CHEMICALLY-MEDIATED FORAGING BY SUBTIDAL MARINE

PREDATORS: A FIELD TEST OF TRITROPHIC SIGNALING

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A Thesis

Presented to the

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Master of Science in Biology

with a Concentration in

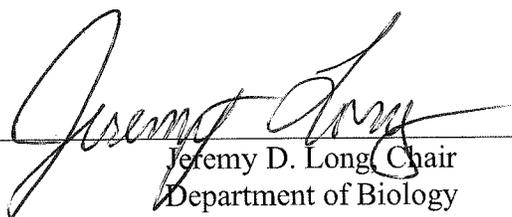Ecology

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by

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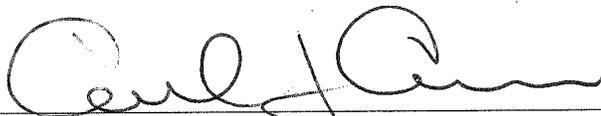
Tritrophic Signaling

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ABSTRACT OF THE THESIS

Chemically-Mediated Foraging by Subtidal Marine Predators: A
Field Test of Tritrophic Signaling
by
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Although predators can use waterborne chemical cues to find dead or damaged prey, the usefulness of distance chemoreception for finding living prey is poorly known in marine systems. In contrast, terrestrial predators like predatory insects and parasitoids commonly use volatile plant compounds released during grazing to find herbivorous prey (i.e. tritrophic signaling). Because few studies have tested for such interactions in marine organisms, it is unclear if marine predators commonly use these fluid-borne cues to find living, herbivorous prey, especially under more realistic settings. Such interactions might occur frequently in the ocean because marine predators often use chemosensory-based foraging, and herbivore-induced chemical responses are common in some algae (especially the Phaeophyceae). To examine the ability of predators to find herbivores using waterborne chemical cues released by grazed algae, we examined the response of marine predators to several bait types using *in situ* trapping and video experiments. These experiments 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) injured herbivores, (3) fresh carrion, (positive control), or (4) negative controls. The majority of predators caught or observed were spiny lobsters. In both trapping and video experiments, actively grazed algae with live herbivores failed to attract predators relative to controls, suggesting that under realistic field conditions, these predators do not use cues from these sources to find living herbivores. Consistent with previous studies, injured herbivores and fresh carrion were highly attractive, with similar predator species contributing to the highest catch rates, visitation rates, and time spent near these treatments. Thus, chemical cues associated with live herbivores, *Macrocystis,* and *Ulva* have little influence on predator foraging strategies, especially relative to cues released from more potent or valuable food sources.

Keywords**:** tritrophic interactions, chemically-mediated, predator foraging, subtidal

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INTRODUCTION

Chemical cues are important mediators of predator-prey interactions in marine communities (Zimmer & Butman 2000, Hay 2009). However, most studies have examined predator responses to cues released by injured or dead prey such as amino acids, sugars, organic acids, nucleotides, or ATP (Zimmer-Faust 1993, Carr et al. 1996, Zimmer et al. 1999, Zimmer & Butman 2000). The relevance of these cues to interactions between predators and live prey is unknown, but there is some suggestion that their release is dramatically suppressed in living, uninjured prey (Zimmer-Faust & Case 1982, Zimmer et al. 1999, Brewer & Konar 2005). Such a result is not surprising given that prey should be under strong selection pressure to avoid the release of chemicals that could be used as predator foraging cues (Dicke et al. 1990, Vet & Dicke 1992). Evidence from terrestrial systems suggests that predatory insects and parasitoids may find live, uninjured herbivores using volatile chemical cues released by plants under grazer attack (i.e. tritrophic signaling; Stowe et al. 1995, Heil 2007). Tests of such interactions are rare in marine settings.

Terrestrial arthropods can find prey using airborne pheromones (Raffs & Dahlsten 1995, Stowe et al. 1995) or herbivore-induced plant volatiles (HIPVs; Heil 2007). HIPVs are released by plants during grazing, and are particularly well known for their ability to influence predator foraging, as well as acting as an indirect defense mechanism for plants (Heil 2007). For example, when given a choice between airborne cues from lima bean plants with or without herbivorous mites, carnivorous mites preferred airborne cues from plants with herbivorous mites (Koveos et al. 1995).

