BEHAVIORAL ECOLOGY

To avoid or deter: interactions among defensive and escape strategies in sabellid worms

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Abstract Numerous studies demonstrate how sessile marine organisms utilize chemical, structural, and nutritional deterrents to persist in predator-rich environments. Little is known, however, about how mobile, more behaviorally complex species minimize predation by integrating avoidance and deterrence strategies. We investigated this using sabellid polychaete worms from the Caribbean and temperate western Atlantic. Sabellids extend their feather-like radioles beyond their protective tubes for feeding and respiration; the body remains inside the tube and the radioles retract when threatened. We used co-occurring consumers to determine the palatability of radioles and bodies for each of the eight species tested. In addition, we examined chemical or structural traits affecting palatability and evaluated predator escape traits, such as tube strength, speed of radiole retraction, completeness of retraction, and sensitivity to a nearby disturbance. All species had unpalatable radioles that were chemically or structurally defended, but only two species had unpalatable bodies. Thus, most species allocated defenses to tissues that were most exposed to predation. The two species with chemically defended bodies, *Bispira brunnea* and *Bispira variegata*, relied less on behavioral escapes than the other species. Their tubes were weak, they did not retract until disturbances were very close, and *B. brunnea* retracted slowly and incompletely even when touched. Other species generally had stronger tubes and/or retracted when disturbances were farther away. This trade-off of deterrence versus escape even occurred within a single species when populations differed in palatability. Populations of *B. variegata* from North Carolina and Georgia were chemically deterrent to both temperate and tropical consumers, while populations from Panama and Florida were palatable. The more palatable Panama population retracted in response to distant movement, while the unpalatable North Carolina population did not retract until nearly touched. Thus, most species utilize a combination of predator avoidance and deterrence strategies, but more deterrent populations of species utilized avoidance less.

Introduction

Numerous studies have demonstrated how organisms, such as terrestrial plants, marine invertebrates, and seaweeds, use chemical and structural (e.g., spines, toughness, spicules) deterrents as defenses against consumers (e.g., Rosenthal and Berenbaum 1992; Hay 1996; McClintock and Baker 2001). In some cases, there appears to be a trade-off between these defensive strategies. As examples: (1) some terrestrial plants show a negative correlation between physical and chemical deterrents (Twigg and Socha 1996); (2) some

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seaweeds have high levels of chemical defenses in tissues that are poorly defended structurally, but decrease chemical defenses as structural defenses increase (Hay et al. 1988); and (3) marine hydroids deter consumers with stinging nematocysts or with chemical defenses, but not with both (Stachowicz and Lindquist 2000).

In other cases, however, chemical and structural defenses are used simultaneously, as occurs for some seaweeds (Paul and Hay 1986; Hay et al. 1994), soft corals (Van Alstyne and Paul 1992), sponges (Hill et al. 2005; Jones et al. 2005), echinoderms (Bryan et al.1997), and terrestrial plants (Ramachandran and Khan 1991). The use of multiple defenses might be selected for in predator-rich environments (such as tropical habitats) where consumer diversity and attack strategies are both high (Lubchenco and Gaines 1981; Hay 1984). In some cases, chemical and structural deterrents may even interact synergistically to enhance resistance to consumers, as has been shown for a soft coral (Gerhart et al. 1988), two seaweed species (Hay et al. 1994), and potentially some sponges (Jones et al. 2005).

While many studies examine structural and chemical defenses in sessile marine species (i.e., seaweeds, sponges, soft-corals, ascidians), few study use or allocation of these defenses in more mobile species that also utilize behavioral escapes from consumers (Stachowicz 2001). Investigations regarding the potential integration of deterrence and avoidance strategies are needed to increase our understanding of defensive tactics used by more behaviorally complex species. Two studies have examined the use of deterrence and refuging in two freshwater vertebrates. Stickleback fish that are structurally unpalatable (due to spines; Hoogland et al. 1957) used refuges less and were faster to emerge from their refuges in the presence of a simulated aerial predator than were palatable minnows (which lack spines or other deterrents) (Krause et al. 2000). Similarly, unpalatable tadpoles often refuged less than palatable tadpoles in the presence of water containing predatory fish (Kats et al. 1988). Terrestrial species may show similar trade-offs. Unpalatable caterpillars tend to be overt, occur gregariously, and feed during the day when predators are active, while palatable species that lack defenses tend to be solitary, cryptic, and refuge from predators by feeding on the undersides of leaves or within rolled leaves (Heinrich 1993). These studies suggest that behavioral avoidance is used more by species lacking predator deterrents and less by species with effective consumer deterrents. More studies on a greater diversity of prey types are needed to determine if this is a general pattern.

Sabellid worms (polychaete annelids) are good model organisms for investigations of how predator deterrence (chemical, structural) and avoidance (behavioral escape, refuge use) strategies may be integrated by marine invertebrates. Sabellids, commonly called "feather duster" worms, are tube-dwelling species that have a crown of feathery radioles for feeding and respiration that project from a protective tube. For most species, the body always remains inside the tube (Fauchald 1977; Fauchald and Jumars 1979) and many species can quickly and completely retract their radioles in response to a physical disturbance, shadow, or approaching consumer. The worm constructs the tube from organic secretions or a mix of mucous secretions and size-selected particles (Brusca and Brusca 1990). Tube-building is an effective antipredation strategy for insects (Hershey 1987; Robinson 2000) and some polychaetes (Matilla 1997; C. Kicklighter, personal observation), so tubes likely protect sabellids as well. Sabellids commonly occur in areas of high consumer activity, such as coral reefs, where chemically defended species such as seaweeds, sponges, soft corals, and ascidians are also common (e.g., Hay and Fenical 1988; McClintock and Baker 2001; Paul et al. 2006). While chemical defenses are uninvestigated in sabellid worms, it seems likely that they might rely on both deterrence and avoidance to persist in predator-rich environments.

We use two ecologically relevant fishes and a crab to examine the palatability, chemical and structural deterrence, and various behavioral and avoidance traits of eight species of sabellid worms from the Republic of Panama, the Bahamas, Florida, Georgia, and North Carolina. We used these data to evaluate how multiple traits were integrated to deter or avoid consumers.

