Limnol. Oceanogr., 50(1), 2005, 354–362

A secondary chemical cue facilitates juvenile–adult postsettlement associations in red sea urchins

Michael T. Nishizaki[1] *and Josef Daniel Ackerman*[2]

Physical Ecology Laboratory, University of Northern British Columbia, Prince George, British Columbia V2N 4Z9, Canada; Bamfield Marine Sciences Centre, Bamfield, British Columbia V0R 1B0, Canada

Abstract

Responses to predator odors or chemical alarm cues or both from conspecifics in aquatic systems generally involve a single chemical cue. We report a secondary chemical cue, released by adult red sea urchins after they detect primary chemical cues from a predatory sea star. This secondary cue, which is detected by juvenile urchins, leads to the aggregation of juveniles underneath adults for protection. In choice experiments, juvenile *Strongylocentrotus franciscanus* moved toward adults in response to a chemical cue produced by adults held downstream of a predatory sea star (*Pycnopodia helianthoides*), but showed no such response to predators presented in the absence of adults or when adults were held upstream of predators. Furthermore, this response was size dependent and not symmetrical, since larger urchins did not respond to the secondary cue. This secondary chemical cue system may confer a selective advantage for juveniles, allowing them to balance risk of predation versus competition with adults. This result underscores the significance of postsettlement processes in the recruitment of mobile benthic invertebrates, which in the case of red sea urchins involves a unique behavioral strategy.

The spatial abundance and distribution of many benthic marine organisms is patchy, and the cause of such variation is not known (Menge 2000). Trophic interactions (Paine 2002), fertilization success in broadcast spawners (Levitan et al. 1992), and variation in larval settlement (Gaines and Roughgarden 1985) may all contribute to this pattern. Although factors affecting early postsettlement juveniles (e.g., predation, competition, disturbance) have been recently identified as potential causes of variation especially in sessile benthic invertebrates such as barnacles, bivalves, and ascidians (*see* Gosselin and Qian 1997; Hunt and Scheibling 1997), the extent to which these conclusions can be generalized to mobile organisms remains to be determined (Menge 2000). For example, juvenile red sea urchins (*Strongylocentrotus franciscanus*) are limited to habitats under the spine canopy of adult urchins (Low 1975; Tegner and Dayton 1977) even though significant genetic variation exists between juveniles and the adults under whom they shelter (Moberg and Burton 2000). Attempts to explain this pattern of juvenile recruitment have focused on three possible mechanisms: (1) larval supply; (2) preferential larval settlement; and (3) postsettlement processes (Cameron and Schroeter 1980). Although larval supply is an important factor in sea urchin recruitment (Miller and Emlet 1997), it does not explain increased juvenile recruitment under adults. Similarly, differential larval settlement does not lead to higher juvenile recruitment under adult urchins in that *S. franciscanus* larvae do not settle in response to any adult-associated cues (Cameron and Schroeter 1980). Conversely, postsettlement processes (i.e., nutrition, predation, fluid dynamics) are likely to be important in recruitment dynamics. In the case of *S. franciscanus*, there does not appear to be a nutritional advantage for juveniles in the aforementioned juvenile–adult associations (Nishizaki and Ackerman 2004); however, protection from predation and fluid dynamics have not been examined. Consequently, an investigation of postsettlement processes for sea urchins, as representative mobile organisms, is warranted.

Predation is among the most well known postsettlement processes (Hunt and Scheibling 1997), and responses to predators in mobile marine organisms include (1) fleeing; (2) aggregation; and (3) refuge use (Witman 1985; Mauck and Harkless 2001). Such strategies, however, may incur a cost in the form of lower access/use of resources (Sih 1980), which in *S. franciscanus* has been attributed to increased competition for food between sheltering juveniles and adults (Nishizaki and Ackerman 2004). Thus, it would be a selective advantage for juvenile sea urchins to assess the need to shelter in an expeditious and accurate manner that optimally balances potential risks (i.e., predation) against potential gains (i.e., growth) (Sih 1980). For many benthic marine organisms, visual cues may be undependable when light is limited, and tactile cues, though more reliable, may not allow an organism to detect a predator over large distances. Conversely, chemical cues transported via advection and diffusion (Zimmer and Butman 2000) may be more reliable while providing advance warning of danger.

---

[1] Present address: Faculty of Environmental Sciences and Departments of Botany and Zoology, University of Guelph, Guelph, Ontario N1G 2W1, Canada.

[2] To whom correspondence should be addressed. Present address: Faculty of Environmental Sciences, University of Guelph, Guelph, Ontario N1G 2W1, Canada (ackerman@uoguelph.ca).

Acknowledgments

We thank the director and staff of the Bamfield Marine Sciences Centre for providing facilities; P. Moore, A. Spencer, M. Loewen, and M. Blouw for commenting on the manuscript; M. Loewen and B. Asher for assistance in developing the PIV system, and several anonymous reviewers for their suggestions. This research was supported in part by Island Scallops, the Pacific Red Urchin Harvesters Association, a Western Canadian Universities Marine Biological Society graduate award to M.T.N., Fisheries Renewal British Columbia, Natural Sciences and Engineering Research Council of Canada, and the Canada Research Chair Program to J.D.A.

Differences in defensive strategies between juvenile and adult red sea urchins, namely, sheltering under adults in the former (Low 1975; Tegner and Dayton 1977) and fleeing in the latter (*see* Chivers and Smith 1998), provide an opportunity to examine the role of postsettlement behavior in juvenile *S. franciscanus*. In this study, we address two issues: (1) whether juvenile *S. franciscanus* use chemical cues to locate the protective shelter of adults and (2) whether there are ontogenetic changes in this postsettlement behavior.

