

Chemically mediated foraging by subtidal marine predators: a field test of tritrophic cues

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ABSTRACT: Terrestrial insect predators and parasitoids commonly use volatile plant compounds released during grazing to find living herbivorous prey (i.e. tritrophic signaling). Because few studies have tested for tritrophic signaling in marine organisms, it is unclear if marine predators commonly use these fluid-borne cues to find living herbivore prey, especially under realistic settings. Such interactions might occur in the ocean because marine predators often use chemosensory-based foraging, and herbivore-induced production of defensive chemistry is common in some algal taxa. To examine the ability of predators to find live herbivores using waterborne chemical cues from grazed algae, we tested the responses of marine predators to several baits using *in situ* trapping and video experiments. We tested the attractiveness of chemical cues released by (1) *Macrocystis pyrifera* and *Ulva* sp. actively grazed by herbivores for short- or long-term periods, (2) crushed herbivores, (3) positive controls containing fish carrion, or (4) negative controls. Most predators trapped and filmed were spiny lobsters *Panulirus interruptus*. Under realistic field conditions, chemical cues from actively grazed algae with live herbivores failed to attract predators, but chemical cues from crushed herbivores and fish carrion were highly attractive. Thus, while marine predators like lobsters may utilize fluid-borne chemical cues to find dead or injured prey, such cues appear less important for finding live herbivore prey. Our literature review of 142 terrestrial studies revealed that tritrophic cues were unrelated to diet breadth of predators or herbivores and that most examples of terrestrial tritrophic signaling contained parasitoid predators. We additionally found that similar field studies in natural, non-agricultural settings such as ours are lacking even in better-studied terrestrial food webs, where they represent only 3% of manipulative experiments.

KEY WORDS: Tritrophic interactions · Chemically mediated · Predator foraging · Subtidal

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INTRODUCTION

Predatory insects and parasitoids commonly find live, uninjured herbivore prey by tracking volatile chemical cues released by grazed plants (a.k.a. 'tritrophic signals' or 'herbivore-induced synomones'; Vet & Dicke 1992, Sabelis et al. 1999, Heil 2008). Interestingly, such tritrophic cues appear rare in the sea, suggesting that either (1) there are fundamental differences between terrestrial and marine organisms or habitats, and/or (2) there is an experimental

bias towards terrestrial systems. Emerging evidence that marine predators can use algal cues to find herbivore prey suggests the latter may be true. For example, phytoplankton release dimethyl sulphide (DMS) upon attack by zooplankton, and this compound is used as a foraging cue by procellariiform birds and African penguins (Nevitt et al. 1995, Wright et al. 2011). Similarly, the seaweed *Ascophyllum nodosum* is more attractive to fish and crab predators when grazed by herbivorous snails compared to ungrazed controls (Coleman et al. 2007).

Despite these examples, additional studies of tritrophic cues in the sea, especially using field experiments, are needed.

Although examples of tritrophic cues are rare in the sea, marine predators commonly exploit waterborne chemical cues to find their prey from a distance. For example, marine invertebrates, especially crustaceans and gastropod mollusks, can find prey by tracking dissolved free amino acids, ATP, nucleotides, and sugars (Zimmer-Faust 1993, Zimmer et al. 1999, Derby et al. 2001). However, these cues are released in such low quantities by living, intact prey, or become so diluted when transported through fluid, that they may be more important for finding dead or injured prey (Derby & Zimmer 2012). In contrast to visual, auditory, and mechanical cues, chemical cues are highly persistent and travel over long distances, making them particularly useful in influencing distance foraging (Weissburg 2012). For example, marine crustacean predators, such as blue crabs and spiny lobsters, respond to and detect live prey cues by rapid sampling of concentrated odor plumes. Such behaviors are believed to improve prey discovery at scales of ~tens of meters (Weissburg & Zimmer-Faust 1993, Zimmer-Faust et al. 1996, Weissburg et al. 2002). Slower moving animals like whelks and nudibranchs appear more effective at chemosensory based foraging over greater distances and higher flow speeds—perhaps because they sample and average multiple concentrations through time (Ferner & Weissburg 2005, Wyeth & Willows 2006, Ferner et al. 2009). Thus, the frequent use of chemosensory-based foraging in marine predators suggests that the rarity of tritrophic cues in the sea does not result from an inability to detect waterborne chemical cues.

The rarity of tritrophic cues in marine systems may result from inherent differences between the sensory capabilities of the organisms found in these ecosystems. For example, the ocean lacks parasitoids (a group that commonly finds prey via tritrophic cues on land; Takabayashi et al. 1998, D'Alessandro & Turlings 2005) and it contains a greater proportion of generalist consumers (Duffy & Hay 2001). Parasitoids may be more likely to exploit tritrophic cues because (1) a single herbivore host has a greater impact on the fitness of a parasitoid than on a predator and (2) assuming similar handling time as predators, parasitoids receive a greater benefit from reducing search time because they use more time searching for prey (Vet & Dicke 1992). Although the link between feeding specialization and the use of infochemicals to find prey has been

debated (Vet & Dicke 1992, Steidle & van Loon 2003), there is evidence that tritrophic cues are less common in terrestrial food webs containing both generalist predators and generalist herbivores (i.e. those most similar to the majority of marine food webs). For example, Steidle & van Loon (2003) conducted a literature review of infochemical use by terrestrial predators and compared food webs differing in feeding specialization (specialist predators+specialist herbivores vs. specialist predators+generalist herbivores vs. generalist predators+generalist herbivores). Their survey suggests that while the general use of infochemicals was common in all food webs, the use of tritrophic cues was rarest in food webs containing both generalist predators and herbivores. Only 14% (4 of 29 studies reporting infochemical use by predators to find prey) of these food webs contained predators that use cues from plants infested with or damaged by their herbivore prey/host. In contrast, 30% (20 of 66 studies) of food webs containing at least one specialist consumer had predators that used these tritrophic cues.

