

INTEGRATING PREY DEFENSIVE TRAITS: CONTRASTS OF MARINE WORMS FROM TEMPERATE AND TROPICAL HABITATS

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Abstract. Marine worms are speciose and numerically prominent members of marine communities where they play critical roles in trophic interactions and in affecting biogeochemical cycles. Despite the ecological importance of this group, little is known about their palatability to, and defenses against, consumers. In addition, most studies of prey defenses in marine organisms have focused on overt, sessile species; few studies have investigated more mobile and behaviorally complex species that could potentially be integrating predator deterrents with refuge use and other escape behaviors. To increase our understanding of consequences of defensive traits among mobile marine prey, we surveyed the palatability of 81 species of worms from the Caribbean and warm-temperate western Atlantic. Thirty-seven percent of the species were unpalatable. Worms with differentially exposed body portions commonly defended exposed feeding appendages with chemical or structural deterrents, while palatable and undefended bodies remained sheltered within structural refuges. Unpalatable worms tended to be brightly colored and sedentary, exposed to epibenthic predators, and to occupy hard substrates. Palatable worms tended to be drab, to live in structural refuges from consumers, to be mobile, and to inhabit unconsolidated sediments. Overall, taxonomy (Sabellidae and Terebellidae) and color were the traits most strongly associated with unpalatability. Unpalatable species appeared less constrained by predation and freer to forage for long periods on higher quality surface sediments or on other invertebrates at the sediment surface (thus, potentially influencing the distribution and abundance of other species). In contrast, palatable species appeared more constrained by predation risk. They fed on lower quality subsurface sediments and foraged at times or locations where consumers were less active. These ecological patterns may be generalized to other soft-bodied prey, such as caterpillars, which show similar trends regarding palatability and lifestyle.

Key words: *antipredator traits; chemical defense; marine worms; polychaete; predator-prey interactions; Sabellidae; soft-sediment species; soft-substrate communities; Terebellidae.*

INTRODUCTION

Marine consumers commonly have dramatic impacts on the distribution and abundance of their prey and on the structure and function of marine communities and ecosystems in general (Duffy and Hay 2001). This strong and persistent effect of consumers selects for defenses among prey (reviewed in McClintock and Baker [2001]), and when selection is for chemical defenses, these defenses may produce community- or ecosystem-wide cascades on ecological and evolutionary processes across broad spatial and temporal scales (Steinberg et al. 1995, Hay and Fenical 1996, Hay and Kubanek 2002).

The role of chemical defenses in deterring consumers and in allowing defended prey to occupy exposed habitats in consumer-rich communities has been rela-

tively well investigated for terrestrial plants (Rosenthal and Berenbaum 1992) and for marine taxa such as seaweeds and sessile invertebrates (e.g., Hay 1996, McClintock and Baker 2001). In contrast, chemical defenses of mobile marine prey have been less commonly investigated (Stachowicz 2001), or have been investigated within taxonomic groups (e.g., nudibranchs) or community types (e.g., pelagic environments) that leave contrasts with previous studies confounded by taxonomy or ecosystem (Cimino and Ghiselin 1998, Bullard and Hay 2002).

Investigations of chemical defenses among organisms from soft-substrate communities are especially rare, but because the worms and small crustaceans from these systems often constitute major foods for fishes (Chao and Musick 1977) and other epibenthic consumers such as crabs (Hsueh et al. 1992), it seems likely that these prey would be under strong selection for defenses that deter consumers or for behaviors that facilitate escape. The paucity of information on palatability and defenses of mobile, more behaviorally complex species limits our ability to evaluate how mobility and behavioral flexibility integrate with chemical, structural, and nutritional

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defenses to affect the ability of a species to avoid or deter consumers. Although some studies have investigated chemical defenses in mobile organisms (reviewed in Stachowicz [2001]), these studies have often focused on only a few species and have rarely addressed how chemical defenses interact with other prey traits or affect the ecological and evolutionary constraints faced by more vs. less palatable species.

To fill these gaps, we investigated the palatability and defenses of 81 species of marine worms from a diverse range of hard- and soft-substrate habitats throughout the tropical (Panama, the Bahamas, the Florida Keys) and warm-temperate portions (Florida, Georgia, North Carolina) of the western Atlantic and Caribbean. Marine worms offer an especially tractable system for addressing how predator deterrents may be integrated with other traits in more mobile species because worms are abundant, occur in a broad diversity of habitats and taxonomic groups, and exhibit considerable variation in their morphology, ecology, and behavior. They range from fully mobile species that move rapidly through sediments or across hard surfaces, to species that live within tubes that can be moved, to species that extend from, or withdraw into, tubes fixed permanently to a single location, to species that are confined to living on, or within, specific hosts. Marine worms possess many of the defensive characteristics of sessile organisms (i.e., defensive chemicals, structures, or low nutritional quality), but they can also actively escape consumers (e.g., retract into a burrow or move deeper into the substrate) or use a physical refuge, such as a tube (that can vary in strength). Using worms as a model system to investigate predator deterrents allows an assessment of how multiple defensive traits interact in more behaviorally complex organisms to affect palatability and susceptibility to predation. Such studies could elucidate potential trade-offs between mobility and defensive chemistry, could more thoroughly elucidate the evolution of predator-prey interactions, and thus help determine how communities are influenced by these interactions.

Because worms are both speciose and cosmopolitan, investigating this group also allows us to contrast the frequency of predator defenses between geographic regions. In the few instances where the palatability of organisms from different geographic areas has been examined, studies have focused on the palatability and defensive traits of higher vs. lower latitude prey because of the assumption that consumer pressure is more intense at lower latitudes and thus selects for greater prey defenses. There are few direct tests of this general theory. Tropical gastropods experience higher predation by fishes than do temperate species (Bertness 1981), tropical seaweeds are less palatable and better defended chemically than are related temperate seaweeds (Bolser and Hay 1996), and southern populations of salt marsh plants are less palatable and more chemically deterrent to both southern and northern herbivores than are more

northern populations of these same plants (Pennings et al. 2001, Siska et al. 2002). In contrast, a recent study found that the palatability of crude extracts from temperate (Mediterranean) vs. tropical (Indo-Pacific) sponges did not differ (Becerro et al. 2003). Thus, studies on a broader variety of organisms will be needed to assess the robustness and generality of geographic patterns.

In this investigation, we address the following questions. (1) Are unpalatable worms or worm body parts (from species with morphologically distinct body parts) more likely to be brightly colored? (2) Are worms or worm body parts that are overt and more exposed to epibenthic predators more frequently unpalatable? (3) Are sedentary worms that cannot move away from consumers more likely to be unpalatable than mobile worms? (4) Do worms from different substrate types differ in their frequency of unpalatability? (5) Are species from tropical areas less palatable than species from nontropical areas? (6) What are the mechanisms responsible for unpalatability? (7) What are the taxonomic patterns of palatability and chemical defense?

METHODS

Collection sites and organisms

Worms (Annelida, Nemertea, Platyhelminthes, and Hemichordata) were collected from August 1999 through October 2002 from coral reefs, coral rubble, other biogenic structures such as oyster reefs, *Halimeda* clumps, sponges, submerged man-made structures such as pilings and jetties, and from intertidal and subtidal unconsolidated sediments (sand flats, mud flats, seagrass beds). Nontropical species were collected from Shackleford Island (34°40' N, 76°37' W) and Middlemarsh (34°41' N, 76°37' W), North Carolina, USA; from areas around Little Tybee Island (31°57' N, 80°55' W), Cabbage Island (31°57' N, 80°58' W), Skidaway Island (31°57' N, 81°00' W), and Altamaha Reef (31°18' N, 81°09' W), Georgia, USA; from Estero Bay, Bonita Springs (26°27' N, 81°56' W), Florida, USA; and from Clam Pass (26°14' N, 81°48' W), Naples, Florida, USA. More tropical species were collected from areas around Key Largo, Florida, including Rodriguez Key (25°08' N, 80°25' W), Pickles Reef (24°60' N, 80°24' W), Blackwater Sound (25°08' N, 80°25' W), and mile marker 110 (25°11' N, 80°25' W); from San Salvador, Bahamas (24°03' N, 74°32' W); and from areas around Bocas del Toro, Republic of Panama (09°21' N, 82°15' W).

Each species was classified according to color, overtness, mobility, type of substrate from which it was collected, and geographic region of occurrence. Due to the large number of species examined and the variety of methods by which they were collected, we chose traits that were relatively easy to assign and did not require extensive observation of species in the field, which was not possible for many of the subsurface species that we collected. In addition, because natural history information on many species of worms is lacking (e.g., regarding

lifespan, diet, behavior) or based on unquantified observations, we chose traits that we could reliably determine for the numerous species we investigated. Coloration of homonomous worms (those without morphologically distinct body parts) and of the different body parts of heteronomous species (those with morphologically distinct portions of the body) was recorded as either "bright" (e.g., red, maroon, green, blue, orange, purple) or "drab" (e.g., brown, reddish-brown, cream, light yellow). We noted whether worms lived exposed to epibenthic predators or in protected habitats with reduced exposure to predators. We based these determinations on our observations while collecting, but our observations parallel those in the literature (e.g., MacGinitie and MacGinitie 1968, Fauchald and Jumars 1979). We also characterized each species as sedentary or mobile. For polychaetes, which comprised ~90% of the species investigated, mobility was based on the former classification of polychaetes into the orders Sedentaria (species that seldom move location) and Errantia (freely crawling or burrowing species). This classification is not perfect, but it is adequate to differentiate those species that rarely move from a fixed location from those that commonly move. The mobility of nonpolychaete worms was based on our field observations. We also recorded the substrate type (e.g., unconsolidated sediments, clumps of algae) and the geographic region from which each species was collected.

Assays of worm palatability

For determining the palatability of worms collected from North Carolina and Georgia, we used the fishes *Fundulus heteroclitus* (mummichog) and *Leiostomus xanthurus* (spot) as well as the crab *Callinectes similis* (lesser blue crab) as consumers, as all three consumers occur in the habitats where worms were collected. For worms collected from Florida, the Bahamas, and Panama we used the fish *Thalassoma bifasciatum* (bluehead wrasse) as the consumer. *Thalassoma bifasciatum* is common on coral reefs, in back reef rubble and sand zones, and often along the margins of seagrass beds and sandy areas where these habitats are intermixed with patches of hard or gorgonian corals. Thus, the fish will commonly co-occur with worms that we collected from sandy and rubble areas near structures, but they would be less frequent consumers of worms that we collected from intertidal sand flats, subtidal sand plains, or seagrass beds without adjacent structures. However, because feeding preferences of bluehead wrasse commonly parallel preferences of other generalist consumers (see Lindquist and Hay 1996, McClintock et al. 1996, Bullard and Hay 2002, Burns et al. 2003), and because this wrasse has commonly been used as a model generalist consumer in other investigations of invertebrate chemical defenses (e.g., Pawlik et al. 1995, Lindquist and Hay 1996, Kubanek et al. 2002, Pisut and Pawlik 2002) we used this species for our bioassays

of palatability. All three fishes and the crab are generalist consumers and invertebrates, including worms, make up the bulk of their diets (Randall 1967).