Materials and methods

Worms were collected September 2000–June 2003 near: (1) Bocas del Toro, Republic of Panama ([09° 21′N, 82° 15′W]—*Anamobaea orstedii*, *Branchiomma nigromaculata*, and *Sabellastarte magnifica* from coral rock and rubble on coral reefs, *Megalomma* sp. from soft-sediments on reefs and reef-flats, and *Bispira variegata* from soft-sediments around mangroves); (2) San Salvador, Bahamas ([24° 03′N, 74° 32′W]—*Bispira brunnea* from weathered coral rock); (3) Key Largo, Florida ([25° 07′N, 80° 25′W]—*Branchiomma* sp. from the alga *Halimeda optunia* around mangroves, and *B. variegata* from mangrove prop roots); (4) Shackleford Island, North Carolina ([34° 40′N, 76° 37′W]—*Bispira variegata* from submerged boulders);

(5) Skidaway Island, Georgia ([31° 57′N, 81° 00′W]—*Sabella* sp. from a floating dock), and (6) near Gray's reef off the coast of Savannah, Georgia ([31° 24′N, 80° 53′W]—*B. variegata* from limestone rock). Palatability of species from North Carolina and Georgia were assayed using the co-occurring fish *Fundulus heroclitus* (mummichog) and the crab *Callinectes similis* (lesser blue crab). Worms from Panama, the Bahamas, and Florida were assayed using the fish *Thalassoma bifasciatum* (bluehead wrasse). This fish is common on coral reefs, in back-reef rubble zones, and sometimes along the margins of seagrass beds and sandy areas when these habitats are intermixed with patches of hard or gorgonian corals. Thus, bluehead wrasse would commonly co-occur with the worms we collected from reefs and reef-flats, but they would be less frequent consumers of worms we collected near mangroves. However, we used this species because feeding preferences of the bluehead wrasse commonly parallel preferences of other generalist consumers (Burns et al. 2003; Kicklighter and Hay 2006), and because this wrasse has commonly been used as a model consumer in similar investigations of chemical defenses (e.g., Pawlik et al. 1995; Lindquist and Hay 1996; Pisut and Pawlik 2002). Mummichog, bluehead wrasse, and the lesser blue crab are generalist consumers, and invertebrates, including worms, make up the bulk of their diets (e.g., Feddern 1965; Baker-Dittus 1978; Hsueh et al. 1992). Mummichogs (4–6 cm long) and lesser blue crabs (4–8 cm carapace width) were collected near Skidaway Island, Georgia. Bluehead wrasse (5–7 cm long) were collected from coral rubble areas around Key Largo, Florida, San Salvador, Bahamas, and Bocas del Toro, Panama.

We collected *B. variegata* in both North Carolina and Panama. Palatability of these populations appeared to differ, but each was tested against its co-occurring consumer, thus potentially confounding preference of the different consumers with differences in worm traits affecting palatability. To remedy this problem, we also fed North Carolina worms to the tropical consumer and Panama worms to the temperate consumers. We also collected *B. variegata* from reefs near Savannah, Georgia and from mangroves near Key Largo, Florida, and fed these to consumers available at these places and times. Thus, *B. variegata* from Panama, North Carolina, and Georgia were fed to the bluehead wrasse, mummichog, and lesser blue crab, while the collection from Key Largo, Florida was fed to the bluehead wrasse alone. To prevent consumer experience with one population from affecting subsequent feeding on another, individual consumers were never offered worms from more than one population. When

we were concerned that the consumers we were using may not have previously encountered the prey and might have needed to learn from an initial encounter, we fed consumers for two consecutive days in order to allow consumer learning. These fishes and crabs will learn to reject defended, novel foods within this time period (Lindquist and Hay 1995; Kicklighter et al. 2004; Long and Hay 2005).

Feeding assays were conducted either at the Georgia Institute of Technology's marine facility on Skidaway Island, Georgia (Georgia collection); the University of North Carolina at Chapel Hill, Institute of Marine Sciences, Morehead City, North Carolina (North Carolina collection); the Smithsonian Tropical Research Institute, Bocas del Toro, Republic of Panama (Panama collection); onboard the R/V Seward Johnson I (Bahamas collection); or at NOAA's National Undersea Research Center in Key Largo, Florida (Florida collection). Assay consumers were kept either individually or in small groups of 4–5 individuals (*Thalassoma*) in recirculating or flow-through seawater systems in either 2.4 l (mummichogs, bluehead wrasse in Georgia), 4.7 l (lesser blue crab), or for the bluehead wrasse, 2.1 l (Key Largo), 38 l (in Panama), or 189 l (on ship) containers. In cases where multiple bluehead wrasse were held together in small groups, separate containers of fish were considered independent replicates. In the laboratory, mummichogs and wrasse were fed frozen brine shrimp (San Francisco Bay Brand, Newark, CA, USA) and crabs were fed frozen squid for at least two days to acclimate them to feeding in the lab and to standardize nutritional conditions. About 1 h before assays with worms or worm extracts, consumers were fed to satiation to ensure that they were not feeding indiscriminately due to unusual hunger levels (Cronin and Hay 1996).

Feeding assays followed standard methodology (e.g., Pawlik et al. 1995; Hay et al. 1998). First, consumers were offered a palatable control food (a brine shrimp for mummichogs and bluehead wrasse or a piece of squid for crabs). If this was eaten, each consumer was then offered fresh worm portions (~0.125 cm³)—either from the body or the radioles. Each offering came from a different individual worm to ensure independence among observations. If the consumer ate the worm tissue (even if it was spat out but then consumed), it was scored as accepted. If the consumer tasted the portion, spat it out, and refused to consume it over the next 30 s, the replicate was scored as rejected. Any consumer rejecting the worm portion was then offered a second control food to ensure it had not rejected the worm due to it being satiated and unwilling to feed on any food. Any consumer that did

not consume either the initial or second control food was excluded from consideration. It was uncommon for the consumer to reject the second control (this occurred in only 11 of 1,044 cases). For a replicate to be included in the statistical analysis, the consumer was required to take the worm portion into its mouth—this ensured that the assessment of palatability was based on taste as opposed to visual discrimination. Consumers were always willing to sample the body, but sometimes individual bluehead wrasse refused to sample the radioles. This behavior occurred when radioles were offered to fish both in the lab and in the field. However, enough fish were always willing to try the radioles that we achieved an adequate sample size ($n=8$–14 for each assay). Fisher's exact test was used to assess feeding on palatable control food versus worm portions, homogenated tissues, or foods containing chemical extracts.

If worm tissues were rejected by consumers, we tested these for the presence of chemical defenses. Freshly collected tissues were placed in acetone equivalent to approximately two times the volume of the tissues and cut into small pieces with scissors. The extract was filtered to remove particulates and solvents were removed with a rotary evaporator. This process was repeated two more times to ensure adequate extraction. The three extracts were combined to form the crude extract for each species. This was done for all collections except for *B. variegata* from North Carolina—see below. Preliminary extractions and bioassays had shown that using dichloromethane (DCM), or DCM mixed with other organic solvents, caused loss of deterrence.