In contrast to terrestrial studies, such interactions appear rare in the sea. However, recent evidence suggests that predator use of algal cues could be widespread in marine systems. For example, grazing-induced release of dimethyl sulphide (DMS) by pelagic phytoplankton is used as a foraging cue by procellariform birds, African penguins, and carangid fishes (Nevitt et al. 1995, DeBose & Nevitt 2007, Wright et al. 2011). Additionally, grazed *Ascophyllum nodosum*, a brown alga, is more attractive to fish and crab predators compared to ungrazed controls, presumably because it provides cues for predators to find snail prey (Coleman et al. 2007).

The study conducted by Coleman et al. (2007) was the first to directly demonstrate predator preferences for waterborne cues released from macroalgae being actively grazed. Similar to many terrestrial studies, this initial assessment of this type of interaction was examined under carefully controlled conditions in a laboratory choice arena (Turlings et al. 1990, Koveos et al. 1995). Given the importance of fluid dynamics in mediating chemosensory-based foraging (Weissburg & Zimmer-Faust 1993, Ferner & Weissburg 2005), additional studies are needed under more realistic flow regimes (e.g. Zimmer-Faust & Case 1982, Zimmer-Faust et al. 1995, Zimmer et al. 1999, Finelli et al. 2000, Brewer & Konar 2005).

Given that marine predators commonly find prey via chemical cues (Weissberg et al. 2002) and algae often respond to grazers with inducible chemical resistance (Toth & Pavia 2000, Rhode et al. 2004, Toth 2007), tritrophic signaling should be common 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.). Predators might be attracted to grazing-associated cues in *Macrocystis* because this alga belongs to an algal division (Phaeophyceae) where inducible chemical responses are common (Pavia & Toth 2000, Toth & Pavia 2000, Rhode et al. 2004, Toth 2007). Similarly, predators might be attracted to grazed *Ulva* because this alga releases DMS upon herbivore attack, and predatory organisms commonly use this volatile chemical (Nevitt et al. 1995, Van Alstyne et al. 2001, Debose & Nevitt 2007). We examined predator attraction to these cues using two approaches, *in situ* trapping, and underwater video recordings.

METHODS

STUDY SPECIES AND SITES

In southern California subtidal communities, algae co-occur with several herbivores and predators with well-described chemosensory-based foraging abilities. To elicit the release of grazing-associated cues, we exposed live algae to their most commonly encountered invertebrate herbivores (Byrnes et al. 2011). *Macrocystis pyrifera* was fed to kelp snails, *Norrisia norrisi,* and purple urchins, *Strongylocentrotus purpuratu;,* and *Ulva* sp. was fed to turban snails, *Chlorostoma (Tegula) aureotincta,* and purple urchins. Algae and herbivores were collected from three 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, benthic traps contained grazed algae with herbivores (treatment) or lacked bait (controls). Mesh bait bags (2 mm^2 pore size) containing treatments were secured in a bait cage (25 x 11 x 12 cm) in the center of a PVC coated crab trap (65 x 49 x 25 cm; 3.8 cm^2 mesh size) with two trap door entrances (16 x 8 cm). The 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 prevented predator access to visual cues. Traps were deployed during the day and retrieved during peak foraging hours of common benthic predators (e.g. lobsters; Lachlan et

al. 2008, Weiss et al. 2008). Traps were retrieved approximately 15 hours after deployment and 3-4 hours before sunrise to avoid trapping during periods of high aggregation (e.g. in lobsters; Zimmer-Faust et al. 1985). Following identification and measurement, all trapped animals were 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.

Short-Term Induction of *Macrocystis pyrifera*

Similar to terrestrial plants, algae may respond differently to varying length of exposure to grazing pressure. For example, the molecular composition or concentration of cues released by plant tissue may differ between an immediate response to grazing (e.g. minutes) and a response to consistent grazing pressure over an extended period (e.g. weeks). This results because heavy grazing pressure elicits inducible chemical cues that become stronger over time and differ from those always present in plant tissues (Turlings et al. 1990, Pavia & Toth 2000). Thus, we tested for potential differences in predator attraction to *Macrocystis* exposed to both short- or long-term grazing periods. To produce short-term grazing (STG) cues, *Macrocystis* was exposed to herbivore grazing for 12-18 hours. These treatments were prepared by placing *Macrocystis* blades (20 ± 1 g) in a mesh bag with two kelp snails and two purple urchins (that were previously starved for 1-2 days) in flow-through seawater tanks overnight (N=35). Before field deployment, herbivores in grazed treatments were replaced with the same amount of new, starved (1-2 days) conspecifics, so that algae grazing would continue in the field without being limited by herbivore satiation.

