The polychaete *Cirriformia punctata* is chemically defended against generalist coral reef predators

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Abstract

The Caribbean cirratulid polychaete, *Cirriformia punctata* (Grube, 1856), is bright orange and burrows just below the surface of fine carbonate sand with only its filamentous tentacles and gills exposed to potential predators. In addition to its conspicuous coloration, *C. punctata* lacks obvious structural and morphological defenses. We hypothesized that *C. punctata* protects itself from predation using secondary metabolites as a chemical defense. Feeding bioassays were performed using whole worms to determine palatability against two generalist coral reef predators: the bluehead wrasse, *Thalassoma bifasciatum*, and the brown anemone, *Aiptasia* sp. Additionally, assays were conducted with hermit crabs of the genus *Paguristes*, but with limited success. In all assays, *C. punctata* was unpalatable to consumers. The tentacles and body of the worm were separately assayed using *T. bifasciatum* to determine whether the defensive mechanism was differentially allocated to body regions that are differently exposed to potential predators. Both the tentacles and body of *C. punctata* were unpalatable to *T. bifasciatum*, indicating no specific allocation of defenses. Crude organic extracts of whole *C. punctata* were unpalatable to *T. bifasciatum*, confirming that the worm defense is chemical rather than morphological. Chemical defenses of *Cirriformia* spp. appear to be generally effective against taxonomically diverse potential consumers.
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1. Introduction

Tropical reef ecosystems are characterized by high levels of herbivory and predation, yet these environments are dominated by fleshy, sessile, benthic invertebrates and algae. Benthic invertebrates in coral reef habitats account for 27–56% of the species in different trophic categories (reviewed in Sano et al., 1984). Consequently, coral reef fish may bite at the benthic substrate over 150,000 times/m^2/day (Carpenter, 1986). This intense predation impacts the distribution and abundance of benthic prey in tropical reef communities (e.g. Pawlik, 1998). Predation is a major selective force in coral reef habitats leading to protective and defensive mechanisms in benthic invertebrates, and important among them are chemical defenses (Paul, 1992; Pawlik, 1993; Hay, 1996; McClintock and Baker, 2001). Several thousand secondary metabolites have been isolated from benthic marine

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invertebrates that flourish in predator-rich communities (reviewed in McClintock and Baker, 2001). Secondary metabolites are known to reduce fouling, inhibit competition, deter microbial pathogens, and assist in reproduction, but have mostly been studied as defenses against predation (e.g. Paul, 1992; Pawlik et al., 1995; Hay, 1996; Lindquist and Hay, 1997; McClintock and Baker, 2001; Barsby et al., 2003; Kicklighter et al., 2004; Kicklighter and Hay, 2006).

Like other marine invertebrates, polychaetes possess a variety of antipredatory mechanisms, including behavioral avoidance, construction of burrow refuges, cryptic coloration, and chemical defenses (e.g. Woodin and Merz, 1987; Kicklighter et al., 2004). Kicklighter and Hay (2006) found that of the 81 species of polychaete worms from the Western Atlantic and Caribbean that they studied, 37% were unpalatable to at least one consumer. Unpalatability correlated most strongly with taxonomy and secondarily with color of the worm, with bright coloration signaling distastefulness. Both the tentacles and body of the brightly colored *Cirriformia tentaculata*, for example, were completely unpalatable to the bluehead wrasse, *Thalassoma bifasciatum* (Kicklighter and Hay, 2006). After determining that crude organic extracts from *C. tentaculata* were unpalatable to *T. bifasciatum*, the chemical defense was isolated as a novel group of 2-*n*-alkylpyrrole sulfamates (Kicklighter et al., 2003).

Cirriformia punctata is found in Caribbean reef habitats in open sandy areas. It is bright orange, 1–4 cm in adult body length, and burrows in soft sand with its tentacles and gills exposed to potential predators (Jones et al., 1986). Like *C. tentaculata*, it lacks obvious structural and morphological defenses. Therefore, we hypothesized that due to its lack of external defenses, occurrence in open sandy areas, bright coloration, and relatedness to *C. tentaculata*, *C. punctata* also protects itself by producing secondary metabolites as a chemical defense. As a means of testing this hypothesis, feeding bioassays were conducted to determine the palatability of *C. punctata* to a variety of generalist predators: the bluehead wrasse, *T. bifasciatum*; the brown anemone, *Aiptasia* sp.; and hermit crabs of the genus *Paguristes*. The bluehead wrasse, *T. bifasciatum*, is a generalist predator from the tropics known to feed mainly on small benthic prey (Pawlik et al., 1995). In addition, this fish species is commonly used as a model consumer in similar experiments on prey palatability (Pawlik et al., 1995; Lindquist and Hay, 1997). The anemone *Aiptasia* sp. has been shown in previous feeding studies to regurgitate pellets containing secondary metabolites (Lindquist and Hay, 1997). In display aquaria, we observed

