SEEING RED: EXAMINING THE POSSIBLE FUNCTIONS OF APYLSIA DACTYLOMELA INK

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Abstract: Many marine mollusks, including cephalopods and some sea slugs, eject ink when disturbed. There have been many studies investigating the possible function of sea hare (*Aplysia* spp.) ink, such as intraspecific communication and predator deterrence, but the results have been inconclusive. We investigated two possible functions of spotted sea hare (*Aplysia dactylomela*) ink: 1) defense against the giant sea anemone (*Condylactis gigantea*) and 2) communication between sea hare individuals in response to the presence of a predator. We found that, although spotted sea hares released ink in response to coming in contact with an anemone, their ink did not prevent anemones from ingesting pieces of spotted sea hare tissue. We found that spotted sea hares respond to ink only over very short distances. This may be ecologically significant as spotted sea hares were often found aggregated in tight mating groups. Thus, ink may function in intraspecific communication. Finally, we also evaluated whether spotted sea hares preferentially ate certain algal species, and determined that their distribution in the field is not driven by the distribution of their preferred food.

Key Words: Aplysia Dactylomela, *communication, defense, ink, mollusca, spotted sea hares*

INTRODUCTION

Marine animals employ a wide range of defenses to avoid predation. Many mollusks, including cephalopods and some sea slugs, eject ink when disturbed. Spotted sea hares (*Aplysia dactylomela*) are nocturnal gastropods that eject clouds of red ink, which they produce from red algae in their diet. Researchers have hypothesized the ink is for defense (DiMatteo 1982), communication (Fiorito and Gherardi 1990), or elimination of sequestered algal toxins (Chapman and Fox 1969). Previous studies have focused on ink's role as a defense against predators such as anemones and decapods, and results have suggested that ink is effective against some, but not all, predators (Tobach et al. 1989, DiMatteo 1982, Rogers et al. 2000).

Little of the published work on the ink of sea hares has focused on the spotted sea hare, which is common on the west back reef of Discovery Bay, Jamaica. Previous studies suggest that the ink of spotted sea hares is unpalatable to crabs (DiMatteo 1982) and may be used to communicate with conspecifics (Hang et. al 1997). We tested whether ink deters predation by the giant sea anemone (*Condylactis gigantea*) or acts as an alarm signal to warn conspecifics of potential danger. If ink is a defense against the giant sea anemone, we expected giant sea anemone individuals exposed to ink to reject intact sea hares or pieces of sea hares. If ink functions as an alarm signal, we expected that spotted sea hares will respond to conspecifics' ink over distances by which they are likely to be separated from one another in the field.

If ink functions in defense or communication, the distribution of spotted sea hares in the field may reflect access to the red algae from which they sequester ink-producing compounds. We tested for

algal preferences in the laboratory and observed in the field whether sea hares were found exclusively near these species. Sea hares may preferentially feed on those red algae from which they produce their ink, in which case their distribution in the field may reflect the distribution of preferred algal species.

METHODS

To test the use of ink as a defense against predators, we offered both live sea hares and pieces of sea hare tissue to giant sea anemones. We collected 23 spotted sea hares from the west back reef of Discovery Bay, Jamaica on 28 February, 2006. We performed the follwing trials: 3 trials offering live sea hares containing ink, 6 trials offering live sea hares drained of all their ink, 2 trials offering approximately 8 cm^3 pieces of sea hare tissue to the anemone without adding ink, 2 trials adding 1.5 ml of ink into the anemone while it was feeding on a tissue piece, 4 trials adding 50 ml of ink into the anemone while it was feeding on a tissue piece, 4 trials adding 5 ml of ink into the anemone without feeding it, and 2 trials adding 10 ml of ink into the anemone without feeding it.

To determine if ink has a function in intraspecific communication, we conducted experiments with 20 individuals in laboratory tanks. We added a 10 mL solution of either saltwater (control), 1% ink, 10% ink, 50% ink, or pure ink with a syringe. We used individual sea hares more than once, but waited at least 6 hours between trials. We added the treatments in close proximity to each individual and observed their responses. Additionally, we performed each treatment once with ink collected from sea hares in the field, and once with ink collected from each particular individual. We waited half an hour between extracting ink from a sea hare and exposing ink to the same individual. We evaluated which treatments spotted sea hares responded most often using a chi-square test.

We investigated the distribution of spotted sea hares within the west back reef of Discovery Bay with two 20-minute searches in three habitats: shallow turtle grass near the shoreline, rocky substrate near the shoreline, and reef within 20 m of the reef crest. We recorded the number of sea hares found and whether individuals were solitary or in groups.

