

Palatability of marine macro-holoplankton: Nematocysts, nutritional quality, and chemistry as defenses against consumers

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Abstract

Chemical defenses against consumers have been hypothesized to be common among marine macro-holoplankton, but few studies have assessed macro-holoplankton susceptibility to predators or the traits affecting palatability. We used generalist fishes to determine the palatability of fresh tissues, freeze-dried homogenates, and chemical extracts from 19 species of macro-holoplankton. Fishes rejected fresh tissues of all the cnidarians, ctenophores, and cyanobacteria we examined but consumed salp and chaetognath tissues. In contrast, fishes consumed homogenates and chemical extracts of all macro-holoplankton except for the cyanobacterium *Trichodesmium* sp. We examined nematocysts and low nutritional quality as mechanisms causing rejection of fresh tissues. Once nematocysts were deactivated, fishes consumed cnidarian tentacles, indicating that nematocysts served as defenses. The nutritional quality of macro-holoplankton varied almost 500-fold among species and was strongly bimodal, with most macro-holoplankton species having ≤ 0.7 mg soluble protein ml⁻¹ or ≥ 7 mg ml⁻¹. In laboratory assays, there was a significant positive relationship between the nutritional quality of artificial foods and their acceptability to fishes. In field assays, reef fishes avoided experimental foods that had a protein content similar to low-quality macro-holoplankton but fed rapidly on higher quality foods. Furthermore, macro-holoplankton that were high in protein content possessed defensive traits that low-protein species lacked. Although fresh tissues of most macro-holoplankton were rejected by generalist fishes, we found evidence of chemical defense only in a cyanobacterium. Thus, chemical defenses were rare among macro-holoplankton, and rejection for >90% of the species we assessed was due to nematocysts or low nutritional quality.

There are conflicting notions regarding the role of predation in shaping the population and community structure of pelagic marine systems. In trying to explain why so many marine invertebrates have evolved complex life cycles with long-lived planktonic larval stages, Strathmann (1985) and Wray (1995) argued that high nearshore predation had selected for larvae that develop offshore in the plankton where the risk of predation was lower. Using ecological data, Morgan (1990, 1997) also concluded that offshore environments

provided juvenile stages of benthic organisms with an escape from high nearshore predation. In contrast, investigators studying holoplankton have suggested that offshore predation pressure might be intense. Hamner (1996) and McClintock et al. (1996) argued that spatial refuges in the open ocean are rare, whereas visual (fishes) and tactile (gelatinous zooplankton) predators can be common. Additionally, high levels of daytime predation in some planktonic systems are thought to select for the daily vertical migrations exhibited by many zooplankton (Gliwicz 1986).

Marine macro-holoplankton (for our purposes defined as holoplankton larger than 2 mm as adults) persist in the pelagic environment throughout their lives. Although some macro-holoplankton might have reduced susceptibilities to predators because of small body size, transparency, diel vertical migration, exploitation of the sea surface, spines, or shells (Cheng 1975; Gliwicz 1986; Lalli and Gilmer 1989; McFall-Ngai 1990; Hamner 1996; McClintock et al. 2001), others have no apparent defenses and are sluggish, are highly pigmented, and have soft bodies. Because species lacking obvious defenses persist in the plankton, it has been hypothesized that chemical defenses might be common among macro-holoplankton (Shanks and Graham 1988; McClintock et al. 1996). Although chemical defenses have been found in a few macro-holoplankton (Shanks and Graham 1988; Bryan et al. 1995), it is unclear whether these cases are unusual exceptions or the norm.

In addition to chemical defenses, other mechanisms might

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also reduce macro-holoplankton susceptibility to predators. Pelagic cnidarians possess nematocysts, complex cells that discharge a tightly coiled venomous thread at high velocity (Mariscal 1974). When fired against another organism, the threads of some nematocysts penetrate the surface of the target organism and inject a venom (Williamson et al. 1996). Penetrating nematocysts are used for both prey capture (Purcell and Mills 1988) and predator deterrence (Shanks and Graham 1988; Stachowicz and Lindquist 2000).

Low nutritional quality can also make it unprofitable for consumers to feed on some macro-holoplankton, such as cnidarians, ctenophores, salps, chaetognaths, and pteropods. Although gelatinous zooplankton can be high in elemental (carbon and nitrogen) and nutritional content (protein, lipid, and carbohydrate concentrations) per unit dry mass, they are also high in water content, hence appreciably diluting their nutritional value (Larson 1986; Clarke et al. 1992; Bailey et al. 1995). If the nutritional quality of a prey item is low enough, the cost of prey capture, handling, and processing can exceed the prey's value to the consumer (Stephens and Krebs 1986). Additionally, because a consumer's gut can hold only a finite volume of material, if a consumer fills its gut with low-quality food, it might not be able to ingest higher quality foods that it encounters later (Slansky 1993). Thus, in environments where prey are patchy and vary in nutritional quality, consumers might avoid low-quality prey to reserve their ability to use higher quality prey as they encounter them.

We examined the roles of chemistry, nematocysts, and nutritional quality in affecting macro-holoplankton susceptibility to consumers. To do so, we conducted laboratory and field assays to determine whether common planktivorous fishes from benthic habitats would consume macro-holoplankton. We then assessed whether macro-holoplankton that had been rejected were avoided owing to chemical defenses, nematocysts, or low nutritional value. This study (also see McClintock et al. 1996) constitutes an initial effort to determine patterns of palatability and defense among common macro-holoplankton. Future studies using pelagic consumers (both fishes and invertebrates) and additional groups of macro-holoplankton would be useful.

Methods

Study organisms—We used three species of benthic fishes as predators in laboratory assays: bluehead wrasse (*Thalassoma bifasciatum*), pinfish (*Lagodon rhomboides*), and sergeant majors (*Abudefduf saxatilis*). Although these fishes are associated with benthic rather than pelagic systems, all three species commonly feed on plankton, have extensive ranges, and occur sympatrically with most of the macro-holoplankton that we examined. Additionally, all three species are generalists that forage on a wide variety of prey and thus should be willing to sample many of the prey they encounter. If a trait deters one species of generalist consumer, it will often, but not always, deter other generalists; however, we note that there can be variance in the responses of generalist consumers to prey items or their defensive traits (Hay and Fenical 1988; Schupp and Paul 1994; Hay 1996). Finally, one of the

only previous surveys of holoplankton palatability (McClintock et al. 1996) used one of these species (*Abudefduf saxatilis*) for their bioassays, thus facilitating contrasts between our data and their earlier investigation.

For most laboratory assays, fishes were held individually in separate containers receiving flow-through seawater. Each individual fish represented a separate, independent replicate. However, for assays conducted during a research cruise (approximately 10% of assays), groups of two to three fishes were held in flow-through aquaria with aeration. To avoid pseudoreplication in assays conducted with groups of fishes, each separate group was considered a replicate. Fishes were held in the laboratory for up to several months and were fed a maintenance diet (composed of frozen blood worms, freeze dried krill, or live or frozen brine shrimp) three times each day. Fishes were not starved between assays; rather, assays were conducted between regular feeding times.

Planktonic organisms were collected over multiple years from locations along the east coast of the United States (North Carolina, Georgia, and Florida) and in the Bahamas. Our main objectives in collecting plankton were to ensure that organisms were not damaged during collection and that they remained alive until processed. The cnidarians *Chrysaora quinquecirrha*, *Carybdea alata*, *Physalia physalis*, and *Stomolophus meleagris* and the ctenophore *Mnemiopsis mccradyi* were collected by lowering buckets over the sides of boats or docks. The cnidarian *Nemopsis bachei* and chaetognath *Sagitta* sp. were collected with a 202- μ m plankton net. The cnidarians *Orchistoma pileus* and *Aurelia auritia*; the ctenophores *Beroe ovata*, *Ocyropsis crystallina*, *Ocyropsis maculata*, and *Bolinopsis vitrea*; the salp *Salpa fusiformis*; and both morphs of the cyanobacterium *Trichodesmium* were collected by hand using suction bottles or plastic bags during blue-water dives on SCUBA or snorkel. The cnidarian *Porpita porpita* was collected from a mass beach stranding near Morehead City, North Carolina. The ctenophore *Pleurobrachia pileus* was collected by A. Sell and L. Madin from the Sargasso Sea using trawls (because fishes were not immediately available for bioassays with this species, this ctenophore was frozen and used only in assays of homogenized tissues and extracts). The benthic cnidarians *Cassiopea frondosa* and *C. xamachana* were collected by snorkelers in mangrove habitats.

