defensive behaviors (i.e. parapodia open/parapodia closed), the only general behaviors reported in Tables 1 and 2 are "crawling" and "eating."

Laboratory response of *A. dactylomela* was examined in the three ink treatments (four sea hares per treatment). We used new individuals for every trial. A trial consisted of the following: placing individual hares into individual tanks, recording the behavior of the hares every 10 sec for five min prior to introducing treatment, injecting 50 ml of the treatment (fresh ink, red dye, or old ink) over the surface of the hare's body, then recording the behavior of the hare again every 10 sec for five min. Field response of *A. dactylomela* was also examined in field animals using the same procedures. We selected the first 12 individual hares we encountered. All trials took place at night since sea hares are nocturnal.

To obtain a rough estimation of the distribution of sea hares in the field, we swam around and tallied all individuals encountered. For each of these focal individuals, we counted the total number of sea hares occurring within 50 cm of this focal sea hare. We also measured the distance from the focal individual to its nearest-neighbor. If the nearest-neighbor distance was greater than one meter, then the focal individual was considered solitary. This distance was chosen based on qualitative observations of the distance ink was dispersed in the field.

Results

Sea hares exhibited different behaviors before and after exposure to fresh ink, artificial red dye, and day-old hare ink (Tables 1 & 2). In the laboratory treatments, the sea hares exhibited defensive behaviors only when exposed to fresh, conspecific ink. On average, these defensive behaviors lasted for approximately 3 min 30 s after ink exposure (Fig.1). Exposure in the laboratory to artificial red dye and day-old conspecific ink elicited absolutely no defensive responses from the hares. After exposure to fresh, conspecific ink in the field, the sea hares exhibited defensive behaviors for approximately 3 min (Fig. 2). We also observed slight defensive responses to the red dye and day-old ink, both of which lasted less than 22 s.

A multivariate repeated measures analysis of interaction which accounted for two between subject factors, location and ink type, revealed statistically significant differences between combined defensive responses before and after exposure to different ink types (Wilks' Lambda; F=8.99, df=18, P=0.008). A multivariate repeated measures analysis having one within subject factor, before/after, revealed no statistically significant differences in combined defensive behavioral responses between the laboratory and field locations (Wilks' Lambda; F=0.13, df=18, P=0.72). However, the analysis revealed that there were statistically significant differences in behavioral responses between ink types in both the laboratory and the field (Wilks' Lambda; F=7.89, df=18, P=0.004).

Of the 15 individual hares observed in the field, 73% were in groups (2 or more individuals) and 27% were solitary. Of the grouped individuals, the mean distance to the nearest neighbor was 11.8 cm. The mean distance to the nearest neighbor for solitary individuals was 181.2 cm.

Discussion

We found that exposure to conspecific ink in *A. dactylomela* ellicits a change in behavior. *A. dactylomela* exhibits behavioral changes, from generalized to defensive, when exposed to fresh, conspecific ink in the laboratory and in the field. If inking is a social behavior, in that it is used in order to warn other sea hares of potential dangers, then we expected to find individual sea hares in groups more often than not. The fact that 73% of *A. dactylomela* were found in groups led us to believe that inking in this species may be a social behavior. Tobach et al. (1965) found that indivduals of *A. dactylomela* are less likely to ink when in groups. The fact that *A. dactylomela* are less likely to ink in groups suggests that the function of the ink as a form of communication between conspecifics is unlikely. However, in the field we did observe inking in groups when one or more individuals were disturbed. Therefore, Tobach's findings do not eliminate the possibility that this inking mechanism is a social behavior. Another possible alternate explanation for finding more individuals in groups could be that sampling occurred during the mating season of *A. dactylomela*.

Results from the field were similar to those found in the laboratory. Although there were slight variations in the degree to which they responded, all *A. dactylomela* in the field exhibited defensive behaviors when exposed to fresh ink. Behavioral responses of *A. dactylomela* in the field may be affected by environmental factors, such as water currents, turbidity, temperature, etc. Squirted fresh-ink, red-dye, or one-day old ink, is more likely to be carried away by the currents and more rapidly diluted in the field. In addition, the observed responses of field animals to dye and old ink may have been affected by the application procedure of the treatments in the field. It was difficult under field conditions to standardize the distance from which the ink-source was administered and some inking may have occurred significantly closer to the body of the hare. Moreover, individuals of *A. dactylomela* may have behaved defensively when they sensed the presence of a foreign body nearby. Nevertheless, the trend of behavioral changes in *A. dactylomela*, from generalized to defensive, was strongest under fresh-ink treatments in both environments (field and laboratory).