Terrestrial and marine contrasts are additionally difficult because many terrestrial studies were conducted under controlled laboratory conditions or artificial agricultural settings. Although laboratory studies have identified key interactions containing tritrophic cues, they poorly recreated important factors (e.g. flow and turbulence) for chemosensory-based foraging (Weissburg & Zimmer-Faust 1993). Such designs may have inadvertently increased the apparent effect size of tritrophic cues by exposing organisms to concentrated cues without temporal or spatial variability (Kessler & Baldwin 2001). Studies of tritrophic interactions under more realistic fluid dynamic settings are needed (Hunter 2002).

Because marine predators commonly find prey via chemical cues (Weissburg et al. 2002) and algae can release potent water-borne chemical compounds in response to grazers (Toth & Pavia 2007), tritrophic cues may be used by marine predators to find living herbivore prey in marine systems. Using field experiments, we tested this hypothesis by examining the attractiveness of grazing-associated cues (e.g. live herbivores and grazed algae) to marine predators. Specifically, we focused on cues released by herbivores or their associated algae (*Macrocystis pyrifera* and *Ulva* sp.). These algae may benefit from attracting predatory animals because, relative to other co-occurring seaweeds, they lack obvious chemical and physical defenses (Steinberg 1985). In addition, predators might be attracted to grazed *Ulva* because this alga releases DMS upon herbivore attack, and pre-

datory organisms commonly utilize this volatile chemical for navigation (Nevitt et al. 1995, Van Alstyne et al. 2001, Wright et al. 2011). We examined the attractiveness of these cues to predators using 2 approaches; *in situ* trapping and videography.

MATERIALS AND METHODS

Literature review

To compare interactions involving tritrophic cues between terrestrial and marine settings, we surveyed 142 manipulative experiments of tritrophic interactions among terrestrial organisms. We performed a database search using Web of Science (1990 to 2013; accessed 22 March 2013) with the terms 'tritrophic interaction*' or 'indirect plant defen*', where * represented wild card search terms to account for differences in spelling (e.g. defence vs. defense). This search found 724 papers. We limited our survey to manipulative experiments that demonstrated direct evidence of predator attraction to plants being actively grazed by herbivores. Only 116 of the original 724 papers met this requirement. This was supplemented with 26 additional references contained within the reference sections of these papers that did not appear in the search, but were relevant to our survey.

To classify the types of dominant consumers in tritrophic interaction studies, we quantified the frequency of terrestrial studies with (1) generalist versus specialist herbivores, (2) generalist versus specialist predators, and (3) parasitoid versus carnivore predators. We also quantified the frequency of studies conducted in laboratories, artificial field settings (e.g. agricultural), or natural field settings. For each study, we determined the diet breadth of each consumer species (specialist or generalist), predator type (carnivore or parasitoid), and experimental venue (laboratory, artificial field, or natural field). Consumer diet breadth was determined from literature searches in Web of Science. Species names were searched and animals were categorized based on previously published classifications. Consumers are generally considered specialists if they are known to prey on few species, or generalists if they feed on a wide range of species (Bernays & Chapman 1994). Predators were categorized as carnivores if they directly kill herbivorous prey by consumption, or parasitoids if they oviposit eggs in or on their hosts (Strand & Obrycki 1996). Each species was counted only once, regardless of the number of times it appeared in the survey.

When diet breadth could not be determined, we counted this species as having an 'unknown' diet breadth.

Experimental venue was categorized as laboratory if it was conducted with wind tunnels, olfactometers, or greenhouses. Venues were considered artificial field if they took place in agricultural or transplanted monocultures of plants. Natural field studies used naturally occurring populations of plants that did not undergo experimental transplantation or manipulation. Each venue within every study (i.e. multiple venues within a single study) was counted in the survey.

Study species and sites

In southern California subtidal communities, algae co-occur with several herbivores and predators with well-described chemosensory-based foraging abilities (e.g. lobsters, crabs, snails, seastars; Zimmer-Faust & Case 1982a,b, Zimmer et al. 1999, Brewer & Konar 2005). To elicit the release of grazing-associated cues, we exposed live algae to some of their most commonly encountered invertebrate herbivores (Byrnes et al. 2011). We tested for tritrophic interactions in 2 food chains that differed with respect to basal macroalgae (*Macrocystis pyrifera* and *Ulva* sp.) and herbivores. For *Macrocystis*, we used herbivorous kelp snails *Norrisia norrisi* and purple urchins *Strongylocentrotus purpuratus*. For *Ulva*, we used herbivorous turban snails *Chlorostoma (Tegula) aureotincta* and purple urchins.

Algae and herbivores were collected from 3 rocky subtidal sites: Point Loma kelp forest (32° 41' 9.20" N, 117° 15' 35.91" W), Mission Bay Channel (32° 45' 37.12" N, 117° 14' 44.21" W), and Bird Rock (32° 48' 52.97" N, 117° 16' 25.11" W). All field experiments took place between July 2011 and August 2012 at the Point Loma and Mission Bay Channel sites.