Consumers were kept in separate containers either individually or in small groups (*Thalassoma*) in recirculating or flow-through seawater systems. In cases where multiple bluehead wrasse were fed in small groups, separate containers of fish were considered independent replicates; separate fish within a container were not. To assure that consumers were not feeding indiscriminately due to unusual hunger levels (Cronin and Hay 1996), mummichogs and bluehead wrasse were fed frozen brine shrimp (San Francisco Bay Brand, Newark, California, USA), spot were fed brine shrimp sticks (Aquatic Ecosystems, Apopka, Florida, USA), and crabs were fed chunks of squid to satiation each morning. Feeding assays with worms or other test foods were conducted about one hour after this initial daily feeding.

We used standard feeding assays to assess worm palatability (e.g., Pawlik et al. 1995). Consumers initially were offered a palatable control food (a brine shrimp for mummichogs and bluehead wrasse, a brine shrimp stick for spot, or a piece of squid for crabs). If this was consumed, then that assay animal was offered a fresh worm (for small species) or worm portion (for larger worms or those with distinctly different body parts, which were assayed separately). Each individual consumer was offered a portion from a separate worm to assure independence among replicates. Consumers rejecting the worm were offered a second control food to ensure that they were not satiated and unwilling to feed on any food. Consumers rejected the second control in only 41 of 3082 offerings (1.3% of the time). Consumers not eating either the initial or second control food were excluded from consideration. For a replicate to be included, the consumer had to take the worm into its mouth, assuring an assessment of palatability based on taste as opposed to vision. Consumers almost always tasted our offerings. These procedures produced sample sizes of 8–14 for each worm–consumer combination.

Bioassay of extracts and homogenized tissues

To determine if rejected worms were chemically defended from consumers, crude extracts from worms, or worm parts that had been rejected as foods, were mixed into a squid-based food and offered to consumers (see methods of Lindquist and Hay 1996). Frequency of acceptance and rejection of these treatment foods was compared to a palatable control consisting of the squid-based food without added extract. For 13 of the 22 species tested for chemical deterrence, we had enough worm mass to also determine the caloric value per volume of the worm so that we could match the value of our artificial squid-based food to that of the worm being investigated (by mixing appropriate ratios of squid and water). Low abundance of some species and the failure of a freezer prevented this determination for the complete set of species. If the caloric content was not

determined, we used a known value from a similar worm.

Extracts of unpalatable worms were added to treatment food at either natural or elevated volumetric concentrations. Some extracts were added at concentrations greater than natural because we suspected that the bioactive compound was volatile or unstable and was being lost during the separation and drying process, as evidenced by diminished activity following multiple separation procedures. Once a deterrent compound was identified, we then determined its true concentration in the worm and retested it at this natural concentration.

For most species, unpalatable worm parts were extracted by placing freshly collected tissues in acetone equivalent to twice their volume and slicing the worm tissue into small pieces with scissors. The extract was then filtered and the solvent removed by rotary evaporation. This process was repeated two more times to ensure efficient extraction and the three acetone extracts were combined to form the crude extract for each species. Further bioassay-guided purification of deterrent crude extracts from species for which we had adequate material was accomplished by partitioning, based on a modified Kupchan et al. (1975) scheme (i.e., producing partitions soluble in hexanes, dichloromethane, ethyl acetate, butanol, or water).

Based on problems with instability of deterrent extracts from several of the species that we investigated early in the study, we modified our chemical procedures for *Cirriformia tentaculata* and for *Bispira variegata* from North Carolina. For these species, tissues were extracted twice with 100% acetone, 100% methanol, and 100% ethyl acetate, and all extracts were filtered to remove particulates. Solvents were combined, removed with a rotary evaporator, and the crude extract, followed by various partitions, was tested in feeding assays.

Because most known chemistry from hemichordates is volatile, we used methods that would minimize loss of volatiles when examining extracts of *Ptychodera bahamensis* and *Saccoglossus kowalevskii*. Worms were placed in methanol equivalent to twice their volume and cut into small pieces. Distilled water (volume equivalent to the methanol added) was then added, and the vial was shaken. An equal volume of hexanes (*Ptychodera*) or pentane (*Saccoglossus*) was added and the vial was shaken several times. The hexanes or pentane layer was drawn off and saved. This procedure was repeated twice. The hexanes or pentane extracts were combined and concentrated by drying under a stream of nitrogen (keeping the vial on ice). The water/methanol extract remaining from the above partition was filtered to remove particulates, and solvent was removed with a "speedvac."

For unpalatable species that did not produce deterrent extracts (and for which we had adequate material), we tested for potential deterrent effects of structural traits by destroying most structural properties and

feeding these altered foods to consumers. Tissues were lyophilized, ground to a fine powder, incorporated into a palatable base of sodium alginate, and bioassayed (see methods in Bolser and Hay 1998) against a palatable control (squid-based food) of the same caloric content/mL.

Nutritional analysis

We constructed our artificial assay foods so that they matched the energy content per volume of the worm species being assayed. To achieve this, the caloric content (calories/g) of homogenized squid paste and all worm species for which we had adequate material was determined by bomb calorimetry ($N = 5-6$ individuals, or replicates of pooled individuals if the species was small and required this pooling to achieve adequate mass) using a Parr 1425 Semimicro bomb calorimeter (Parr Instruments, Moline, Illinois, USA). Values were converted to J/mL of tissue based on the dry mass per volume conversion for each species. Ash-free dry mass (AFDM) per volume also was determined for all species for which we had adequate material.

Statistical analyses

Fisher's exact test (Statview, Version 5.0, SAS Institute, Cary, North Carolina, USA) was used to assess frequency of consumer acceptance of control food vs. worm portions, worm extracts, and worm homogenates. It was also used to examine trends in worm palatability vs. coloration, overtness to predators, mobility, type of substrate the worm was found in or on (unconsolidated sediments vs. biogenic or human-made structure), and region (tropical vs. nontropical) from which the worm was collected. To examine the interaction between worm palatability, type of substrate, and the geographic region of collection, log-linear models were used (Systat, Version 9.0, SPSS, Chicago, Illinois, USA), as this analysis is commonly used to analyze multidimensional contingency tables (Sokal and Rohlf 1995). Because only models containing a three-factor interaction term were significant, we made separate two-way tests of independence for palatability of worms from nontropical vs. tropical locations from either unconsolidated sediments or structure (Sokal and Rohlf 1995). A G test (Statview, Version 5.0) examined trends in worm palatability across geographic regions (species from Georgia vs. Florida vs. Panama). To determine whether the five traits were independent, we analyzed all pairs of traits via a Pearson χ^2 test (Agresti 1996).

To assess the traits (i.e., coloration, overtness, mobility, substrate type, region) that were most frequently associated with unpalatability, we used logistic regression because our dependent variable was categorical (palatable vs. unpalatable) and logistic regression does not assume a linear relationship between independent and dependent variables and does not require normally distributed variables (Agresti 1990). Specifi-

cally, we used multinomial logistic regression (SPSS, Version 12.0) because it is more general than the SPSS standard logistic regression analysis and can produce more valid goodness-of-fit tests and informative residuals when all predictors are categorical (see manual for SPSS, Version 12.0). The Pearson χ^2 analysis indicated that mobility and overtiness, and substrate and region, were not independent. We, therefore, used overtiness (but not mobility) and substrate (but not region) in the analysis to avoid problems with multicollinearity. We used overtiness over mobility because several of the sedentary species that we examined had body parts that varied in overtiness. Substrate was used instead of region because it appeared to be more closely related to worm natural history than the region of collection. In addition, because worms from the polychaete families Sabellidae, Terebellidae, and Amphinomidae accounted for 70% of the unpalatable worms surveyed, we included taxonomy (Sabellidae vs. Terebellidae vs. Amphinomidae vs. other) as a trait. Thus, the independent variables included in the logistic regression analysis were color, overtiness, substrate, and taxonomy. To avoid counting species with morphologically distinct body parts twice in terms of taxonomy, analyses were performed using data for anterior or posterior body parts only. To identify a best-fit model, forward stepwise analysis was used, with a significance level of 0.05 for entering a variable into the stepwise logistic regression and 0.1 for retaining that variable in the analysis (Glantz and Slinker 2001). For the North Carolina and Georgia worms assessed in these analyses, we used only data from the mummichog feeding assay because all worms were fed to this consumer. We did not include measures of calories or ash-free dry mass in these analyses because information on these traits was available for only 23–41% of the species that we investigated due to failure of a freezer used for storing samples for these analyses and due to problems with collecting adequate material for some rare species.

Ash-free dry mass (g/mL) and calories (J/mL) for palatable vs. unpalatable worms, or worm parts, were compared using a Mann-Whitney U test because sample variances were heterogeneous and transformation was unsuccessful at making them homogeneous.

RESULTS

Of the 81 species investigated, 37% were in whole or in part (for heteronomous worms) unpalatable to at least one consumer (Figs. 1–4). Different consumers usually reacted similarly to a species of worm in terms of its palatability. Thirty-nine species were fed to multiple consumers; in 34 of these instances, all consumers fed similarly (Figs. 1–4). For five species, palatability varied as a function of consumer. The crab consumed *Notopygos* sp., *Armandia agilis*, and *Saccoglossus kowalevskii*, while fishes rejected these species (Figs. 2–4). Spot did not reject *Terebella rubra* tentacles at significant frequencies, while the lesser blue crab and

mummichog did (Fig. 1B). Finally, both the crab and spot consumed tentacles of *Thelepus setosus*, while the mummichog would not (Fig. 1B).

Of the heteronomous worms, 62% had body parts that differed in palatability, with the more overt and exposed parts commonly being less palatable than the sheltered parts. This was especially true for the Sabellidae (Fig. 1A) and Terebellidae (Fig. 1B). For the sabellids, only three of 10 species had unpalatable bodies, but all 10 species had unpalatable radioles (the tentacular crown used for feeding and respiration). The pattern for terebellids was similar, only one of 10 had an unpalatable body while nine of 10 had unpalatable tentacles. For these families, the bodies are protected in tubes or beneath the sediments, while the tentacles and radioles are usually exposed as they feed beyond these refuges.

The majority of unpalatable worms (24 out of 30) were in the polychaete families Sabellidae (feather duster worms, 10 of 10 species unpalatable; Fig. 1A), Terebellidae (spaghetti worms, nine of 10 species unpalatable; Fig. 1B), and Amphinomidae (bristle worms, three of three species unpalatable; Fig. 2). The other unpalatable species were two hemichordates, a platyhelminth, a nemertine, and various polychaetes in other families.