For bioassays testing the effects of chemical extracts, we used a squid-based food in which we could include or exclude the crude extract of the test worm. Food was made by reconstituting lyophilized homogenated squid mantle with 0.03 g sodium alginate per ml of squid. The squid paste was drawn into a 50 μl pipet and extruded into a 0.25 M calcium chloride solution, which caused the paste to harden to the consistency of cooked pasta. After about 30 s, the squid noodle was removed from the calcium chloride and cut into small pieces that were fed to consumers. For a test food, a natural volumetric concentration of crude extract was solubilized in ethanol, transferred to a 2.0 ml microcentrifuge tube, and the solvent removed under a stream of nitrogen or air, where nitrogen was unavailable. Squid-paste that matched the caloric value of the worm was then added and mixed to ensure even dispersal. This was then formed into a noodle as described above and fed to the fishes and crab versus a palatable control noodle. Control foods were made the same way (including the solvent carrier) but without addition of

the crude extract (methods of Lindquist and Hay 1996).

So we could match the caloric value of our squid-based food to that of the worm being investigated (by adding different ratios of squid and water), we determined the caloric value of radioles and bodies of each worm species tested for deterrence. The caloric contents (calories g^{-1}) of the radioles of all species ($n=4$–6), the bodies of unpalatable species ($n=4$–6), and of homogenized squid mantle ($n=6$) were determined by bomb calorimetry of 0.03–0.05 g dry mass of lyophilized tissue in a Parr 1425 Semimicro bomb calorimeter (Parr Instrument Co., Moline, IL, USA). Values were converted to calories ml^{-1} of tissue based on our determined dry mass volume^{-1} conversion for each species and for the squid-based food.

Bioassay-guided fractionations of deterrent crude extracts from each species (except *Bispira variegata* from North Carolina) were accomplished by partitioning based on a modified Kupchan et al. (1975) scheme. This scheme yielded five partitions that differed in solubility: these were soluble in hexanes, dichloromethane, ethyl acetate, butanol, or water. All fractions from each species were bioassayed for chemical deterrence using a co-occurring consumer.

Bispira variegata from North Carolina was investigated after examining the other species and, based on problems with maintaining the activity of deterrent extracts from other species, we altered our extraction procedures for this worm. Tissues were extracted with 100% methanol, 100% acetone, and 100% ethyl acetate. The filtered solvents were combined and solvent was removed with a rotary evaporator. Crude extracts from the radioles and the body were then tested separately in feeding assays. Because crude extracts from both radioles and the body deterred feeding by mummichogs and crabs, whole (radioles + body) *B. variegata* were extracted and further purified by partitioning based on a modified Kupchan et al. (1975) scheme, producing three partitions: hexanes, ethyl acetate, and water.

Unpalatable radioles might be defended either chemically or structurally. If defense was structural, then destroying the structure should alter the palatability. If defense was chemical and not degraded by tissue damage, then destroying the structure should not alter palatability. To test these possibilities, these tissues were lyophilized, ground to a fine powder (to eliminate structural traits such as toughness, spines, etc.), incorporated into alginate as a gelling agent, and offered to consumers along with a palatable control food that was matched for caloric content (see Methods in Lindquist and Hay 1996).

To investigate whether defensive traits and predator avoidance strategies might be integrated, we examined tube toughness and escape behavior for each species (or population). Presumably, a tube that is tough would provide greater protection from consumers. As a measure of toughness, the weight necessary to tear a tube was measured by clamping the tube, 6 mm from the top, in a 1.9 cm binder clip, which was attached to a 473 ml plastic cup by 30 cm of monofilament line. The opposite end of the tube was held between two fingers while the cup was slowly filled with sand until the tube broke. After this had occurred, the binder clip + fishing line + cup + sand were then weighed.

Worms that can sense an approaching predator and retract their radioles should avoid consumption more effectively than those that retract more slowly, less completely, or only when a consumer is very near. To measure the distance at which worms retracted into their tubes in response to an approaching physical disturbance (such as an approaching predator), a diver swam slowly toward a worm extending a 0.37 kg hammer toward the worm. When the worm retracted, the hammer was placed onto the substrate and the distance to the nearest 0.5 cm measured between the head of the hammer and the worm. For standardization, the same person performed all measurements for each species.

The faster a worm retracts its radioles in response to a consumer, the less susceptible it should be to consumers. Retraction time was measured by video taping while shooting water at a worm from a pipet held 2–3 cm from the radioles. In some cases, worms retracted their radioles before the diver could get close enough to shoot water from the pipet. If this occurred, the diver waited for at least 1 min after the worm fully emerged from its tube before prompting the worm to retract. Individual video frames were captured on a computer and analyzed with video analysis software (Perception Video Recorder, Leitch Corp., Florence, KY, USA) to determine the number of frames and thus the time it took for each worm to stop retracting its radioles—this occurred when the radioles were no longer visible, as they had been pulled completely into the tube, or when two subsequent frames showed the radioles at the same location, indicating retraction had stopped before the radioles were completely within the tube.

How completely the radioles are pulled into the tube will also affect the worm's susceptibility to consumers. The percent radiole length retracted was measured using the video footage taken for retraction time analysis. Video frames of the worm radioles before the disturbance (thus, with radioles fully projecting from the tube) and after the disturbance, when the radioles were no longer being pulled into the tube, were captured with video analysis software. The length of worm radioles in these two frames was measured using image analysis software (ImageJ, http://rsb.info.nih.gov/ij/), and the percent radiole length retracted was determined.

For tube strength, the residuals were not normal but the variances were homogeneous (Bartlett's test). Because ANOVA is robust to departures from normality, especially with equal sample sizes (Underwood 1997), we analyzed our data using ANOVA. Tukey–Kramer post hoc analyses identified differences among species (and populations for *B. variegata*) in the mass required to tear the tubes. For all other analyses (time to retract radioles, distance from physical disturbance at the time of radiole retraction, and percent radiole length retracted), the residuals were not normal and variances were heterogeneous (Bartlett's test); transformation was unsuccessful at making them homogeneous. For these analyses, we used Kruskal–Wallis followed by nonparametric multiple comparison analyses (Zar 1999) to identify differences among the species.