Trapping experiments

To test the attractiveness of herbivore-induced algal cues to subtidal predators, we compared the trapping success of benthic traps baited with grazed algae with herbivores versus bait-free controls. Mesh bait bags (2 mm² pore size) were secured in a bait cage (25 × 11 × 12 cm) in the center of a PVC coated crab trap (65 × 49 × 25 cm; 3.8 cm² mesh size) with 2 trap door entrances (16 × 8 cm). The minimum distance between the outside of the trap and the bait

treatment was 19 cm. Permeable bait bags allowed the release of chemical cues, and the small mesh size removed visual cues for predators. Trapped animals were identified, counted, and released at the point of capture. We determined treatment attractiveness by comparing treatment and control traps with respect to: (1) the number of predators trapped and (2) the percentage of empty traps (e.g. those that did not trap any predators). The empty trap percentage provided an estimate of the attractiveness of a bait that was unbiased by the presence of previously trapped predators.

To examine the influence of mesh size on bait attractiveness, we conducted a preliminary experiment that used a larger mesh opening (100 mm²). We compared the relative attractiveness of traps baited with *Macrocystis* plus grazing herbivores (2 kelp snails and 2 purple urchins) to traps baited with ungrazed *Macrocystis* and non-grazing herbivores. As with smaller mesh bait bags, we did not find a significant difference in the attractiveness of these baits (paired *t*-test, $t = -0.73$, $p = 0.474$) and very few predators were caught in these traps (0.95 ± 0.25 and 1.32 ± 0.41 predators per trap for *Macrocystis* plus grazing herbivores and ungrazed *Macrocystis* plus non-grazing herbivores, respectively). All other experiments used bait bags with small mesh openings (2 mm²) to remove the influence of visual cues on bait attraction.

Short-term induction of *Macrocystis*

Similar to terrestrial plants, algal responses to grazing may depend upon length of exposure to grazing. For example, grazing-induced production of algal chemical defenses can take minutes (Van Alstyne & Houser 2003) or weeks (Pavia & Toth 2000, Toth & Pavia 2007). Thus, we tested for potential differences in the attractiveness of *Macrocystis* exposed to both short- or long-term grazing periods. To produce short-term grazing (STG) cues, *Macrocystis* blades (20 ± 1 g) were exposed to 2 kelp snails and 2 purple urchins (that were previously starved for 1 to 2 d) in the trap mesh bags in flow-through seawater tanks overnight (N = 35). For all of our experiments using purple urchins and kelp snails, we used small urchins and large snails (both ~4 cm in diameter) to standardize cue release among treatments. Before field deployment, herbivores in grazed treatments were replaced with the same amount of new, starved (1 to 2 d) conspecifics, so that algal grazing would continue in the field

without being limited by herbivore satiation. For controls, we exposed *Macrocystis* to flow-through seawater without herbivores, then prior to field deployment, placed non-grazed *Macrocystis* in traps with live non-feeding herbivores, separating the algae and herbivores into 2 compartments to prevent grazing during deployment (N = 35). We chose this type of control to better isolate the effects of grazing-associated cues from ambient algal and herbivore cues that could also provide useful cues to predators. However, all other experiments used unbaited traps for controls to maximize the probability of detecting a treatment bait effect.

Long-term induction of *Macrocystis*

To produce long-term grazing (LTG) cues, *Macrocystis* was grazed for 2 wk; a grazing period that commonly induces chemical defenses of brown algae (Pavia & Toth 2000, Borell et al. 2004, Toth et al. 2005). Blades were submerged in 10 cm of seawater, and grown in outdoor mesocosms with 2 kelp snails and 2 purple urchins (N = 28). Mesocosms (35 × 21 × 13 cm) containing treatments were supplied with individual flow-through seawater. After 2 wk, *Macrocystis* (10 ± 1 g) was placed in mesh bags with 2 new, starved (for 1 to 2 d) conspecific herbivores, and inserted into traps immediately before deployment. Treatment attractiveness was measured by comparing catch in baited versus unbaited traps.

Short-term induction of *Ulva*

We used similar techniques described above to test the attractiveness of *Ulva* when grazed by purple urchins and turban snails. Because DMS can be a useful foraging cue, and its release was expected to be immediate upon herbivore grazing damage, only STG *Ulva* responses were tested with no overnight grazing period before deployment. On the day of deployment, *Ulva* (10 ± 1 g), 2 purple urchins, and 4 turban snails (starved for 2 to 3 d) were placed in mesh bags and immediately inserted into traps (N = 56). Because turban snails are approximately half the size of kelp snails (2 cm and 4 cm diameter, respectively) and we wanted to attempt to standardize cue release among experiments, we doubled the number of turban snails in this experiment. Treatment attractiveness was compared to unbaited control traps (N = 56).

Crushed herbivores and fish carrion

Kelp forest predators may be attracted to chemical signals from damaged or freshly killed prey, perhaps because these cues indicate prey vulnerability or that cues from these sources provide stronger signals that are more concentrated and persist longer in the environment (Zimmer-Faust & Case 1982a, Zimmer et al. 1999, Brewer & Konar 2005). To test predator attraction to injured prey, we conducted a trapping experiment with treatments containing crushed (i.e. freshly killed) herbivores using similar methods described above (N = 40). Two purple urchins and 4 turban snails were placed in mesh bags of treatment traps and were crushed with a hammer immediately before placement in traps. It should be noted that 2 treatment traps were lost (buoys vandalized) during the deployment periods, reducing the sample size of treatment traps from 40 to 38.