C. punctata that had crawled or drifted onto tentacles of *Aiptasia* sp. being rejected (JRP, pers. obs.). Hermit crabs of the genus *Paguristes* are omnivorous opportunists that have also been used in previous assays of invertebrate chemical defenses (e.g. Pawlik et al., 1986; Waddell and Pawlik, 2000a).

2. Materials and methods

2.1. Collection and maintenance of worms

Laboratory cultures of *C. punctata* were established from a stock culture maintained by J.R. Pawlik (University of North Carolina Wilmington, Center for Marine Science, North Carolina, USA). Worms were held at 26 °C, 30–35‰, 13 h light: 11 h dark photoperiod, and constant aeration in three plastic containers (27 cm × 15 cm × 10 cm) containing fine coral sand. Complete water changes were conducted followed by feeding (TetraMarin© flake food) three times per week throughout the course of the experiment. Terebellid worms, *Neoleprea* sp., were collected from floating docks at Wrightsville Beach, North Carolina, USA (34°12.46′ N, 77°47.66′ W), and used as controls in feeding experiments because they have a size and tentaculate morphology similar to *C. punctata*. The control worms were used in feeding assays immediately after collection and did not require any maintenance. Worms used in assays were ∼2 cm in length.

2.2. Whole worm assays with T. bifasciatum

Feeding assays were conducted as described by Pawlik et al. (1995) and Kicklighter et al. (2004). Worms were presented to 10 groups of bluehead wrasses, *T. bifasciatum*, (2–4 wrasses per group) with each group held in separate partitions of laboratory aquaria. Groups of fish were first offered a palatable control worm, *Neoleprea* sp. If it was consumed, the fish were then offered an experimental worm, *C. punctata*. If one or more fish ejected a worm from their mouth after a minimum of 3 feeding attempts, or if the worm was approached and ignored after one such attempt, the worm was considered rejected. If the worm was rejected, a second control worm was offered to verify willingness to feed. Three replicate assays were performed, each with 10 groups of different fish.

2.3. Whole worm assays with Aiptasia sp.

Sixty anemones were placed in aquaria and given a day to attach to 350 ml glass containers (one per container) in

an aquarium (26 °C and 30–35‰). For approximately one month, until the anemones were assayed, they were fed frozen brine shrimp three times per week. Whole worm assays were performed using both *C. punctata* ($N=30$) and *Neoleprea* sp. ($N=30$) to determine palatability. Anemones were assayed separately by first offering them a specimen of *C. punctata* and then *Neoleprea* sp. A worm was considered rejected if it was not ingested or regurgitated by an anemone during a 10-minute period.

2.4. Whole worm assays with Paguristes sp.

Feeding choice experiments employing *Paguristes* sp. were conducted as described in Pawlik et al. (1986) with minor modifications. Thirty crabs were assayed individually in a 350 ml glass arena by placing the crab in the center and one *C. punctata* and one *Neoleprea* sp. on either side and about 2.5 cm away from the crab. The behavior of the crab was monitored for 20 minutes and feeding on either of the two worms was recorded. A worm was considered rejected if it was not fed upon by the crab.

2.5. Assays to test for differential allocation of defense

The tentacles and bodies of *C. punctata* were separately subjected to feeding assays employing *T. bifasciatum*. All worms were chilled in a refrigerator (4 °C) for 10 min to slow their movements, then the tentacles were severed from the worm body with a scalpel. Feeding assays were conducted as described above, with the fish first being offered the tentacles or body of the control worm (*Neoleprea* sp.) followed by the tentacles or body of the experimental worm.