Results

Radioles of all eight species were unpalatable to sympatric consumers, while the bodies of only two species were unpalatable (Fig. 1). Bodies of *B. brunnea* and *B. variegata* (from North Carolina but not from Panama) were unpalatable. The North Carolina versus Panama difference for *B. variegata* could be due to different traits of the worms or due to different feeding preferences of the co-occurring consumer species used in the different locations. To assess this, we fed bodies from both locations to the same consumers. When *B. variegata* bodies from North Carolina were fed to tropical bluehead wrasses, they were immediately rejected (Fig. 2a, $P<0.001$). In contrast, when the body tissues from populations in North Carolina, Georgia, or Panama were fed to temperate mummichogs, they were readily consumed on day one, but mummichogs that ate the tissues from temperate populations on day one rejected these foods on day two, while mummichogs that were fed tissues from the tropical population consumed these readily on both days (Fig. 2a–c). Mummichogs consuming tissues from North Carolina or Georgia worms on day one almost all regurgitated the worm portion, but not the palatable control food, within 20–30 min of consuming these tissues (North Carolina: $n=10$, $P<0.001$; Georgia: $n=11$, $P=0.004$).
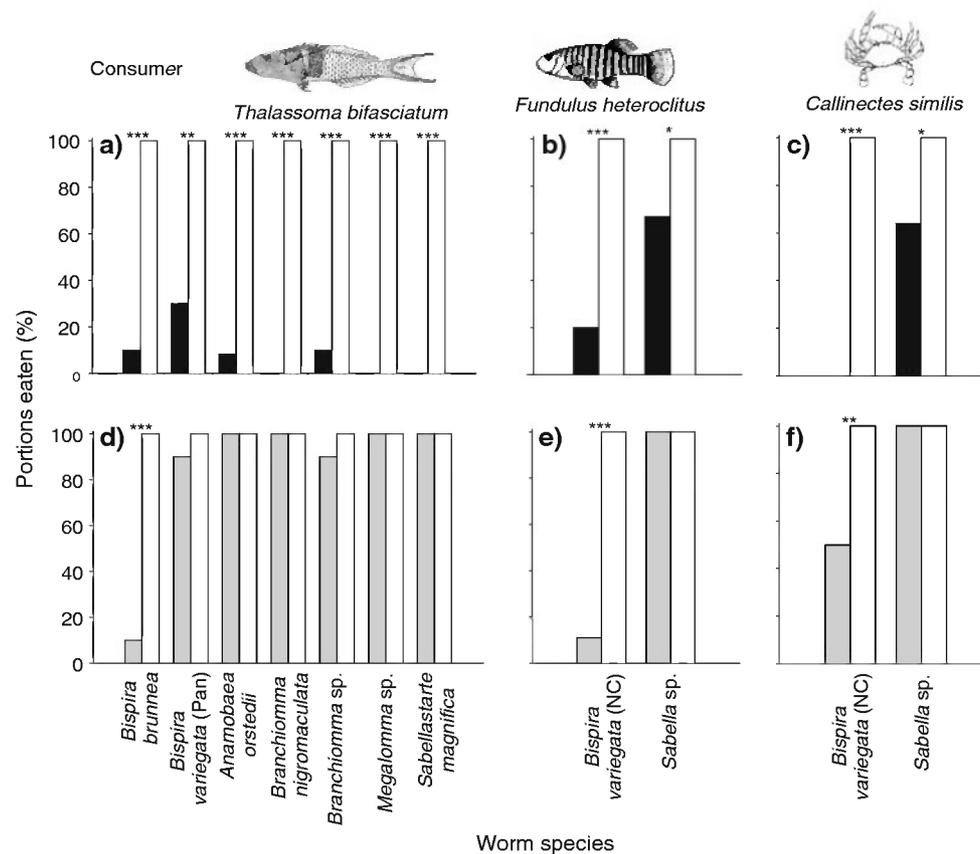
Fig. 1a–f Consumption of portions of (**a–c**) worm radioles (*black bars*) and (**d–f**) worm bodies (*gray bars*) by the (**a, d**) bluehead wrasse *Thalassoma bifasciatum*, (**b, e**) mummichog *Fundulus heteroclitus*, and (**c, f**) lesser blue crab *Callinectes similis*. Control food portions (*open bars*) consisted of brine shrimp for bluehead wrasse and mummichog or squid chunks for the lesser blue crab. *Asterisks* (*) indicate significant differences (*$P<0.05$, **$P<0.01$, ***$P<0.001$), as determined by Fisher's exact test, $n=8–14$

Those feeding on tissues from the Panama population did not regurgitate, and did not avoid consumption of worm tissue the next day. Thus, avoidance of tissues on day two was associated with regurgitation on day one. Temperate crabs found tissues from all populations unpalatable; crabs feeding on tissues from the Georgia or Panama populations rejected these immediately, while those feeding on tissues from North Carolina rejected tissues only on the second day of feeding. We never observed crabs regurgitating.

We also collected *B. variegata* on mangrove roots in Key Largo, FL. Nine of ten bluehead wrasse immediately consumed these worms when offered; this did not differ from the consumption of the palatable control food (*P>0.999*). These worms were uncommon in Florida, so we were unable to collect enough worms to conduct this assay with temperate consumers. Thus, *B. variegata* from reef habitats in North Carolina and Georgia were unpalatable to fishes, while tropical populations from mangrove areas of Panama and Florida were palatable (Fig. 2).

When the radioles from *B. brunnea*, *B. nigromaculata*, *Branchiomma* sp., *Megalomma* sp., *Sabella* sp., and *S. magnifica* were homogenized and reconstituted into a gel-based food, all were palatable (Fig. 3), suggesting that either homogenization compromised their chemical defenses, or that their initial unpalatability was due to structural traits. Radioles from *B. variegata* (from both Panama and North Carolina) and *A. orstedii*, and bodies of *B. brunnea* and *B. variegata* (from North Carolina) were still unpalatable as homogenized tissues (Fig. 3), suggesting chemical deterrents. Radioles from the six species that yielded palatable tissue homogenates produced palatable crude extracts. The four tissues that were unpalatable as homogenates (*B. brunnea* body, *B. variegata* body from North Carolina, and radioles from *A. orstedii* and *B. variegata* from both North Carolina and Panama) produced deterrent crude extracts (Fig. 3).