As a positive control for our algal and live-herbivore associated cue experiments, we conducted a trapping experiment comparing the attraction of fish carrion and unbaited controls. Freshly thawed Pacific sardines *Sardinops sagax caerulea* were placed within mesh bags in traps (N = 20), and attractiveness was compared to unbaited control traps (N = 20).

Underwater video experiments

QCS system

Because experiments using traps to examine bait attractiveness may create bias by selective trapping of predator species or predator sizes (Jury et al. 2001), we captured continuous digital recordings of treatments and control baits placed outside of traps using a quadrapod camera system (QCS). A GoPro HD Hero camera with a flat lens housing (Eye of Mine Action Cameras) was mounted on a quadrapod frame pointing downward towards a bait cage. The deployed quadrapod rested on the seafloor. Light was supplied by 2 mounted Underwater Kinetics eLED Light Cannons (825 lumens) covered with diffusers and red film (Rosco Roscolux Light Red Lighting Filter). Red film minimized lighting effects on predator behavior, as crustacean predators have a low sensitivity to red light (Weiss 2006). A bait cage (24 × 12 × 9 cm; 0.635 cm² mesh opening) was secured to the bottom of the quadrapod, 28 cm from the camera. The area of the field of view was 36 × 48 cm.

Video trials

Video trials tested the attractiveness of the same treatments used in trapping experiments; (1) LTG *Macrocystis*, (2) STG *Macrocystis* and *Ulva*, (3) crushed herbivores, and (4) fish carrion, and were compared to unbaited controls. All treatments (N = 6) were prepared using the same pre-deployment procedures as described above in the trapping experiments, with the exception of STG algae. Because we found no significant attraction to either STG *Macrocystis* or *Ulva* in trapping experiments, we combined them into a single treatment for video trials; however, pre-grazing periods for each alga remained the same as in trapping experiments. Two QCS units were deployed in a single trial, a treatment and a control. Using video from these trials, we determined (1) the number of times each predator species entered the field of view (= predator visit) and (2) the duration of each visit. Because individual predators were not identifiable, we quantified 'predator visits' as the number of times a predator species entered and exited the field of view. We used the number of predator visits as a proxy for treatment attractiveness. Attractiveness was also quantified by measuring the percent of trial time predators spent in the field of view (duration of visit/total trial time).

Spatial and temporal arrangement

Trapping experiments

Traps were deployed during the day (between 09:00 h and 12:00 h) and retrieved during peak foraging hours of common benthic predators (e.g. lobsters; MacArthur et al. 2008, Weiss et al. 2008). Traps were retrieved approximately 15 h after deployment, 3 to 4 h before sunrise, to avoid trapping during periods of high aggregation (e.g. lobsters aggregate shortly before sunrise; Zimmer-Faust et al. 1985).

STG *Macrocystis* traps were deployed in 6 to 12 m of water in the rocky reefs of the Point Loma kelp forest over 2 separate deployment periods in July 2011. Treatment and control traps were deployed in pairs to account for spatial heterogeneity in predator abundance, especially lobsters (California Department of Fish and Game 2006). Deployment locations were randomly selected within the kelp forest. Each pair was separated by 250 to 300 m, and traps within each pair were separated by 100 m. LTG *Macrocystis* traps were deployed over 3 separate deployment

periods in September 2011 in the same way as STG *Macrocystis* trials.

STG *Ulva* traps were deployed in the Mission Bay Channel, which contains the same community of organisms as the Point Loma kelp forest. Treatment and control traps were paired and separated by 50 m. Traps were deployed in linear array in 6 to 7.5 m of water in rocky reef kelp habitat over 3 separate deployment periods in August and September 2011. Crushed herbivores and fish carrion traps were also deployed in the Mission Bay Channel, in a similar way as STG *Ulva*. Treatment and unbaited control traps were placed randomly in a linear array, separated by 50 m. Crushed herbivore experiments took place over 2 separate deployment periods in February 2012, and fish carrion was deployed over 1 deployment period, also in February 2012.

Video experiments

QCS units were randomly deployed 50 m apart in 6 to 8 m of water in the Mission Bay Channel at night during peak predator foraging (21:00h to 24:00 h), and retrieved after 1 h of continuous recording. Deployment sites were located in the same general area where trapping experiments in the channel occurred; however for video trials, we specifically targeted the flat, sandy substrate immediately adjacent to kelp habitat for maximum visibility during video analysis. These experiments took place between June and August 2012.

Statistical analyses

Literature review results were compared using chi-square tests to determine differences in consumer diet breadth (specialists vs. generalists) and predator type (carnivores vs. parasitoids) from an expected frequency of 50%. This allowed us to determine if one mode was more likely to be associated with tritrophic signaling than the other. We excluded consumer species whose diet breadth could not be identified as specialist or generalist (13% of prey and 25% of predators). Experimental venues were not compared statistically because expected frequencies are unknown.