Palatability of fresh tissues—To determine the palatability of fresh macro-holoplankton to fishes in the laboratory, feeding assays were conducted in a manner similar to Bullard et al. (1999). During assays, a palatable control food (a piece of defrosted blood worm or a live or defrosted brine shrimp) was offered to fishes followed by a similarly sized portion of fresh macro-holoplankton tissue. Fishes could consume or reject offered items. Because we were interested in assessing the palatability of tissues, rather than apparency or avoidance, a rejection was only scored if a food item was taken into the fish's mouth and then spit out. If a fish would not sample a food item, that fish was excluded from the assay. Occasionally, a fish would sample a food item repeatedly by taking it into its mouth and spitting it out multiple times. In instances of multiple samplings, we allowed a fish to sample the item as many times as it wished before we recorded its

willingness to consume the item. If the majority of the food item was consumed during multiple samplings, we considered the fish to be willing to eat the item. If most of the food item remained after sampling, or if the item was broken into multiple small fragments (which the fish thereafter ignored), we considered this a rejection. We did not use satiation controls during these assays because previous experience had demonstrated that fishes rarely (if ever) become satiated during these types of feeding assays (e.g., Bullard et al. 1999). Differences in the consumption of fresh macro-holoplankton tissues versus palatable control foods were compared using Fisher's exact tests.

The macro-holoplankton species we assessed were highly variable in size and coloration. Some macro-holoplankton were small enough so that the entire organism could be sampled by fishes (*Nemopsis bachei*, *Orchistoma pileus*, *Sagitta* sp., and both morphs of *Trichodesmium*). Other macro-holoplankton were too large for our predators to sample the entire organism. For these larger species, bite-sized portions were cut from the organisms and presented to fishes during assays. Large cnidarians possessed distinct bell and tentacle portions, and the palatability of these tissues were assessed separately. Additionally, some species of macro-holoplankton were highly transparent (*Nemopsis bachei*, *Mnemiopsis mccradyi*, *Ocyropsis crystallina*, *Bolinopsis vitrea*, and *Sagitta* sp.). When transparent organisms were initially presented, fishes appeared to be unable to see the organisms and did not attack them. To assess the palatability, as opposed to the apparency of these organisms, we used commercial food coloring to dye both the macro-holoplankton and the control foods. Once dyed, these macro-holoplankton were readily sampled by fishes. Because fresh macro-holoplankton were offered to fishes soon after collection, most fresh macro-holoplankton collected in tropical locations were offered only to bluehead wrasses (11 spp.), and most macro-holoplankton collected in temperate locations were offered only to pinfish (four spp.).

Nutritional quality—We assessed the nutritional quality of our macro-holoplankton for several reasons. First, so that we could ensure that our macro-holoplankton homogenates and chemical extracts were incorporated into assay foods having a similar nutritional quality as the prey organisms from which they were derived. Second, so that we could determine whether low nutritional quality was associated with unpalatability for some macro-holoplankton. We used soluble protein content (mg ml^{-1}) as our surrogate for nutritional quality because it previously has been used as a measure of nutritional quality in feeding assays with fishes (Duffy and Paul 1992) and because protein content correlates significantly and positively with lipid and carbohydrate content in gelatinous zooplankton ($R^2 = 0.71, 0.34; P < 0.001, 0.040, n = 28, 20$, respectively) (derived from Bailey et al. 1995).

To measure soluble protein content, multiple individuals of each macro-holoplankton species were frozen and lyophilized following determination of wet displacement volume. Individuals of small species were used whole, whereas pie-shaped sections of roughly equal volumetric proportions were combined from multiple individuals of larger species. Once this material had been lyophilized, it was ground into

a fine powder with a mortar and pestle and soluble protein levels were measured using the Bradford (1976) colorimetric assay. Crossman et al. (2000) recently suggested that the Bradford assay may yield inaccurate results when used to assess the soluble protein content of seaweeds. However, this method has previously been considered to be one of the more robust measures of protein content (Davis 1988), and its extensive use in ecological studies provides a wealth of data against which our results can be compared.

Palatability of tissue homogenates—To separate structural traits from the chemical and nutritional traits that might deter feeding, we lyophilized macro-holoplankton tissue and ground them to a fine powder (to destroy differences in structural traits) before incorporating these into artificial foods and offering them to laboratory fishes (Hay et al. 1998). For cnidarians, lyophilization destroyed the nematocysts, but chemicals from the nematocysts may have remained. To assess the palatability of homogenates to predators, food pellets were prepared by incorporating finely powdered lyophilized material into a sodium alginate-based food prepared at the ratio of 0.2 g of sodium alginate to 10 ml distilled water (hereafter called "alginate food"). We added homogenates to alginate food at a natural dry mass per volume basis. Thus, if 2 ml of alginate food was prepared, the mass of lyophilized material contained in 2 ml of that macro-holoplankton was added. For controls, alginate foods were prepared with an equivalent protein concentration to the macro-holoplankton species being examined by adding different amounts of lyophilized squid paste (pureed squid mantle mixed with distilled water [Lindquist and Hay 1996]). To ensure the macro-holoplankton homogenates and controls had a similar appearance, commercial food coloring was added to both foods. Once prepared, alginate foods were injected into a 0.25-M solution of calcium chloride (CaCl_2) using a 5-ml disposable syringe. After approximately 2 min in the CaCl_2 , the alginate food hardened to the consistency of cooked pasta. The gelled alginate foods were then removed from the CaCl_2 , cut into bite-sized food pellets, and offered to fishes in palatability assays. During assays, fishes were first offered a control pellet followed by a homogenate pellet. Fishes could consume or reject (as described above) the offered pellets. Differences in the consumption of control versus homogenate pellets were compared using Fisher's exact tests. Unlike fresh macro-holoplankton tissue, homogenates (as well as chemical extracts, see below) did not have to be assayed immediately after collection. Thus, most homogenates could be offered to all three species of fishes. For some of the rarer species, however, we did not have enough material to perform homogenate palatability assays.

Palatability of chemical extracts—Results from our tissue homogenate assays suggested that few of our macro-holoplankton possessed defensive chemistry. However, some compounds can be altered during lyophilization (Hay et al. 1998). Thus, to determine whether our macro-holoplankton species possessed extractable chemical defenses, we offered laboratory fishes alginate foods infused with crude chemical extracts of macro-holoplankton. To prepare extracts, frozen macro-holoplankton samples were ground in a blender and

extracted three times with 2:1 dichloromethane:methanol using a total volume of solvent equal to 15 times the macro-holoplankton volume. After completing extraction with the first solvent series, the remaining macro-holoplankton residue was extracted three times with 70:30 methanol:distilled water using the same volume of solvent as above. Solvents were removed under vacuum on a rotary evaporator. The resulting extracts were then resolubilized with 100% dichloromethane and 100% distilled water and partitioned into lipophilic and water-soluble extracts by gravity separation. Feeding assays were performed using total crude extracts made by combining appropriate portions of the lipophilic and water-soluble extracts. Initially, lipophilic and water-soluble compounds were extracted separately because we expected that some of our macro-holoplankton would possess deterrent extracts. Thus, we hoped to simplify further fractionation and isolation of deterrent compounds by following initial assays of total crude extracts with separate assays of the water- versus lipid-soluble extracts. Because few of our macro-holoplankton possessed deterrent chemistry, this procedure proved to be unnecessary. However, we retained our initial extraction procedure to ensure methodological consistency across all species tested.

Chemical extracts were incorporated into assay foods based on natural volumetric concentration. Thus, if 2 ml of alginate food was prepared, the extract from 2 ml of macro-holoplankton was added. To prepare alginate foods, lipophilic extract was placed onto a watch glass or in a scintillation vial and dried with nitrogen gas (N_2) or under vacuum. Once the lipophilic extract had dried, the water-soluble extract was added to the same glass or vial and dried. The resulting crude extract was then incorporated into an alginate food (made as above, but with fresh squid paste instead of lyophilized squid paste) that had a soluble protein concentration equivalent to the macro-holoplankton being examined. To incorporate extracts into the alginate foods, 100% ethanol (approximately $25 \mu\text{l ml}^{-1}$ of food volume) was added to the dried crude extract to help dissolve the lipophilic extract. Prepared alginate food equivalent to the wet volume of macro-holoplankton from which the crude extract was acquired was then added to the watch glass or vial, and the extract and alginate food were mixed together with a spatula until the extract was dispersed evenly throughout the alginate food. Control foods were prepared in an identical manner but did not have extracts incorporated into them. Once prepared, extract and control food pellets were hardened with CaCl_2 and presented to fishes as described above. Differences in the frequency of consumption of control versus extract-treated pellets were compared using Fisher's exact test.

Assays of deactivated nematocysts—To determine whether nematocysts rendered cnidarians unpalatable to fishes, we conducted assays with cnidarian tentacles in which the nematocysts had been discharged or removed. The nematocysts of some species of cnidarians can be discharged by exposing them to freshwater (Mariscal 1974; Williamson et al. 1996). Freezing also ruptures, or physically removes, the nematocysts of many cnidarian species. Thus, to remove nematocysts from cnidarian tentacles we either dipped the tissue

into a bath of distilled water for 30 s or froze the tissue for at least 24 h. To assess the palatability of cnidarian tissues without nematocysts, laboratory fishes were first offered a palatable control food (brine shrimp or pieces of blood worms) followed by a piece of cnidarian tentacle without nematocysts. Differences in fish consumption of controls versus cnidarian tentacles without nematocysts were examined using Fisher's exact test. Results from these assays were contrasted with previous assays using fresh tentacles with intact nematocysts.