Relationships between palatability and other traits

When examining the relationship between palatability and worm traits, we observed that some traits appeared to be correlated (e.g., species on hard substrates were usually tropical). When all possible trait pairs were contrasted, the only trait pairs that were not independent were mobility and overtiness and substrate and region ($\chi^2 = 6.436$, $P = 0.001$; $\chi^2 = 19.04$, $P < 0.001$, respectively). Thus, color was the only trait we assessed that was independent of all other traits. When palatability was analyzed according to color, 64% of brightly colored homonomous worms were unpalatable, while only 7% of drab homonomous worms were unpalatable ($P < 0.001$; Fig. 5A). When body parts of heteronomous worms were considered, 67% of brightly colored body parts were unpalatable while only 35% of drab body parts were unpalatable ($P = 0.043$; Fig. 5B). If the palatability of worms and parts was examined with respect to exposure to epibenthic predators (i.e., overt on surfaces as opposed to sheltered in sediments or other refuges), 63% of overt species were unpalatable, while only 19% of sheltered species were unpalatable ($P < 0.001$; Fig. 5C). Of sedentary species, 49% were unpalatable ($P = 0.034$; Fig. 5E) while only 22% of mobile species were unpalatable. Because overtiness and mobility were correlated, this suggested that most sedentary species were overt. However, when considering only heteronomous worms (all of which are sedentary), 76% of overt body parts were unpalatable, while only 26% of sheltered body parts were unpalatable

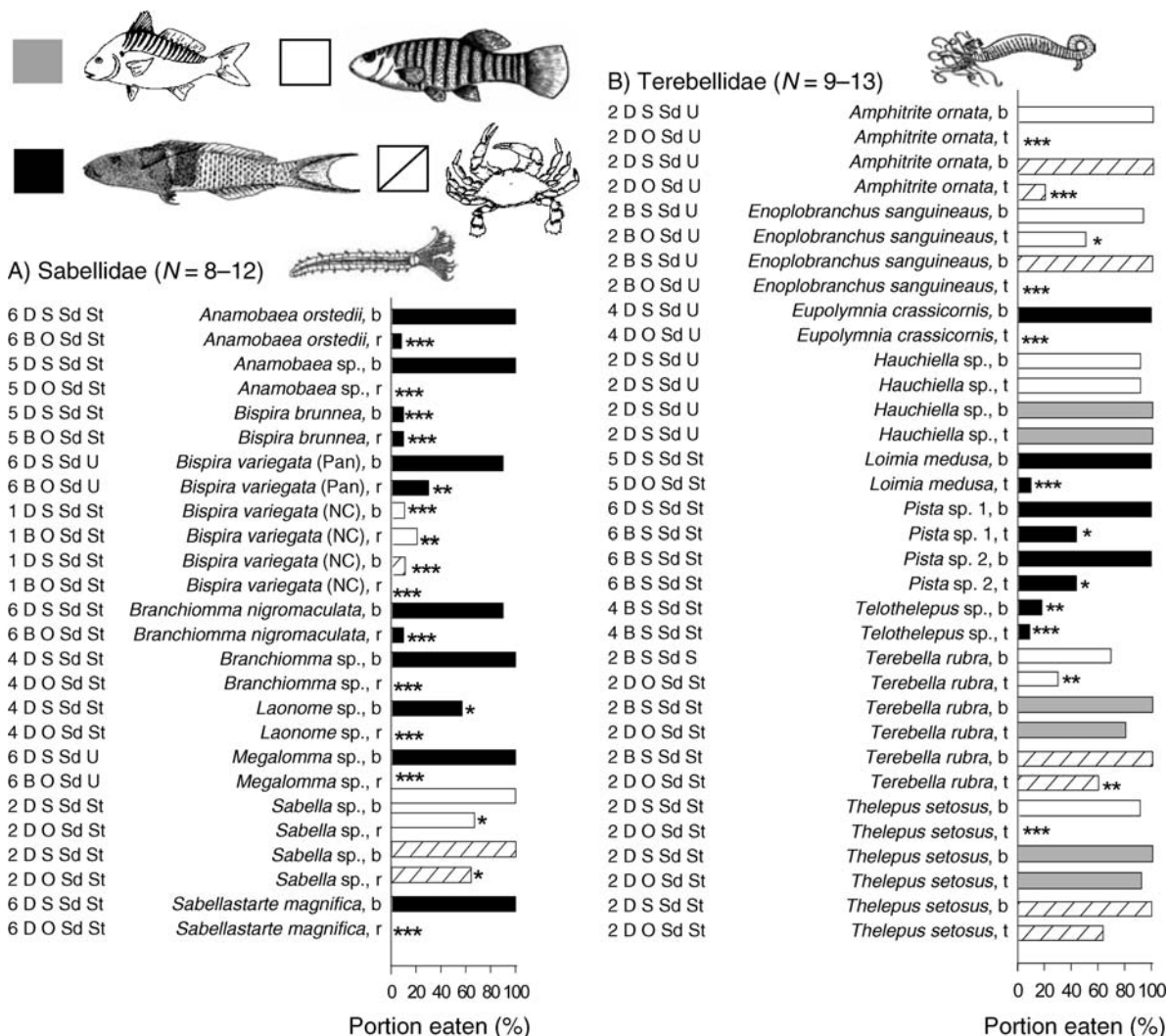


FIG. 1. Palatability of (A) sabellid and (B) terebellid worms to three species of fish and a crab (see Fig. 7). The palatable control food paired with each worm portion was always eaten (data are not shown, but all are at 100%). Numbers represent collection locations: 1, North Carolina; 2, Georgia; 3, Bonita Springs/Naples, Florida; 4, Key Largo, Florida; 5, the Bahamas; 6, Panama. Letters indicate worm characteristics: D, drab; B, brightly colored; S, sheltered; O, overt; Sd, sedentary; M, mobile; U, unconsolidated sediments; St, structure. Body parts eaten are: b, body; r, radioles; t, tentacles. Probability is indicated as * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$ by Fisher's exact test.

($P < 0.001$; Fig. 5D), indicating a relationship between overtiness and palatability independent of mobility.

Habitat type and geographic area were also associated with variation in palatability. Species living on some form of structure (e.g., mangrove roots, pilings, oyster reefs) were more frequently unpalatable (59% of species) than were species living in unconsolidated sediments (20% of species; $P < 0.001$; Fig. 6A). In addition, species from tropical areas were more likely to be unpalatable than species from nontropical areas (54% vs. 21%; $P = 0.003$; Fig. 6B). When we compared the frequency of unpalatable worms and worm parts among geographic locations where we collected enough species to allow meaningful contrasts (i.e., Georgia, $N = 35$; Key Largo, Florida, $N = 19$; and Panama, $N = 15$; but not North

Carolina, $N = 4$; Naples/Bonita Springs, Florida, $N = 4$; or the Bahamas, $N = 5$), frequency of palatability differed among locations ($P = 0.036$, G test). Worms from Georgia were less commonly unpalatable (23% of species) than were worms from Panama (60% of species; $P = 0.021$, Fisher's exact test). Worms from Key Largo showed an intermediate frequency of unpalatability (42%), which did not differ significantly from the frequency for either Georgia ($P = 0.212$) or Panama ($P = 0.491$). Because substrate and geographic region are not independent, this pattern parallels patterns observed when considering the substrate from which worms were collected. Of Georgia worms, 76% were from sediments and 24% were from structure, while only 14% of Panama species were from sediments, but 86% were from

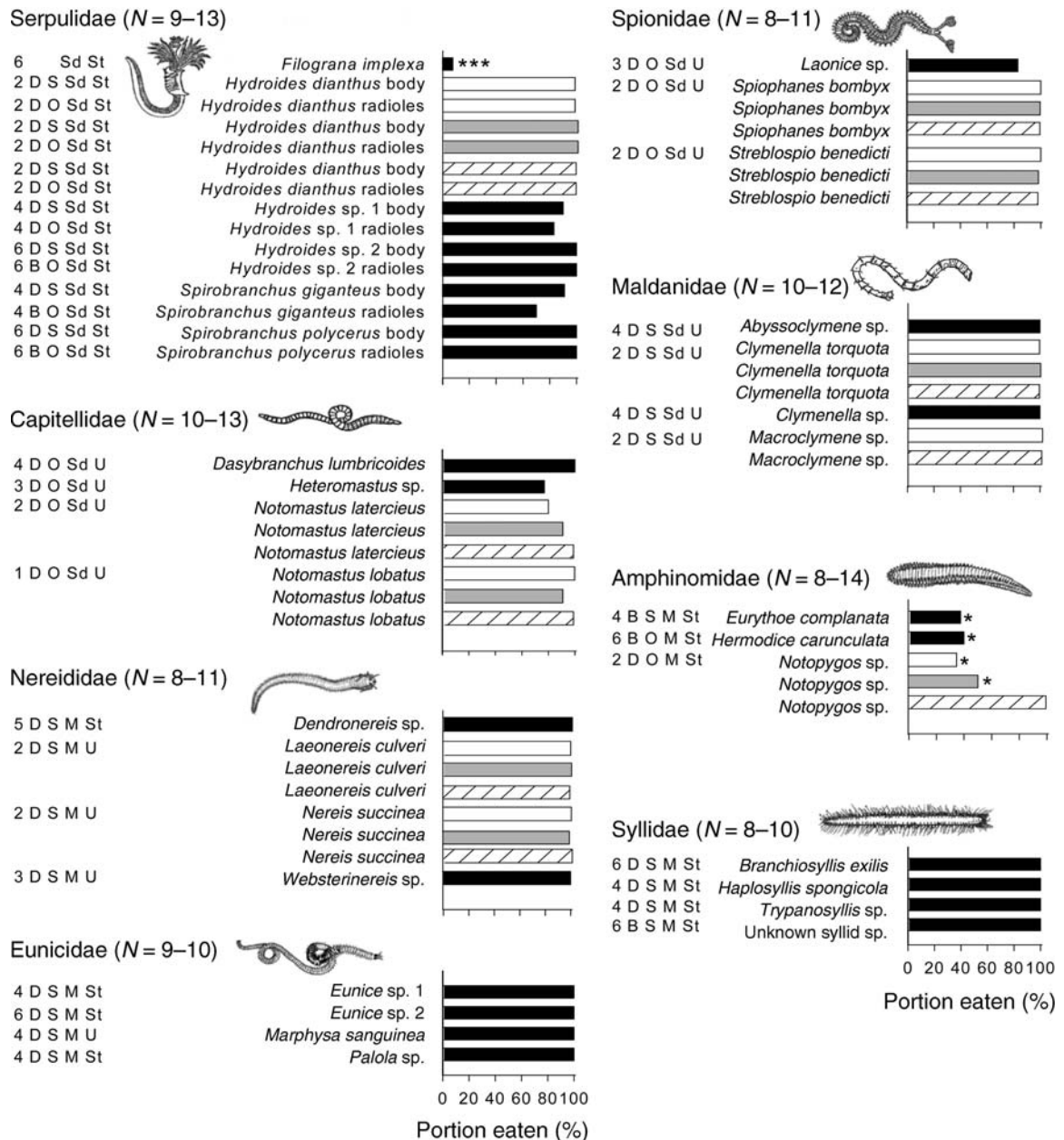


Fig. 2. Palatability of polychaete families not included in Fig. 1 where three or more species were investigated. Methods, symbols, and analysis are as in Fig. 1. *Filograna implexa* was omitted from the analyses of color and overtness because, even though the heteronomous body parts differed in overtness, the individual body parts were too small to be seen during feeding assays; therefore, consumers were fed entire worms.

structure. Key Largo shows an intermediate level of species from sediments vs. structure (39% vs. 61%, respectively).