For controls, we placed non-grazed (NG) *Macrocystis* in traps with live non-feeding herbivores, separating the algae and herbivores into two compartments to prevent grazing during deployment (N=35). The purpose of this control was to isolate the effects of grazing-associated cues from ambient algal and herbivore cues when compared simultaneously to grazed algal treatments. However, for the remaining experiments described below, the control traps were unbaited to maximize the probability of detecting a treatment bait effect.

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

Long-Term Induction of *Macrocystis pyrifera*

To produce long-term grazing (LTG) cues, *Macrocystis* was grazed for two weeks; a grazing period commonly used to trigger algae to induce chemical defenses (Borell et al. 2004, Toth et al. 2005, Pavia & Toth 2000). These blades were grown in outdoor mesocosms with two kelp snails and two purple urchins (N=28). Mesocosms (35 x 21 x 13 cm) containing treatments were supplied with individual flow-through seawater. After two weeks, *Macrocystis* (10 ± 1 g) was placed in mesh bags with the same number of new, starved (for 1-2 days) conspecific herbivores, and inserted into traps immediately before deployment. Treatment attractiveness was compared to a control of an unbaited trap. Traps were deployed in the Point Loma kelp forest over three separate deployment periods (September 2011) in the same way as STG *Macrocystis* trials.

Short-Term Induction of *Ulva* sp.

We used similar techniques described above to test the attractiveness of *Ulva* when grazed by purple urchins and turban snails. Because DMS release was expected to be immediate upon herbivore grazing damage (Van Alstyne et al. 2009), only short-term grazed (STG) *Ulva* responses were tested with no overnight grazing period before deployment. This differed from STG *Macrocystis*, in that STG *Macrocystis* was exposed to grazing prior to deployment. On the day of deployment, *Ulva* (10 ± 1 g), two purple urchins, and four turban snails (starved for 2-3 days) were placed in mesh bags and immediately inserted into traps (N=56). Treatment attractiveness was compared to a control of an unbaited trap (N=56). Traps were deployed in the Mission Bay Channel, which contains the same community of organisms as the Point Loma kelp forest. Traps were randomly deployed in 6-7.5 m of water in rocky reef kelp habitat over three separate deployment periods (August-September 2011). Treatment and control traps were paired and separated by 50 meters.

Injured Herbivores and Fresh Carrion

Predators may be attracted to chemical signals from injured prey, perhaps because these cues indicate prey vulnerability or that cues from these sources provide a stronger concentration gradient to follow (Zimmer-Faust & Case 1982, Zimmer et al. 1999, Brewer & Konar 2005). To test this hypothesis, we conducted a trapping experiment with treatments containing injured herbivores using similar methods described above. Two purple urchins and four turban snails were placed in mesh bags and injured by crushing them with a hammer immediately before placement in traps. Treatment and unbaited control traps were deployed randomly in the Mission Bay Channel over two separate deployment periods (February 2012,

N=40). During the deployment periods, two treatment traps were lost (buoys vandalized), reducing the treatment replicate size (N=38).

As a positive control, we conducted a trapping experiment comparing the attraction of fresh fish carrion and unbaited controls. Freshly thawed Pacific sardines (*Sardinops sagax caerulea*) were placed within mesh bags in traps (N=20) and deployed in the Mission Bay Channel over a single deployment period (February 2012) in the same manner as injured herbivores. Fresh carrion attractiveness was compared to unbaited control traps (N=20).

UNDERWATER VIDEO EXPERIMENTS

Because experiments using traps to examine bait attractiveness may create bias (e.g. 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, Figure 1).

QCS System

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 (Figure 1A). Light was supplied by two 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 (Zimmer-Faust et al. 1996, Weiss et al. 2006). A bait cage (24 x 12 x 9 cm; 0.635 cm^2 mesh opening) was secured to the bottom of the quadrapod, 28 cm from the camera. The area of the field of view was 36 x 48 cm (Figure 1B).