Assays of nutritional quality—To assess how nutritional quality affected feeding, we offered laboratory fishes a range of alginate foods differing in concentration of soluble protein. Soluble protein content was adjusted by adding different amounts of lyophilized squid paste (as above) to a standard volume of artificial food. Fishes in numbered containers were presented with food pellets in series of "rounds," with the number of rounds being equal to the number of protein concentrations being assessed during that assay. During each round, each fish was offered a food pellet of a randomly selected protein concentration. Fish could either consume or reject (take into the mouth and then spit out) the offered food pellet. Each fish was offered a pellet of each protein concentration only once. Thus, after all of the rounds had been completed, each fish had been offered one pellet of each protein concentration in a randomized sequence. For assays with bluehead wrasses, we assessed 10 protein concentrations ranging from 0.0 to 4.2 mg ml^{-1} ; for pinfish, we assessed six protein concentrations ranging from 0.0 to 1.5 mg ml^{-1} ; and for sergeant majors, we assessed seven protein concentrations ranging from 0.0 to 1.2 mg ml^{-1} . The different upper limits of protein content were selected because fishes demonstrated species-specific differences in the protein concentration at which their feeding saturated (i.e., they became willing to consume all assay pellets). For example, in our laboratory assays, bluehead wrasses consumed all pellets once soluble protein concentration reached about 4 mg ml^{-1} . The percentage of pellets consumed for each species of fish was plotted against soluble protein concentration and a linear regression fitted to these values.

To test how food nutritional value affected susceptibility to consumption by reef fishes under field conditions, we modified the methods of Harvell et al. (1988). Carrageenan-based foods of differing soluble protein content were prepared by mixing different amounts of fresh squid paste into preheated carrageenan mixtures (1.5 g of type 1 carrageenan in 60 ml of distilled water). The resulting mixture was poured into molds that had been laced with cotton string. After hardening, the mixture was cut into strips ($5 \times 1 \times 0.63 \text{ cm}$), each with a central cotton string running its length. In the field, carrageenan food strips were individually placed into polypropylene ropes and deployed at a depth of 7 m near Pickles Reef, Key Largo, Florida. Once deployed, food strips were continuously monitored for 3 h. Two separate assays were performed. In the first, foods of two low nutritional qualities were deployed (0.0 and 0.3 mg ml^{-1} of protein). Ropes anchoring different treatments were deployed in pairs ($n = 18$ pairs) with 0.25 m between members of each pair and 1.5 m between pairs. All food strips survived the

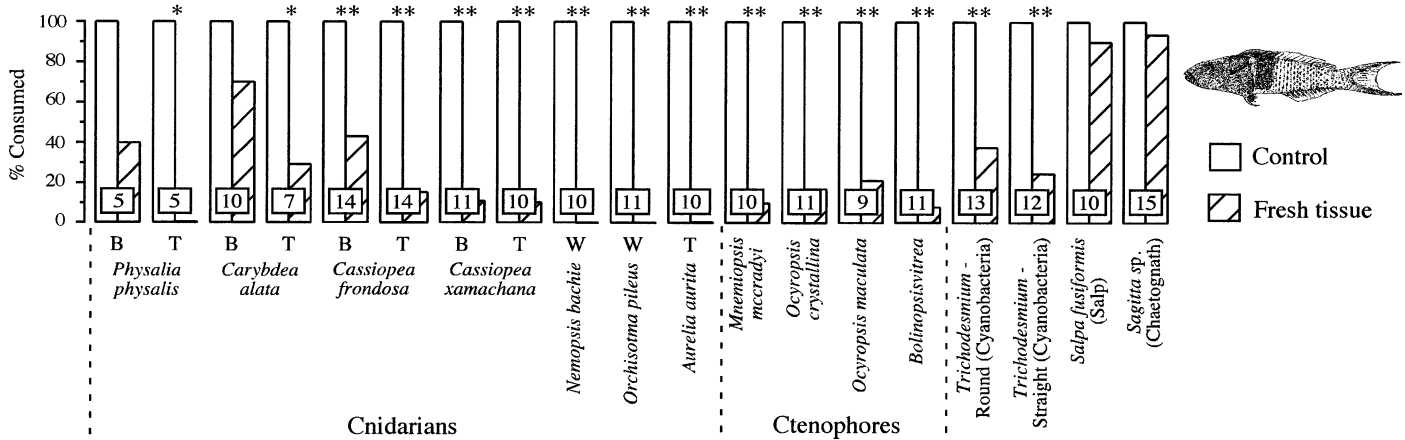


Fig. 1. The percentage of fresh macro-holoplankton tissues consumed by bluehead wrasses compared to an equal number of palatable control foods (brine shrimp or blood worms). Sample size is shown at the base of each set of bars. An asterisk or double asterisk indicates a significant difference (Fisher's exact test, $P \leq 0.05$ or $P \leq 0.01$, respectively) between macro-holoplankton and control foods. For cnidarians, the letter at the base of the bars in the histogram indicates the type of tissue being offered; B, bell; T, tentacles; W, whole organism.

3-h exposure period. At the end of this time, the remaining length of each food strip was measured and the percentage of food consumed from each food strip was recorded to the nearest 10%. Most strips were consumed from the top down, making it easy to measure loss of food as a change in length. On the few strips where some loss occurred along the margin, the missing area along the side was estimated and subtracted from the length. If 10% of the food strip had not been consumed but some consumption was apparent, we recorded this as 5% consumption. The percent consumed for each food strip was converted to a rate of consumption (cm h^{-1}) and a Wilcoxon paired-sample test was used to determine whether the rates of consumption were different between treatments of differing nutritional quality.

In the second assay, four food treatments were deployed (0.3, 3.0, 6.0, and 9.0 mg ml^{-1} of protein). Treatment ropes were placed in blocks with a distance of 0.25 m between members of each block and 1.5 m between blocks ($n = 18$ blocks). When approximately 50% of the total food had been consumed from a food strip, the food strip was removed from the block and the percentage of food consumed and the time the food strip was removed were recorded. At the end of the 3-h assay, all remaining food strips were retrieved and scored for the amount of food eaten as described above. Percent consumption for each food strip was transformed to rate of consumption (cm h^{-1}). Differences in the rate of consumption among treatments of differing nutritional quality were examined using a Kruskal-Wallis test.

Results

Fresh tissues of macro-holoplankton were unpalatable to fishes (Figs. 1, 2). Of the 18 species of macro-holoplankton we tested in the fresh tissue assays, 16 species (89%) had at least some body parts that were consumed at significantly lower levels than controls. Fishes rejected the fresh tentacles or whole organisms of all 10 of the cnidarian species we tested. In contrast, they rejected the fresh bell tissues of only

four of seven cnidarian species (Figs. 1, 2), although our sample size for *Physalia physalis* was very low ($N = 5$), constraining our power to detect avoidance for the bell of this species. Fresh tissues of all five species of ctenophores and both morphs of the cyanobacterium *Trichodesmium* sp. were also rejected by fishes relative to control foods (Figs. 1, 2). However, fresh tissues of the salp and chaetognath were readily consumed (Fig. 1).

In contrast to fresh tissues, fishes consumed freeze-dried homogenates of most macro-holoplankton at levels similar to control foods of equivalent nutritional quality (Fig. 3). Homogenates of the cyanobacterium *Trichodesmium* sp. were rejected by bluehead wrasses and sergeant majors, but not by pinfish (Fig. 3). Homogenates of all other macro-holoplankton were consumed at levels similar to control foods. In many instances, fishes consumed less than 100% of control and homogenate food pellets.

The palatability of macro-holoplankton chemical extracts showed similar patterns to homogenates (Fig. 4). Two of three fishes avoided foods containing extracts of *Trichodesmium* sp., whereas extracts of all other species did not affect feeding (Fig. 4).

Although fishes had rejected the fresh tentacles of all of the cnidarian species we tested (Figs. 1, 2), when we offered fishes six species of cnidarian tentacles with deactivated nematocysts, fishes consumed the deactivated tentacles at levels similar to control foods (Fig. 5).