For each separate trapping experiment, differences in predator catch per trap for each algal species were compared between the treatment and corresponding control using 2-tailed, paired *t*-tests, because trap deployments were paired. For trapping experiments

with crushed herbivore and fish carrion as treatments, we used 2-tailed, 2-sample *t*-tests to test against their associated controls. Differences in the number of traps without predators were compared to traps with predators using Pearson chi-square analyses for each separate trapping experiment. For each underwater video experiment, predator visits and percent of trial time predators spent in the field of view (duration of visit/total trial time) were compared between treatments and controls using paired *t*-tests. To determine if different predator types were attracted to crushed herbivores or fish carrion treatments, the composition of predator species captured were compared between treatments for both trapping and video experiments using Pearson chi-square analyses.

Between-treatment differences in predator catch and predator visits were also compared qualitatively within trapping and underwater video experiments, respectively, to examine relative differences in attractiveness. No statistics were conducted to quantitatively compare differences between treatments because experiments were done during different times and in different locations, due to logistical constraints. Because these experiments were conducted at different times, treatments were standardized by transforming predator catch using the formula: $T/(C + 1)$, where *T* is predator catch (trapping experiments) or predator visits (video experiments) for treatment bait, and *C* is predator catch or predator visits for the paired control bait. Since treatment and control replicates in the fresh carrion trapping experiments were unpaired and balanced, control replicates for this comparison were randomly paired with treatments to calculate the ratio. For crushed herbivore trapping treatments where treatments and controls were unpaired and unbalanced (2 treatment replicates lost), an equal amount of control replicates were randomly excluded (2), and the remaining controls were randomly paired with treatments.

RESULTS

Literature review

In 142 studies that found evidence of tritrophic interactions in terrestrial settings (see Supplements 1 & 2 at www.int-res.com/articles/suppl/m498p161_supp.pdf), 50% of herbivores used were feeding specialists, 37% were generalists, and 13% had unknown (to our knowledge) diet breadths (Fig. 1A, Supplement 1). After excluding unknown herbivore types, 57% of herbivores were specialists and 43%

were generalists. For predators surveyed, 38% were specialists, 37% were generalists, and 25% had unknown diet breadths (Fig. 1B, Supplement 1). After excluding unknown predator types, 51% were specialists and 49% were generalists. When com-

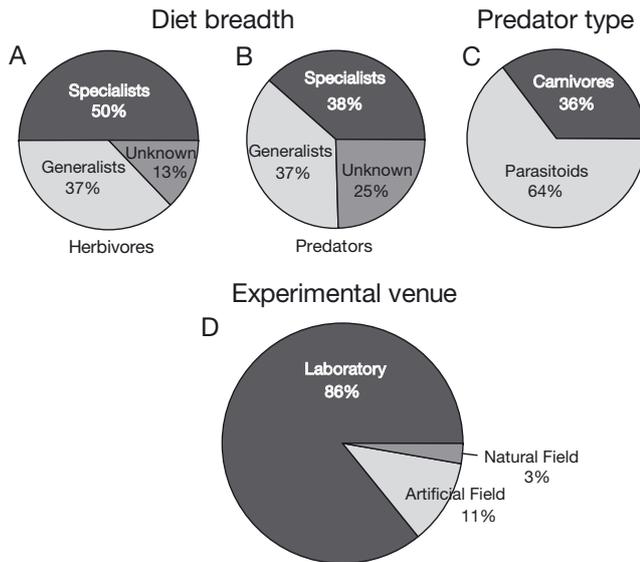


Fig. 1. Proportion of (A) herbivore and (B) predator species involved in terrestrial tritrophic signaling that are specialists, generalists, or have an unknown diet breadth. (C) Proportion of predators involved in terrestrial tritrophic signaling that are carnivorous or parasitoids. (D) Proportion of experiments testing for tritrophic signaling using laboratory, artificial field, or natural field venues

pared against a 50:50 expected frequency, tritrophic cues in terrestrial settings were not more common for specialist herbivores ($\chi^2 = 1.19$, $p = 0.276$) or predators ($\chi^2 = 0.02$, $p = 0.886$). However, tritrophic interactions were reported more often for studies with parasitoids ($\chi^2 = 5.88$, $p = 0.015$; Fig. 1C, Supplement 1). Only 36% of predators used in experiments were carnivorous, but 64% were parasitoids. Studies were heavily skewed towards laboratory tests, with 86% lab experiments, 11% artificial field experiments, and 3% natural field experiments (Fig. 1D, Supplement 1).

Trapping experiments

Both trapping and video experiments caught predatory animals that typically use chemoreception to locate their prey. These included lobsters (*Panulirus interruptus*), crabs (*Cancer antennarius*, *Portunus xantusii*, and *Loxorhynchus grandis*), fish (*Scorpaena* spp., *Sebastes* spp., *Paralabrax clathratus*, *Embiotoca jacksoni*, and *Paralichthys californicus*), rays (*Urolophus halleri* and *Myliobatis californica*; video only), and whelks (*Kelletia kelletii*; traps only). Traps baited with *Macrocystis* that had been grazed for 2 wk (i.e. LTG) and live herbivores were not more attractive than controls (Fig. 2A, $t = -0.90$, $p = 0.375$; Fig. 2F, $\chi^2 = 0.82$, $p = 0.365$). This pattern persisted when *Macrocystis* was only grazed for 12 to 15 h (STG; STG;