Materials and methods

Adult *S. franciscanus* (A. Agassiz, 1863) (94 ± 3 mm [mean ± 1 SE] test diameter) were collected by self-contained underwater breathing apparatus (SCUBA) from Barkley Sound, British Columbia, Canada, and juveniles (7.1 ± 0.3 mm test diameter) were obtained from an experimental hatchery (Island Scallops). Adults and juveniles were maintained separately for up to 3 months at the Bamfield Marine Sciences Centre, British Columbia, Canada, in unfiltered seawater drawn from a 25-m depth (temperature = 8.80°C to 13.20°C, salinity = 25.93 to 34.66, pH 7.50 to 8.30, dissolved oxygen = 43.20% to 100% saturation). Urchins were fed kelp (*Macrocystis integrifolia*) and starved for a minimum of 2 weeks before experiments.

Response to chemical cues—A Y-shaped maze, constructed of clear Plexiglas (Fig. 1), was used to examine whether juveniles moved in response to various chemical cues. Water was supplied directly into the center of the left and right arms of the Y maze, 2 cm from the bottom via 2.5-mm diameter flexible hosing regulated with hose clamps. The maze was filled to a depth of 3.5 cm, and the flow conditions were unidirectional with a mean velocity, measured by timed dye releases, in the main arm of 3.3 ± 0.2 cm s^{-1} for all juvenile treatments and the adult experiment involving an adult downstream of a predator. This corresponded to a chamber Reynolds number (Re) of 1.5×10^3 where the hydraulic diameter of the main chamber was used as the length scale. The remaining adult treatments were operated at 2.1 ± 0.1 cm s^{-1} corresponding to a chamber Re = 9.4×10^2. The spatial pattern of flow in the Y maze was measured at a mean velocity of 3.3 ± 0.2 cm s^{-1} in three ways. First, a single potassium permanganate crystal was placed in each arm near the entrance to the main arm, 15 cm lateral from the centerline of the main arm (*see asterisk symbols in Fig. 1*). The path of the dye streakline from each crystal was tracked at 10-cm intervals in the downstream direction. These dye measurements, which were repeated 10 times, confirmed that water flowed evenly out of each arm with little recirculation or mixing between the two sides of the chamber. Second, different colored vegetable dyes (Club House) were released in the left and right arm of the Y maze. Images taken with a digital camera (Coolpix 995, Nikon Corporation) indicated that water flowed evenly out of both arms with little mixing until near the outlet end of the Y maze (i.e., >80 cm from the target; Fig. 1). Finally, friction velocity (u_*) was determined along the centerline and 15 cm lateral from the centerline at 10, 30, and 60 cm downstream (*see filled circles in Fig. 1*). In this case, u_* was taken as the von Karman's constant ($\kappa = 0.4$) multiplied by the slope obtained from a graph of velocity (u) versus height ($\ln z$) in the logarithmic region of the boundary layer (Ackerman and Hoover 2001). The velocity profiles were obtained using sil-

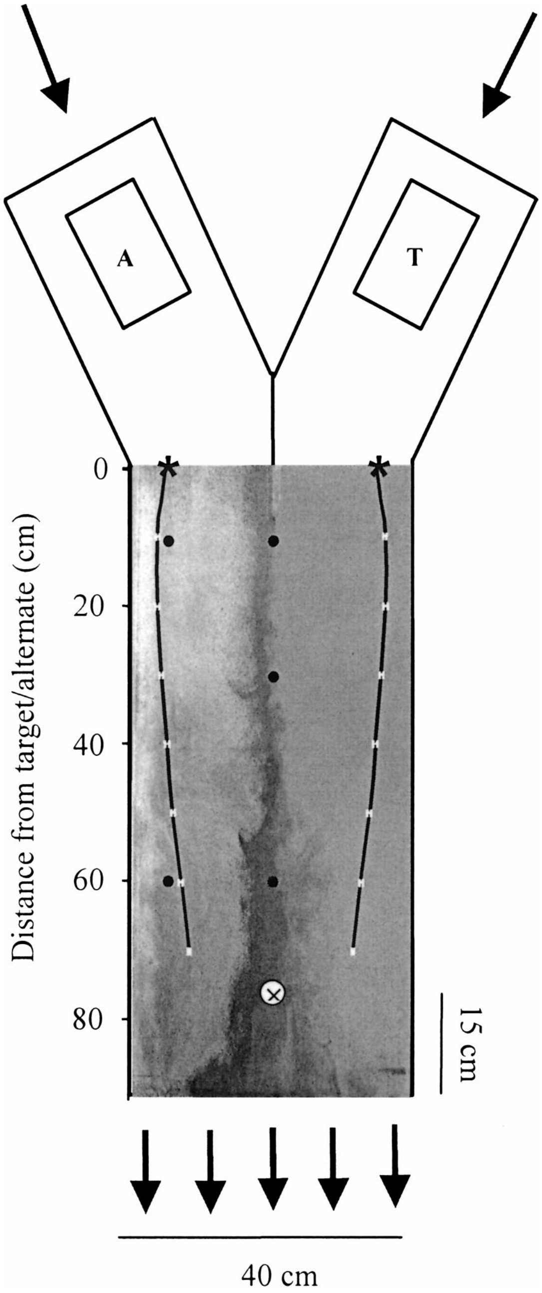

Fig. 1. Fluid dynamic conditions in the Y maze chamber. Solid arrows indicate the direction of water flow. Photographic image represents the release of different colors of vegetable dye in the left and right arms of the Y maze. The circled X represents the release point for urchin subjects, T represents the target cage and A represents the alternate cage. The black dots represent locations where velocity profiles were obtained. The asterisks indicate the location of potassium permanganate crystals; the black lines connect the mean spatial location of the potassium permanganate streaklines at 10-cm downstream intervals; and the error bars indicate ±1 SE (in many cases error bars are smaller than the symbol); $n = 10$ trials.