The nutritional value of macro-holoplankton, as estimated by soluble protein content, varied more than 476-fold among species (Table 1). Protein concentration per volume of organisms ranged from $<0.1 \text{ mg ml}^{-1}$ for the ctenophore *Bolinopsis vitrea* to as high as 47.6 mg ml^{-1} for one of the morphs of *Trichodesmium* sp. By comparison, pureed squid mantle tissue, which is known to be highly palatable to fishes, had a protein content of 34.5 mg ml^{-1} . Ctenophores were uniformly low in protein content, whereas Cnidarians were more variable and exhibited a 180-fold range in protein content. Although most of the transparent species (*Nemopsis*

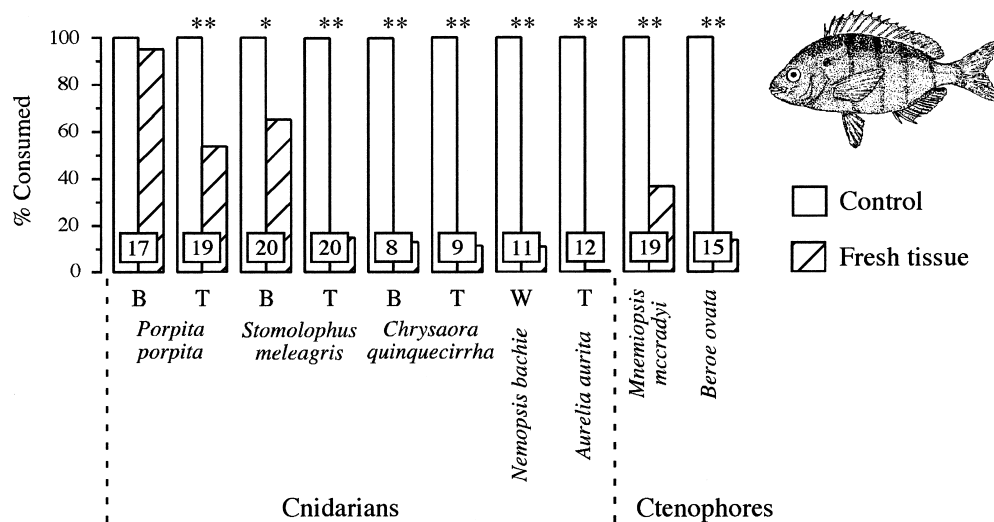


Fig. 2. The percentage of fresh macro-holoplankton tissues consumed by pinfish compared to an equal number of palatable control foods (brine shrimp or blood worms). An asterisk or double asterisk indicates a significant difference (Fisher's exact test, $P \leq 0.05$ or $P \leq 0.01$, respectively) between macro-holoplankton and control foods. For cnidarians, the letter at the base of the bars in the histogram indicates the type of tissue being offered; B, bell; T, tentacles; W, whole organism.

bachei, *Mnemiopsis mccradyi*, *Ocyropsis crystallina*, *Bolinopsis vitrea*) were low in protein content, the highly transparent chaetognath *Sagitta* sp. had two orders of magnitude more protein than some of these species.

For our experimental foods made with carrageenan or alginate, palatability was positively correlated with nutritional quality. In laboratory assays conducted with alginate-based foods of different protein concentrations, there was a significant positive relationship between the protein content of food pellets and the percentage of food pellets consumed for all three species of fishes (Fig. 6). In field assays, foods with higher concentrations of soluble protein were consumed at significantly faster rates than foods with lower protein content. During a reef assay conducted with foods containing 0.0 and 0.3 mg ml⁻¹ of soluble protein (similar to about 50% of the macro-holoplankton species we tested), very little of either food was eaten (Fig. 7A), even though we observed that foods were commonly sampled by fishes of the families Lutjanidae, Haemulidae, Acanthuridae, Labridae, and Scaridae. During our second reef assay, carrageenan-based foods with 6.0 and 9.0 mg ml⁻¹ of protein were consumed at significantly higher rates than carrageenan foods with 0.3 mg ml⁻¹ of protein (Fig. 7B); carrageenan foods with 3.0 mg ml⁻¹ of protein were consumed at intermediate rates that did not differ significantly from any other foods (Fig. 7B).

Discussion

We found minimal support for the hypothesis (Shanks and Graham 1988; McClintock et al. 1996) that extractable chemical defenses are common among macro-holoplankton. We note, however, that our study focused mainly on cnidarians and ctenophores. As with the study by McClintock et al. (1996), we found that fresh tissues of most of our macro-holoplankton species (16 of 18) were unpalatable to fishes (Figs. 1, 2). However, except for the cyanobacterium *Tri-*

chodesmium sp., all of the species we examined became as palatable as control foods of equivalent nutritional quality when homogenized and incorporated into alginate-based foods (Fig. 3). Similarly, chemical defenses could be demonstrated only for *Trichodesmium* sp. (Fig. 4). These results contrast with studies from benthic communities where unpalatable soft-bodied organisms are often chemically defended. For example, many soft corals, gorgonians, sponges, seaweeds, and benthic invertebrate larvae produce chemical extracts that deter fishes in assays much like the ones we performed (Hay and Fenical 1988; Paul 1992; Pawlik 1993; Hay 1996; Lindquist and Hay 1996; McClintock and Baker 2001).

Of the 19 macro-holoplankton species we examined, only the cyanobacterium *Trichodesmium* sp. produced a deterrent chemical extract. The whole organism, lyophilized homogenates, and chemical extracts of both morphs of *Trichodesmium* sp. were rejected by bluehead wrasses and by sergeant majors (Figs. 1, 3, 4). McClintock et al. (1996) also noted that sergeant majors would not consume intact colonies of *Trichodesmium* sp., and *Trichodesmium* sp. has been shown to be toxic to copepods and penaeid shrimp larvae (Hawser et al. 1992; Preston et al. 1998). However *Trichodesmium*'s defense is not universal. Pinfish were not deterred by either homogenates or extracts of *Trichodesmium* sp. (Figs. 3, 4), and a few specialized harpacticoid copepods use *Trichodesmium* as a food source and as a substratum for depositing eggs (O'Neil 1998). In addition, we also have observed large amounts of *Trichodesmium* sp. in the guts of the salp *Salpa fusiformis* off North Carolina. Overall, however, *Trichodesmium* appears to experience minimal consumption and to be chemically defended against several generalist consumers. *Trichodesmium* is abundant world-wide and plays an important role in oceanic nutrient cycling (Capone et al. 1997). Its chemical arsenal could help explain its importance in pelagic ecosystems.

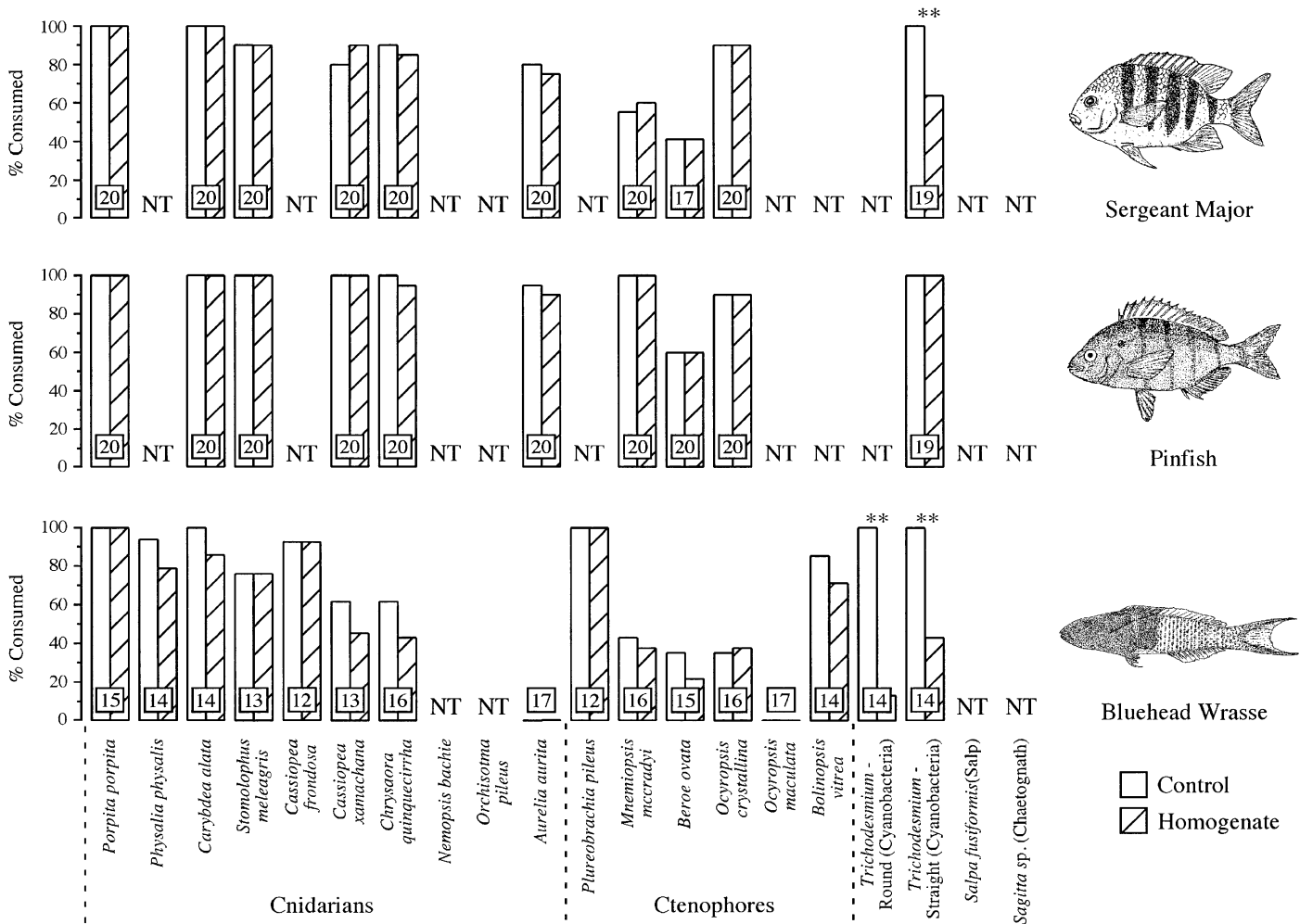


Fig. 3. The percentage of whole organism homogenates from macro-holoplankton consumed by fishes compared to control foods of equal protein content. An asterisk or double asterisk indicates a significant difference (Fisher's exact test, $P \leq 0.05$ or $P \leq 0.01$, respectively) between macro-holoplankton and control foods. NT, not tested.