This imbalance, with most soft-sediment species being collected from nontropical areas and most structure-associated species being collected from the tropics, occurred despite extensive efforts to find more hard-substrate species in temperate areas and more soft-substrate species in tropical areas. When we analyzed the relationship between palatability, substrate, and region

with log-linear models, the three-way interaction among palatability, region, and substrate was necessary to create a model that was not statistically different from a model incorporating all possible interactions ($G^2 = 1.57$, $P = 0.955$; $\chi^2 = 1.57$, $P = 0.955$; see Appendix A). Because a three-factor interaction term was significant, the degree of association between any two of the variables would depend on the third. Therefore, we analyzed the palatability of nontropical and tropical worms from unconsolidated sediments and structure

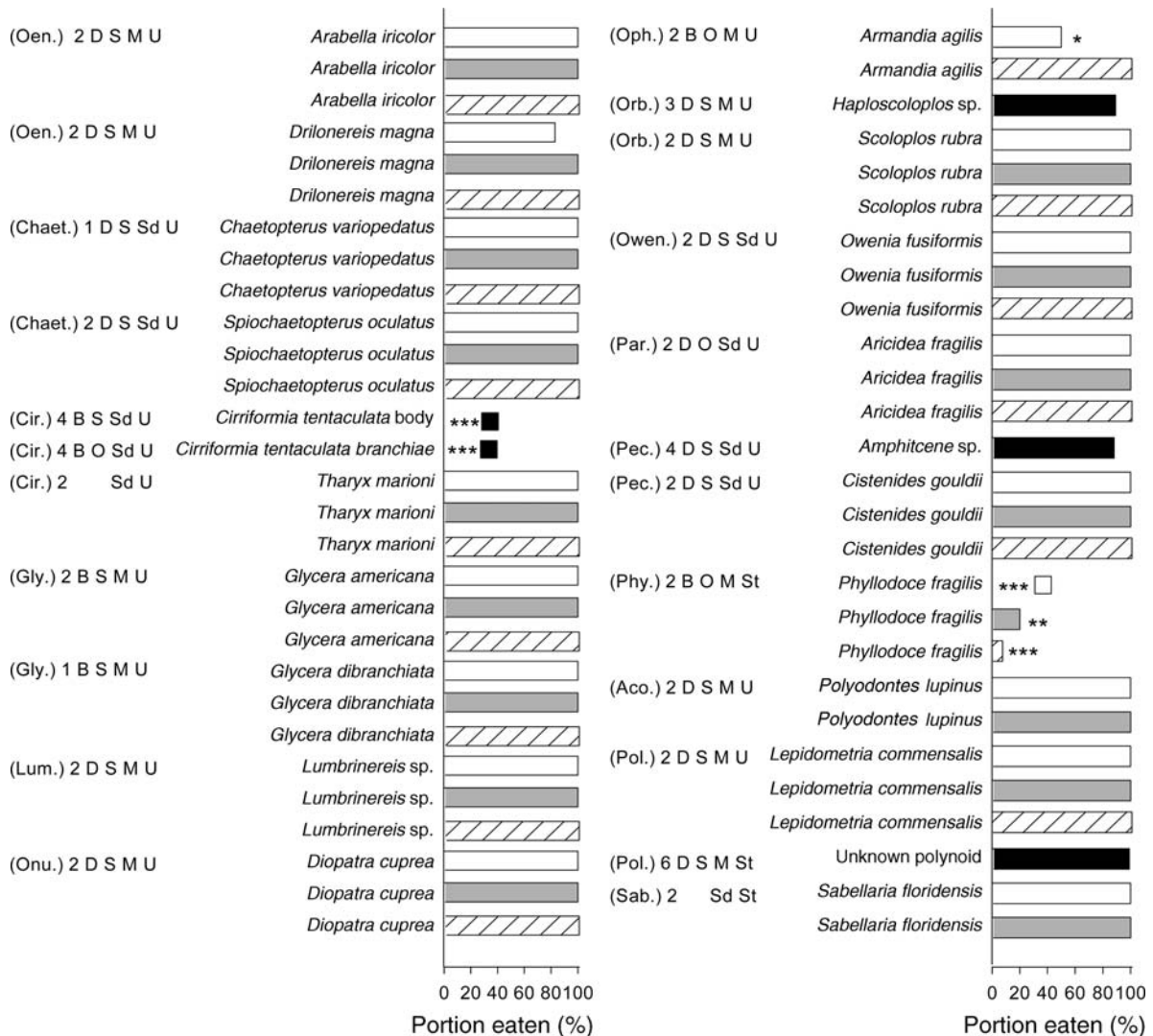


FIG. 3. Palatability of polychaete families not included in Fig. 1 where fewer than three species were investigated ($N = 8-13$ per species). Abbreviations in parentheses give the family: Oen., Oeonidae; Chaet., Chaetopteridae; Cir., Cirratulidae; Gly., Glyceridae; Lum., Lumbrineridae; Onu., Onuphidae; Oph., Opheliidae; Orb., Orbiniidae; Owen., Oweniidae; Par., Paronidae; Pec., Pectinariidae; Phy., Phyllodocidae; Aco., Acoetidae; Pol., Polynoidae; Sab., Sabellariidae. For assays in which 0% was eaten, squares above the y-axis indicate the consumer that was used. Methods, symbols, and analysis are as in Fig. 1. *Sabellaria floridensis* and *Tharyx marioni* were omitted from the analyses of color and overtness because, even though their heteronomous body parts differed in color and overtness, the individual body parts were too small to see during feeding assays; therefore, consumers were fed entire worms.

separately. For species that lived in soft substrates, tropical species showed a higher frequency of unpalatability than nontropical species (50% vs. 11%; $P = 0.017$; Fig. 6C). In contrast, a similar comparison for species from more structured habitats indicated no difference between regions; 52% of tropical species from structured habitats and 75% of nontropical species from structured habitats were unpalatable ($P = 0.431$; Fig. 6D).

When the traits color, overtness to epibenthic consumers, substrate, and taxonomy were considered together as determinants of worm palatability, logistic regression indicated that taxonomy was often the most

important predictor of palatability, with color and overtness playing lesser but significant roles. Substrate was never a significant predictor of palatability. When anterior portions of Sabellidae and Terebellidae were analyzed with either the anterior or the posterior portions of other worms, Terebellidae was the most important predictor of palatability, with Sabellidae playing a slightly less important role. In addition, color and overtness were also significant predictors (Table 1). When posterior portions of Sabellidae and Terebellidae were analyzed with the anterior portions of other worms, color and the family Sabellidae were the only

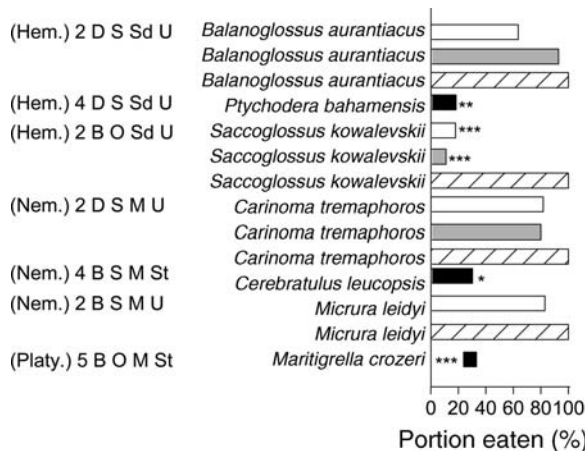


FIG. 4. Palatability of worms in the phyla Nemertea, Platyhelminthes, and Hemichordata ($N = 9-14$ per species). Methods, symbols, and analysis are as in Fig. 1.

significant predictors. When the posterior portions of Sabellidae and Terebellidae were analyzed with the posterior portions of other worms, Sabellidae, color, and overtness were the traits most closely associated with palatability (Table 1). When considering only heteronomous worms, the analysis of anterior portions

of Sabellidae and Terebellidae with either anterior or posterior portions of other worms showed that Sabellidae and Terebellidae were the only predictors of palatability (Table 1). In contrast, there were no valid models when Sabellidae and Terebellidae posterior portions were analyzed with the anterior or posterior portions of heteronomous worms (Table 1). The trait most correlated with palatability for homonomous worms (which does not include the Sabellidae or Terebellidae) was color, with overtness playing a slightly less important role (Table 1). If data for sabellids, terebellids, and amphinomids were not included in the analysis, the only predictor of palatability for all other worms and parts was color (Table 1).

When palatability of polychaete families was considered with respect to a cladogram of the Polychaeta (from Rouse and Fauchald 1997; including only the families that we investigated, but omitting the Family Oeonidae as this was not included in the Rouse and Fauchald cladogram), predator deterrence occurred in multiple clades and was not clustered only within a few related groups (Appendix B).

Bioassay of worm extracts and tissue homogenates

For 22 of the 30 unpalatable species, we had adequate quantities of worm tissue with which to test the effect of

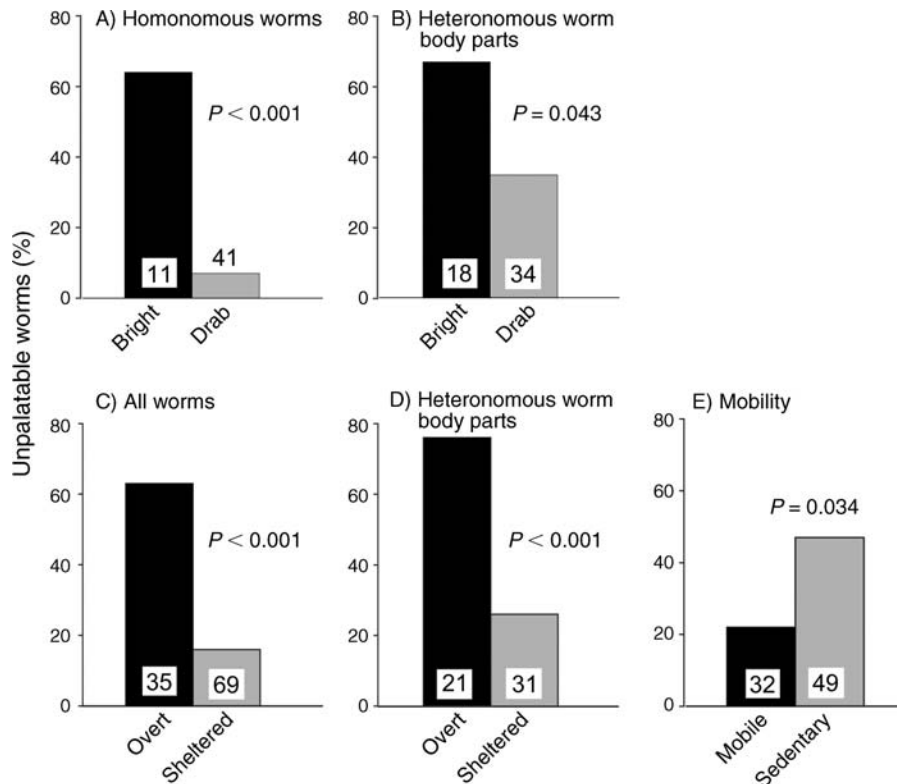


FIG. 5. Frequency of unpalatability in relation to coloration of (A) homonomous worms and (B) the differentiated parts of heteronomous worms. The frequency of unpalatability in relation to exposure to epibenthic predators for (C) all worms and for (D) heteronomous worm body parts. (E) The frequency of unpalatability in relation to worm mobility. Numbers inside the histograms indicate sample size. Analyses are by Fisher's exact test.