ver-coated hollow glass spheres (mean diameter 16 μm, density 2.0 g cm^{-3}, Potters Industries), which were tracked in a particle image velocimetry (PIV) system (Lasiris Magnum SP diode laser, StockerYale; Model 500 Pulse Generator, Berkeley Nucleonics; TM-9701 Progressive Scan Camera, PULNiX America; Video Savant Version 3.0, IO Industries; PIVView 2.1, PivTec GmbH), and measured velocities, based on 150 image pairs, were obtained in 1-mm vertical resolution. The average friction velocity ranged from 0.39 ± 0.07 to 0.42 ± 0.07 cm s^{-1} from 10 to 60 cm downstream, respectively, and from 0.32 ± 0.02 to 0.44 ± 0.03 cm s^{-1} on the centerline and sidewall locations, respectively (Fig. 1). The average friction velocity for all points was 0.38 ± 0.03 cm s^{-1}.

Targets (i.e., five juvenile urchins, an adult urchin, a predator, or kelp) and alternates (i.e., physical control = adult-sized rocks), which were placed in plastic cages (20 cm × 10 cm × 15 cm) with mesh siding, were positioned in one of the arms of the Y maze (targets in "T," alternates in "A"; Fig. 1). The placement of targets and alternates was switched among trials. For adult-downstream-of-predator and adult-upstream-of-predator treatments, both targets were placed in the same arm of the chamber in separate cages. Test subjects (i.e., three juveniles [test diameter = 9.93 ± 0.54 mm] or one adult) were placed on the centerline of the chamber 15 cm from the outlet end (circled X in Fig. 1), and their position was recorded after 12 h for juveniles and 45 min for adults. This was based on observations that juveniles typically remained stationary after 4–10 h, whereas adults remained stationary after ~30 min of their introduction into the chamber (trials were run for up to 72 and 12 h for juveniles and adults, respectively). The locations in the Y maze were designated as target side (on the same side as the target cage), alternate side (on the same side as the alternate cage), upstream (locations <77 cm from target/alternate arm; i.e., upstream of the release point), and downstream (locations >77 cm from target/alternate; i.e., downstream of release point). Juveniles and adults were exposed to chemical cues from (1) food (kelp); (2) predators (sea stars, *Pycnopodia helianthoides*); (3) conspecifics; and (4) controls (physical shelter = urchin-sized rocks). We also examined the response of juveniles exposed to various combinations of adult and predator stimuli (adult vs. predator; adult upstream of predator vs. rock; adult downstream of predator vs. rock).

The data from the Y maze experiment were analyzed in two ways. First, the null hypothesis of an equal distribution of urchins among the four quadrants at the end of the experiment (12 h for juveniles and 45 min for adults) was examined using a *G*-test. In a further analysis of these data, we examined the relationship between the movement of the subjects relative to the type of targets/alternates. Specifically, we tested the null hypothesis that the spatial distribution of juveniles and adults (i.e., distance from target/alternate, on the target side vs. the alternate side of the Y maze) was equal in the treatments and control (i.e., rock; note that the data for juveniles and adults were pooled in the control treatment).

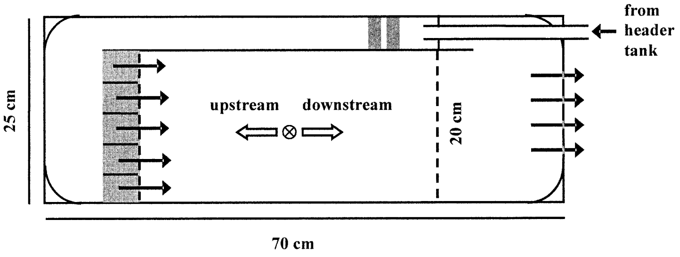

Fig. 2. Racetrack flow chamber. Scale diagram of flow chamber where solid arrows indicate direction of water flow, and circled X represents the release point of juvenile urchin subjects. Shaded area indicates flow straighteners placed upstream of the test section, and open arrows indicate the direction of urchin movement.

Ontogenetic patterns in juvenile orientation and crawling speed—A second experiment was undertaken using a "racetrack" flow chamber (Fig. 2) to examine the orientation and crawling speeds (based on the distance traversed over the duration of the experiment) of juvenile sea urchins of different sizes in response to (1) no stimuli (control); (2) predators; and (3) adults downstream of predators. Predators were held in a 20-liter header tank that supplied water into the main test chamber at a rate of 6 L min^{-1}. In the adult-downstream-of-predator treatment, predators were held in a second header tank (20 liters) that supplied water to the first header tank containing adults. The test section of the racetrack chamber was 40 cm long, 20 cm wide, and the water depth was 1.5 cm. Dye releases indicated unidirectional flow without areas of recirculation within the test section, and water velocities of 3.3 ± 0.1 cm s^{-1} were measured by timed dye releases, corresponding to chamber $Re = 1.4 \times 10^3$. Juveniles were placed on the crossed hollow circle symbol in the racetrack (Fig. 2), and experiments were terminated when juveniles contacted any edge of the chamber or after 1 h.