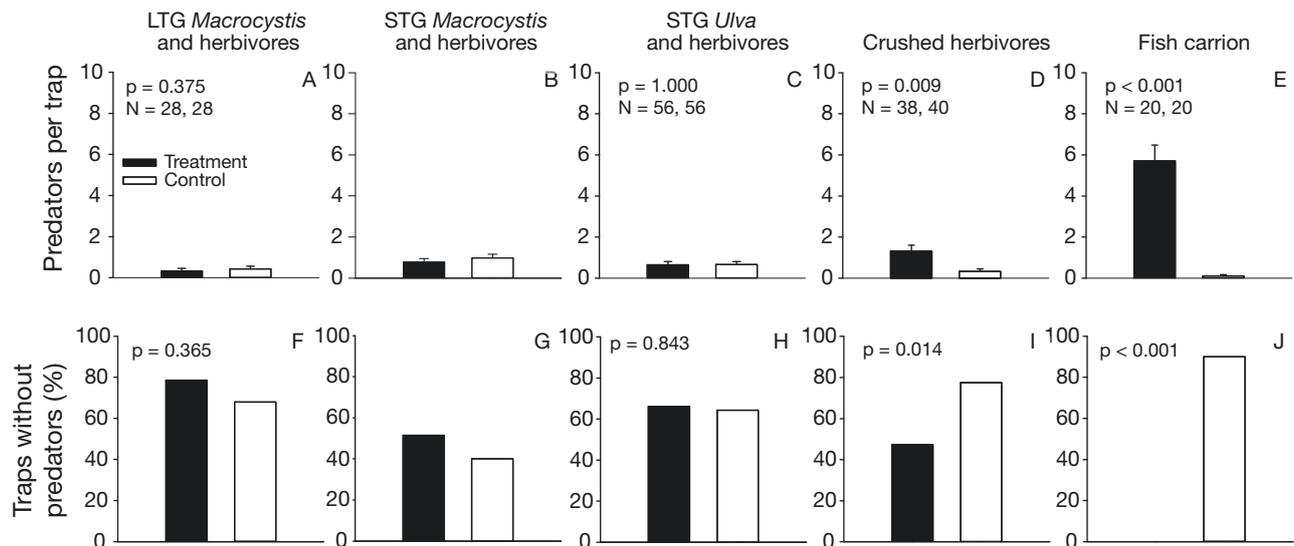


Fig. 2. (A–E) Number of predators caught per trap and (F–J) frequency of empty traps for traps placed *in situ* and baited with (A,F) long-term grazed (LTG) *Macrocystis* and herbivores, (C,H) short-term grazed (STG) *Ulva* and herbivores, (D,I) crushed herbivores, or (E,J) fish carrion, and tested against a control with no cue. (B,G) STG *Macrocystis* was tested against a control of non-grazed (NG) *Macrocystis* and non-grazing herbivores. p-values indicate significant differences as determined by (A–C) paired *t*-tests, (D,E) 2-sample *t*-tests, or (F–J) Pearson chi-square tests. Values are means \pm SE

Fig. 2B, $t = -0.68$, $p = 0.498$; Fig. 2G, $\chi^2 = 0.92$, $p = 0.337$). Traps baited with *Ulva* actively being grazed by herbivores also were not more attractive than controls (Fig. 2C, $t = 0.00$, $p = 1.000$; Fig. 2H, $\chi^2 = 0.04$, $p = 0.843$).

In contrast to traps containing algae and live herbivores, traps baited with crushed herbivores and fish carrion caught more predators than unbaited traps. Traps containing crushed herbivores caught approximately 3 times more predators than unbaited traps (Fig. 3D, $t = 2.57$, $p = 0.010$; Fig. 2I, $\chi^2 = 6.05$, $p = 0.014$), and traps containing fish carrion caught 57 times more predators than unbaited traps (Fig. 2E, $t = -7.20$, $p < 0.001$; Fig. 2J, $\chi^2 = 32.73$, $p < 0.001$). The composition of predator type caught was similar between crushed herbivores and fish carrion traps ($\chi^2 = 7.21$, $p = 0.079$). Of the 46 animals trapped using crushed herbivores, 80% were lobsters, 17% were whelks, and 2% were crabs. Traps baited with fish carrion caught a total of 114 animals, of which 84% were lobsters, 6% were whelks, 5% were crabs, and 4% were fish. No rays were caught in traps, most likely due to their large body size. The success of predator attraction with these treatments suggests that our traps effectively released cues from a bait source that were detectable by predators from a distance.

Across all trapping experiments, catch rates depended upon treatment type (Fig. 4A). Predator catch was higher in traps baited with fish carrion relative to all other treatments, while traps using crushed herbivores as bait caught more predators than LTG *Macrocystis* and STG *Ulva*. Finally, LTG *Macrocystis*, STG *Macrocystis*, STG *Ulva* were all similar in attracting the fewest number of predators to traps.

Underwater video experiments

The results from our video experiments largely agreed with results from our trapping experiments. There were no differences between total predator visits to (Fig. 3A, $t = -1.39$, $p = 0.224$) or amount of time spent near (Fig. 3E, $t = -1.35$, $p = 0.235$) LTG *Macrocystis* treatments compared to controls. Results for STG *Macrocystis* and *Ulva* were similar, with no difference between predator visits (Fig. 3B, $t = -0.76$, $p = 0.480$) or time spent near (Fig. 3F, $t = -0.04$, $p = 0.971$) treatments and controls.