Although we found few chemical defenses in the macro-holoplankton we examined, our work focused mainly on cnidarians and ctenophores. Chemical defense may be important for other species or groups of macro-holoplankton. For instance, the gymnosome pteropod *Clione antarctica* is chemically defended against sympatric fishes (Bryan et al. 1995; McClintock et al. 2001). Gymnosome pteropods are in the same subclass as benthic nudibranchs, a group that contains many chemically defended species (Karusu 1987; Faulkner 1992). In many ways nudibranchs and gymnosomes are morphologically similar. Both groups have lost the molluscan shell, are fairly slow moving, and often are conspicuously colored (Lalli and Gilmer 1989; Faulkner 1992). These observations suggest that chemical defenses could be more common among gymnosomes.

Because many of the macro-holoplankton we examined were rejected by fishes but did not possess deterrent chemistry, other mechanisms must account for their unpalatability. Within the cnidarians, it appears that nematocysts function defensively. The rejection of fresh cnidarian tentacles with intact nematocysts (Figs. 1, 2) and acceptance of ten-

tacles once nematocysts were inactivated (Fig. 5) suggests that the nematocysts, or the compounds they inject when fired, are important in defending these plankton. Although the defensive qualities of nematocysts have rarely been assessed for pelagic cnidarians (*but see* Shanks and Graham 1988), Stachowicz and Lindquist (2000) have demonstrated that some benthic hydroids use penetrating nematocysts to deter predators. There are many kinds of nematocysts, only some of which are capable of penetrating tissues. Of the cnidarians we examined, six species (*Carybdea alata*, *Porpita porpita*, *Physalia physalis*, *Chrysaora quinquecirrha*, *Aequorea aequorea*, and *Aurelia auritia*) possess penetrating nematocysts (atrichous isorhizas, basitrichous isorhizas, microbasic mastigophores, and/or stenotele nematocysts) (Marsical 1974; Purcell and Mills 1988; Shostak 1995). Additionally, *Cassiopea andromeda* possesses penetrating atrichous isorhizas nematocysts, suggesting that the congeners *C. frondosa* and *C. xamachana* might also possess these nematocysts. Although it is unclear whether the microbasic euryteles nematocysts possessed by *Stomolophus meleagris* (Shostak 1995) can penetrate tissues, Shanks and Graham

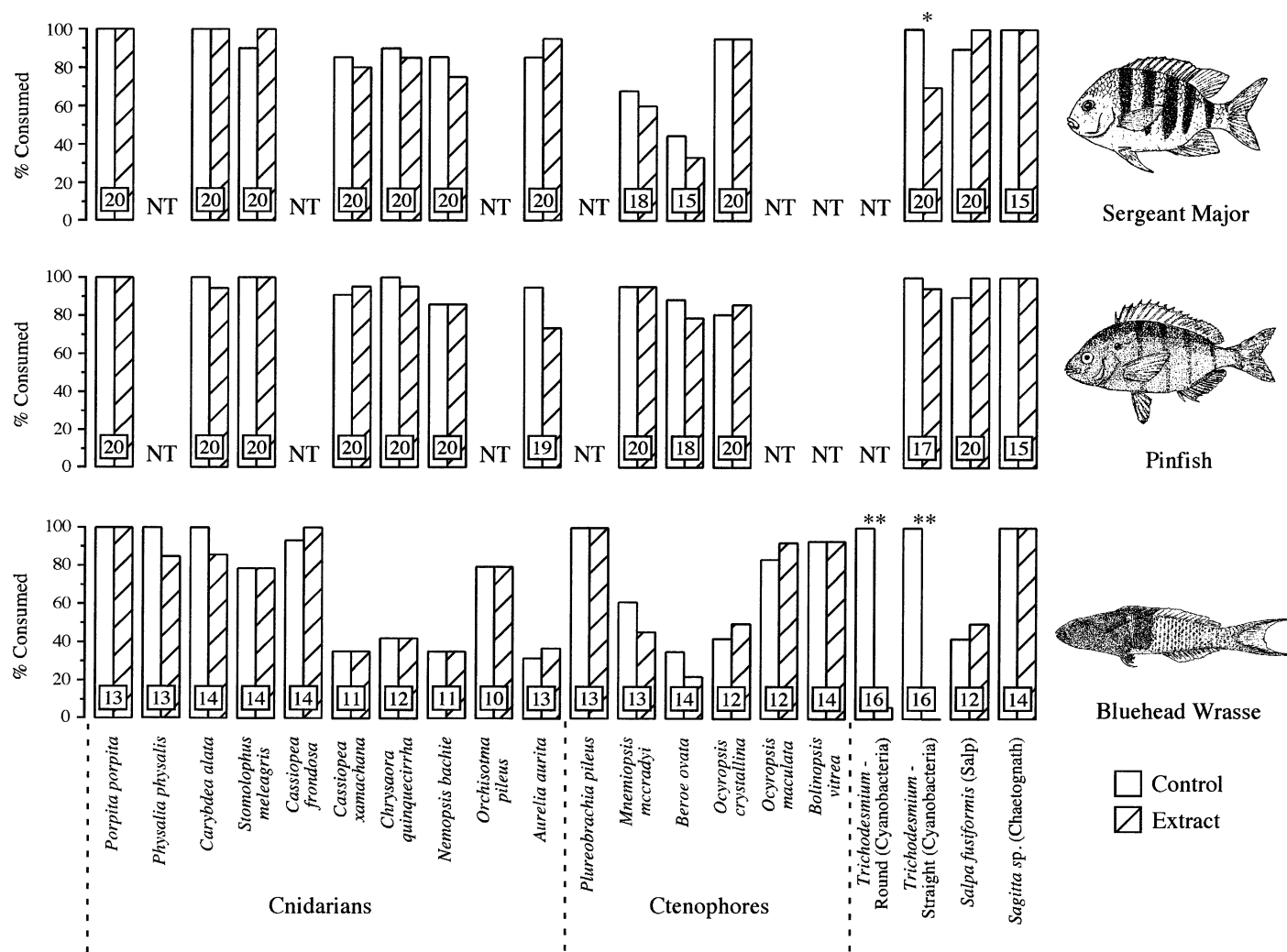


Fig. 4. The percentage of food containing chemical extracts from macro-holoplankton that were consumed by fishes compared to control foods of equal protein content. An asterisk or double asterisk indicates a significant difference (Fisher's exact test, $P \leq 0.05$ or $P \leq 0.01$, respectively) between macro-holoplankton and control foods. NT, not tested.

(1988) have shown that *S. meleagris* might use their nematocysts to deter predation by fishes. Thus, many, if not all, of the cnidarians we examined have nematocysts capable of injecting venom into potential predators (the nematocysts have not been characterized for *Orchistoma pileus* or *Nemopsis bachei*).

Because penetrating nematocysts function defensively and inject a toxin, it could be argued that cnidarians possess a form of chemical defense (e.g., Shanks and Graham 1988). Indeed, Shanks and Graham (1988) found that when disturbed, *S. meleagris* released a cloud of nematocyst-laden mucus capable of killing small fishes in enclosed containers. They speculated that the fishes were killed by hemolytic and cardiovascular toxins that entered the bloodstream of the fishes when the nematocysts discharged against the gills (Shanks and Graham 1988). However, compounds from nematocysts apparently function only when injected because the lyophilized homogenates and chemical extracts of all of our pelagic cnidarians (including *S. meleagris*) were palatable to benthic fishes (Figs. 3, 4). This suggests that com-

pounds from nematocysts are not deterrent when ingested rather than injected or that these compounds degrade rapidly once nematocysts are ruptured. Thus, the mechanism of defense of nematocysts and their toxins appears to differ from ingestion-based chemical defenses described for benthic invertebrates, including benthic hydroids that lack penetrating nematocysts (Stachowicz and Lindquist 2000). This conclusion should be tempered, however, by the recognition that we did not specifically identify and test the injected compounds from any of our species and thus cannot assure that our extraction procedure did not degrade metabolites.