A Yates-corrected *G*-test was employed to examine the null hypothesis that the frequency of juveniles in the upstream versus downstream directions was equal for each size class in the various treatments. The influence of different chemical stimuli on juvenile crawling speed was analyzed using analysis of covariance (ANCOVA). Slopes of the regressions for juvenile crawling speed were compared using body size as the covariate and chemical stimuli/juvenile orientation as the main effects. Statistical analysis was conducted using SPSS 10.0.5 for Windows software (SPSS).

Results

Response to chemical cues—Both juvenile and adult sea urchins showed statistically significant behavioral responses in the Y maze flow chamber (Table 1). In all treatments, a portion of juveniles (i.e., ≥17%) tended to move upstream, toward the target arm of the Y maze (black bars in Fig. 3b). Juvenile sea urchins were significantly attracted to food (78% moved toward the target, i.e., kelp; *G*-test = 32.95, $p < 0.001$) but not to physical controls (i.e., rocks; 24% and 33%, note that rocks were included in the alternate cage; *G*-test = 1.78, $p = 0.637$). In addition, juveniles were not significantly attracted to adults (30%; *G*-test = 1.02, p =

Table 1. Summary of *G*-test analysis for the response of juvenile and adult *Strongylocentrotus franciscanus* to chemical cues in the Y maze experiment. Bold indicates significant differences.

Test organism	Treatment	*n*	*G*	*p*
S. franciscanus (juveniles)	Kelp	23*	32.95	<**0.001**
	Adult	27	1.02	0.796
	Predator	24	3.32	0.344
	Adult vs. predator	27	5.75	0.124
	Adult upstream of predator	30	2.27	0.518
	Adult downstream of predator	30	16.94	<**0.001**
	Control	21	1.78	0.637
S. franciscanus (adults)	Kelp	24	36.97	<**0.001**
	Juvenile	20	1.31	0.737
	Predator	23	15.63	**0.001**
	Adult downstream of predator	14	17.57	**0.001**
	Control	15*	1.90	0.593

* Trial where one juvenile escaped.

0.796), nor were they attracted to predators: (1) predator (17%; *G*-test = 3.32, $p = 0.344$); (2) adult versus predator (30% vs. 7%; *G*-test = 5.75, $p = 0.124$); and (3) adult upstream of predator (20%; *G*-test = 2.27, $p = 0.518$). Surprisingly, a significant proportion of juveniles were attracted to adults in the adult-downstream-of-predator treatment (60%; *G*-test = 16.94, $p < 0.001$).

The response of adult sea urchins was similar to juveniles in that adults were significantly attracted to food (79%; *G*-test = 36.97, $p < 0.001$) but not to physical controls (i.e., rocks; 20% and 13%; *G*-test = 1.90, $p = 0.593$; Fig. 3c). Similarly, adults were not attracted to juveniles (25%; *G*-test = 1.31, $p = 0.737$), yet, unlike juveniles, adults avoided predators (0%; *G*-test = 15.63, $p = 0.001$) and adults downstream of predators (7%; *G*-test = 17.57, $p = 0.001$).

When the locations of the subjects are considered by position in the chamber (i.e., distance from target/alternate), several behavioral responses become evident (Fig. 4). First, both juveniles and adults exhibited a neutral response to rocks with a large proportion of subjects found near and downstream of the release point (77 cm) on both sides of the Y maze (Fig. 4a). In contrast, both juveniles and adults showed a positive response to kelp, consistently moving upstream (~75 cm) to the arm containing kelp (*G*-test = 52.08, $p < 0.001$; *G*-test = 24.67, $p < 0.001$, respectively). Adult subjects exposed to juveniles showed neutral patterns that were not significantly different from controls (*G*-test = 2.11, $p = 0.716$). Similarly, juvenile subjects exposed to adults exhibited a neutral response (*G*-test = 1.89, $p = 0.756$). In the presence of predators, however, neither adults nor juveniles differed significantly from their response to controls (*G*-test = 4.26, $p = 0.372$; *G*-test = 3.39, $p = 0.495$), although adults tended to avoid the side of Y maze containing the predator, especially in the upstream locations (Fig. 4d) and in the adult-downstream-of-predator treatment (*G*-test = 4.39, $p = 0.356$). In contrast and as noted above, juveniles showed a significant, positive response upstream and toward the adult downstream of predator (*G*-test = 25.97, $p < 0.001$).

Ontogenetic patterns in juvenile orientation and crawling speed—The behavioral response of juveniles was examined in greater detail with respect to orientation, crawling speed (based on the distance traversed over the duration of the experiment), and body size in the racetrack flow chamber (Fig. 2). Differences in orientation or in size-specific crawling speed of juveniles were not detected in the absence of a chemical cue (i.e., control; Fig. 5a). Juveniles in the control showed no directed orientation (*G*-test results $p > 0.05$ for all size classes) or difference in crawling speed in the upstream versus downstream directions (ANCOVA; $F_{1,10} = 0.01$, $p = 0.896$; Fig. 5a). Size-related differences in orientation and crawling speeds were, however, found among juveniles in response to chemical cues. Specifically, larger juveniles in the predator treatment oriented in the downstream direction (*G*-test = 17.07, $p < 0.001$), and their crawling speeds tended to increase with body size when oriented in the downstream direction (ANCOVA; $F_{1,10} = 4.75$, $p = 0.054$; Fig. 5b). In the adult-downstream-of-predator treatment, smaller juveniles oriented in the upstream direction more frequently (*G*-test = 32.51, $p < 0.001$ and *G*-test = 57.71, $p < 0.001$ for the two smallest size classes), although no significant differences in crawling speed were detected in the upstream versus downstream directions (ANCOVA; $F_{1,10} = 0.15$, $p = 0.708$; Fig. 5c). In terms of the different treatments, juveniles in the adult-downstream-of-predator treatment had significantly faster upstream crawling speeds than in the predator treatment (ANCOVA; $F_{1,10} = 35.34$, $p < 0.001$; $F_{1,10} = 24.93$, $p = 0.005$, respectively), but no differences were detected in the downstream direction ($F_{1,10} = 0.01$, $p = 0.923$; Fig. 5b,c).

