

## ECOLOGICAL FUNCTION OF CHEMICAL DETERRENTS IN A TROPICAL POLYCHAETE, *EUPOLYMNIA CRASSICORNIS* (ANNELIDA, TEREPELLIDAE), IN BELIZE

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### ABSTRACT

The chemical ecology and trophic interactions of a tropical polychaete, *Eupolymnia crassicornis* (Annelida, Terebellidae), were investigated to determine presence and function of chemical deterrents. Field assays indicated that bluehead wrasses, *Thalassoma bifasciatum*, and green clinging crabs, *Mithrax sculptus*, rejected worm tentacles and feeding pellets treated with extracts from the tentacles. Body parts of the worms were not deterrent in the same assays. This was the first study to use field assays to demonstrate function of chemical defenses in polychaetes, and the first to document presence of chemical defenses in *Eupolymnia*. The study supports basic paradigms concerning presence of chemical defenses within taxonomic groups, biogeographic variability in chemical defenses, and allocation of chemical defenses to vulnerable portions of organisms.

Terebellid polychaetes (Annelida) are sessile benthos that inhabit tubes and extend their tentacles across the sediment surface during feeding. These tentacles are spread in full view of potential consumers, making terebellids excellent candidates for studies of predator-prey interactions. Many polychaetes are known to produce secondary metabolites to deter interactions with their competitors (Chen et al., 1991; Woodin, 1991; Yoon et al., 1994; Chen et al., 1996; Hay, 1996; Fielman et al., 1999; Cowart et al., 2000), and terebellid polychaetes in particular have been the focus of some chemical-ecology studies (Woodin et al., 1987; Goerke and Weber, 1990 1991; Goerke et al., 1991).

Numerous investigations over the past two decades demonstrated that secondary metabolites of benthic organisms serve as defenses against consumers (Bertness et al., 1981; Carpenter, 1986; Hay, 1991, 1996; Steinberg, 1992). Although many taxa produce secondary metabolites, the ecological functions of these compounds have been ascertained in only a few species (Paul, 1992). Much of the early research into these organic compounds centered on tropical marine species and the potential of these compounds as feeding deterrents (reviewed by Pawlik, 1993; Hay, 1996). There is a growing body of evidence that biogeography is an important factor in distribution of prey chemical defenses (Bertness et al., 1981; Carpenter, 1986; Steinberg, 1992; Bolser and Hay, 1996; Hay 1996). Secondary metabolites may be especially common among tropical benthos due to increased consumer activity at lower latitudes (Pawlik, 1993; Pawlik et al., 1995; Hay, 1996).

Previous studies established that not all terebellids produce secondary metabolites, but it is unknown whether terebellids with secondary metabolites allocate them to their entire bodies or concentrate defenses in their exposed tentacles. Seaweeds, gorgonians, soft corals, and bryozoans apparently concentrate secondary metabolites in the most 'apparent' or exposed and actively growing regions of their tissues (reviewed by Hay, 1996), but previous studies of terebellids did not test tentacles and bodies separately (Woodin et al., 1987; Goerke and Weber, 1991; Goerke et al., 1991; Woodin et al., 1993; Fielman et al., 1999).

Secondary metabolites in terebellids may serve numerous functions other than defense. For instance, Woodin et al. (1993) reported that the terebellid, *Thelepus crispus*, along the Washington coast contaminated sediments around its tube with brominated-aromatic metabolites, evidently to discourage establishment by larvae of competitors. Woodin et al. (1987) noted that another terebellid, *Eupolymnia heterobranchia*, co-occurred with *T. crispus*, but did not produce detectable levels of halogenated compounds. Several other studies reported secondary metabolites (bromophenols) in terebellids, but none were detected in the congeners, *E. nebulosa* and *E. nesidensis*, collected in the German Bight and English Channel (Goerke and Weber, 1990, 1991; Goerke et al., 1991). We studied a congener of these species, *E. crassicornis*, that inhabits tropical shallow waters of Belize. We wanted to determine (1) if this organism contained chemical defenses, (2) if those defenses provided deterrents against potential predators, and (3) if the defenses were equally allocated to the tentacles and body.