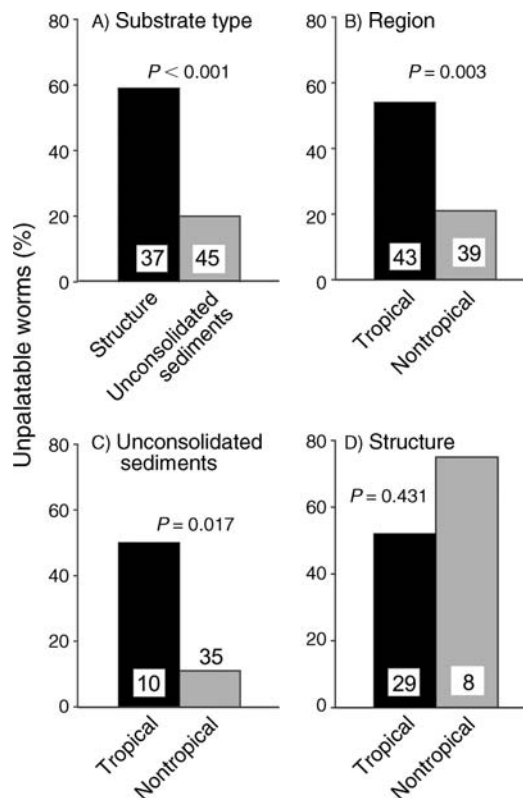


FIG. 6. Frequency of unpalatability in relation to (A) substrate type and (B) region. Regional contrasts are also presented separately for species found in (C) unconsolidated sediments vs. (D) more physically structured habitats. Analyses are by Fisher's exact test.

crude chemical extracts on consumer feeding. For 10 of the 22 species, crude extracts from unpalatable worms or worm parts deterred consumer feeding (Fig. 7). Species with chemical defenses were *Anamobaea orstedii*, *Bispira brunnea*, *Bispira variegata*, *Cirriformia tentaculata*, *Eupolymnia crassicornis*, *Loimia medusa*, *Maritigrella crozeri*, *Ptychodera bahamensis*, *Saccoglossus kowalevskii*, and *Terebella rubra*. For nine of these 10 species, we attempted to further purify deterrent metabolites; in all cases, lipid-soluble fractions were deterrent, and in two of the nine species (*Ptychodera bahamensis* and whole *Bispira variegata* from North Carolina but not from Panama), water-soluble extracts were also deterrent (Appendix C). Further purification was often unsuccessful due to inadequate amounts of worm tissue for further chemical work or due to the repeated loss of deterrence as extracts were subjected to additional separation and purification procedures. These losses of activity suggest that worm chemical defenses commonly may be unstable or volatile. We were, however, able to identify natural products that serve as chemical defenses for two species. The hemichordate *Saccoglossus kowalevskii* is defended by 2,3,4-tribromopyrrole (Kicklighter et al. 2004), and the annelid *Cirriformia tentaculata* is

defended by a novel group of 2-*n*-alkylpyrrole sulfamates (Barsby et al. 2003, Kicklighter et al. 2003).

Of the 12 species in Fig. 7 whose extracts were not deterrent, we had adequate biomass of worm tissue to bioassay homogenates from nine worms or worm parts, plus a tenth species (*Sabella* sp. radioles), which was unpalatable (Fig. 1) but whose extract was not tested. These assays suggest that nine of these 10 worms could possess structural defenses (Fig. 8); their tissues became palatable following destruction of their structural traits during homogenization. Alternatively, chemical defenses could have been compromised by our methods of tissue destruction (freeze drying and grinding). If chemical defenses were volatile, as we know is the case for some species (Kicklighter et al. 2004), then the compounds would be lost under the vacuum of freeze drying. Although extracts of *Amphitrite ornata* tentacles did not deter feeding (Fig. 7), homogenates of its tissues were still strongly avoided by both mummichogs and the crab (Fig. 8B, C), suggesting a chemical defense that may have been compromised or was not extracted by our chemical procedures.

Nutritional quality of worms

Palatable worms and worm parts were a significant 56% richer in ash-free dry mass (AFDM) per volume than unpalatable worms (0.064 ± 0.007 [mean \pm SE] g/mL vs. 0.041 ± 0.005 g/mL; $P = 0.014$; Appendix D). When considering only body parts of heteronomous worms, palatable body parts contained a significant 36% more AFDM/mL than unpalatable body parts (0.053 ± 0.01 vs. 0.039 ± 0.01 ; $P = 0.034$; Appendix D). There was no significant difference in AFDM/mL between palatable and unpalatable homonomous worms ($P = 0.420$; Appendix D), but the low sample size ($N = 4$) for unpalatable homonomous worms limited the statistical power of this analysis. Caloric content per volume of palatable worms and worm parts (3901 ± 239 [mean \pm SE] J/mL) vs. unpalatable worms and worm parts (3248 ± 213 J/mL) did not differ significantly ($P = 0.078$, Appendix E). However, when the mean caloric content of palatable vs. unpalatable body parts within heteronomous worms was compared, palatable body parts were a significant 22% higher in caloric content (3968 ± 197 J/mL vs. 3156 ± 197 J/mL; $N = 10$ and 14 , respectively; $P = 0.026$; Appendix E). There was no significant difference in mean caloric content for palatable vs. unpalatable homonomous worms ($P = 0.724$; Appendix E).

DISCUSSION

Worms play major roles as both consumers and prey in marine food webs (e.g., Randall 1967, Fauchald and Jumars 1979), and they affect biogeochemical processes and sediment characteristics in a diversity of sedimentary environments (reviewed in Hutchings [1998]). They also form important trophic connections as they consume both benthic production and detrital fall from

TABLE 1. Significant predictors of palatability from logistic regressions with the independent variables of color, overtness, and taxonomy (Sabellidae vs. Terebellidae vs. Amphinomidae vs. other worms).

	Variable information			Model fit		Nagelkerke pseudo- R^2
	β (\pm SE)	(Exp) β	P	χ^2	P	
Sabellidae and Terebellidae anterior portions†						
All worms (anterior portions)						
Terebellidae	5.323 (1.569)	204.998	0.001			
Sabellidae	5.233 (1.636)	187.354	0.001			
Color	3.276 (1.230)	26.472	0.008			
Overtness	1.922 (0.891)	6.833	0.031	77.200	<0.001	0.834
All worms (posterior portions)						
Terebellidae	4.310 (1.321)	74.440	0.001			
Sabellidae	4.142 (1.399)	82.434	0.003			
Color	2.634 (0.937)	13.927	0.005			
Overtness	2.009 (0.826)	7.455	0.015	64.523	<0.001	0.753
Heteronomous worms (anterior portions)						
Sabellidae	3.689 (1.533)	40.005	0.016			
Terebellidae	3.584 (1.537)	36.017	0.020	9.883	0.007	0.479
Heteronomous worms (posterior portions)						
Sabellidae	3.912 (1.517)	50.000	0.070			
Terebellidae	3.807 (1.520)	45.015	0.020	12.293	0.002	0.537
Sabellidae and Terebellidae posterior portions						
All worms (anterior portions)						
Color	3.468 (1.155)	32.081	0.003			
Sabellidae	3.025 (1.276)	25.129	0.018	21.698	<0.001	0.399
All worms (posterior portions)						
Sabellidae	3.851 (1.481)	47.040	0.009			
Color	2.634 (0.937)	13.927	0.005			
Overtness	2.009 (0.826)	7.455	0.015	64.523	<0.001	0.753
Heteronomous worms (anterior portions)						
No valid model						
Heteronomous worms (posterior portions)						
No valid model						
Homonomous worms						
Color	3.351 (1.191)	28.524	0.005			
Overtness	1.984 (1.031)	7.271	0.054	19.283	<0.001	0.529
All worms and parts except Sabellidae, Terebellidae, and Amphinomidae‡						
Color	3.355 (1.121)	28.636	0.003	14.534	<0.001	0.384

Notes: Analyses were conducted separately using data first for anterior and then for posterior portions of sabellids, terebellids, and other heteronomous worms. For each independent variable, $\beta \pm$ SE is the unstandardized logistic regression coefficient. (Exp) β is the odds ratio and can be used to rank the relative importance of independent variables in terms of their effect on the dependent variable. P is the significance value for each variable. The test for the overall fit of the model is the log-likelihood ratio, χ^2 . P values < 0.05 indicate that the model is a good fit of the data. Nagelkerke R^2 is an estimation of the variance in the dependent variable explained by the independent variables in the model.

† Sabellidae and Terebellidae are heteronomous worms (those with morphologically distinct portions of the body).

‡ Amphinomidae are homonomous worms (those without morphologically distinct body parts).

the water column and convert this to body mass that may then be consumed and dispersed by water column fishes and by epibenthic invertebrates such as crabs and shrimp. Given their prominent role in trophic transfer within marine systems, it is surprising that so much remains unknown regarding the specifics of both their feeding biology (Fauchald and Jumars 1979) and their palatability to, and defenses against, consumers. Although there have been careful studies of unusual secondary metabolites produced by marine worms (e.g., Ashworth and Cormier 1967, Fielman et al. 1999), and observations that some worms were rejected by some

consumers (e.g., Prezant 1980, Yoshiyama and Darling 1982), there have been few direct tests of worm palatability followed by a rigorous determination of how crude extracts or known secondary metabolites from these worms affected consumer feeding (but see Gaston and Slattery 2002, Kicklighter et al. 2003, 2004).

Unlike broad surveys on the palatability and chemical defenses of sessile organisms like seaweeds (Paul et al. 2001), sponges (Pawlik et al. 1995, Burns et al. 2003), and gorgonian corals (Koh et al. 2000, O'Neal and Pawlik 2002) that provide foundations for our understanding of how consumers have shaped the traits of