The deterrent metabolite(s) of *B. brunnea* body and *B. variegata* radioles (from Panama only) were dichloromethane-soluble, while deterrent metabolites
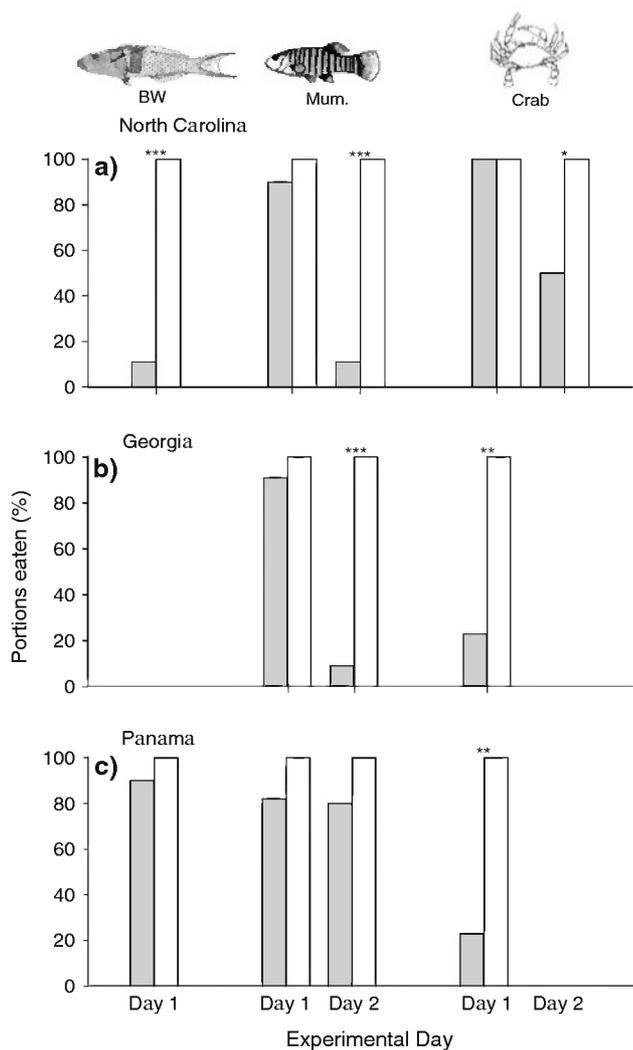
Fig. 2a–c Consumption of body portions (*black bars*) of *Bispira variegata* from **a** North Carolina, **b** Georgia, and **c** Panama versus control food portions (*open bars*) when fed to bluehead wrasses (BW), mummichogs (Mum.) or lesser blue crabs (Crab) over two days. It was not possible to feed *B. variegata* from Georgia to bluehead wrasse, as they were unavailable. Worm bodies rejected by consumers on day 1 were not offered on day 2. *Asterisks* (*) indicate significant differences (*$P<0.05$, **$P<0.01$, ***$P<0.001$), as determined by Fisher's exact test, $n=9–13$

from *A. orstedii* radioles were hexane-soluble (Fig. 4a–c). We were unable to identify the active metabolite(s) in these fractions due to repeated loss of deterrence following further purification. Because of lost activity in the Panama collection, we modified our chemical procedures for investigating the North Carolina population. For whole *B. variegata* (from North Carolina), both the ethyl acetate- and water-soluble extracts deterred crab and mummichog feeding (Fig. 4d, e); however, despite repeated efforts, we were unable to identify the metabolites mediating

unpalatability due to loss of deterrence following various steps in purification.

Calories ml^{-1} (± 1 SE) of worm radioles were as follows: *B. brunnea*, 431±9; *B. variegata* (North Carolina), 602±20; *B. variegata* (Panama), 631±15; *A. orstedii* 645±13; *B. nigromaculata* 845±30; *Branchiomma* sp. 832±23; *Megalomma* sp. 956±9; *Sabella* sp. 772±21; *S. magnifica* 1,031±20. Values for the bodies were 529±5 for *B. brunnea* and 882±19 for *B. variegata* (North Carolina). Homogenized squid mantle was 834±11. These values are from material where numerous individual worms were pooled before freezing the sample; the variance is thus due to methods of processing and is not a good measure of variance among individuals.

Tubes varied considerably in the weight required to tear them. *B. brunnea*, *B. variegata* (from North Carolina and Panama) and *Branchiomma* sp. had weak tubes that all tore at about 30–70 g (Fig. 5a). *Anamobaea orstedii*, *Megalomma* sp., and *S. magnifica* had the strongest tubes (Fig. 5a); they withstood >200 g. *B. nigromaculata* and *Sabella* sp. were intermediate in strength, withstanding 140–160 g before tearing.

Species differed dramatically in the distance at which they responded to an approaching object (Fig. 5b). *B. brunnea*, *B. variegata* (from North Carolina), *Sabella* sp., and *S. magnifica* did not retract until touched or nearly touched. Other species retracted 4–6 cm before the objected reached them. Most species retracted radioles into their tubes within 84–154 ms; however, *B. brunnea* took much longer to retract than all other worms—an average of 239 ms. Most species retracted ≥92% of their radiole length into their tubes (Fig. 5d). Only *B. brunnea* and *Sabella* sp. retracted less completely, leaving ~40–50% of their radiole length exposed.