*E. crassicornis* (Schmarda, 1861) occurs widely in shallows of the Caribbean. It is a conspicuous terebellid that spreads its tentacles on the surrounding rocky substrate to deposit feed. Although it withdraws tentacles when disturbed, it is often seen with tentacles fully extended for up to a meter. Behavior of this 'spaghetti worm' has not been described in detail and almost nothing is known about its interactions with consumers or its chemical ecology. The habit of spreading its tentacles in full view of predators led us to hypothesize that *E. crassicornis* produces chemical deterrents, and concentrates them in its tentacles.

#### MATERIALS AND METHODS

The study was conducted near South Water Caye, Belize (16° 49.2'N, 88° 4.9'W), a 6.0-ha (15-acre) island located about 32 km SW of Dangriga and 1.6 km north of the Smithsonian's Caribbean Coral Reef Ecosystems research facility on Carrie Bow Caye. South Water Caye sits astride the Belize Barrier Reef and is bordered on the west by extensive turtlegrass beds (*Thalassia testudinum*) of the barrier reef lagoon, and on the east by the coral foreereef. South Water Caye supports a modest human population throughout the year, and the marine ecosystems surrounding the island remain nearly pristine.

Field data for this study were collected during February–May 2000. Laboratory data were collected during May 2000. A study area about 100 m by 50 m was established in the backreef area at the northern end of South Water Caye where the worms were relatively abundant. Habitats of this area (described by Gaston and Hall, 2000) were 90% coral rubble, mostly cemented together by coralline algae, and 10% bare sand (coarse sand; primarily derived from coralline algae, *Halimeda opuntia*). The region was an ecotone between the dynamic reef crest (to the east) and an extensive turtlegrass bed (to the west).

Density estimates were made with a m<sup>2</sup> quadrat. Specimens of *E. crassicornis* were collected for assays and laboratory analyses by hand from the backreef, where the organisms occupied sand-grain tubes beneath rock and rubble. Specimens for laboratory analyses were frozen in seawater and returned to the University of Mississippi. Live specimens were sampled in the field or maintained in aquaria on South Water Caye to allow us to observe activity of the worms. Stereomicroscopy provided magnified viewing of tube-building behavior.

Feeding assays were conducted on the tentacles and bodies of worms and the extracts of these tissue groups. Tentacles and bodies were cut into small pellets or extracted for the assays. One set of the feeding assays was conducted using worm tentacles and bodies, and tentacle and body models. The models were produced from alginate extruded through a disposable syringe into a solution of 1 M CaCl<sub>2</sub>; this provided acceptable shaped model worm tentacle or body pellets (as per Bryan

et al., 1995; Slattery et al., 1999). For the second feeding assay, tentacles or body pieces were suspended in seawater within a graduated cylinder to assess displacement volume, then extracted with 5 ml of 1:1 dichloromethane-methanol, dried under reduced pressure, and weighed. The worm extract was added to the liquid alginic acid in a dichloromethane carrier solvent at worm volumetric concentrations and 2% dried krill (soluble protein = 15%, approximating worm levels: Slattery unpubl. data). Control pellets were treated identically except the carrier solvent contained no extract.

Two common species of the study region, the omnivorous (Pawlik et al., 1995) bluehead wrasse, *Thalassoma bifasciatum*, and the omnivorous (Colin, 1978; Delbeek and Sprung, 1997: 279) green clinging crab, *Mithrax sculptus*, were utilized as feeding models in field assays. Each species was offered a control and a treated pellet in a random assignment ( $n = 10$  each) and response was scored as positive if the organism ate the pellet or negative if the organism rejected the pellet. A third control pellet was always offered to the organism to control for satiation with the expectation that a trial would be discounted should the final pellet be refused; this never happened in any of the trials performed. No organism was exposed to a treatment pellet more than once in any set of experiments. These data were analyzed using Fisher's Exact Test (Zar, 1998).

## RESULTS

Both the bluehead wrasses (Fig. 1) and the green clinging crabs (Fig. 2) refused most offerings of the worm tentacles (first assay) and food pellets treated with extract (second assay), and accepted a significant ( $P < 0.05$ ) number of control (untreated) models. The first feeding bioassay was repeated at additional patch reefs near South Water Caye by offering bluehead wrasses the tentacles and tentacle models. The wrasses accepted more tentacle models than tentacles in all observations, and refused tentacles from *E. crassicornis* even when they were mixed with bits of small fish (silversides, *Atherinomorus stipes*). The extract from the worms contained characteristic halogen profiles (by GCMS; Slattery, unpubl. data), but there was so little material that the compound could not be identified further.