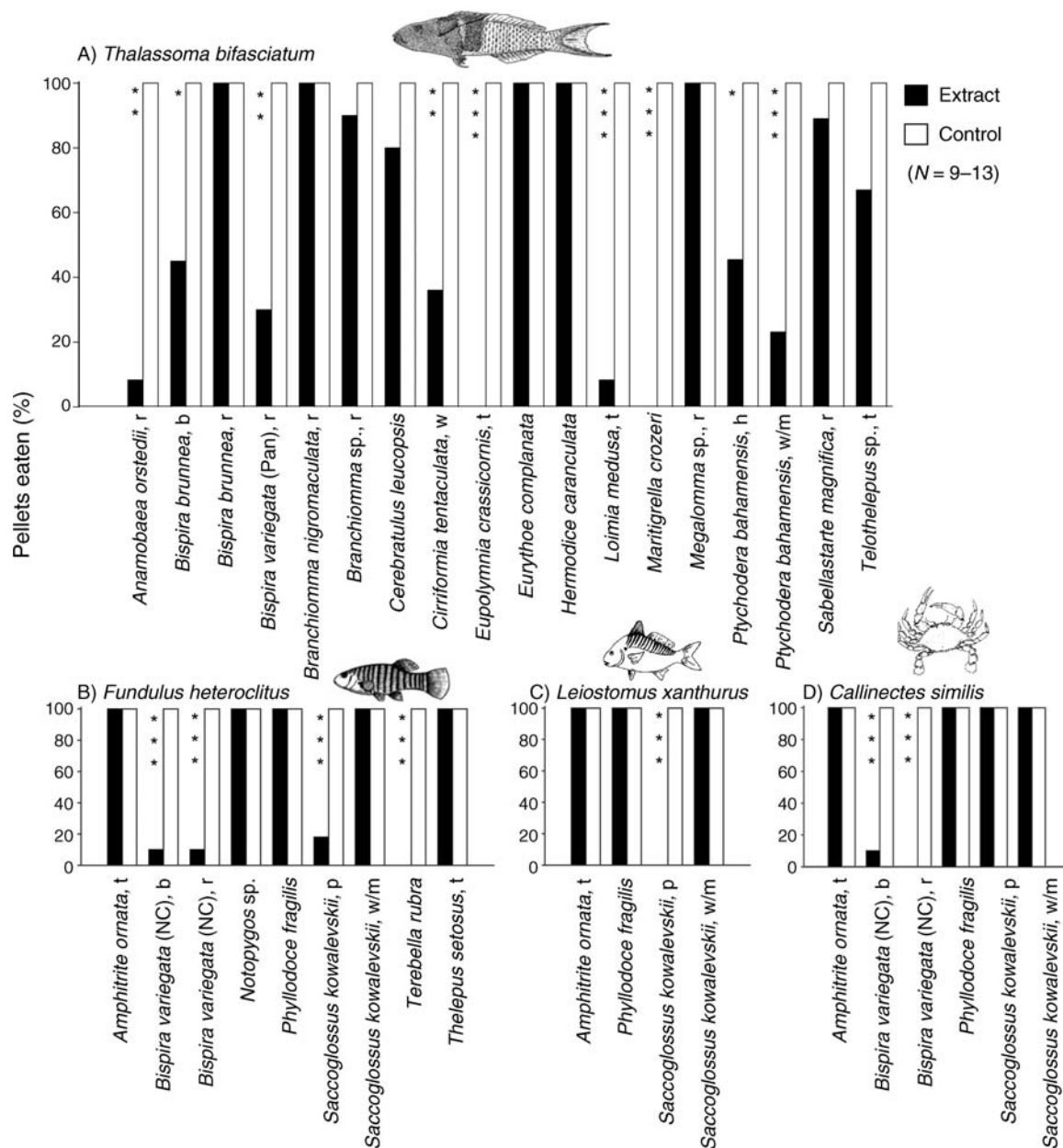


FIG. 7. Effects of extracts from unpalatable worms collected in (A) tropical regions or (B–D) nontropical regions when fed to co-occurring consumers. All extracts were tested at natural concentration except for the following: *Amphitrite ornata* (5×), *Phyllodoce fragilis* (5×), *Saccoglossus kowalevskii* (2×), and *Thelepus setosus* (5×). Exceptions were due to perceived problems of active compound degradation or loss for these species (see Kicklighter et al. 2004). Key to abbreviations for body parts eaten or extracts are: b, body; r, radioles; t, tentacles; w, whole (body + tentacles); h, hexanes; w/m, water/methanol; p, pentane. Probability is indicated as * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$ by Fisher's exact test.

sessile marine species, no similarly extensive data are available for any group of mobile marine prey (reviewed in Stachowicz [2001]). Thus, the degree to which predation on marine worms, or other mobile invertebrates, may have selected for defenses against consumers, and especially for chemical defenses, is unclear, as is the role that such defenses could play in determining patterns of distribution and behavior among mobile marine invertebrates. Our broad survey of 81 species of

worms from four phyla and 25 polychaete families fills this gap by systematically examining the palatability and defenses of worms that are representative of a wide range of morphologies, habitats, lifestyles, and geographic origins.

Patterns of palatability

Several patterns of palatability emerged when we considered predator consumption of worms in relation

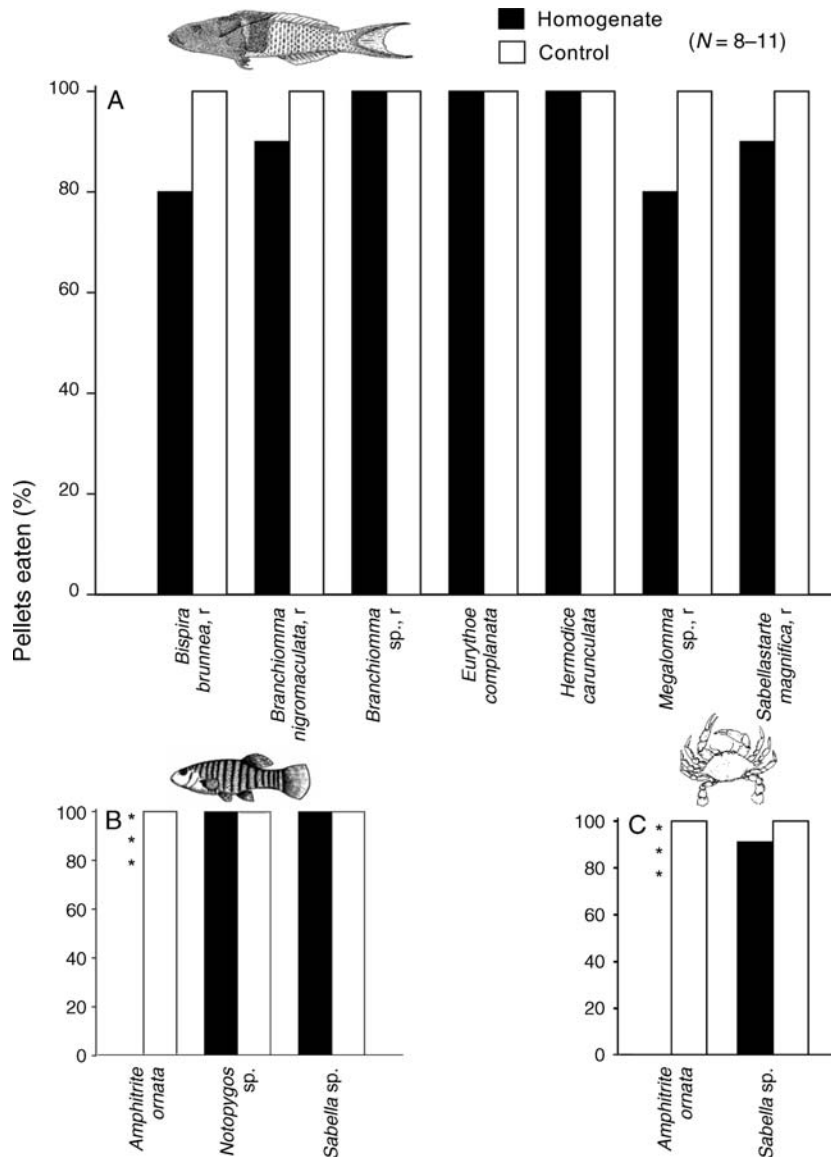


FIG. 8. Palatability of homogenized worm tissues from (A) tropical species and (B and C) nontropical species. We included only worms that were unpalatable when whole but whose extracts were palatable. Pellets composed of radioles are indicated by “r”. Methods, species abbreviations, and analysis are as in Fig. 7 (except for Sab sp., *Sabella* sp.).

to taxonomy, color, overtness, mobility, substrate, and geographic region. Some traits, such as taxonomy and color, appeared to be more important correlates of distastefulness than others. Overall, 37% of the species we investigated had at least one body part that was unpalatable to sympatric predators (Figs. 1–4). Unpalatable species occurred in each of the four phyla and within seven of the 25 families of polychaete annelids that we investigated.

Although the use of predator deterrence occurs in a range of polychaete families, it appears to be phylogenetically constrained at this taxonomic level. If one considers only family level contrasts where we collected three or more species in a family (thus providing some

minimal ability to assess frequency of unpalatability within families), the frequency of unpalatability appears bimodal, with almost all members of a family being either palatable or unpalatable. For example, 100% of Sabellidae (10 of 10), 90% of Terebellidae (nine of 10), and 100% of Amphinomidae (three of three) were unpalatable while only 17% of Serpulidae (one of six) and none of the Capitellidae ($N = 4$), Eunicidae ($N = 4$), Maldanidae ($N = 4$), Nereididae ($N = 4$), Spionidae ($N = 3$), and Syllidae ($N = 4$) were unpalatable. Seventy percent of unpalatable species were from only three groups: the polychaete families Sabellidae, Terebellidae, and Amphinomidae (Figs. 1–4).

Some polychaete families that appeared very similar in morphology and lifestyle differed considerably in palatability. For example, polychaetes in the families Sabellidae and Serpulidae are very similar in that they are both heteronomous and both have radioles that extend from a tube for filter feeding and respiration. Despite this similarity, all sabellids had unpalatable radioles (and a few had unpalatable bodies) while only one of six serpulids had any unpalatable body part (Fig. 2). A major difference between the unpalatable sabellids and the palatable serpulids is the strength and refuge value of the tube. Serpulids inhabit a hard calcium carbonate tube that is difficult to break into, while sabellids produce soft tubes that offer much less physical resistance to tearing. The strong serpulid tube, coupled with their more rapid and more complete retraction into the tube (C. Kicklighter, *unpublished data*), may alleviate the need for chemical deterrence among these species. Sabellids appear to rely more on predator deterrence than on behavioral avoidance via escape into a strong refuge. Thus, predator deterrence, avoidance, and refuge characteristics may be integrated differently in species with similar morphologies and lifestyles, but with differentially effective chemical or morphological defenses against consumers.

When considering whether exposure to consumers and/or other worm traits were closely associated with unpalatability for all worms surveyed, we observed that various traits tended to occur in clusters associated with either palatable or unpalatable worms. It seems reasonable to propose that unpalatable worms could forage and live more overtly once they were rarely attacked by epibenthic consumers, and that they might benefit from advertising their unpalatability via bright coloration. Warning coloration is well-known among terrestrial organisms, such as insects, amphibians, and reptiles (e.g., Gittleman and Harvey 1980), but it is less clear how often color functions as a warning among marine species. Lindquist and Hay (1996) found that brightly colored invertebrate larvae were less palatable than drab or clear larvae, but Pawlik et al. (1995) and Dunlap and Pawlik (1996) found no relationship between sponge color and the palatability or deterrence of sponge extracts. For homonomous worms, frequency of unpalatability among brightly colored species was ninefold higher than among drab species (Fig. 5A). This pattern also held within individuals for heteronomous worms; brightly colored parts were about twice as likely to be unpalatable as were drab body parts (Fig. 5B). Previous experiments by Young and Bingham (1987) demonstrated that a predatory fish could rapidly learn the connection between a brightly colored larva and its chemical defenses and begin avoiding the larva on the basis of visual cues alone. Thus, warning coloration could be adaptive in clear-water marine habitats where consumers can forage using visual cues (e.g., coral reefs, sea grass beds, mangroves). Warning coloration should

be less adaptive in darker or more turbid habitats (i.e., turbid mudflats, abyssal plains).

In addition to being brightly colored, less palatable worms also tended to be more overt in that they were exposed on open substrates during daylight hours. The frequency of unpalatability among overt worms was nearly fourfold greater than among more sheltered species (Fig. 5C). Similarly, unpalatability for sedentary species was more than twofold higher than for mobile species (Fig. 5E). Because overtness and mobility did not occur independently, overt unpalatable worms also tended to be sedentary. This suggests that mobile species that can behaviorally escape predators are less likely to invest in potentially costly chemical (Baldwin 1998) or morphological (Bronmark and Miner 1992) defenses against consumers. However, when we considered palatability vs. exposure to predation of the different body parts of heteronomous worms (all of which are sedentary), frequency of unpalatability was about threefold higher in more exposed body parts (Fig. 5D), demonstrating that overtness is not necessarily dependent upon mobility for all species. For both sabellids and terebellids, the bodies, which are sheltered in tubes and are often nestled among other structures or below the sediment surface, are commonly palatable while the exposed radioles or tentacles are almost uniformly unpalatable (Fig. 1). Thus, defenses are being allocated preferentially to those body parts most exposed to consumers. In the marine environment, there are few examples of differential allocation of defense resulting in variation in palatability of exposed vs. protected, or more vs. less valuable, tissues within an individual. This has been demonstrated with some mollusks (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 and Van Alstyne 1988, Pavia et al. 2002). In two cases, attacked seaweeds have been shown to preferentially induce defenses in those tissues that result in greatest loss to the seaweed if attacked further (Taylor et al. 2002, Toth et al. 2004).