Figure 1. (A) Quadrapod camera system (QCS) for *in situ* continuous recording of predator behavior. (B) View of study area taken from video camera placed above bait box.

Video Trials

Video trials tested the attractiveness of the same treatments used in trapping experiments; (1) LTG *Macrocystis,* (2) STG *Macrocystis* and *Ulva*, (3) injured herbivores, and (4) fresh 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. Two QCS units were deployed in a single trial, a treatment and a control. QCS units were randomly deployed 50 m apart in 6-8 m of water in the Mission Bay Channel at night during peak predator foraging (21:00-24:00), and retrieved after an hour of continuous recording. Deployment sites were located in the same areas as trapping sites, however for video trials, we specifically targeted the flat, sandy substrate immediately adjacent to kelp habitat for maximum visibility during video analysis. Following QSC trials, videos were analyzed and predator behavior was recorded. We recorded (1) the number of times each predator species entered the study area (= predator visit) and (2) the duration of each visit. Because predators were not individually identifiable, we assumed that predators entered and re-entered the study area multiple times. Thus, "predator visits" were used as our measure of treatment attractiveness. The duration of each visit was divided by the total trial length and reported as proportional time spent in the study area (duration of visit/total trial time). These experiments took place between June and August 2012.

STATISTICAL ANALYSES

For trapping experiments, differences in predator catch per trap were compared between treatments and controls using two-tailed, paired t-tests for *Macrocystis* and *Ulva* trials (paired trap deployments), and two-tailed, two-sample t-tests for injured herbivore and fresh carrion trials (unpaired trap deployments). Differences in the number of traps without predators were compared using Pearson chi-square analyses for all trapping experiments. For the underwater video experiments, predator visits and proportional time spent in the study area (duration of visit/total trial time) were compared using paired t-tests. The composition of predator species captured with injured herbivore and fresh carrion treatments were compared between treatments for both trapping video experiments using Pearson chi-square analyses.

Between-treatment differences in predator catch and predator visits were also compared within trapping and underwater video experiments, respectively, using a one-way fixed factor PERMANOVA, followed by pair-wise permutation post hoc tests if significant differences were found. This was done statistically rather than experimentally because treatments were not compared against one another at the same time in the field. 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. Because treatments and control replicates in the fresh carrion trapping experiments were unpaired and balanced, control replicates were randomly paired with treatments to calculate the ratio. For injured herbivore trapping treatments where treatments and controls were unpaired and unbalanced (two treatment

replicates lost), an equal amount of control replicates were excluded (two), and the remaining

controls were randomly paired with treatments.

RESULTS

TRAPPING EXPERIMENTS

Trapping experiments caught predatory animals that typically use chemotaxis to locate their prey (Zimmer-Faust et al. 1996, Ferner & Weissburg 2005). These included lobsters (*Panulirus interruptus*), crabs (*Cancer antennarius, Portunus xantusii, Loxorhynchus grandis*), fish (*Scorpaena* spp., *Sebastes* spp., *Paralabrax clathratus, Embiotoca jacksoni, Paralichthys californicus*), rays (*Urolophus halleri, Myliobatis californica*), and whelks (*Kelletia kelletii*). Traps baited with long-term (2 weeks) grazed (LTG) algal cues from *Macrocystis* and live herbivores (Figure 2) were not more attractive than controls (Figure 2A, t=-0.90, p=0.375; Figure 2F, x^2=0.82, p=0.365). This pattern persisted when *Macrocystis* was grazed for a short-term (STG, 12-15 hrs, Figure 2) period relative to non-grazed *Macrocystis* controls (Figure 2B, t=-0.68, p=0.498; Figure 2G, x^2=0.92, p=0.337). Baiting traps with short-term grazed *Ulva* and living, uninjured herbivores (Figure 2) also did not enhance predator catch relative to controls (Figure 2C, t=0.00, p=1.000; Figure 2H, x^2=0.04, p=0.843).