Discussion

Juvenile sheltering among conspecific adults is a unique postsettlement process in red sea urchins facilitated by chemical cues (Figs. 3–5). The most striking result from this study is that juveniles moved toward adults only when adults were downstream of a predator but not when adults were alone or when they were upstream from the predator. This result can be explained only by the existence of a secondary chemical cue released by conspecific adults after they were exposed to a primary chemical cue from the upstream predator.

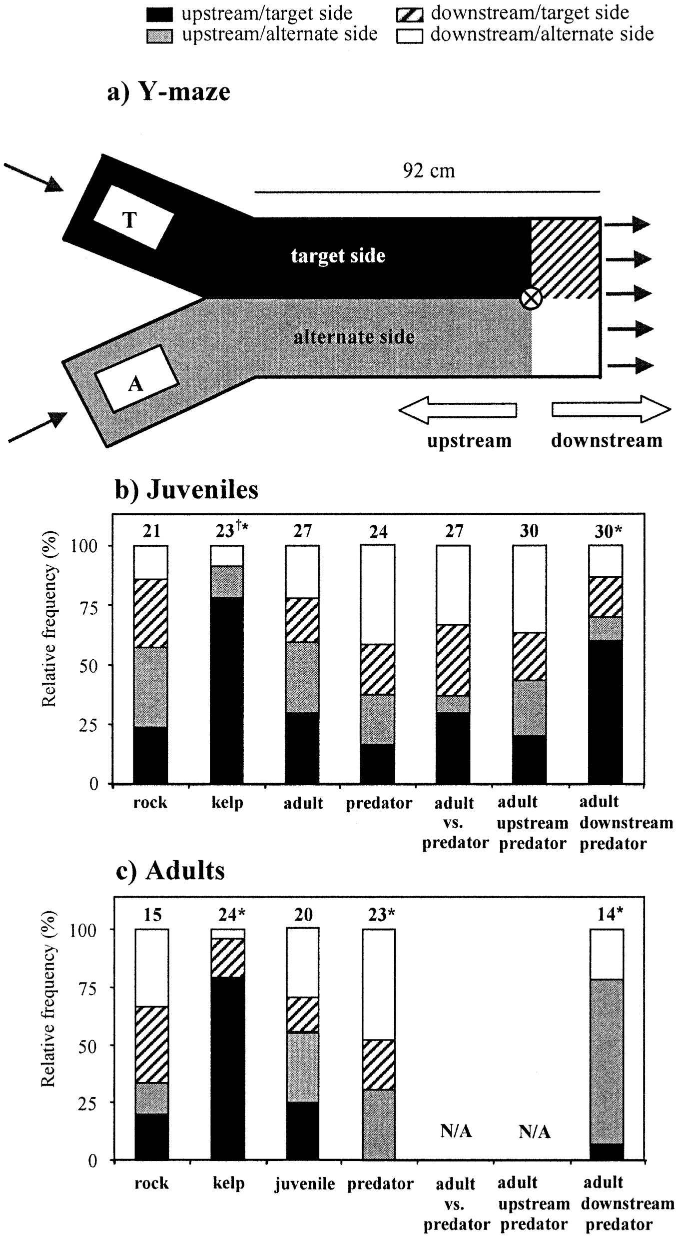

Fig. 3. The response of juvenile and adult *Strongylocentrotus franciscanus* to chemical cues in the Y maze experiment: (a) Scale diagram of Y maze where solid arrows indicate the direction of water flow, the crossed empty circle symbol represents the release point of the urchin subjects, T represents target cage whose contents are listed below each bar in parts (b) and (c), A represents the alternate cage, which contained urchin-sized rocks except in the adult versus predator treatment when it contained *Pycnopodia helianthoides*, and open arrows indicate the direction of urchin movement. Note that the placement of targets and alternates were switched among trials. Distribution of (b) juveniles and (c) adults, where bars represent frequencies of subjects, numbers indicate sample size, asterisks indicate nonrandom patterns (*G*-test, $p < 0.0001$), and † indicates one escaped juvenile.

This response cannot result merely from a synergism between two separate chemical cues since adults upstream from predators did not cause the juvenile response. The observed sheltering is a postsettlement phenomenon associated with juveniles, since adult *S. franciscanus* responded differently to the secondary cue. It is doubtful that adult urchins produce an alarm pheromone to benefit unrelated juveniles (e.g., Sherman 1977); it is more likely that juvenile urchins developed the ability to detect a cue, perhaps some metabolic byproduct, from stressed adults. This provides experimental evidence of a secondary chemical cue that involves a predator → adult → juvenile interaction.