There were five exceptions to the trend for sabellids and terebellids to have a palatable body and unpalatable radioles or tentacles. For the terebellids, both the body and tentacles of *Hauchiella* sp. were palatable, while both the body and tentacles of *Telothelep* sp. were unpalatable (Fig. 1B). These exceptions to the general trend may arise from differing natural history traits of these species. *Hauchiella* sp. was unique among the Terebellidae that we investigated in that it lived completely subsurface and did not expose its tentacles on the surface to feed. In contrast, *Telothelep* sp. was attached to the undersides of coral rubble in sandy depressions on shallow coral reefs where consumer pressure is high (Dunlap and Pawlik 1996, Hay 1997). When this rubble is turned and redistributed during storms, *Telothelep* sp. would be exposed to consumers, possibly selecting for an unpalatable body as well as tentacles. The sabellid *Laonome* sp. was similar to

Telothelopus sp. in having both an unpalatable body and radioles and in occurring in similar rubble areas that are disturbed during storms.

Bispira brunnea from the Bahamas and *B. variegata* from North Carolina differed from most other sabellids by having both unpalatable bodies and radioles (Fig. 1A). Unlike other sabellids, these species appeared to be more exposed to consumers and to rely more on chemical defenses and less on behavioral escape (C. Kicklighter and M. Hay, *unpublished manuscript*). These species had tubes attached to hard flat surfaces into which they could not escape. The entire worm and its soft tube were easily plucked from the substrate, and the worms retracted only slowly, and sometimes not at all, when disturbed as we harvested them.

Numerous investigators have hypothesized that tropical prey experience greater rates of attack than prey from higher latitudes and are therefore under greater selection for defenses (e.g., Vermeij 1978, Bolser and Hay 1996, Siska et al. 2002). When we examined the palatability of nontropical vs. tropical worms, we detected the hypothesized pattern; the frequency of unpalatability was 2.6-fold higher for tropical than for nontropical species (Fig. 6B). Unfortunately, this latitudinal contrast is confounded by substrate type because 65% of our tropical species occurred on structured substrates, while only 18% of our nontropical species were from this habitat. When we examined the frequency of unpalatability of nontropical and tropical worms from unconsolidated sediments, tropical species were a significant 4.5 times as likely to be unpalatable ($P = 0.017$; Fig. 6C). However, the frequency of unpalatability did not differ for species from structured habitats ($P = 0.431$; Fig. 6D).

The worms we contrasted from tropical vs. nontropical habitats were almost always different species. However, we did examine populations of *Bispira variegata* from both a mangrove habitat in Panama and a subtidal rock jetty in North Carolina that supported dense populations of fishes. This allowed a direct comparison of geographic variation in palatability for this single species, from these single locations in each region. Interestingly, the North Carolina population was more unpalatable than the tropical population. This pattern contrasts with earlier, more highly replicated, studies of seaweeds (Bolser and Hay 1996), salt marsh plants (Pennings et al. 2001, Siska et al. 2002), and terrestrial trees (Coley and Aide 1990), which have all shown reduced palatability and increased defenses for the more tropical species or populations. Studies of how palatability and defenses of marine invertebrates vary geographically are less common, and less clear (Stachowicz and Hay 2000, Becerro et al. 2003).

Despite the predation pressure from stingrays, crustaceans, and fishes in soft-substrate communities (Peterson 1979, Quammen 1984), the majority of worms from these habitats were palatable; the frequency of unpalatability was threefold higher for species from structured habitats than for species from unconsolidated

soft sediments (Fig. 6A). This pattern could be due to soft sediments serving as a refuge through which mobile worms can move and forage while reducing exposure to epibenthic predators. Many species burrow deeply in the sediments (>15 cm) and will burrow more deeply in response to disturbance (C. Kicklighter, *personal observation*). That more deeply burrowing worms are less impacted by consumers is suggested by Virnstein's (1977, 1979) findings that the abundance of deeply burrowing polychaetes did not change when epibenthic predators were excluded by cages, but that more shallow-dwelling species increased following consumer exclusion. With the exception of a few groups that bore into hard substrates and lived in tubes protected within corals or rocks (e.g., some Cirratulidae, Nereididae, Sabellidae, and Serpulidae), most worms living on hard structure can't retreat into it to seek shelter. Thus, these species may be under greater selection for traits producing distastefulness.

When each of the five qualitative traits (color, overtness, mobility, substrate, region) were considered separately, unpalatable worms and worm parts were most likely to be brightly colored, overt to epibenthic predators, sedentary, on structured habitats, and from tropical locations. Conversely, palatable worms and worm parts tended to be drab, sheltered from epibenthic predators, in unconsolidated sediments, and in nontropical locations. However, some of these traits were not independent.

To determine which traits, or combinations of traits, were most frequently associated with palatability, and whether these traits tended to co-occur in predictable clusters, we employed logistic regression. Because 70% of the unpalatable worms occurred in only three polychaete families, we added taxonomy (Sabellidae vs. Terebellidae vs. Amphinomidae) to the analysis to determine its importance as a predictor of palatability. When logistic regression was used to consider color, overtness, substrate, and taxonomy for all worms and worm parts examined, taxonomy, color, and overtness were significant predictors of palatability, with Sabellidae and Terebellidae having the greatest impact in terms of taxonomy (Table 1). For heteronomous worms, Sabellidae and Terebellidae were the only predictors, which is not surprising, because 20 of 27 heteronomous species were from these two families. Color was the most important predictor for homonomous worms (which would include only the Amphinomidae and not the Sabellidae or Terebellidae) and also for non-sabellid, terebellid, and amphinomid worms (Table 1). Thus, although the polychaete families Sabellidae and Terebellidae influenced the palatability patterns in our data set, color was a significant predictor of palatability in the absence of these two groups. Therefore, unpalatable worms from a diversity of polychaete families (Appendix B) and phyla (Fig. 4) appear to convey their distastefulness by being brightly colored.

Traits affecting palatability

Of the 30 worms that had at least one unpalatable body part, we were able to assay chemical extracts from 22. The crude extracts from 10 of these species significantly deterred consumer feeding (Fig. 7), demonstrating the presence of chemical defenses against consumers. We attempted to further separate and purify the deterrent extracts from nine of these 10 species in order to identify the deterrent metabolite(s). The exception was *Terebella rubra*, due to lack of material. A natural concentration of 2,3,4-tribromopyrrole defended *Saccoglossus kowalevskii* from predation by the mummichog *Fundulus heteroclitus* and spot *Leiostomus xanthurus*, but this compound was ineffective against the lesser blue crab *Callinectes similis* (Kicklighter et al. 2004). *Cirriformia tentaculata* was defended from the bluehead wrasse by a mixture of three novel 2-*n*-alkylpyrrole sulfamates (Barsby et al. 2003, Kicklighter et al. 2003). Although numerous species of marine worms are known to produce unusual secondary metabolites (Ashworth and Cormier 1967, Fielman and Targett 1995, King et al. 1995) hypothesized to function as defenses against consumers, the above studies by Barsby et al. and Kicklighter et al. appear to be the only direct tests demonstrating that marine worms are defended by known metabolites at natural concentrations.

All of the other seven species that we investigated were defended by lipid-soluble compounds (Appendix C; e.g., those soluble in organic solvents like hexanes, dichloromethane, and ethyl acetate), with two species (*Ptychodera bahamensis* and *Bispira variegata* from North Carolina) also containing deterrent compounds that were water soluble. *Ptychodera bahamensis* is likely defended by multiple metabolites, as water-, ethyl acetate-, and hexane-soluble extracts were all deterrent. However, we were unsuccessful at isolating and identifying deterrent pure metabolites from species other than *S. kowalevskii* and *C. tentaculata* because the deterrent activity of the extracts degraded during the repeated chemical procedures needed to progress to the final stages of purification.

To assess the possibility of deterrence due to structural traits, we lyophilized, ground into a fine powder, and reconstituted into a gel-based food the tissues from 10 unpalatable worms or worm parts that did not yield deterrent crude extracts. If defensive metabolites are stable, this process produces a food with most of the chemical and nutritional traits of the worm tissue, but with its structural traits destroyed. Tissues from nine of these species became palatable following this treatment (Fig. 8), suggesting that these species could have deterred consumers via structural traits that were destroyed by our grinding process. Even with structural traits destroyed, tissues from the tentacles of *Amphitrite ornata* were still strongly rejected by both the mummichog and lesser blue crab (Fig. 8). This suggests that this species is chemically defended but

that our initial extraction process either degraded the deterrent compounds in this worm or did not adequately extract the deterrent compounds from the tissues.

Although the lack of deterrent extracts coupled with increased palatability following destruction of tissue structural properties suggests that several of the deterrent worms we assayed could be defended by structural traits, it is also possible that volatile or unstable chemical deterrents were lost or inactivated during lyophilization, so we cannot confidently exclude the possibility of chemical defenses among these species. However, some species do have clear structural defenses that would have been destroyed during the production of our gel-based foods. For example, *Notopygos* sp., *Hermodice carunculata*, and *Eurythoe* sp. all have sharp hairs (setae) that they erect when disturbed. These hairs easily detach, penetrate into tissues, and cause a burning sensation.

The radioles of six sabellid species increased in palatability following destruction of their structural traits (Fig. 8). This result is not surprising because these tissues were difficult to grind into a fine powder due to their fibrous nature. Radioles of the other two species investigated (*Anamobaea orstedii*, *Bispira variegata*) were chemically defended (Fig. 7), but it is possible that they had structural traits that also lessened their palatability, as they also had a fibrous nature. Variation in the use of chemical vs. structural deterrents in the sabellids may be possible because this group can also avoid predators by retracting quickly and completely into their tubes and, thus, may not be as reliant on deterrent defenses as less quickly responding species that cannot retract into a tube. In other species that do not seem as reliant on behavioral avoidance, such as terebellids, we found little variation in palatability (i.e., nine of 10 species had unpalatable tentacles; Fig. 1B).

The structural deterrents employed by sabellids and fireworms (*Notopygos* sp., *H. carunculata*, *Eurythoe* sp.) are effective against small predatory fishes (bluehead wrasse, mummichog, spot, which were numerous in several areas where worms were collected; C. Kicklighter, *personal observation*), but crustaceans may be less deterred by this defensive strategy. *Callinectes similis* was not deterred by *Notopygos* sp. or *Armandia agilis* while fishes were, and *C. similis* was deterred by whole *Sabella* sp. radioles, but found the homogenate palatable. Thus, the effectiveness of structural deterrents may vary according to consumer.