2.6. Assay to test for chemical defenses

To differentiate between morphological and chemical defenses, whole *C. punctata* were extracted in organic solvents and the extracts reconstituted into artificial food at the same volumetric concentration as in worm tissue and subjected to feeding assays employing the bluehead wrasse, *T. bifasciatum* (Pawlik et al., 1995). For each sample, 20–30 worms with a volume of ∼5 ml was measured by displacement of seawater in a 10 ml graduated cylinder. The water was then drained and the tissue was extracted with 1:1 methanol:dichloromethane using standard techniques (Pawlik et al., 1995). A mixture of 3.0 g alginic acid and 5.0 g of freeze-dried, powdered squid mantle was made with 100 ml distilled water. A volumetric equivalent of the squid mixture was then added to the vial containing crude extracts and vigorously stirred to homogenize and suspend the extracts into the food matrix. The mixture was then loaded into a 5 ml syringe and extruded into a beaker containing 25 ml of 0.25 M calcium chloride, thereby forming a spaghetti-like strand. After a few minutes, the hardened strand was removed and cut into 4 mm long pellets with a razor blade. Control pellets were made the same way, but without the addition of organic extract. A small amount of food coloring was added to match the color of the control pellets to the color of the extract-treated pellets. Assays with the pellets were conducted in the same fashion as previously described for the whole worms.

2.7. Data analysis

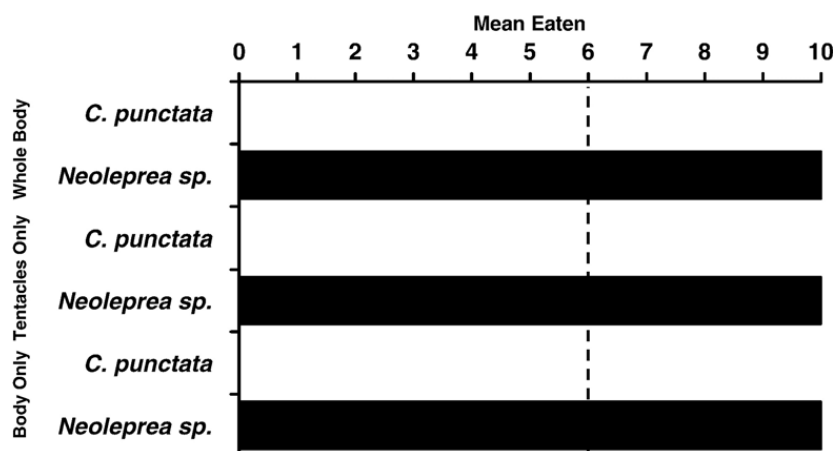The differences in consumption of experimental worms versus control worms in feeding assays employing

Fig. 1. Palatability of the polychaetes *Cirriformia punctata* and *Neoleprea* sp. to the bluehead wrasse, *Thalassoma bifasciatum*. Separate assays were performed on whole worms and separated worm tentacles and worm bodies. For an individual assay, treatments were considered deterrent if the number eaten was less than or equal to 6 ($p<0.043$, Fisher exact test, 1-tailed) as indicated by the dotted line. Three replicate assays were performed.

Fig. 2. Palatability of *Cirriformia punctata* and *Neoleprea* sp. to the brown anemone *Aiptasia* sp. (*N*=30) and to hermit crabs of the genus *Paguristes* (*N*=30) (*p*<0.05 for both comparisons, binomial test).

T. bifasciatum were evaluated using the Fisher exact test (see Pawlik et al., 1995). For any single assay of 10 replicates, the experimental worm was significantly unpalatable if 6 or more were rejected (*p*<0.043, Fisher exact test, 1-tailed). The data from experiments with *Aiptasia* sp. and *Paguristes* sp. were analyzed using a binomial test.

3. Results

In assays of whole worms, *C. punctata* was unpalatable to the bluehead wrasse *T. bifasciatum* relative to the control worm (*Neoleprea* sp.) (*p* ≤ 0.043, Fisher exact test, 1-tailed; Fig. 1), as were the tentacles and bodies of *C. punctata* when assayed separately (*p* ≤ 0.043, Fisher exact test, 1-tailed; Fig. 1). The brown anemone, *Aiptasia* sp., consumed a significantly greater number of *Neoleprea* sp. (27/30) than *C. punctata* (4/30) (*p* ≤ 0.05;

Fig. 2). Hermit crabs of the genus *Paguristes* consumed a significantly greater number of *Neoleprea* sp. (3/30) than *C. punctata* (0/30) (*p* ≤ 0.05; Fig. 2). Food pellets containing a volumetrically equivalent concentration of crude organic extract from *C. punctata* were unpalatable to *T. bifasciatum* relative to control pellets (Fig. 3).