As indicated above, juvenile sea urchins aggregate and shelter under adults in response to predators, but in so doing they decrease foraging opportunity due to competition with adults (Nishizaki and Ackerman 2004). Balancing these risks against opportunities requires that juveniles detect aggregation cues (i.e., in the presence of a predator) simultaneously with being able to locate adult urchins under which to shelter. In this case, the secondary chemical cue provides a parsimonious mechanism whereby juvenile urchins can both identify danger and locate the safety of the adult spine canopy. Moreover, the response is dependent on the size of the postsettlement juveniles in that small individuals (<10 mm) appear more sensitive to secondary chemical cues from adults (i.e., increased movement toward adults) and less sensitive to predator stimuli (i.e., no fleeing response). This higher response for smaller juveniles, however, may be offset by their slower crawling speeds. The potential coupling of juveniles crawling toward adults (Fig. 3b) and adults fleeing predators (Fig. 3c) would facilitate the location of adults by juveniles. In some urchin species, adult aggregation behavior around food supersedes any fleeing response from predators (Vadas et al. 1986), which may provide a means for juvenile sheltering to occur.

Marine organisms are exposed to a variety of chemical signals (Zimmer and Butman 2000), and many possess morphological, physiological, and behavioral defenses that rely on the detection of these chemical cues (Appleton and Palmer 1988; Atema 1995; Toth and Pavia 2000). Under natural conditions organisms are faced with chemical cues from multiple sources, and thus single chemical cues are often not sufficient to explain behavior (Relyea 2003). Although the effect of chemical cues from multiple sources may be additive (Nystroem and Abjoernsson 2000), synergistic (Hazlett 1999), or suppressive (Johnson et al. 1985; Zimmer-Faust 1993; Simon and Derby 1995), these conclusions are based on responses to mixtures of chemical cues (Relyea 2003). Our results show a response, not simply to the presence of multiple cues, but to an ordered set of chemical cues (i.e., primary from the predator and secondary from the adult).

Chemical cues involved with predation risk may originate directly from predators (Chivers and Smith 1998; Schneider and Moore 2000) or indirectly from conspecifics (Schneider and Moore 2000). These indirect cues can be delineated into (1) damage-released alarm cues, which occur after attack (Zimmer and Butman 2000; Wisenden 2000) and have been widely reported and (2) disturbance cues, which do not involve injury/damage to the sender (Schneider and Moore

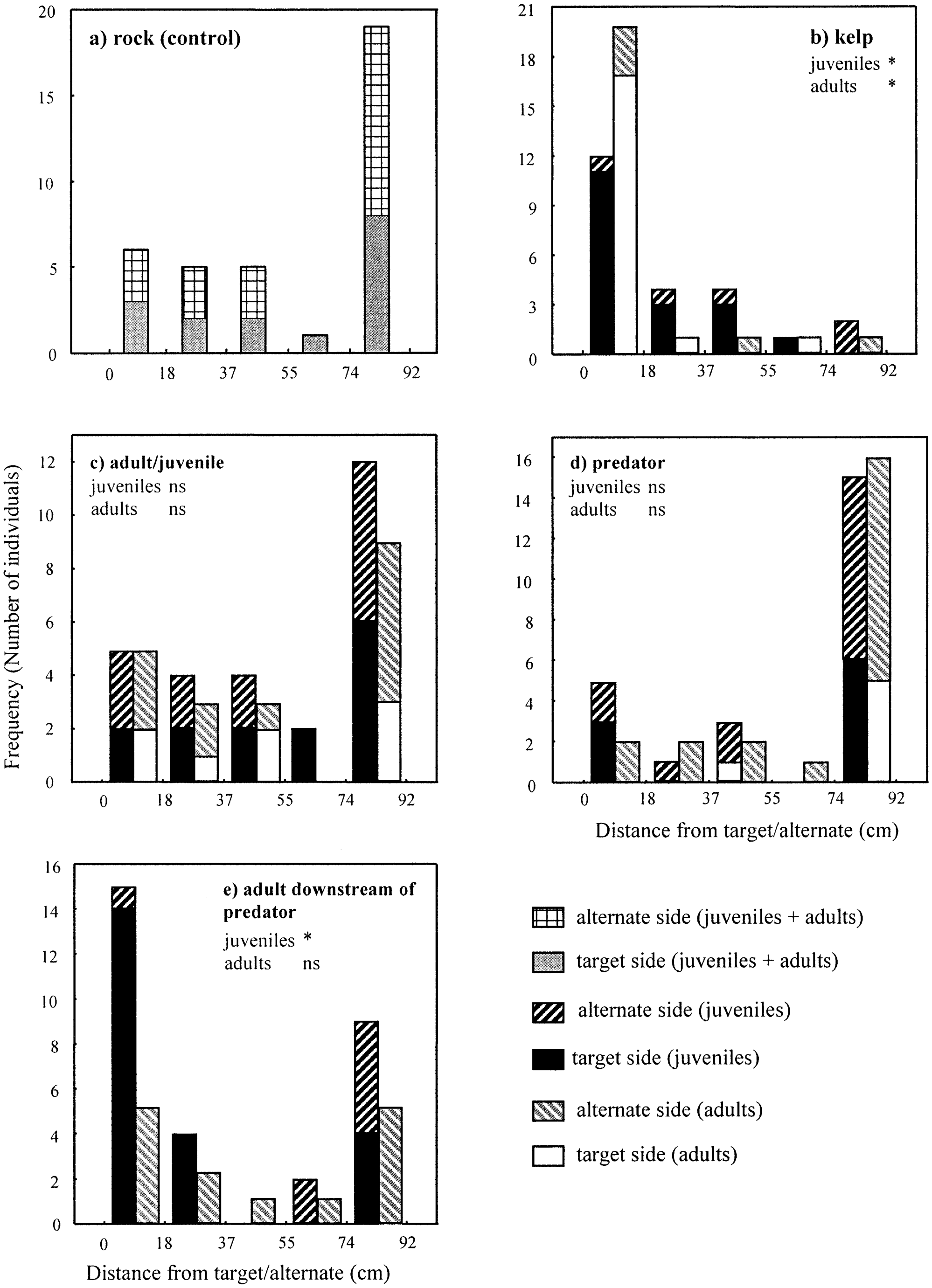

Fig. 4. Spatial distribution of juvenile and adult *Strongylocentrotus franciscanus* in the Y maze experiment. Histograms of distances from the targets/alternates (cm) by juveniles and adults in response to (a) rock (i.e., control), (b) kelp, (c) adult (for juvenile subjects) or juveniles (for adult subjects), (d) predator (i.e., *Pycnopodia helianthoides*), and (e) adult-downstream-of-predator stimuli. Asterisks indicate patterns that differ significantly from the control (*G*-test, $p < 0.0001$), and ns is not significant ($p > 0.05$).