In contrast to the algal treatments, injured herbivores and fresh carrion were highly attractive to predators, resulting in significantly higher predator catch rates than unbaited traps (Figure 2). Injured herbivores caught approximately 3 times more predators than unbaited traps (Figure 2D, t=2.57, p=0.010; Figure 2I, x^2=6.05, p=0.014), and fresh carrion caught 57 times more predators than unbaited traps (Figure 2E, t=-7.20, p<0.001; Figure 2J, x^2=32.73, p<0.001). The composition of predator type caught was similar between injured herbivores and fresh carrion treatments (x^2=7.21, p=0.079). Of the 46 animals trapped using
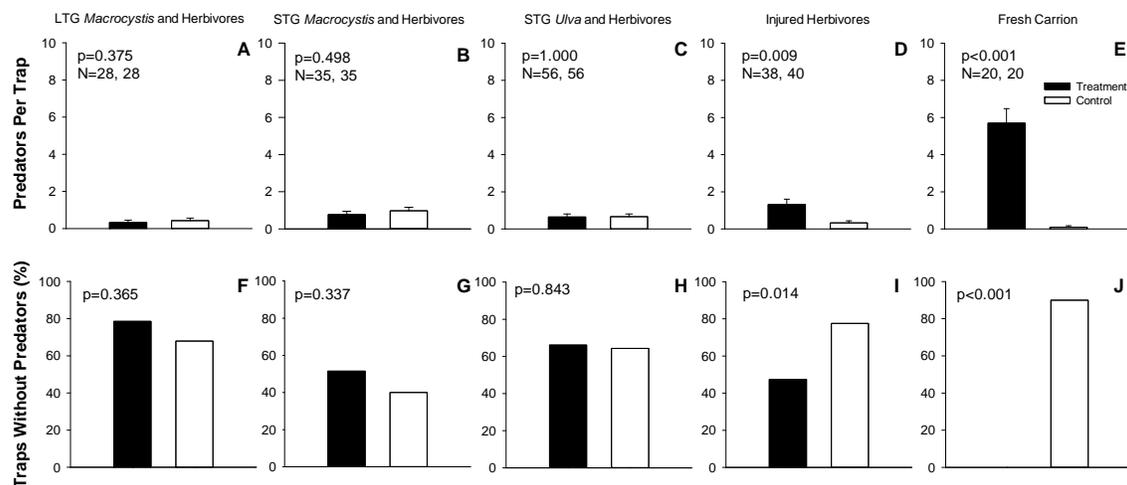
Figure 2. Number of predators caught per trap (A-E, mean ± SE) and frequency of empty traps (E-J) for traps placed *in situ* and baited with long-term grazed (LTG) *Macrocystis* and herbivores (A, F), short-term grazed (STG) *Ulva* and herbivores (C, H), injured herbivores (D, I), or fresh carrion (E, J), and tested against a control with no cue. STG *Macrocystis* (B, G) was tested against a control of non-grazed (NG) *Macrocystis* and non-grazing herbivores. *P*-values indicate significant differences as determined by paired t-tests (A-C), two-sample t-tests (E, D), or Pearson chi-square tests (F-J).

injured herbivores, 80% were lobsters, 2% were crabs, and 17% were whelks. Traps baited with fresh carrion caught a total of 114 animals, of which 84% were lobsters, 5% were crabs, 4% were fish, and 6% were whelks. 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.