Four other species that did not produce deterrent extracts (*Cerebratulus leucopsis*, *Phyllodoce fragilis*, *Telothelopus* sp. tentacles, and *Thelepus setosus* tentacles; Fig. 7) could not be tested in gel-based foods due to inadequate amounts of worm tissue being available or because the tissue homogenate would not gel (*Phyllodoce fragilis*). It is unlikely, however, that these species were unpalatable due to structural traits, as they do not have sharp setae and do not share characteristics with structurally defended worms (like amphinomid or

sabellids). Since the tentacles of three terebellids did produce deterrent extracts (*Eupolymnia crassicornis*, *Loimia medusa*, *Terebella rubra*; Fig. 7A, B), it seemed likely that the tentacles of *Amphitrite ornata*, *Telothelopus* sp., and *Thelepus setosus* might be chemically defended as well, but by metabolites that degraded during extraction. However, extractions using several different methods and solvents (targeting proteins, volatile compounds) were never successful at producing deterrent extracts.

As an additional analysis, *Amphitrite ornata* tentacles and *Phyllodoce fragilis* tissues were analyzed for the presence of heavy metals, such as copper and vanadium, which are known to occur in some worms and are thought to be responsible for the distastefulness of some invertebrate species (Stoecker 1978, Gibbs et al. 1981). Neither species contained high concentrations of heavy metals.

Prezant (1980) investigated the palatability of the congener *Phyllodoce mucosa*, which was unpalatable to several species of fish. This worm produced copious amounts of mucous that deterred fish feeding when coated onto palatable worms. *Phyllodoce fragilis* also produced copious amounts of mucous so we investigated the palatability of this mucous by coating it onto freeze-dried krill and fresh palatable worms. These offerings were always readily eaten. Mucous was also removed from live worms by blotting them with a paper towel, but these specimens retained their unpalatability. Thus, we were unable to determine the mechanism of unpalatability for this species. The other seven unpalatable species (*Anamobaea* sp., *Armandia agilis*, *Enoplobranchus sanguineus*, *Filograna implexa*, *Laonome* sp., *Pista* sp. 1, or *Pista* sp. 2) were small or rare, and we lacked adequate tissue mass for further investigations.

We analyzed nutritional value (as ash-free dry mass [AFDM]/mL and J/mL) of worm species for which we had adequate amounts of tissue. For heteronomous worms, palatable portions were significantly higher in AFDM/mL and J/mL than were the unpalatable portions (Appendices D and E, respectively). For homonomous worms, AFDM/mL was 54% higher for palatable than for unpalatable species; for caloric content, this difference was only 3%. However, neither difference was statistically significant (Appendices D and E, respectively). When data for heteronomous and homonomous worms were pooled, AFDM was significantly higher in palatable vs. unpalatable tissues, but there was no difference in caloric content.

None of the worms we investigated were so depauperate in nutritional quality that they should have been rejected by our consumers due to nutrition alone (see Bullard and Hay 2002), as consumers were always willing to eat our palatable control foods, which were made to match this same caloric content. However, lower caloric content of unpalatable parts may interact with other defensive traits to enhance deterrence (Cruz-Rivera and Hay 2003). Based on our caloric analysis of

sabellids (the group for which we have the most complete data set), unpalatable radioles always contained fewer calories/mL than palatable bodies (Appendix E). This pattern could occur due to selection for reducing nutritional investment in body parts exposed to consumers, but could also be generated by simple mechanical needs of the worm. The (usually) palatable body has to move the worm up and down the tube to extend or retract the radioles; a muscular body is required for this and may mandate increased caloric and organic content. In addition, chemically defended radioles had fewer calories/mL (2520–2700 J/mL) than radioles that appeared to be structurally defended; radioles from most structurally defended species had values slightly below, or even above, homogenized squid (3491 J/mL; Figs. 7, 8; Appendix E). This suggests that species with chemically defended radioles may be selected to lower their energetic value to consumers as a way of increasing the relative effect of the deterrent chemicals. Chemical defenses are more effective when they are in lower value prey (Duffy and Paul 1992, Pennings and Paul 1992, Hay et al. 1994, Cruz-Rivera and Hay 2003). Similarly, structural defenses, such as calcification, can also be more effective in prey offering less nutritional reward to the consumer (Hay et al. 1994). These types of interactions between defenses and nutritional reward of the prey may occur because a consumer can overcome the negative effects of the defensive metabolites or structures if the prey is nutritionally rich and this extra food value can be allocated to metabolizing, degrading, or processing the deterrent compounds or structures.

Ecological patterns

In comparison to the few studies that investigated the palatability of other marine organisms using methods similar to ours, the frequency of distastefulness for worms (37%) was much less than the frequency of unpalatability for echinoderms (95%), sponges (69%), ascidians (94%), gorgonians (100%), seaweeds (71%), holoplankton (89%), and large larvae from marine invertebrates (74%; Paul and Hay 1986, Pawlik et al. 1995, Lindquist and Hay 1996, Bryan et al. 1997, Bullard and Hay 2002, O'Neal and Pawlik 2002, Pisut and Pawlik 2002). These frequencies could be conservative compared to ours, given that (1) most of these studies did not examine the palatability of multiple tissues separately, as in our study (and we considered species unpalatable if any tissue type was unpalatable), and (2) four of the studies (echinoderms, sponges, ascidians, gorgonians) only tested the palatability of chemical extracts. For this second reason, it is possible that some species were deemed palatable due to extracts from palatable tissues diluting the effects of extracts from unpalatable tissues. Testing only extracts may also underestimate the frequency of unpalatability due to unstable metabolites decomposing in the extraction process or due to the organism being defended by

nonchemical traits that would not be assessed in these assays (i.e., structural defenses). Most of these groups (with the exception of echinoderms), are sedentary and overt or likely cannot exhibit behavioral escape (holoplankton, larvae), and thus may rely heavily on predator deterrence strategies. The ability of many of the worms we investigated to employ behavioral escapes by retracting into tubes and by moving into deeper, three-dimensional substrates may account for their lower frequency of distastefulness. Thus, worms may be freer in their integration of various antipredation strategies than less behaviorally complex, sessile species.

Chemical and structural defenses that deter predation on worms should interact with selection for behavior and niche use, just as Lindquist and Hay (1996) found for palatable vs. chemically defended larvae of marine invertebrates. In their study, chemically defended larvae tended to be brightly colored and released during the day when predators were actively foraging, just as our unpalatable worms are brightly colored and overt in areas and times when consumers are actively foraging. In contrast, palatable larvae were generally smaller, clear, or less colorful, and released at night when foraging by visually hunting fishes was reduced, just as our palatable worms were more commonly drab and living in shelters as opposed to being overt. Lindquist and Hay (1996) suggested that chemically defended species, which had been released from the constraints imposed by consumers, were freer to respond to other important evolutionary constraints.

A similar pattern can also be seen among terrestrial organisms. Caterpillars are similar to worms in that they are soft-bodied prey that are highly susceptible to predation (Heinrich 1993, Montllor and Bernays 1993). Unpalatable caterpillars tend to be brightly colored, overt, possess chemical and structural deterrents, and to occur gregariously (Heinrich 1993). In contrast, palatable caterpillars lack chemical and structural deterrents (such as hairs and spines), occur as solitary individuals, are cryptic and remain motionless during the day (Heinrich 1993); they tend to feed at night when vertebrate predators are less active (Heinrich 1979). Palatable caterpillars that do forage during the day tend to feed on the undersides of leaves or within rolled or tied leaves, and they limit their movement in order to decrease their apparency to predators (Heinrich 1993). Thus, they commonly consume an entire leaf (including less nutritious leaf tissue, such as veins) rather than moving to a fresh leaf after the most nutritious parts have been consumed. This also serves to avoid leaving partially eaten, damaged leaves, which predators may use to find their prey (reviewed in Heinrich [1993]). Feeding on less nutritious vs. more nutritious leaf tissue can lead to decreased growth (Damman 1987), as can foraging only at times and in ways that decrease apparency (Herrebut et al. 1963, Schultz 1983). Conversely, unpalatable species are less constrained in their foraging habits and behavior. They can feed both

during the day and night and tend to feed on only the most nutritious leaf portions. They are seldom cryptic, and they more readily relocate to a new leaf when the most favorable portions have been exhausted (reviewed in Heinrich [1993]).

Our data for marine worms suggest a similar ecological release. Unpalatable species appear to have more access to food because they can extend their feeding appendages at the sediment surface where they have access to newly arriving (organic rich) food. More palatable species are often restricted to feeding on subsurface sediments of lower organic content, and may experience lower growth and reproduction than surface feeders when the concentration of organic matter in subsurface sediments is too low, as has been demonstrated for *Capitella* sp. I (Forbes et al. 1994). Being unpalatable also may allow some worms to openly forage for specific, and more valuable, prey (e.g., *Hermodice carunculata* feeds on cnidarians; Fauchald and Jumars 1979) and to do so during periods when predators are active. Predator deterrents also allow unpalatable species, such as *Cirriformia tentaculata*, *Hermodice carunculata*, *Saccoglossus kowalevskii*, sabellids, and terebellids to feed and respire at the surface overtly during the day despite the presence of visually orienting consumers. In contrast, palatable worms may have to feed more opportunistically on foods available in times and places where worm predators are less active.

Predator defenses may play critical roles in allowing unpalatable species to increase their densities in places or times when consumers are common and have suppressed the densities of more palatable competitors. As a possible example, the chemically defended hemichordate *Saccoglossus kowalevskii* not only persists but increases in abundance on mudflats during seasons when predation pressure is high; palatable worms in these habitats decline dramatically during the same time period (Kicklighter et al. 2004).

Finally, chemically defended surface-feeding species, such as members of the polychaete family Terebellidae, may differentially affect local community structure by consuming settling larvae because the distastefulness of their tentacles allows them to forage for extensive periods of time and to extend their appendages far from burrow openings with lessened risk of loss. All nine of the surface-feeding terebellids we examined had unpalatable tentacles, suggesting that unpalatable tentacles are common among members of this family. Terebellids often include diatoms, other unicellular algae, and small invertebrates, including larvae, in their diets (Fauchald and Jumars 1979). These feeding patterns coupled with their high densities (Woodin 1974, Woodin et al. 1993; C. Kicklighter, *personal observation*) could allow their feeding to strongly impact meiofaunal communities. This is supported by an experiment demonstrating that the survivorship of polychaete larvae was lowered by terebellid feeding (Wilson 1980). In addition, Warwick et al. (1986) found that meiofaunal density was lowest

and diversity highest in the feeding areas of a terebellid, while meiofaunal density was highest and diversity lower in terebellid fecal mounds. Thus, worm consumer deterrents may also have cascading effects on other organisms with which these worms interact.

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APPENDIX A

Loglinear models examining the relationship between palatability of worms and the substrate and region from which they were collected (*Ecological Archives* M076-007-A1).

APPENDIX B

A figure showing a Polychaeta cladogram of the families that we investigated (*Ecological Archives* M076-007-A2).

APPENDIX C

A table showing further purification of deterrent crude extracts for all species for which we had adequate material (*Ecological Archives* M076-007-A3).

APPENDIX D

A table showing mean ash-free dry mass per worm volume for palatable and unpalatable worms and worm body parts (*Ecological Archives* M076-007-A4).

APPENDIX E

A table showing calories for palatable and unpalatable worms and worm body parts (*Ecological Archives* M076-007-A5).