2000) and are less commonly reported. For sea urchins, there are behavioral responses to direct odors from predators (Campbell et al. 2001; Hagen et al. 2002) and indirect chemical alarm cues from damaged conspecifics (Campbell et al. 2001). The experimental results reported here, however, indicate the presence of a conspecific disturbance cue, which does not involve tissue damage. Furthermore, as indicated above, this secondary chemical cue is dependent on the presence of a predator-associated cue, and the order of the chemical releasers influences juvenile behavioral responses. It is doubtful that such an effect is due to a concentration-dependent response of the adult signal in that there would be minimal dilution over the 5-cm distance between adult urchins and predators (i.e., in the adult-upstream-of-predator and adult-downstream-of-predator treatments) given the 139 cm total length of the chamber (i.e., cages to outlet). Unfortunately, we do not have information on the efficacy of the response in deterring juvenile predation, which is an issue that would warrant future research on both the chemistry of the unidentified cues and the response of juveniles. Unlike some responses that are common to both juvenile and adult urchins (i.e., attraction to kelp), this secondary chemical cue appears to be specific to early postsettlement juveniles paralleling ontogenetic shifts in chemical sensitivity observed in some freshwater organisms (Quirt and Lasenby 2002).

Implications for juveniles in the field—It is reasonable to explore the physical conditions under which juvenile sheltering occurs to better understand the potential transport and detection of the secondary chemical cue system. Adult and juvenile red sea urchins are usually found in the vicinity of kelp forests, where they are significant members of kelp ecosystems in that they feed on kelp holdfasts and other benthic organisms (Tegner et al. 1995). These kelp forests are located on hard substrates in the subtidal region, at densities of ~3 to 13 individuals m^{-2} (Carr 1994; Dean et al. 2000), corresponding to an interalgal distance of ~0.08 to 0.33 m. Kelp forests have long been known to dampen water currents (Okubo et al. 2002), and average velocities have been reported to range between 0.2 and 24 cm s^{-1} within ~1 m of the bottom (e.g., 15–24 cm s^{-1} at 1.5 m from the bottom, Eckman et al. 1989; 0.2–4.7 cm s^{-1} at 0.1 to 0.5 m, Levitan et al. 1992; 4.70–6.79 cm s^{-1} at 1 m, Gagnon et al. 2003). Given the logarithmic nature of the benthic boundary layer, it is likely that near-bottom velocities within a few centimeters of the seafloor are on the same order as those used in this study (i.e., ~3 cm s^{-1}). Moreover, assuming a rough turbulent boundary layer, we would expect friction velocity to lie within the range of 0.02 to 2.4 cm s^{-1} (i.e., $u_* \sim 1/10$ of free stream velocity), which includes the friction velocity measured in our experiments (0.38 ± 0.03 cm s^{-1}). This would indicate that the laboratory flow conditions fall within the range found in the field at the lower end of the range of velocities (Nowell and Jumars 1984). Given these conditions, chemical detection near the bottom may be possible up to 1 m from the source, assuming a constant and detectable signal. If adults can detect predators from a similar distance it is likely that the secondary cue operates on scales of the same order in the field. The situation in the field is more complex in that each of the organisms and the chemical signals (i.e., primary and secondary) are moving relative to one another. For example, data from unidirectional flow conditions in the laboratory indicate that juvenile