UNDERWATER VIDEO EXPERIMENTS

Video experiments demonstrated similar results in treatment attractiveness as trapping experiments (Figure 3). There were no differences between total predator visits to (Figure 3A, t=-1.39, p=0.224) or amount of time spent near (Figure 3E, t=-1.35, p=0.235) LTG *Macrocystis* treatments compared to controls. Results for STG *Macrocystis* and *Ulva*
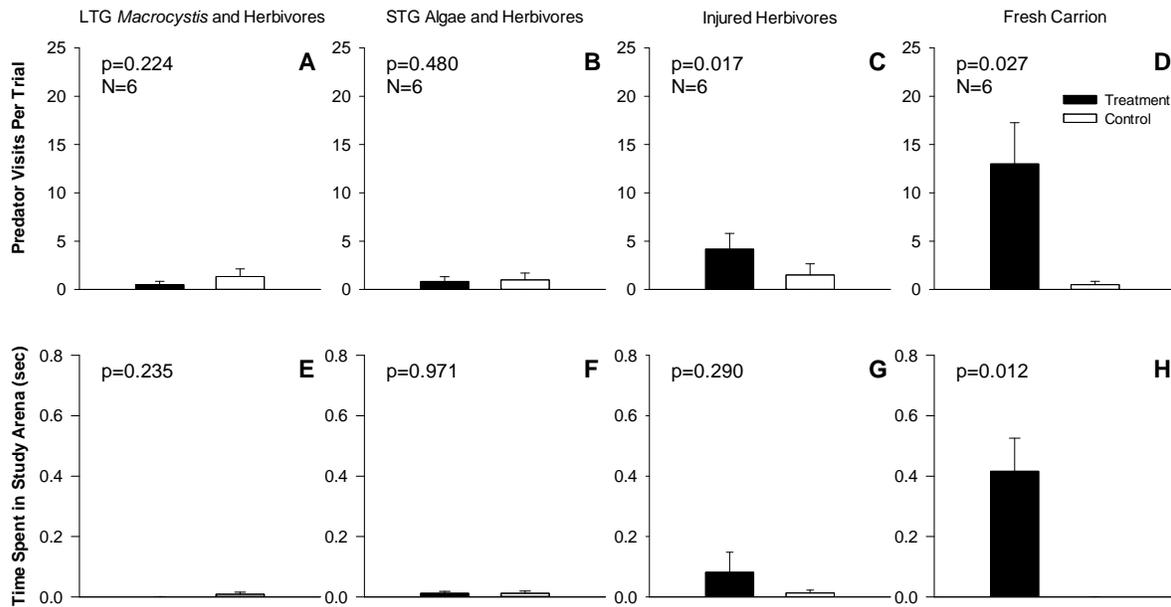
Figure 3. Number of visits by predators to the study area (A-D, mean ± SE) during video trials and time (sec) spent in the study area (E-H, mean ±SE) per total trial time, for *in situ* underwater video experiments. Study areas were baited with long-term grazed (LTG *Macrocystis* and herbivores (A, E), short-term grazed (STG) *Macrocystis* and *Ulva* (algae) and herbivores (B, F), injured herbivores (C, G), or fresh carrion (D, H), and tested against no cue controls. *P*-values indicate significant differences as determined by paired t-tests.

were similar, with no difference between predator visits (Figure 3B, t=-0.76, p=0.480) or time spent in the study area (Figure 3F, t=-0.04, p=0.971) between treatments and controls.

The number of predator visits to injured herbivore treatments was significantly different from the unbaited controls (Figure 3C, t=3.51, p=0.017), but time spent near this treatment was not (Figure 3G, t=1.18, p=0.290). For treatments containing fresh carrion, number of visits (Figure 3D, t=3.09, p=0.027) were significantly different from controls, as was time spent in the study area (Figure 3H, t=3.81, p=0.012). Injured herbivores attracted approximately 3 times more predators than controls, while fresh carrion attracted 26 times more predators than controls. The composition of predator types that visited injured herbivore and fresh carrion treatments were similar (x^2=2.64, p=0.406). Of the 25 visits to

injured herbivore bait treatments, 76% were lobsters, 16% were crabs, 4% were fish, and 4% were rays. For fresh carrion bait 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 carrion and injured herbivore treatments, they were also frequently the first to arrive (fresh carrion, 67% of first visitors; injured herbivores, 83% of first visitors), appearing as soon as 23 seconds into a trial, with an average appearance time of 1198.22 ± 356.15 seconds. No whelks were recorded entering the study area for any video trials, most likely due to the short, 1-hour trial periods and their relatively slow movement.

BETWEEN TREATMENT COMPARISONS

When comparisons were made between all trapping experiment treatments (Figure 4), catch rates were found to depend upon treatment type (Figure- 4A, pseudo-$F_{4,171}=34.45$, p=0.001). Predator catch was significantly higher in traps baited with fresh carrion relative to all other treatments, while traps using injured herbivores as bait caught significantly more predators than LTG *Macrocystis* and STG *Ulva*. LTG *Macrocystis*, STG *Macrocystis*, STG *Ulva* were all similar in attracting the fewest number of predators to traps.