Discussion

Sabellids expose their radioles for feeding and respiration while sheltering their bodies within tubes. Of the eight species we investigated, all had unpalatable radioles; only two had unpalatable bodies (Fig. 1). Most sabellids, thus, allocate more defenses to body parts that are more exposed to consumers. In the marine environment, there are few examples of differential allocation of defense resulting in variation in palatability of exposed versus protected tissues within an individual. This has only been demonstrated with molluscs (Avila and Paul 1997; Pennings et al. 1999), a brachiopod (Mahon et al. 2003), an annelid (Gaston and Slattery 2002), and some seaweeds (Hay et al. 1988; Paul
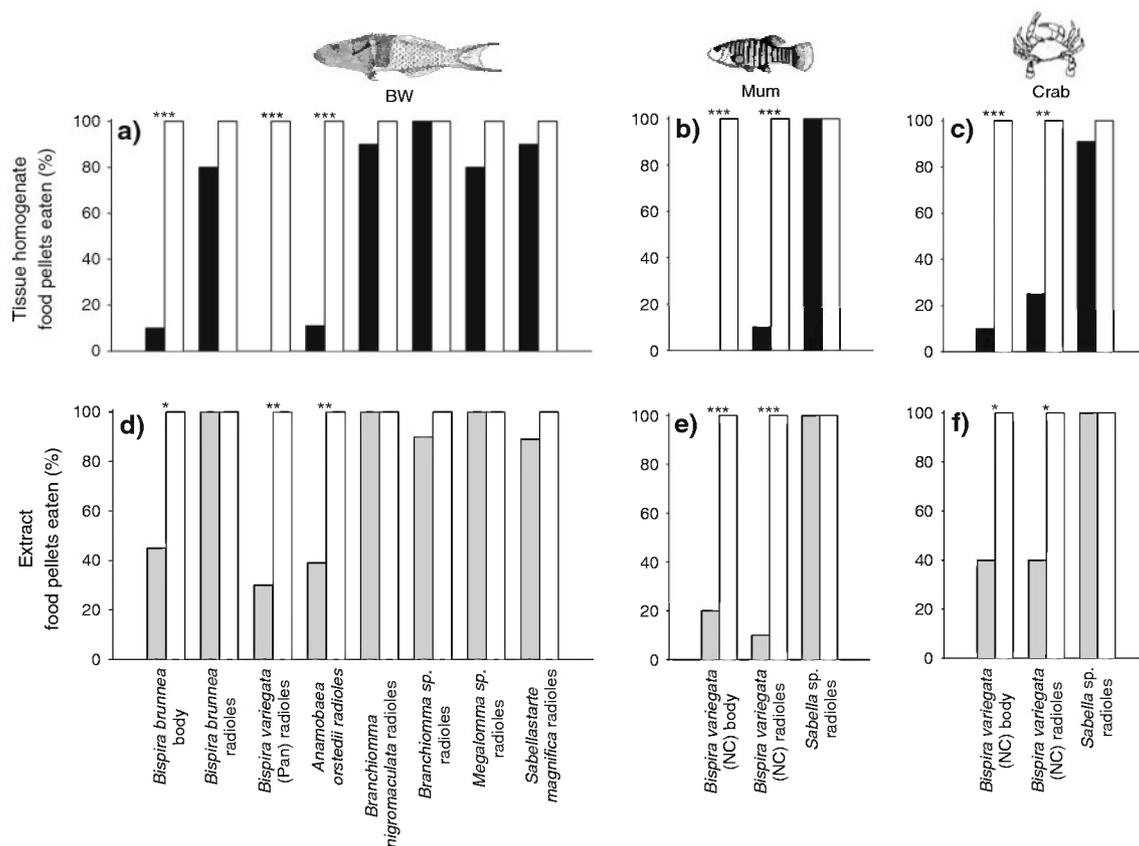
Fig. 3a–f Consumption of food pellets made of (**a–c**) tissue homogenates (*black bars*) or (**d–f**) extracts from tissues (*gray bars*) from unpalatable worm portions versus control food pellets (*open bars*) when fed to (**a, d**) bluehead wrasse (BW), (**b, e**) mummichog (Mum.), and (**c, f**) lesser blue crab (Crab). Worm portions were determined to be unpalatable when tested as fresh tissues (Fig. 1). Control food is squid pellets. *Asterisks* (*) indicate significant differences (*P<0.05, **P<0.01, ***P<0.001) as determined by Fisher's exact test, n=8–14

and van Alstyne 1988; Pavia et al. 2002; Taylor et al. 2002).

The two species that are exceptions to the general pattern of bodies being palatable (*B. brunnea* and *B. variegata* from North Carolina) reinforce the idea that susceptibility to predation influences investment in other traits that promote escape. The tubes of these two unpalatable species were soft and easy to tear, their radioles did not retract until nearly touched, and *B. brunnea* retracted both slowly and incompletely (Fig. 5a). The worms and their tubes were very easily collected by plucking the intact worm and tube from the flat, hard substrates to which they were attached. Thus, they would have been easier for consumers to detach and eat than other species; however, it appears that their chemical defenses prevent them from being a desirable choice for consumers.

For the six species with palatable bodies, the bodies were often in tougher tubes and these tubes were more difficult to collect because they were commonly more sheltered from epibenthic consumers due to residing at least partially under the sediment, in crevices, among other organisms, or under coral rubble. For all of these palatable species, half or more of the length of the tube was sheltered. For *A. orstedii* and *Sabella* sp., more than half of the length of the tube was sheltered. In addition, species with palatable bodies commonly retracted rapidly, completely, and at a considerable distance from an approaching object (Fig. 5). Thus, the pattern of trade-offs between deterrence and avoidance strategies previously reported for two freshwater vertebrates and caterpillars (Kats et al. 1988; Heinrich 1993; Krause et al. 2000) also occurs for the marine worms studied here.

Variation in exposure to consumers may also explain variation in body palatability among different populations of the same species. Body tissues of *B. variegata* from North Carolina and Georgia were unpalatable to
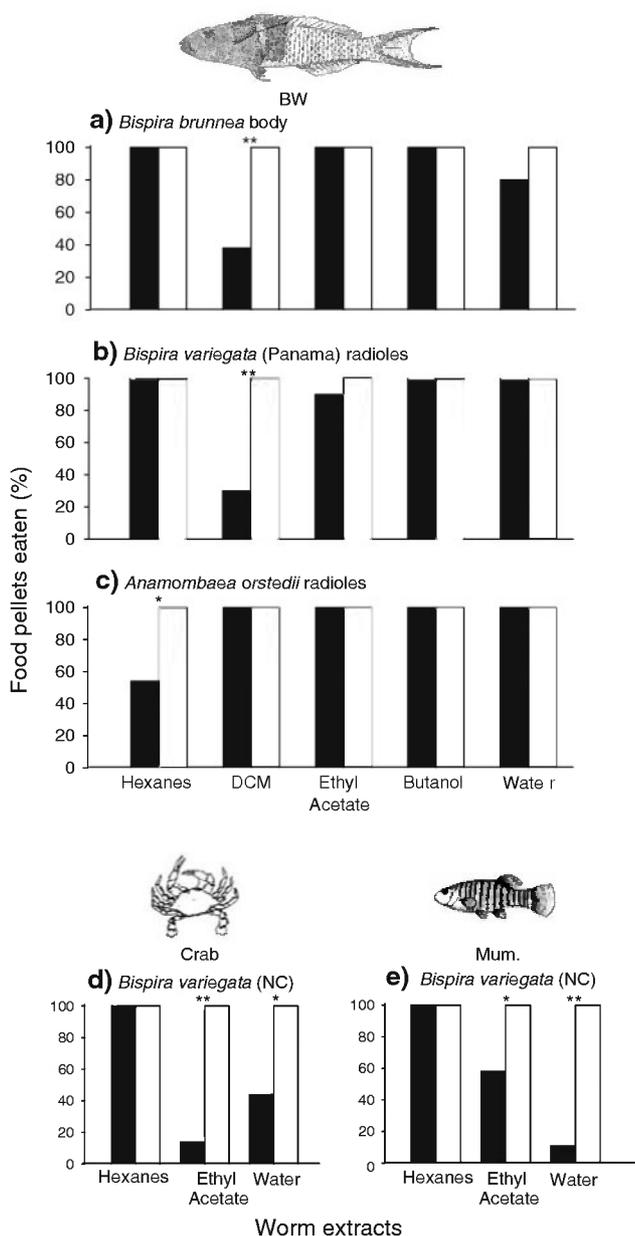
Fig. 4a–e Consumption of food pellets treated with partitions (hexanes, DCM ethyl acetate, butanol, and water; *solid bars*) from the crude extract from the **a** body of *Bispira brunnea*, **b** radioles of *B. variegata* from Panama, **c** radioles of *Anamobaea orstedii*, and **d**, **e** radioles and body of *B. variegata* (North Carolina) versus control food (*open bars*) when fed to (**a–c**) bluehead wrasse (BW), (**d**) mummichog (Mum.) or (**e**) lesser blue crab (Crab). *Asterisks* (*) indicate significant differences (*P<0.05, **P<0.01, ***P<0.001) as determined by Fisher's exact test, n=9–13