These worms were most common in water less than 1.0 m deep of the study region. They were conspicuous because of their long tentacles, and evenly distributed ( $0.7 \text{ ind m}^{-2}$ ). Tentacles of adjacent specimens were never seen overlapping. Laboratory observations indicated that they are intolerant of contact with other specimens of their species, and moved to reestablish tubes if crowded. Tube building required 8–10 h for specimens 8.0-cm long (excluding tentacles). Specimens of this length could extend tentacles up to 1.0 m from their tubes. Another terebellid, *Loimia medusa*, replaced *E. crassicornis* as the habitat graded from backreef coral rubble to turtlegrass beds. The two species were distributed in distinct zones that did not overlap.

## DISCUSSION

*E. crassicornis* apparently contains defensive chemical compounds which deter predation. Our data demonstrated that the worm extracts functioned as defenses against consumers, although we acknowledge that these compounds may have other functions as well (reviewed by Hay, 1996), such as deterrents to settling larvae (Pawlik, 1992; Woodin et al., 1993; Fielman et al., 1999) antibiotic action (King, 1986), or shields to ultraviolet

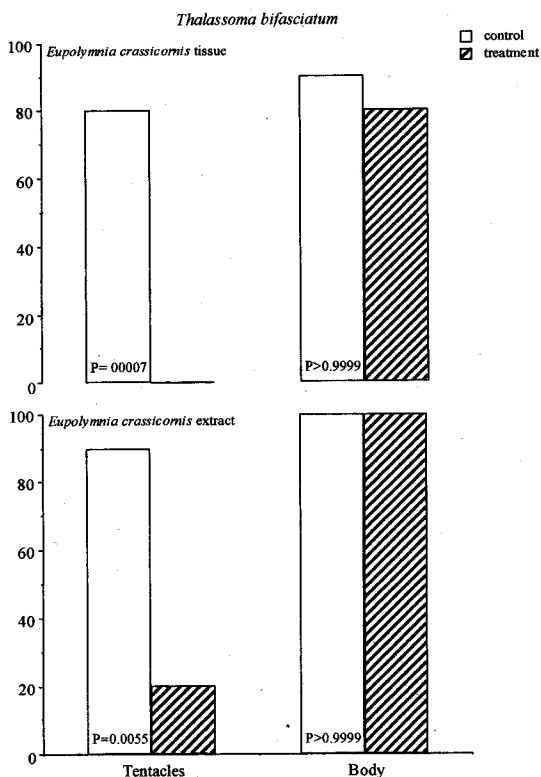


Figure 1. Field feeding assays conducted in Belize on the bluehead wrasse (*Thalassoma bifasciatum*) using tissues and extracts from the polychaete, *Eupolymnia crassicornis*. Bits of polychaete tentacles and portions of polychaete bodies were tested against model control pellets (upper graph). Tentacle and body extracts (in model pellets) and appropriate controls were also tested (lower graph). Percentage of food pellets consumed is indicated by bars.

radiation (Pawlik, 1993). Woodin et al. (1993) provided insight into the complexity of these relationships in their research on sediment-dwelling terebellid polychaetes (*T. crispus*) and motile nereidid polychaetes (*Nereis vexillosa*). The terebellid contaminated sediments around its tube with brominated-aromatic metabolites, evidently to discourage establishment by larvae of the nereidid.

The presence of chemical deterrents in *E. crassicornis*, but not in its congeners (*E. heterobranchia*, *E. nebulosa* and *E. nesidensis*) bolsters the increasing body of evidence that chemical deterrents are not necessarily characteristic of taxonomic groups (Woodin et al., 1987; Goerke and Weber, 1991; Goerke et al., 1991). Even though our understanding of the molecular phylogeny of terebellid polychaetes is in its infancy (McHugh, 1998, 2000; Westheide et al., 1999), it is clear that closely related terebellids may vary widely in their production of secondary metabolites. Goerke and Weber (1991) sampled eleven species of terebellids in the German Bight and English Channel and reported both intra- and interspecific variations in concentrations of bromophenols. Levels they observed were independent of animal size, sex, and season.