Predator visitation was also compared between all treatments (Figure 4) for underwater video experiments, and like trapping experiment treatments, was found to be significantly different between treatment type (Figure 4B, pseudo-$F_{3,20}=15.31$, p=0.001). Predator visits to fresh carrion was significantly higher than all other treatments. Injured herbivore visitation was significantly higher than LTG *Macrocystis*, but similar to that of STG algae. LTG *Macrocystis* and STG algae were similar in attracting the lowest amount of predator visitors.
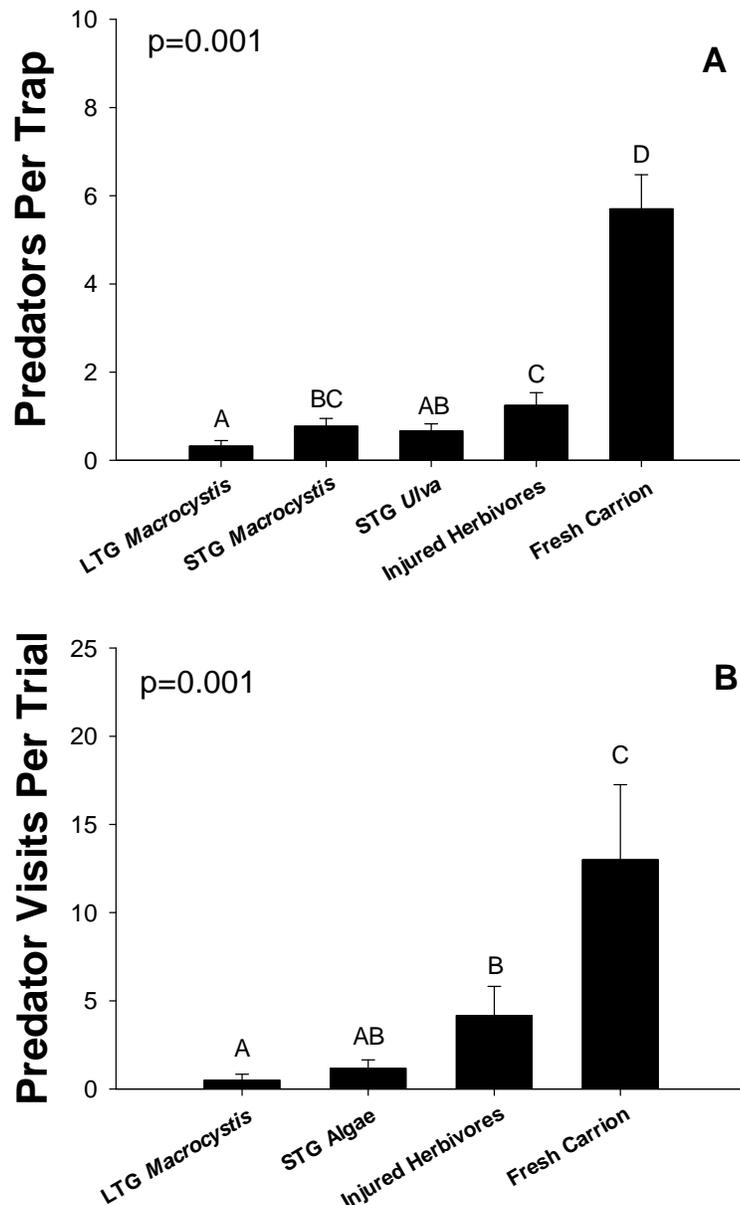
Figure 4. Number of predators caught per trap during trapping experiments (A, mean ± SE) and number of visits by predators to the study area (B, mean ± SE) during underwater video trials for treatment bait types only. *P*-values indicate significant differences as determined by one-way fixed factor PERMANOVAs. Letters indicate significant differences.

DISCUSSION

Unlike terrestrial systems where tritrophic signaling appears common, chemical cues from living, uninjured herbivores and grazed algae did not attract dominant kelp forest predators in the field. In contrast, chemical cues from injured herbivores were significantly attractive to these same predators. Consistent with previous studies, chemical cues from fresh carrion were more attractive than injured herbivores, which in turn were more attractive than all other cues tested. Our results were robust as we observed this same hierarchy using both trapping and video recording experiments. Thus, it appears that kelp forest predators do not exploit tritrophic signals to find live herbivore prey.