The number of predator visits to crushed herbivore treatments was significantly different from the unbaited controls (Fig. 3C, $t = 3.51$, $p = 0.017$), but time spent near this treatment was not (Fig. 3G, $t = 1.18$,

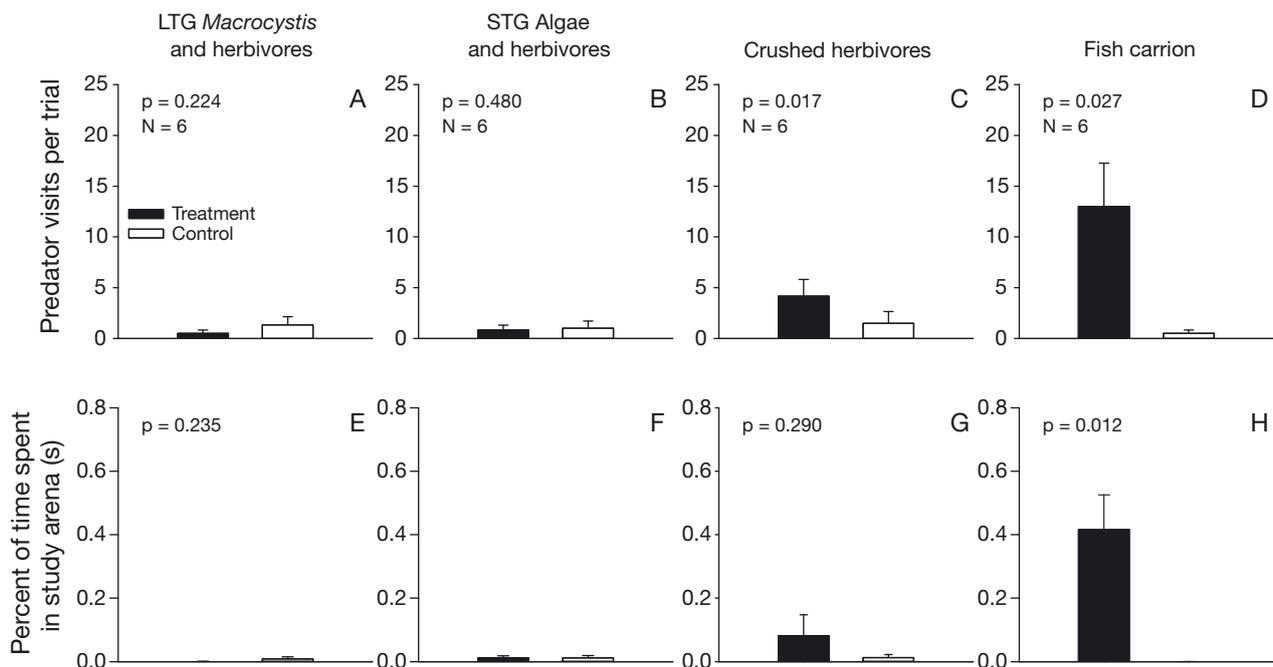


Fig. 3. *In situ* underwater video experiments showing (A–D) number of visits by predators to the study area during video trials, and (E–H) the percent of trial time (s) predators spent in field of view per total trial time. Study areas were baited with (A,E) long-term grazed (LTG) *Macrocystis* and herbivores, (B,F) short-term grazed (STG) *Macrocystis* and *Ulva* (algae) and herbivores, (C,G) crushed herbivores, or (D,H) fish carrion, and tested against no cue controls. p-values indicate significant differences as determined by paired *t*-tests. Values are means \pm SE

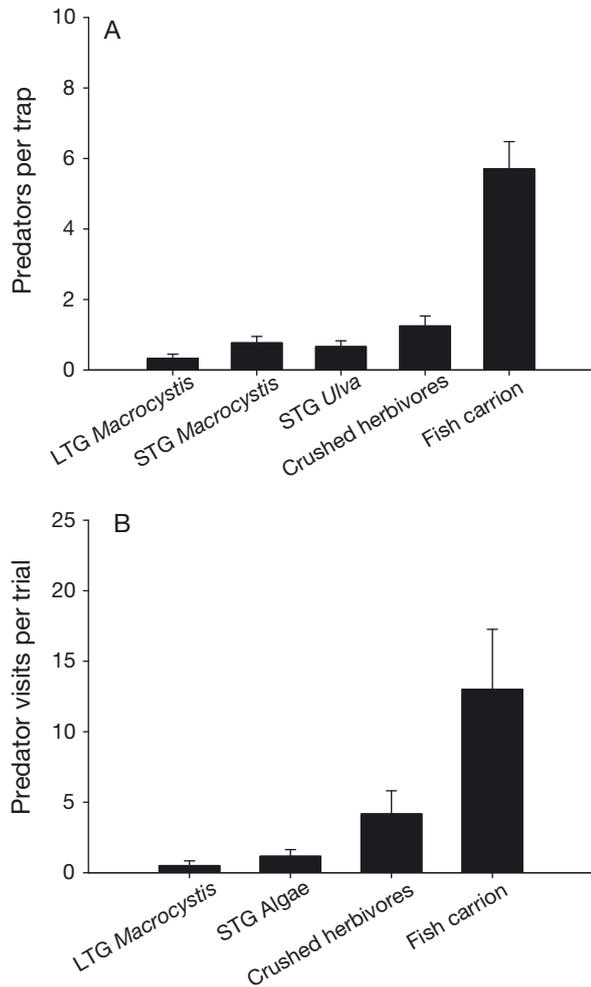


Fig. 4. (A) Standardized number of predators caught per trap during trapping experiments and (B) number of visits by predators to the study area during underwater video trials for treatment bait types only. Treatment values were standardized by transforming predator catch/visits (T) with control values (C) using the formula $T/(C + 1)$ to correct for temporal variability between experiments. Values are means \pm SE