Low nutritional quality also appears to reduce the susceptibility of some holoplankton to consumers, including species that lack nematocysts such as ctenophores. The frequency distribution of protein concentrations for the holoplankton species we examined was dramatically bimodal, and many species were very low in soluble protein content. Of the species we examined, 68% had soluble protein concentrations of 0.7 mg ml^{-1} or less, 26% had approximately 7 mg ml^{-1} or more, and only one species fell into

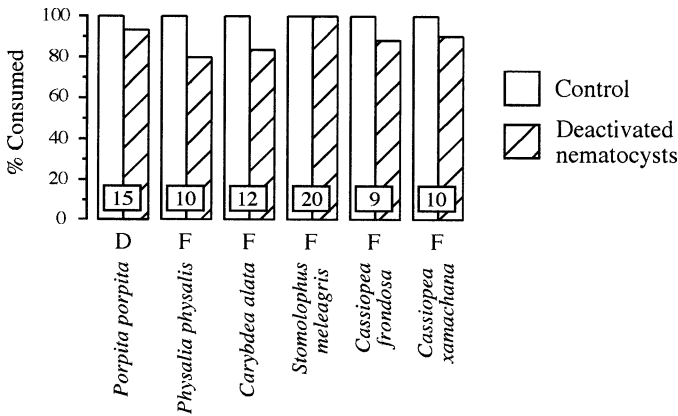


Fig. 5. The percentage of cnidarian tentacles without nematocysts consumed by fishes compared to an equal number of palatable control foods (brine shrimp or blood worms). Pinfish were used as predators for *Porpita porpita*; for all others, bluehead wrasses were the predators. The letter at the base of the bars in the histogram indicates how the nematocysts were removed; D, dipped in distilled water for 30 s; F, frozen for at least 24 h. Fisher's exact test indicated no significant differences ($P < 0.05$) for any of these contrasts.

the order of magnitude gap between these values (Table 1). In laboratory assays, feeding by all three of our benthic fish species was significantly and positively correlated with soluble protein content (Fig. 6), and reef fishes avoided foods of 0.3 mg ml^{-1} or less, while consuming foods of $>3.0 \text{ mg ml}^{-1}$ during field assays (Fig. 7). Although both laboratory and field assays suggest that benthic fishes select foods based on their nutritional quality, field assays might be better predictors of the true effects of nutritional quality because fishes in the lab had been trained to feed on the pellets provided. Thus, the training process probably biased the laboratory fishes in favor of accepting lower quality foods and might explain why laboratory sergeant majors and pinfish still consumed 40–60% of pellets that contained no protein, whereas reef fishes in the field avoided foods of low nutritional quality (Figs. 6, 7).

Previous investigations also found that low nutritional

Table 1. Mean soluble protein content ($\pm 1 \text{ SE}$) of holoplankton. NS = SE is $<0.1 \text{ mg ml}^{-1}$.

Species	Protein (mg ml^{-1})	N
<i>Porpita porpita</i> (cnidarian)	18.0 ± 0.4	4
<i>Physalia physalis</i> (cnidarian)	9.5 ± 2.2	4
<i>Carybdea alata</i> (cnidarian)	6.8 ± 0.4	4
<i>Stomolophus meleagris</i> (cnidarian)	2.0 ± 0.2	4
<i>Cassiopea frondosa</i> (cnidarian)	0.7 ± 0.1	4
<i>Cassiopea xamachana</i> (cnidarian)	0.7 ± 0.1	4
<i>Chrysaora quinquecirrha</i> (cnidarian)	$0.7 \pm \text{NS}$	4
<i>Nemopsis bachei</i> (cnidarian)	0.4	1
<i>Orchistoma pileus</i> (cnidarian)	$0.1 \pm \text{NS}$	3
<i>Aurelia aurita</i> (cnidarian)	$0.1 \pm \text{NS}$	4
<i>Pleurobrachia pileus</i> (ctenophore)	0.4 ± 0.1	4
<i>Mnemiopsis mccradyi</i> (ctenophore)	$0.2 \pm \text{NS}$	4
<i>Beroe ovata</i> (ctenophore)	$0.2 \pm \text{NS}$	4
<i>Ocyropsis crystallina</i> (ctenophore)	0.1 ± 0.1	4
<i>Ocyropsis maculata</i> (ctenophore)	0.1	2
<i>Bolinopsis vitrea</i> (ctenophore)	$<0.1 \pm \text{NS}$	4
<i>Trichodesmium</i> , round (cyanobacteria)	47.6 ± 4.5	4
<i>Trichodesmium</i> , straight (cyanobacteria)	28.7 ± 1.5	4
<i>Salpa fusiformis</i> (salp)	0.1	2
<i>Sagitta</i> sp. (chaetognath)	10.9 ± 1.5	4
Squid paste	34.5 ± 3.4	4

quality could deter consumers (Duffy and Paul 1992), although some consumers compensated for low nutritional quality by consuming more prey (Cruz-Rivera and Hay 2000). Because gelatinous macro-holoplankton often occur in dense swarms, it has been suggested that predators may be able to compensate for the low nutritive value of macro-holoplankton by consuming large numbers of them (McClintock et al. 1996). In contrast, our data suggest that benthic fishes commonly reject foods with the soluble protein content of many of the macro-holoplankton species we investigated. However, our data were generated using benthic fishes and it is possible that some pelagic consumers might have different nutritional needs and metabolic capabilities. Thus, some pelagic consumers might feed on low-quality prey items. This strategy seems possible for consumers with

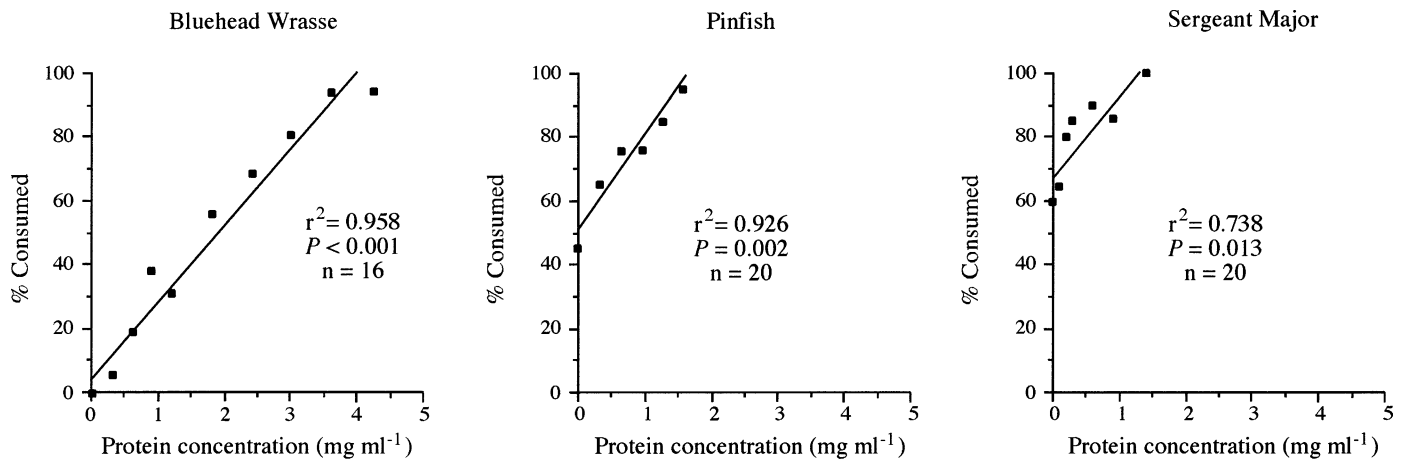


Fig. 6. Consumption of food pellets of differing nutritional value (soluble protein content) by fishes in the laboratory.