As a result, kelp forest predators must either use other cues to find living herbivore prey (e.g. visual or contact), or living herbivores are unimportant prey types for these predators. Although historical documentation, field and lab observations, and gut content analyses provide evidence that predators, such as lobsters, consume live, uninjured prey like sea urchins (Tegner & Dayton 1981, Tegner & Levin 1983, Castaneda-Fernandez-De-Lara et al. 2005), predation on these herbivores may be limited relative to other prey types, perhaps because of strong predator avoidance behaviors of these prey to these predators (Matassa 2010). As further evidence of this hypothesis, urchins and lobsters frequently occupy the same dens (pers. obs.). While we did not see evidence of tritrophic signaling using abundant and ecologically important organisms, it is possible that other kelp forest species interact this way. Indeed, tritrophic signaling in terrestrial settings can be species-specific (Heil 2007). 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.

Tritrophic signaling might be predicted to be rare in kelp forests because of the dominance of generalist consumers. In terrestrial communities, tritrophic signaling is most effective in food chains containing specialist consumers - both herbivores and predators (Vet & Dicke 1992, Steidle & van Loon 2003, Heil 2007). Although there are exceptions where generalists are capable of learning the association between specific plant cues and prey, host plants being consumed by specialist herbivores release specific cues that are more frequently used by specialist predators. In fact, some specialist predators become dependent on these cues, forming mutualistic relationships with plants (Heil 2007). Because specialist herbivores can circumnavigate the direct chemical defenses of plants (Krieger et al. 1971), tritrophic signaling also works as a form of indirect defense for plants unable to reduce grazing directly (Dicke & Sabelis 1988, Dicke et al. 1990, Turlings et al. 1990, Heil 2007). The absence of dominant specialist consumers in kelp forests may prevent the evolution of this foraging strategy in kelp forest predators. More broadly, one might predict tritrophic signaling to occur less frequently in the ocean compared to land given the relative lack of specialist consumers in the ocean. If feeding mode (specialist vs. generalist) is critical to these interactions, then examples from the sea might center around food chains containing specialists. Although specialist predators are rare in the sea, specialist primary consumers are well-known (e.g. predatory whelks that prefer barnacles or mussels, or mesograzers that

specialize on chemically defended algae). To test the relevance of feeding mode to tritrophic signaling, future marine studies should consider focusing on habitats containing specialist consumers.

Additionally, tritrophic signaling might be found in food chains containing plants or algae displaying inducible chemical resistance (e.g. an induced chemical defense that lowers an alga's palatability). For example, grazing-associated volatiles from terrestrial plants that are attractive to predators can also suppress direct grazing by herbivores (Pare & Tumlinson 1999, Kessler & Baldwin 2001, Heil 2004). We predicted that kelp forest predators might use similar cues because *Macrocystis* is a member of the Phaeophyceae that (1) is the best-studied algal division for inducible resistance and (2) contains *Ascophyllum nodosum*, one of the only algae known to be involved in tritrophic signaling (Coleman et al. 2007). Similarly, we predicted that these predators would use cues from *Ulva* because *Ulva* damage converts DMSP to two feeding deterrents, DMS and acrylic acid (Van Alstyne et al. 2009), and DMS is a potent olfactory cue for a variety of predators (Nevitt et al. 1995, DeBose & Nevitt 2007, Wright et al. 2011). Although it is unknown whether these species produce inducible defenses in response to herbivory, herbivores readily consumed these algae after both long- and short- term exposure to grazing (unpublished data). This suggests that *Ulva* and *Macrocystis* do not produce chemicals to directly deter herbivory, and thus may have no link between direct defense and tritrophic signaling.

While knowledge of tritrophic interactions in terrestrial settings continues to advance, studies in aquatic ecosystems are still lacking. This study represents one of the first attempts to test tritrophic signaling under realistic conditions in marine communities. Although we found no evidence supporting tritrophic signaling in our system, systems containing both

specialized consumers and chemically defended algae (e.g. the tropics) may be more likely to display this interaction. In an environment where chemically-driven mechanisms are essential to mediating basic life processes, the ability of predators to utilize algal cues may be more common in the ocean than currently known (Hay 2009). More studies examining these unknown trophic links will allow us to gain a better understanding of how predators find their herbivorous prey, and the role that these interactions play in influencing overall marine community structure.

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