$p = 0.290$). The number of visits to treatments containing fish carrion (Fig. 3D, $t = 3.09$, $p = 0.027$) was significantly different from controls, as was time spent in the field of view (Fig. 3H, $t = 3.81$, $p = 0.012$). Crushed herbivores attracted approximately 3 times more predators than controls, while fish carrion attracted 26 times more predators than controls. The composition of predator types that visited crushed herbivore and fish carrion treatments were similar ($\chi^2 = 2.64$, $p = 0.406$). Of the 25 visits to crushed herbivore bait treatments, 76% were lobsters, 16% were crabs, 4% were fish, and 4% were rays. For fish carrion treatments, of 78 total visits, 64% were lobster, 15% were crab, 4% were fish, and 17% were rays. In

addition to lobsters contributing the most to visitation rates for fish carrion and crushed herbivore treatments, they were also frequently the first to arrive (fish carrion, 67% of first visitors; crushed herbivores, 83% of first visitors), appearing as soon as 23 s into a trial, with an average appearance time of 1198.22 ± 356.15 s. No whelks were recorded entering the field of view for any video trials, most likely due to their relatively slow movement and the short, 1 h trial periods.

Across all video experiments, predator visitation depended upon treatment type (Fig. 4B). Predator visits to fish carrion was higher than all other treatments. Crushed herbivore visitation was higher than LTG *Macrocyctis*, but similar to that of STG algae. LTG *Macrocyctis* and STG algae treatments had the fewest predator visitors.

DISCUSSION

In a natural field setting, kelp forest predators did not exploit tritrophic chemical cues to find live herbivore prey. Yet these same predators were attracted to chemical cues from crushed herbivores and fish carrion—a finding consistent with previous studies (Zimmer-Faust 1993, Zimmer et al. 1999). Fish carrion was qualitatively more attractive than crushed herbivores, which in turn were qualitatively more attractive than all other cues tested. Our results were robust as we observed this same pattern using both trapping and video recording experiments. Importantly, our study is one of only 5 studies in any system (either terrestrial or marine) to test for tritrophic cues in a natural setting in the field.

The discovery that neither chemical cues from live herbivores nor those associated with herbivore grazing attracted kelp forest predators suggests that either (1) these predators use non-chemical cues to find herbivores (e.g. visual or contact), (2) they use herbivore or tritrophic chemical cues across shorter distances than those we could assess with our experiment (<19 cm for our trapping study), or (3) living, mobile herbivores are less important prey for these predators. The present study is unable to distinguish between these different mechanisms. However, the observation that lobsters commonly consume live herbivores (e.g. sea urchin prey; Tegner & Levin 1983, Shears & Babcock 2002) does not necessarily require that lobsters find these herbivores using chemosensory based foraging across long distances.

Our results do not preclude the possibility that other kelp forest species interact via tritrophic cues

because these interactions can be species-specific (Sabelis & van de Baan 1983). For example, when grazed by spider mites *Tetranychus utricae*, apple leaves release volatile blends preferred by predatory mites *Phytoseiulus persimilis*. In contrast, when grazed by a different species of spider mite, *Panonychus ulmi*, apple leaves release different volatile blends that are preferred by the predatory mites *Amblyseius finlandicus* and *A. andersoni* (Sabelis & van de Baan 1983). However, the lack of responses of dominant predators to cues released by dominant grazers on dominant algae suggests that in kelp forests, the ecological importance of these interactions is minor relative to other interaction types.

Although tritrophic cues can be used by marine species (e.g. Coleman et al. 2007), it remains unclear whether these interactions are common or occur under complex field conditions. The strong bias in research effort towards terrestrial systems clearly contributes to this uncertainty. However, 2 important observations suggest that tritrophic cues may be rare in the sea. First, marine systems are dominated by food webs containing generalist predators feeding on generalist consumers, and these food webs appear to have proportionately fewer examples of predators using tritrophic cues (Steidle & van Loon 2003). Using the literature review by Steidle & van Loon (2003), we found 5 times as many examples of tritrophic cues in food webs containing at least one specialist consumer versus those containing only generalist consumers (151 vs. 30, respectively). Although the specific mechanism underlying this pattern is unknown, selection for tritrophic cues may differ between these food webs. Second, seaweeds lack a vascular system that would facilitate a systemic release of tritrophic cues (Lobban & Harrison 1994, Cronin & Hay 1996, Toth & Pavia 2000). In contrast, plants possess efficient systemic transport systems that rapidly carry chemical cues throughout the plant, inducing the release of volatile cues in tissues other than the wound site, thereby maximizing the release of tritrophic cues (Karban & Baldwin 1997, Guerrieri et al. 1999, Hiltbold et al. 2011). This may severely limit the quantity of potential cues released by seaweeds, and diffusion may quickly reduce the scale at which these cues may be useful for foraging predators.

Together, these observations suggest that the dominance of marine food webs by generalists and marine primary producers limited in their ability to systemically release cues may explain the relative rarity of tritrophic cues in the sea. However, the clear bias in research effort makes such conclusions premature.

We encourage additional field studies that test for these interactions in marine settings, particularly for food webs containing specialist consumers and marine vascular plants.

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