both temperate and tropical consumers, while bodies from *B. variegata* from Panama and Key Largo, Florida were readily consumed (Figs. 1, 2). *B. variegata* from Panama and Florida were collected around mangroves, where both densities and species richness of

consumers are lower than on reefs (Wulff 1997; Dunlap and Pawlik 1996). Conversely, the North Carolina and Georgia populations were from a subtidal jetty and rock reef, respectively, where consumer pressure can be high (Hay 1986; Miller and Hay 1998; Stachowicz and Hay 1999) due to high densities of omnivorous and predatory fishes (e.g., *Lagodon rhomboides, Diplodus holbrooki, Centropristis striata*), and where tubes were exposed to consumers on the flat rock surfaces of these habitats. Thus, despite the general notion that tropical habitats experience greater consumer pressure than more temperate habitats (Vermeij 1978; Bolser and Hay 1996; Pennings et al. 2001; Siska et al. 2002; Kicklighter and Hay 2006), there can also be considerable variance in these pressures within geographic regions (e.g., Hay 1985, 1997), and the North Carolina and Georgia populations from hard substrates may have experienced more intense predation than the tropical populations from mangrove areas, potentially selecting for, or inducing, a more deterrent body.

Because we tested the palatability of *B. variegata* bodies from Panama using the co-occurring bluehead wrasse and the palatability of North Carolina worms using mummichogs and crabs, our contrasts of palatability were confounded by consumer type. When we corrected this by feeding the various populations of *B. variegata* to each consumer type, both temperate and tropical fish rejected bodies from the northern population of worms, but readily fed on bodies from the Panama population (Fig. 2). The lesser blue crab, however, rejected tissues from all populations. These differences were not confined to the particular populations we found in Panama and North Carolina. A population from temperate Georgia was unpalatable, while a more tropical population from Florida was palatable. *B. variegata* from both North Carolina and Georgia appeared to be more noxious. Tissues from these populations caused regurgitation and rejection in the mummichog, and after these assays mummichogs refused to eat any novel foods, including worms that were routinely consumed by other mummichogs that had never been offered *B. variegata*. This behavioral avoidance of all novel foods was not observed following feedings on other sabellid species; we also did not note regurgitation following other feedings. Additionally, not all consumers were affected so strongly; crabs continued to try novel foods even after rejecting worm bodies from North Carolina and Georgia. Thus, consumers vary in their tolerance to some prey defenses, and some consumers (i.e., mummichog, lesser blue crab) must learn to reject some foods, as has been demonstrated in a limited number of other studies (Lindquist and Hay 1995; Kicklighter et al. 2004; Long and Hay 2005).
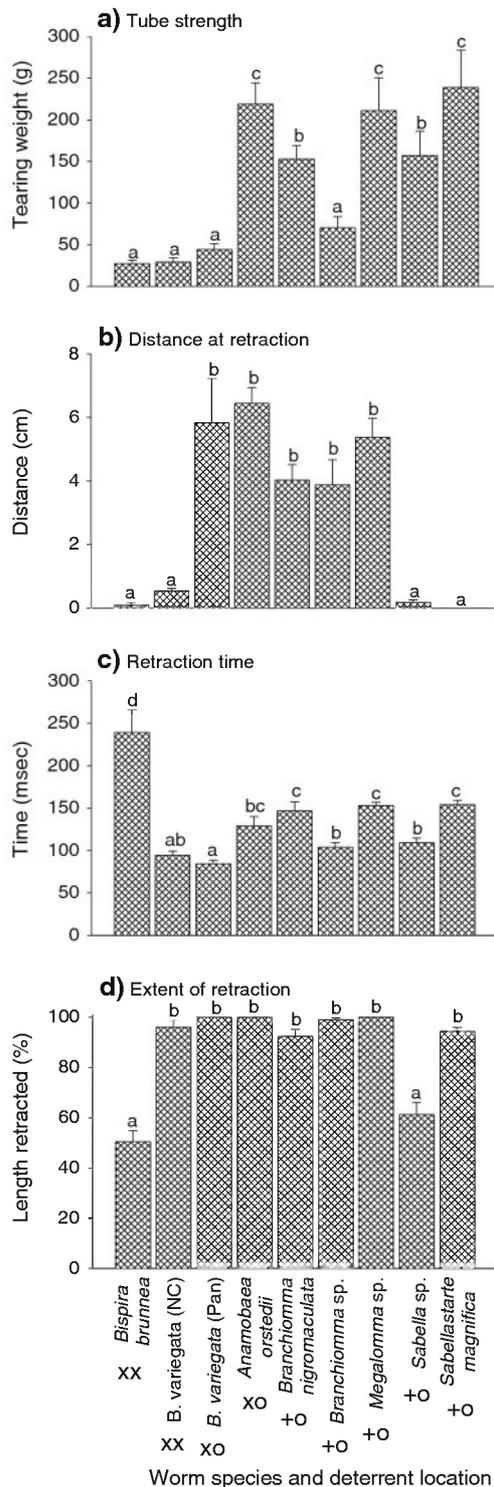
Fig. 5a–d Predator avoidance traits (means + 1 SE) by worms with structural deterrents (+), chemical deterrents (X), or no deterrents (0, palatable) in radioles (*symbol on left*) and body (*symbol on right*). Avoidance traits measured are: **a** tube strength as measured by the tearing weight, the mass necessary to tear the tube (*n*=12 for *Bispira brunnea*; *n*=20 for all other species); **b** retraction distance, the distance from an approaching physical disturbance at which the worm retracts (*n*=20); **c** retraction time, the length of time in milliseconds it takes worms to retract their radioles (*n*=18); and **d** extent of retraction, measured as the percent radiole length pulled into the tube when retracted (*n*=20). Data for tube strength were analyzed utilizing ANOVA and Tukey–Kramer post hoc analyses. All other avoidance data were analyzed utilizing Kruskal–Wallis analysis and nonparametric multiple comparisons. *Letters* indicate significant differences (*P*<0.05) between bars