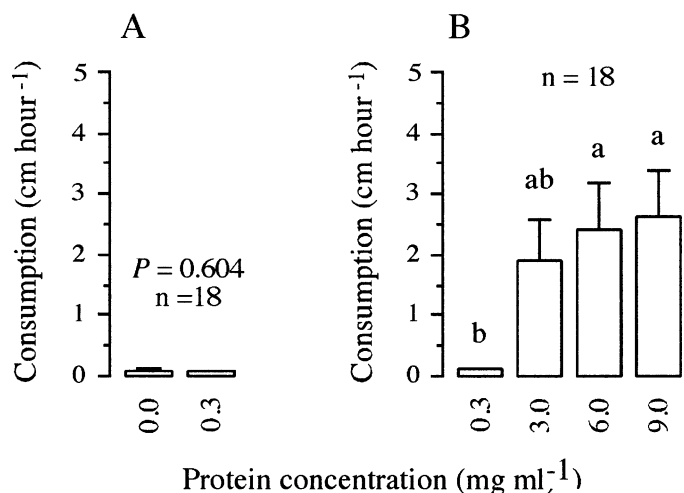


Fig. 7. Relative rate of consumption (± 1 SE) of carrageenan food strips of differing nutritional value (protein content) by fishes on reefs near Key Largo, Florida. (A) Comparison of food strips containing 0.0 and 0.3 mg ml⁻¹ of protein. Analysis is by Wilcoxon paired-sample *t*-test. (B) Comparison of feeding on food strips ranging from 0.3 to 9.0 mg ml⁻¹ of protein. Analyses were by Kruskal–Wallis test. Letters denote significant differences ($P \leq 0.05$) among treatments.

low metabolic rates, such as ocean sunfish (*Mola mola*) or sea turtles (both of which are known to consume cnidarians), but it seems unlikely for more metabolically active pelagic consumers that forage over large areas (e.g., mackerel and tuna). However, specific tests of the acceptability of foods with low protein content to pelagic predators are needed before generalizations can be drawn about the susceptibility of prey with low food value to pelagic consumers.

Although many of the species we examined had very low soluble protein concentrations, five species (the cnidarians *Porpita porpita*, *Physalia physalis*, and *Carybdea alata*; the chaetognath *Sagitta* sp.; and both morphs of the cyanobacteria *Trichodesmium* sp.) had protein content more than an order of magnitude higher than the other species. Because feeding by benthic fishes in the field was strongly related to soluble protein concentration of the food (Fig. 7), it might be expected that species having higher protein concentrations would be at greater risk of predation. Additional support for the idea that nutritionally rich prey are at greater risk of predation is provided by the observation that four of our high-protein organisms exhibited defensive traits that the low-protein species lacked. Both morphs of *Trichodesmium* sp. were chemically repellent, whereas none of the other 18 species we examined produced chemical deterrents. *Porpita porpita* and *Physalia physalis* float on the sea surface (Cheng 1975), making them less apparent to, and hence potentially less at risk from, visually foraging fishes (Hamner 1996). *Physalia physalis* (the Portuguese Man-of-War) and *Carybdea alata* (the sea wasp) possess more toxic nematocysts than other pelagic cnidarians (Humann 1992). Finally, *Carybdea alata* is the only species of gelatinous zooplankton we examined that has been described as being nocturnal (Humann 1992); because planktivory appears less intense at

night (Hobson 1991), *Carybdea alata* might escape day-active predators.

An exception to the pattern of high-food value prey having different defenses from low-food value prey was the chaetognath *Sagitta* sp., which appeared to avoid consumer detection by being extremely transparent, as did some other species. Transparency is believed to help macro-holoplankton escape detection by visual predators (McFall-Ngai 1990; Hamner 1996), and our observations were consistent with this hypothesis. Transparency is not an exceptional trait, however, because several low-protein content species were also highly transparent.

Fishes did not reject intact portions of all macro-holoplankton species. Fresh tissues of the salp *Salpa fusiformis* and the chaetognath *Sagitta* sp. were palatable to bluehead wrasses (Fig. 1). For *S. fusiformis*, these results are in contrast to those of McClintock et al. (1996) who found that intact pieces of this species were rejected by sergeant majors. We pose three potential reasons why our results might differ from those of McClintock et al. (1996). First, we assessed the palatability of *S. fusiformis* using bluehead wrasses rather than sergeant majors and there may be an interspecific difference in prey choice among these fishes. Second, *S. fusiformis* might occasionally possess a dietarily derived chemical deterrent. During a collection of *S. fusiformis* made off of North Carolina, large amounts of the chemically deterrent cyanobacterium *Trichodesmium* sp. occurred in the guts of *S. fusiformis*. When we offered sections of *S. fusiformis* guts to fish, they rejected portions from guts containing *Trichodesmium* sp. but consumed portions with guts lacking *Trichodesmium* sp. (S. Bullard pers. obs.). Thus, it is possible that our results differed from McClintock et al. (1996) because of the recent feeding histories of the prey offered. Finally, our feeding assay of fresh *S. fusiformis* might have been biased because we offered wrasses sections of salps that contained the pigmented gut. *S. fusiformis* had a mean protein content of 0.1 mg ml⁻¹, and laboratory bluehead wrasses typically rejected foods with this protein content (Fig. 6). However, Madin and Harbison (1977) have shown that hyperiid amphipods often consume the guts of salps but do not consume the outer tunic. Thus, the guts may be of higher nutritional quality than the remaining portions of the salp. If so, the portions of *S. fusiformis* we offered to fishes might have been higher in protein content than *S. fusiformis* as a whole.

Our findings do not resolve the question of whether pelagic systems are subject to minimal predation and serve as spatial escapes from consumers or whether they are areas of intense predation where prey will be selected to be deterrent. Although almost all prey we evaluated were relatively unpalatable to fishes, the mechanisms of deterrence (mainly nematocysts for cnidarians and low food value for cnidarians, ctenophores, and salps) could have evolved for reasons other than defense. Nematocysts play a prominent role in food capture, and their value as consumer deterrents could be fortuitous rather than evolved. In a similar way, low food value could be due to ecological constraints other than consumer deterrence. For example, large size could be selected for in macro-holoplankton in order to maximize the surface area for food capture or to slow the sinking rate; dispersing

proteins widely within lower value tissues could be an effective way for macro-holoplankton to increase size in a nutrient-poor environment. Finally, transparency could have multiple advantages. Although transparency would make prey less obvious to consumers, it might also enhance the ability of transparent predators to approach their prey. It is clear, however, that the types of extractable chemical defenses that commonly occur in benthic systems (Paul 1992; Pawlik 1993; Hay 1996; McClintock and Baker 2001) are not common among the groups of macro-holoplankton we examined.

Macro-holoplankton are among the most abundant organisms in the pelagic environment (Hamner et al. 1975; Mills 1995; Pages et al. 1996) and might contribute significantly to large-scale ocean processes, including nutrient cycling (Romeo et al. 1992; Capone et al. 1997; Andersen 1998). Additionally, the abundance of gelatinous zooplankton might be increasing in many ecosystems (Mills 1995; Brodeur et al. 1999). However, the benthic fishes we tested were unwilling to consume most macro-holoplankton because of the presence of nematocysts, low nutritional quality, or both. If generalist pelagic fishes are similar to the generalist benthic fishes used in our assays, then our data suggest that pelagic fishes might avoid consuming most gelatinous zooplankton (*but see* Arai 1988; Harbison 1998). If future studies of pelagic fishes support our observations that fishes are unable to control macro-holoplankton populations, it might be difficult to remove macro-holoplankton from pelagic systems once they become dominant (Mills 1995). Additionally, if macro-holoplankton are typically not consumed by fishes, much of the carbon located in macro-holoplankton could enter detrital rather than pelagic food webs.