We evaluated the food preferences of spotted sea hares in the laboratory to see if there is a relationship between distributions of preferred algal species and spotted sea hares. We performed one cafeteria experiment with algae collected from the back reef and one with algae collected from near the shoreline. In these we placed 20 g (wet mass) each of 15 different algal species into tanks containing three spotted sea hares, and after 24 hours we removed and weighed the algae again to determine mass eaten. We also placed 20 g of each algal species in a control tank without spotted sea hares to determine changes in algal mass not due to sea hare grazing.

RESULTS

Feeding trials with live spotted sea hares indicated that sea hares were equally capable of escaping giant sea anemones regardless of inking ability. The three

spotted sea hares capable of releasing ink when introduced to giant sea anemones did release ink upon contact, yet even spotted sea hares without ink managed to escape without apparent injury. However, sea hare tissue was a palatable food item for giant sea anemones. In all eight trials in which sea hare tissue was fed to an anemone, the anemone consumed the entire portion. The presence of spotted sea hare ink did not influence giant sea anemone feeding: anemones did not respond to ink in any concentration.

Spotted sea hares did respond to the presence of ink, and were significantly more likely to respond to ink at 100% and 50% concentrations than 10% and 1% concentrations (X^2 = 41.4, df = 3, P < 0.001; Fig. 1). Responses consisted of withdrawing the head, contracting the body, and closing the parapodia. Individual spotted sea hares responded similarly (X^2 = 17.8, df = 19, P = 0.53) and did not respond differently to their own ink than to the ink of others (X^2 = 1.48, df = 1, P = 0.22).

We found significantly more spotted sea hares near the shoreline than on the back reef (X^2 = 19.9, df = 2, P < 0.001), with 13 spotted sea hares found on rocky substrate near the shoreline, 16 found in habitat dominated by turtle grass near the shoreline, and 0 found on the back reef. Thirteen of the 29 spotted sea hares observed were aggregated, and all but 2 of these were mating.

Lab experiments found that spotted sea hares selectively feed on algae (X^2 = 131.1, df = 14, P <0.001; Fig. 2), preferring mostly noncalcareous red algae and some noncalcareous green algae. They did not feed on any brown algae. The change in the mass of the control algae was negligible. Preferred algal species were abundant in all areas sampled.

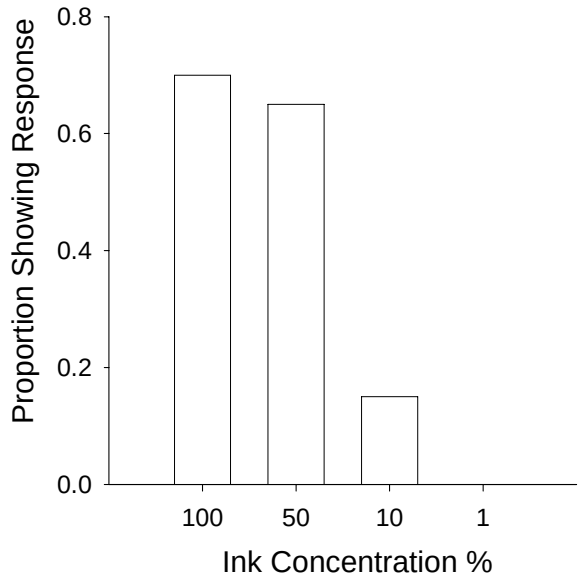

Figure 1. The percentage of *Aplysia dactylomela* that responded to being squirted with ink at different concentrations. A response was defined as a sudden change in behavior that involved withdrawing its head, balling up, or closing its parapodia.

DISCUSSION

Ink likely functions as a defense, but was ineffective against the giant sea anemone. All individuals with ink ejected it upon contact with a giant sea anemone. However, giant sea anemones did not consume whole adult spotted sea hare regardless of whether they released ink, and ink did not deter them from eating smaller pieces of spotted sea hare tissue. Ink may function as a general predator deterrence method and is released against all perceived threats, regardless of efficacy. If ink serves as an alarm signal to conspecifics, our findings suggest that its role is likely limited. Individuals retracted

their heads and other body parts when exposed to high concentrations of either their own or other individuals' ink, suggesting that ink could be used to induce a defensive posture in nearby conspecifics. Alternatively, posture changes in response to ink may occur in response to the noxious compounds in ink and not reflect anti-predator behavior. Because spotted sea hares did not respond to low concentrations of ink, there seems to be a limited range (centimeters) over which ink may be used to communicate between individuals. Moreover, individuals did not display different responses to their own ink than to the ink of conspecifics, suggesting that the alarm signal is not directed. We found spotted sea hares aggregated in nearly half of our field observations, suggesting that they are often close enough to be able to detect one another's ink. However, the majority of these individuals were mating, which may occur only at certain periods.