A basic paradigm in the study of chemical defenses is that chemical deterrents are more common in tropical organisms, perhaps because of increased predation pressure in tropi-

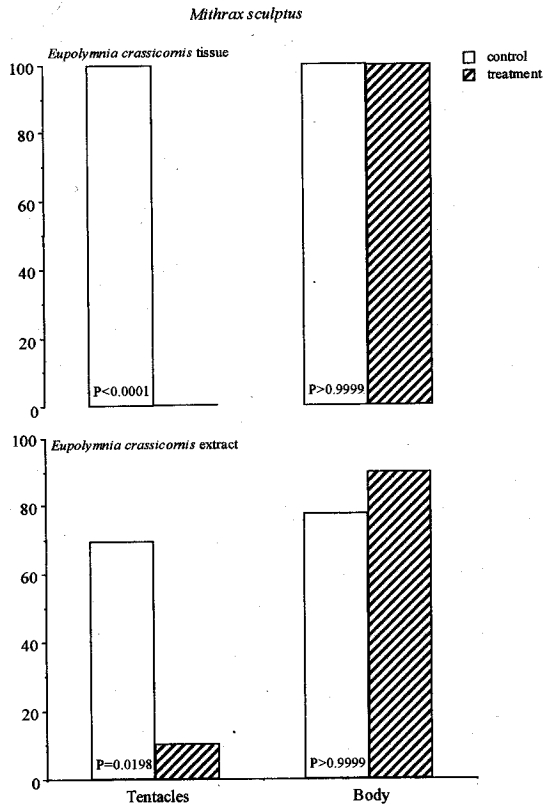


Figure 2. Field feeding assays conducted in Belize on the green clinging crab (*Mithrax sculptus*) using tissues and extracts from the polychaete, *Eupolymnia crassicornis*. Bits of polychaete tentacles and portions of polychaete bodies were tested against model control pellets (upper graph). Tentacle and body extracts (in model pellets) and appropriate controls were also tested (lower graph). Percentage of food pellets consumed is indicated by bars.

cal ecosystems (Steinberg, 1992; Sarma et al., 1993; Pawlik et al., 1995; Hay, 1996). Presence of secondary metabolites in *E. crassicornis*, but not its temperate congeners (Woodin et al., 1987; Goerke and Weber, 1991; Goerke et al., 1991), supports this premise.

Defensive chemicals were not detected in the body of our organisms, but were present in tentacles. Plant apparency models predict that the most visible tissues will be preyed upon first, and therefore should be most heavily defended (Herms and Matson, 1992). Similarly, seaweeds, gorgonians, soft corals, and bryozoans allocated defenses to the vulnerable outer portions of the organisms (reviewed by Hay, 1996). Previous studies of terebellids apparently did not test for differences in tentacle concentrations (Woodin et al., 1987; Goerke and Weber, 1991; Goerke et al., 1991; Woodin et al., 1993; Fielman et al., 1999).

Our primary goal was to determine whether these tropical terebellid polychaetes contained chemical deterrents. We demonstrated that the worms concentrated chemical defenses in their tentacles, and that extracts of these compounds served as deterrents. This was the first study to use field assays to demonstrate function of chemical defenses in polychaetes, and the first to document the presence of feeding deterrents in *Eupolymnia*.

The study supports basic paradigms concerning presence of chemical defenses within taxonomic groups, biogeographic variability in chemical deterrents, and allocation of chemical defenses to vulnerable portions of organisms.

#### ACKNOWLEDGMENTS

Funding was provided by the University of Mississippi (UM) and American Universities International Program (AUIP). This project was part of the sabbatical research of the senior author. L. Shaffer (UM) and E. Marsh (UM) helped collect data. Logistical support was provided by A. Belisle (Belmopan, Belize), L. Tomson (AUIP), R. Aukerman (AUIP), and R. Gill (Dangriga, Belize). Specimens for this study were collected under the auspices of Scientific Permit No. 19770 from the Belize Ministry of Fisheries.

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DATE SUBMITTED: February 28, 2001.

DATE ACCEPTED: January 18, 2002.

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