References

- ANDERSEN, V. 1998. Salp and pyrosomid blooms and their importance in biogeochemical cycles, p. 125–137. *In* Q. Bone [ed.], *The biology of pelagic tunicates*. University of Oxford Press.
- ARAI, M. N. 1988. Interactions of fish and pelagic coelenterates. *Can. J. Zool.* **66**: 1913–1927.
- BAILEY, T. G., M. J. YOUNGBLUTH, AND G. P. OWEN. 1995. Chemical composition and metabolic rates of gelatinous zooplankton from midwater and benthic boundary layer environments off Cape Hatteras, North Carolina, USA. *Mar. Ecol. Prog. Ser.* **122**: 121–134.
- BRADFORD, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal. Biochem.* **72**: 510–514.
- BRODEUR, R. D., C. E. MILLS, J. E. OVERLAND, G. E. WALTERS, AND J. D. SCHUMACHER. 1999. Evidence for a substantial increase in gelatinous zooplankton in the Bering Sea, with possible links to climate change. *Fish. Oceanogr.* **8**: 296–306.
- BRYAN, G. J., W. YOSHIDA, J. B. MCCLINTOCK, AND B. J. BAKER. 1995. Ecological role of pteroenone, a novel antifeedant produced by the conspicuous Antarctic pteropod *Clione antarctica* (Gymnosomata: Gastropoda). *Mar. Biol.* **122**: 271–277.
- BULLARD, S. G., N. L. LINDQUIST, AND M. E. HAY. 1999. Susceptibility of invertebrate larvae to predators: How common are post-capture larval defenses? *Mar. Ecol. Prog. Ser.* **191**: 153–161.
- CAPONE, D. G., J. P. ZEHR, H. W. PAERL, B. BERGMAN, AND E. J. CARPENTER. 1997. *Trichodesmium*, a globally significant marine cyanobacterium. *Science* **276**: 1221–1229.
- CHENG, L. 1975. Marine pleuston—animals at the sea–air interface. *Oceanogr. Mar. Biol. Ann. Rev.* **13**: 181–212.
- CLARKE, A., L. J. HOLMES, AND D. J. GORE. 1992. Proximate and elemental composition of gelatinous zooplankton from the Southern Ocean. *J. Exp. Mar. Biol. Ecol.* **155**: 55–68.
- CROSSMAN, D. J., K. D. CLEMENTS, AND J. S. COOPER. 2000. Determination of protein for studies of marine herbivory: A comparison of methods. *J. Exp. Mar. Biol. Ecol.* **244**: 45–65.
- CRUZ-RIVERA, E., AND M. E. HAY. 2000. Can quantity replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. *Ecology* **81**: 201–219.
- DAVIS, E. M. 1988. Protein assays: A review of common techniques. *Am. Biotechnol. Lab.* **6**: 28–37.
- DUFFY, J. E., AND V. J. PAUL. 1992. Prey nutritional quality and the effectiveness of chemical defenses against tropical reef fishes. *Oecologia* **90**: 333–339.
- FAULKNER, D. J. 1992. Chemical defenses of marine molluscs, p. 119–163. *In* V. J. Paul [ed.], *Ecological roles of marine natural products*. Comstock.
- GLIWICZ, M. Z. 1986. Predation and the evolution of vertical migration in zooplankton. *Nature* **320**: 746–748.
- HAMNER, W. M. 1996. Predation, cover, and convergent evolution in epipelagic oceans, p. 17–35. *In* P. H. Lenz, D. K. Hartline, J. E. Purcell, and D. L. McMillan [eds.], *Zooplankton: Sensory ecology and physiology*. Gordon and Breach.
- , L. P. MADIN, A. L. ALLDREDGE, R. W. GILMER, AND P. P. HAMNER. 1975. Underwater observations of gelatinous zooplankton: sampling problems, feeding biology, and behavior. *Limnol. Oceanogr.* **20**: 907–917.
- HARBISON, G. R. 1998. The parasites and predators of Thaliacea, p. 187–214. *In* Q. Bone [ed.], *The biology of pelagic tunicates*. Oxford Univ. Press.
- HARVELL, C. D., W. FENICAL, AND C. H. GREEN. 1988. Chemical and structural defenses of Caribbean gorgonians (*Pseudopterogorgia* spp.). I. Development of an in situ feeding assay. *Mar. Ecol. Prog. Ser.* **49**: 287–294.
- HAWSER, S. P., G. A. CODD, D. G. CAPONE, AND E. J. CARPENTER. 1992. Toxicity of blooms of cyanobacterium *Trichodesmium* to zooplankton. *Appl. Phycol.* **4**: 79–86.
- HAY, M. E. 1996. Marine chemical ecology: What is known and what is next? *J. Exp. Mar. Biol. Ecol.* **200**: 103–134.
- , AND W. FENICAL. 1988. Marine plant–herbivore interactions: The ecology of chemical defense. *Annu. Rev. Ecol. Syst.* **19**: 111–145.
- , J. J. STACHOWICZ, E. CRUZ-RIVERA, S. G. BULLARD, M. S. DEAL, AND N. LINDQUIST. 1998. Bioassays with marine and freshwater macroorganisms, p. 39–141. *In* K. F. Haynes and J. G. Miller [eds.], *Methods in Chemical Ecology*. Chapman and Hall.
- HOBSON, E. S. 1991. Trophic relationships of fishes specialized to feed on zooplankters above coral reefs, p. 69–95. *In* P. F. Sale [ed.], *The ecology of fishes on coral reefs*. Academic Press.
- HUMANN, P. 1992. Reef creature identification, Florida, Caribbean, Bahamas. New World Publications.
- KARUSO, P. 1987. Chemical ecology of the nudibranchs, p. 31–60. *In* P. J. Scheuer [ed.], *Bioorganic marine chemistry*. V. 1. Springer-Verlag.
- LALLI, C. M., AND R. W. GILMER. 1989. Pelagic snails: The biology of holoplanktonic gastropod mollusks. Stanford Univ. Press.
- LARSON, R. J. 1986. Water content, organic content and carbon and nitrogen composition of medusae from the northeast Pacific. *J. Exp. Mar. Biol. Ecol.* **99**: 107–120.
- LINDQUIST, N., AND M. E. HAY. 1996. Palatability and chemical

- defense of marine invertebrate larvae. *Ecol. Monogr.* **66**: 431–450.
- MADIN, L. P., AND G. R. HARBISON. 1977. The association of Amphipoda Hyperiididae with gelatinous zooplankton. 1. Associations with Salpidae. *Deep-Sea Res.* **24**: 449–463.
- MARISCAL, R. N. 1974. Nematocysts, p. 129–178. *In* L. Muscatin, and H. M. Lenhoff [eds.], *Coelenterate biology*. Academic Press.
- MCCCLINTOCK, J. B., AND B. J. BAKER [EDS.]. 2001. *Marine chemical ecology*. CRC Press.
- , D. P. SWENSON, D. K. STEINBERG, AND A. A. MICHAELS. 1996. Feeding-deterrent properties of common oceanic holoplankton from Bermudian waters. *Limnol. Oceanogr.* **41**: 798–801.
- , B. J. BAKER, AND D. K. STEINBERG. 2001. The chemical ecology of invertebrate meroplankton and holoplankton, p. 195–225. *In* J. B. McClintock and B. J. Baker [eds.], *Marine chemical ecology*. CRC Press.
- McFALL-NGAI, M. J. 1990. Cypsis in the pelagic environment. *Am. Zool.* **20**: 175–188.
- MILLS, C. E. 1995. Medusae, siphonophores, and ctenophores as planktivorous predators in changing global ecosystems. *ICES J. Mar. Sci.* **52**: 575–581.
- MORGAN, S. G. 1990. Impact of planktivorous fishes on dispersal, hatching, and morphology of estuarine crabs. *Ecology* **71**: 1639–1652.
- . 1997. Planktivorous fishes as selective agents for reproductive synchrony. *J. Exp. Mar. Biol. Ecol.* **209**: 89–101.
- O'NEIL, J. M. 1998. The colonial cyanobacterium *Trichodesmium* as a physical and nutritional substrate for the harpacticoid copepod *Macrosetella gracilis*. *J. Plankton Res.* **20**: 43–59.
- PAGES, F., M. G. WHITE, AND P. G. RODHOUSE. 1996. Abundance of gelatinous carnivores in the nekton community of the Antarctic Polar Frontal Zone in the summer of 1994. *Mar. Ecol. Prog. Ser.* **141**: 139–147.
- PAUL, V. J. 1992. Ecological roles of marine natural products. *Comstock*.
- PAWLIK, J. R. 1993. Marine invertebrate chemical defenses. *Chem. Rev.* **93**: 1911–1922.
- PRESTON, N. P., M. A. BURFORD, AND D. J. STENZEL. 1998. Effects of *Trichodesmium* spp. blooms on penaeid prawn larvae. *Mar. Biol.* **131**: 671–679.
- PURCELL, J. E., AND C. E. MILLS. 1988. The correlation between nematocyst types and diets in pelagic hydrozoa, p. 463–485. *In* D. A. Hessinger and H. M. Lenhoff [eds.], *The biology of nematocysts*. Academic Press.
- ROMEO, M., M. GNASSIABRELLI, AND C. CARRE. 1992. Importance of gelatinous plankton organisms in storage and transfer of trace-metals in the northwestern Mediterranean. *Mar. Ecol. Prog. Ser.* **82**: 267–274.
- SCHUPP, P. J., AND V. J. PAUL. 1994. Calcification and secondary metabolites in tropical seaweeds: Variable effects on herbivorous fishes. *Ecology* **75**: 1172–1185.
- SHANKS, A. L., AND W. M. GRAHAM. 1988. Chemical defense in a scyphomedusa. *Mar. Ecol. Prog. Ser.* **45**: 81–86.
- SHOSTAK, S. 1995. Nematocyst database. <http://www.pitt.edu/~sshostak/cnidocyst/database> (We have archived a copy of this database.)
- SLANSKY, F., JR. 1993. Nutritional ecology: The fundamental quest for nutrients, p. 29–91. *In* N. E. Stamp and T. M. Casey [eds.], *Caterpillars: Ecological and evolutionary constraints on foraging*. Chapman and Hall.
- STACHOWICZ, J. J., AND N. LINDQUIST. 2000. Hydroid defenses against predators: The importance of secondary metabolites vs. nematocysts. *Oecologia* **124**: 280–288.
- STEPHENS, D. W., AND J. R. KREBS. 1986. *Foraging theory*. Princeton Univ. Press.
- STRATHMANN, R. R. 1985. Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. *Ann. Rev. Ecol. Syst.* **16**: 339–361.
- WILLIAMSON, J. A., P. J. FENNER, J. W. BURNETT, AND J. F. RIFKIN. 1996. *Venomous and poisonous marine animals*. Univ. of New South Wales Press.
- WRAY, G. A. 1995. Evolution of larvae and developmental modes, p. 413–447. *In* L. R. McEdward [ed.], *Ecology of marine invertebrate larvae*. CRC Press.

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