We found no evidence that the distribution of spotted sea hares is correlated with the distribution of noncalcalareous red algae, their preferred food. We did not find spotted sea hares in the back reef, even though individuals readily ate algae collected from there. This suggests that their absence in this region is more likely the result of other biotic conditions such as predation of young or adults or abiotic conditions such as greater water turbulence.

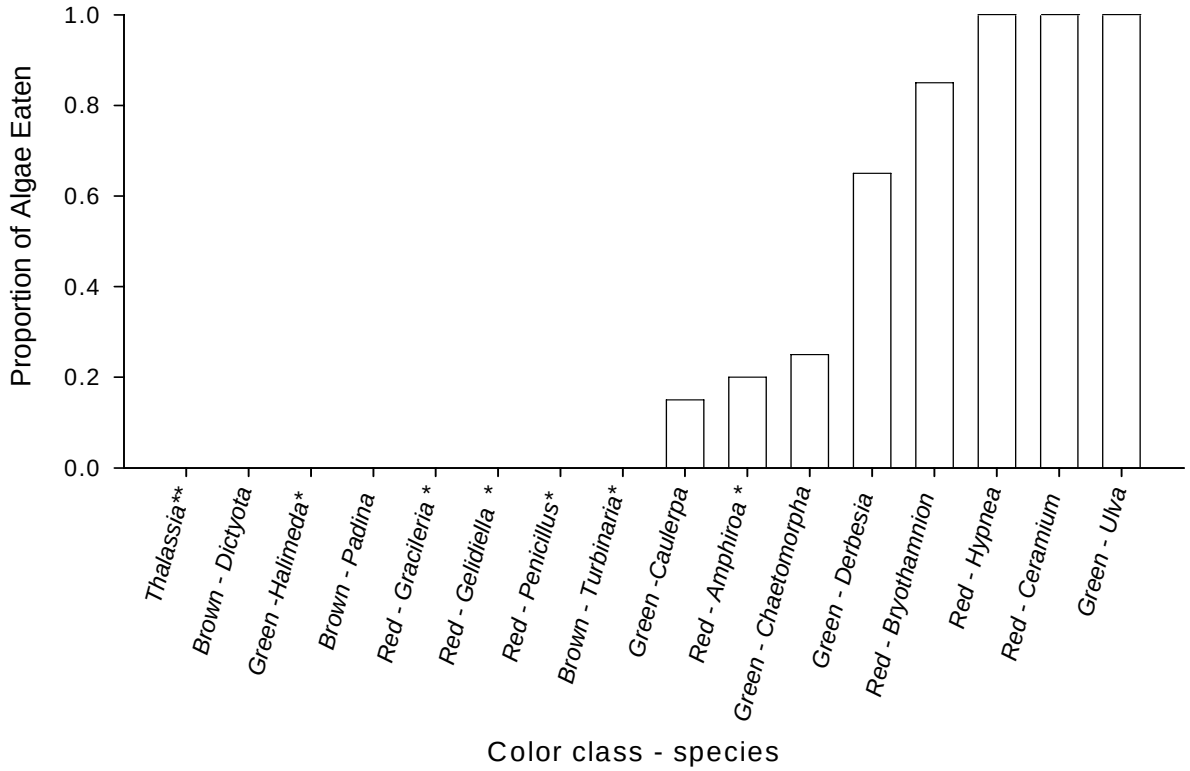

Figure 2. The proportion of algae eaten by *Aplysia dactylomela* after 24 hours in a lab setting exposed to 20 grams of each type of algae. An asterisk denotes calcareous algae, while all others are noncalcareous. ***Thalassia* is a true plant rather than an algae.

LITERATURE CITED

Chapman, D.J. and D.L. Fox. 1969. Bile pigment metabolism in the sea-hare *Aplysia*. J. Exp. Mar. Biol. Ecol. 4: 71-78.

Dimattero, T. 1982. The ink of *Aplysia Dactylomela* and its role as a defensive mechanism. Marine Biological Ecology 57: 196-180.

Fiorito, G. and F. Gheradi. 1990. Behavioral changes induced by ink in *Aplysia fasciata*: Evidence for a social signal role of inking. Marine Behavior and Physiology 17: 129-135.

Hang, P.T.X., K.R. Kroenlein, A.J. Siegel and J.P. Zak. 1997. Inking as a potential social behavior in the sea hare *Aplysia Dactylomela*. Dartmouth Studies in Tropical Ecology p. 161-165.

Rogers, C.N., R. DeNys, T.S. Charlton and P.D. Steinberg. 2000. Dynamics of algal secondary metabolites in two species of sea hare. Journal of Chemical Ecology 26: 721-742.

Torbach, E., A. Zafares and L. Migenis-Lopez. 1989. *Aplysia* Ink and Opaline: Exploration of their relation to predation. Bulletin of Marine Science 45: 664-670.