marine species that have investigated among-population variance in palatability suggest that large differences among populations could be common (e.g., Bolser and Hay 1996; Taylor et al. 2003). The pattern of higher latitude populations (Georgia and North Carolina) being more unpalatable than lower latitude populations (Florida and Panama) is in opposition to a commonly held belief that consumer pressure is more intense at lower latitudes and that this selects for better developed defenses in the tropics (e.g., Vermeij 1978; Menge and Lubchenco 1981). Studies using large numbers of seaweed and marsh plants indicate that higher latitude species are commonly more palatable (Bolser and Hay 1996; Pennings et al. 2001; Siska et al. 2002), but exceptions do occur, such as the high frequency of unpalatable seaweeds in Antarctica (Amsler et al. 2005). In the only survey to examine geographic variation in worm palatability, Kicklighter and Hay (2006) found that tropical species were more frequently unpalatable than temperate species if they contrasted species living in soft substrates, but not if they focused on species from more structured habitats such as reefs. Their comparisons were based almost entirely on contrasts among different species from the different areas, so more data on patterns of palatability for the same species occurring in both high- and low-latitude habitats would be useful.

When we investigated the traits producing the unpalatability of sabellid body parts, most radioles (six of eight species—*B. brunnea*, *B. nigromaculata*, *Branchiomma* sp., *Megalomma* sp., *S. magnifica*, and *Sabella* sp.) became palatable following destruction of their physical traits (Fig. 3). This was not surprising, given that these radioles had a fibrous nature and were difficult to grind into a fine powder with a mortar and pestle. However, it is also possible that deterrent chemistry was degraded by freeze-drying and grinding. In contrast to the above pattern, radioles of *A. orstedii* and of *B. variegata* from both Panama and North Carolina,

Our contrast of North Carolina versus Panama populations of *Bispira variegata* was facilitated by finding adequate numbers of individuals in both locations. Similar contrasts for other species were not possible because we found those species only in single geographic areas. However, the few other studies of

as well as body tissues of *B. brunnea* and *B. variegata* (North Carolina only), were unpalatable due to chemical defenses (Fig. 3). These chemically defended radioles may also possess structural traits that lessen their palatability, because their radioles also had a fibrous nature and were difficult to grind into a fine powder.

For species where we demonstrated deterrent crude extracts (Fig. 4) and followed these further via bioassay-guided separations, the deterrent effects were dispersed among different fractions, suggesting the presence of multiple deterrent metabolites. However, we were unable to identify the compounds responsible for these effects because further purification based on polarity (normal and reversed phase chromatography) and size (gel filtration) always resulted in loss of deterrence. This suggests that the metabolites may be stabilized by compounds (e.g., antioxidants) in the crude extract but that the active metabolites become unstable as these stabilizing metabolites are removed during purification. To our knowledge, the only defensive metabolites isolated from any marine polychaete worm (including sabellid worms) are sulfamated pyrroles from the cirratulid *Cirriformia tentaculata* (Barsby et al. 2003; Kicklighter et al. 2003). These metabolites are unstable and oxidize easily. The lack of identified defensive molecules from marine worms may be due to difficulties with maintaining deterrent activity during the purification process, as we experienced here, and in other efforts (Kicklighter and Hay 2006).

In addition to direct feeding deterrence due to structural and chemical defenses, it is also possible that chemcial and structural defenses act together with nutritional quality to enhance deterrent effects. Studies with sponges (Jones et al. 2005), a gorgonian (Gerhart et al. 1988), and seaweeds (Duffy and Paul 1992; Pennings and Paul 1992; Hay et al. 1994; Cruz-Rivera and Hay 2003) demonstrate that nutritional quality, structure, and chemical defenses can interact additively or synergistically to deter consumers. Based on our nutritional analysis of radioles, chemically defended species did contain fewer calories ml^{-1} (an average of 602–645 calories ml^{-1}) than most structurally deterrent species, which had values just slightly below or even above homogenized squid (834 calories ml^{-1}). Thus, the deterrence of chemically defended radioles could be enhanced by their low caloric content, as well as by their tough structure.

Sabellid worms rely on deterrence strategies (i.e., chemical, structural, nutritional), just like many sponges, ascidians, and seaweeds. However, unlike these sessile species, sabellids can integrate deterrence with escape behavior by retracting into their tubes. Most investigations of the interaction among antipre-dation strategies have focused only on deterrence strategies (see reviews by Hay 1996; McClintock and Baker 2001; Paul et al. 2006). Our study also investigated the use of predator avoidance (i.e., refuge use and behavioral escape) in conjunction with deterrence. Among the sabellids we investigated, considerable among-species variance occurred in the relative use of deterrence and avoidance characteristics. Two species relied primarily on deterrence and much less on avoidance. *B. brunnea* would be poor at avoidance due to its weak tube, slow and incomplete retraction, and insensitivity to a disturbance nearby. The six other species appeared to rely more on predator avoidance, despite having unpalatable radioles.

While the integration of multiple antipredation strategies appears to be important for most of the species we investigated, how the strategies are integrated varied among worms within the same genus and even intraspecifically. For example, *B. nigromaculata* and *Branchiomma* sp. both have structurally deterrent radioles, similar retraction distances and percent radiole lengths retracted, but differed significantly in tube strength and time to retract the radioles. *B. nigromaculata* retracted more slowly than *Brachiomma* sp., but was retracting into a tube that was twice as strong (Fig. 5a, c). Similarly, *B. brunnea* retracted more slowly and less completely than *B. variegata* from either Panama or North Carolina, and the less palatable populations of *Bispira* also let a disturbance come much closer to their radioles before seeking refuge in the tube (Fig. 5). Thus, those worms that were chemically defended relied less on avoiding consumers.

Investigation of the variation in, and integrated use of, defensive and avoidance strategies may provide an insight into both the ecological and evolutionary processes that shape the connections among escape, defense, and fitness. Patterns among sabellid worms suggest that escaping and deterring consumers can both be effective strategies, that greater deterrence may allow reduced investment in escape behaviors, but that worms can also succeed via partial investment in each strategy. Our data show that closely related species, and even different populations of the same species, can integrate deterrence and avoidance strategies differently. Thus, when considering how a given guild of prey may avoid or deter consumption, it cannot be assumed that all members will utilize similar strategies.

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