Although these behavioral changes were observed in *A. dactylomela* directly after exposure to fresh ink from conspecifics, other studies have suggested that the primary purpose of the ink secretion mechanism is to protect oneself from predators. In fact, studies have shown a strong tendency of crabs, one of the potential predators of *A. dactylomela*, to avoid the ink secreted by *A. dactylomela* (DiMatteo 1982). It has also been documented that the ink released by *A. dactylomela* contains some type of distasteful and/or noxious component, and that *A. dactylomela* is able to emit a large cloud of ink which may be sufficiently unpalatable or noxious to predators to cause them to avoid the ink (Paul et al. 1991). Over the course of evolution, individuals of *A. dactylomela* may have learned to sense the presence of conspecific ink by way of olfactory or chemical cues, and have developed defensive response behaviors to protect themselves from the predators in the vicinity that induced the inking response.

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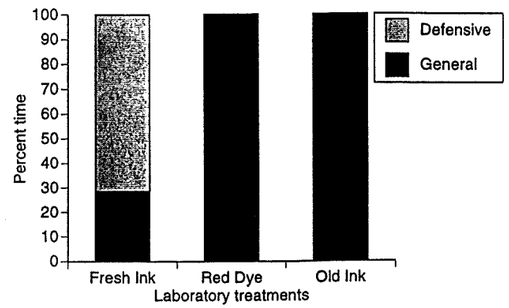

FIG. 1. Relative percentages of time spent exhibiting general and defensive behaviors after exposure to fresh hare ink, artificial red dye, and day-old hare ink in the laboratory. Behavior was observed for a total of 5 min after ink exposure (N= 4 sea hares per treatment.)

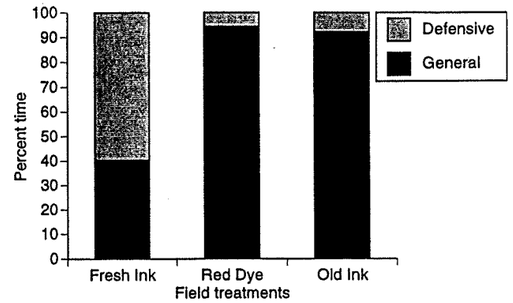

FIG. 2. Relative percentages of time spent exhibiting general and defensive behaviors after exposure to fresh hare ink, artificial red dye, and day-old hare ink in the field. Behavior was observed for a total of 5 min after ink exposure (N= 4 sea hares per treatment.)

Table 1. Mean percentages (±S.E.) of total time spent exhibiting general (GEN) and defensive (DEF) behaviors in the laboratory 5 min before and 5 min after exposure to fresh sea hare ink, red dye, or old sea hare ink (N= 4 per treatment.) (Note: Mean percentages of particular defensive behaviors may not add up to 100 percent due to the fact that behaviors may occur simultaneously.)

		Lab- Ink Before	After	Lab- Dye Before	After	Lab- Old Before	After
GEN	Crawling	55.25±20.49	19.07±8.52	35.38±12.13	9.50±3.76	16.50±10.89	58.63±21.
	Eating	44.79±20.54	28.29±12.78	64.63±12.13	90.50±3.76	83.50±10.89	41.38±21.
DEF	Balled-Up	0.00	47.44±17.59	0.00	0.00	0.00	0.00
	Parapodia Closed	0.00	47.44±17.59	0.00	0.00	0.00	0.00
	Head Withdrawal	0.00	35.32±8.94	0.00	0.00	0.00	0.00
	Rhinophores Crossed	0.00	12.88±7.47	0.00	0.00	0.00	0.00
	Siphon Withdrawal	0.00	14.63±14.63	0.00	0.00	0.00	0.00
	% General	100	28.45±9.52	100	100	100	100
	% Defensive	0	71.55±9.52	0	0	0	0

Table 2. Mean percentages (±S.E.) of total time spent exhibiting general (GEN) and defensive (DEF) behaviors in the field 5 min before and 5 min after exposure to fresh sea hare ink, red dye, or old sea hare ink (N= 4 per treatment). (Note: Mean percentages of particular defensive behaviors may not add up to 100 percent due to the fact that behaviors may occur simultaneously.)

		Field- Ink Before	After	Field- Dye Before	After	Field- Old Before	After
GEN	Crawling	50.00±16.75	47.39±18.61	46.50±17.97	48.30±21.85	81.00±11.78	62.89±11.
	Eating	50.00±16.75	17.25±15.01	53.50±17.97	50.68±21.28	19.00±11.78	34.65±12.
DEF	Balled-Up	0.00	39.53±20.37	0.00	1.77±1.02	0.00	2.69±1.74
	Parapodia Closed	0.00	39.53±20.37	0.00	1.77±1.02	0.00	2.69±1.74
	Head Withdrawal	0.00	39.53±20.37	0.00	1.77±1.02	0.00	2.69±1.74
	Rhinophores Crossed	0.00	31.88±23.62	0.00	0.00	0.00	0.00
	Siphon Withdrawal	0.00	36.13±22.03	0.00	0.89±0.89	0.00	0.87±0.87
	% General	100	40.47±15.23	100	94.38±3.32	100	92.11±4.7
	% Defensive	0	59.53±15.23	0	5.62±3.32	0	7.89±4.75