4. Discussion

In assays against three potential consumers, *C. punctata* was unpalatable relative to a control worm, *Neoleprea* sp. (Figs. 1 and 2). Food choice assays conducted with the hermit crab *Paguristes* sp. provided a very low number of trials in which either worm was chosen as food by an assay crab (3 of 30, Fig. 2), but in all of those cases, *Neoleprea* sp. was chosen over *C. punctata*. Crude organic extracts of whole *C. punctata* were unpalatable to *T. bifasciatum* relative to control
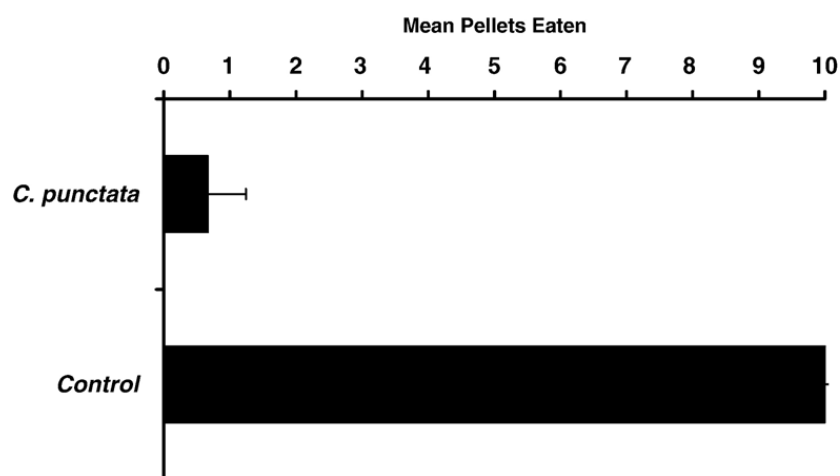

Fig. 3. Palatability of food pellets containing volumetric equivalent concentrations of whole worm crude organic extracts of *Cirriformia punctata* to the bluehead wrasse, *Thalassoma bifasciatum*. For an individual assay, extracts were considered deterrent if the number of pellets eaten was less than or equal to 6 (*p*<0.043, Fisher exact test, 1-tailed) as indicated by the dotted line. Three replicate assays were performed.

pellets (Fig. 3), confirming that unpalatability was due to secondary metabolites rather than morphological defenses. When assayed separately, both the tentacles and body of *C. punctata* were unpalatable to *T. bifasciatum* (Fig. 1). Therefore, chemical defenses in *C. punctata* are not differentially allocated to body regions that are exposed to predation. This lack of differential allocation may be due to the small size of the worm and its tendency to crawl on or near the sediment surface; predatory fishes could easily ingest a whole worm in one bite. In contrast, larger tube-dwelling worms such as *Eupolymnia crassicornis* differentially allocate chemical defenses to exposed tentacles (Kicklighter et al. 2003).

It is tempting to conclude that the chemical defense of *C. punctata* is tied to aposematic coloration, but the case for this is not an easy one to make (see Pawlik et al. 1995). No relationship has been demonstrated between chemical defense and color in Caribbean sponges, and bright red and orange colors are only visible at the shallowest depths in marine environments. It has been suggested that the bright red and orange colors of some marine organisms are more the result of sequestered dietary or respiratory pigments, and less the result of selection for warning coloration (Edmunds, 1991; Pawlik et al., 1995).

A congeneric species of *C. punctata*, *C. tentaculata*, produces secondary metabolites that deter feeding by predatory fishes (Barsby et al. 2003; Kicklighter et al. 2003). In a study by Kicklighter and Hay (2006) palatability of polychaetes was most strongly correlated with taxonomic relatedness. The present study extends the work of Kicklighter et al. (2004) by demonstrating that other species within the genus *Cirriformia* are chemically defended, and that these defenses are also effective against invertebrate consumers as well as fish predators. Previous work on the chemical defenses of Caribbean sponges has shown that chemical defenses against fishes (Pawlik et al., 1995) are also generally effective against hermit crabs (Waddell and Pawlik, 2000a) and seastars (Waddell and Pawlik, 2000b). The present study corroborates the hypothesis that chemical defenses of marine invertebrates are generally effective against a taxonomically diverse group of potential consumers (Waddell and Pawlik, 2000a,b).

Acknowledgements

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