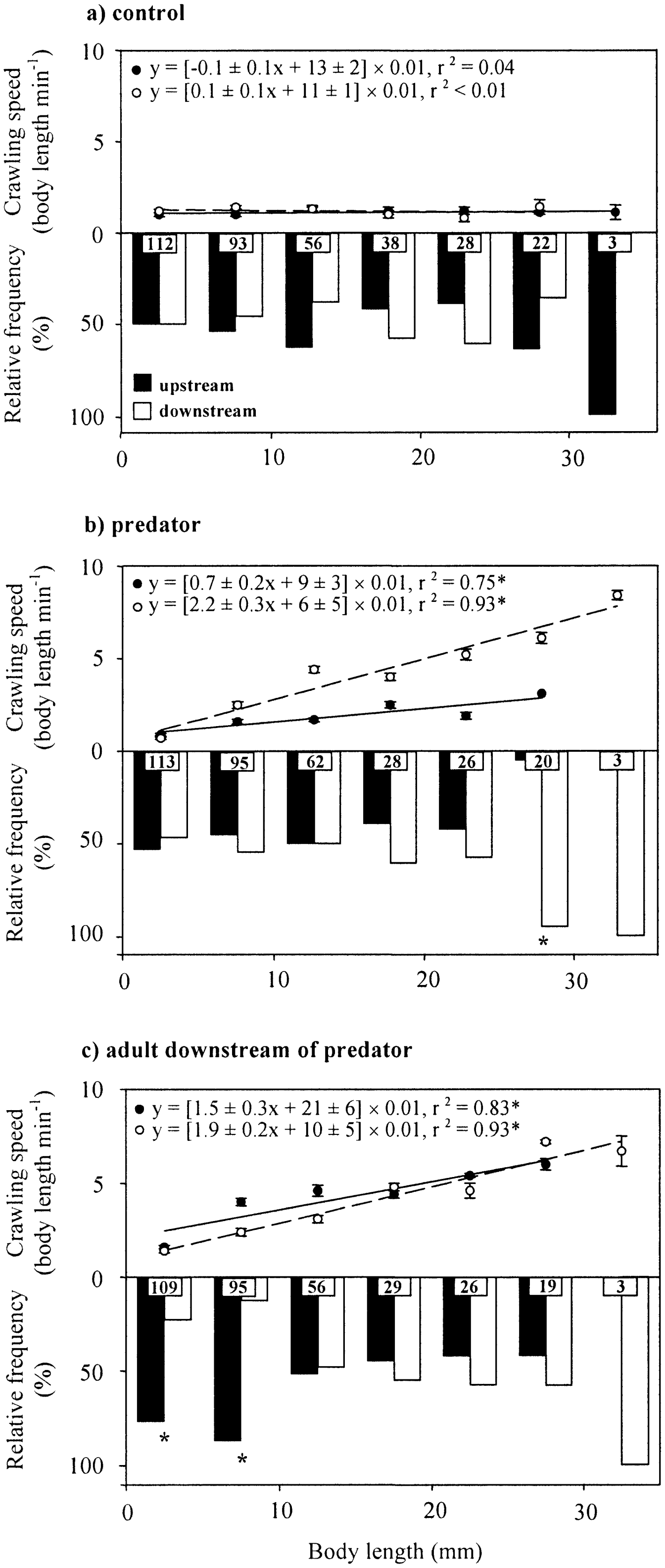

Fig. 5. Ontogenetic patterns in juvenile *Strongylocentrotus franciscanus* orientation and crawling speed in the racetrack experiment. Scatterplots of crawling speed (body length min^{-1}) versus body length (i.e., test diameter) for (a) control, (b) predator (i.e., *Pycnopodia helianthoides*), and (c) adult-downstream-of-predator treatments. Juveniles moving upstream are represented by the solid regression lines, and juveniles moving downstream are represented by dashed regression lines. Paired bars and numbers indicate the frequency distribution of responses and sample size, respectively, within each size class. Asterisks indicate significant linear regression ($p < 0.05$) for scatterplots and nonrandom patterns (*G*-test, $p < 0.0001$) in the direction of movement for histograms. No tests were performed on juveniles >30-mm test diameter due to limited sample size.

Strongylocentrotus franciscanus move much slower (~0.17 cm s^{-1}) than either adults (~0.42 cm s^{-1}), or predators such as *Pycnopodia helianthoides* (~0.83 cm s^{-1}, Fig. 5; Nishizaki and Ackerman unpubl. data). However, in shallow, food-rich habitats, presumably in the absence of predators, adults move very little (i.e., 8.7×10^{-5} cm s^{-1} or 7.5 cm d^{-1}, Mattison et al. 1977; Rogers-Bennett et al. 1995).

We estimated the distance over which this secondary cue system might operate using the speeds reported above. In this case, we assume the water flow to be unidirectional (i.e., tidal) at a mean velocity of 3.3 cm s^{-1}, the chemical signals to be transported in the flow without degradation, and the reaction time for the detection or release or both of the advected chemical cue to be zero (predator → adult → juvenile). Under these circumstances, a predator 100 cm upstream from an adult urchin would overtake the adult moving downstream 77 cm from the adult's original position. At the same time, a juvenile urchin would move 26 cm upstream where it would encounter the adult urchin and the predator. Hence the maximum distance between adult and juvenile would be ~103 cm, recognizing the aforementioned assumptions, and the difficulty associated with extending laboratory results to field conditions. It is doubtful that this secondary chemical cue would operate under the highly energetic and turbulent conditions characteristic of some subtidal regions, and the intertidal region, where turbulence (e.g., variation in speed and direction) and oscillatory surface wave-driven flow (Gaylord 1999) would likely limit the efficacy of the secondary chemical cue system to small distances. Clearly additional research into the transport of chemical cues under field conditions is warranted.

Postsettlement processes can be the primary factor determining distribution patterns in both sessile (i.e., ascidians, barnacles) and mobile (i.e., crabs) benthic invertebrates (Stoner 1990; Heck et al. 2001; Delany et al. 2003). Factors such as predation, disturbance, extreme weather events, and ecological interactions (e.g., competition for space, succession) affect postsettlement mortality for both sessile and mobile organisms. The only defensive strategies available to sessile invertebrates are to take refuge in numbers, size, or elevation in the intertidal. Conversely, mobile invertebrates can also take advantage of behavioral strategies such as fleeing, aggregation, and refuge in space to reduce the impact from these postsettlement factors (Hunt and Scheibling 1997). Juvenile sheltering in red urchins provides an example of a complex postsettlement behavior, which balances the risks of predation versus the cost of intercohort competition. That this strategy evolved to use a secondary chemical cue speaks to the important role of postsettlement processes in the life history of mobile benthic invertebrates.

References

Ackerman, J. D., and T. M. Hoover. 2001. Measurement of local bed shear stress in streams using a Preston-static tube. Limnol. Oceanogr. **46:** 2080–2087.

Appleton, R. D., and A. R. Palmer. 1988. Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. Proc. Natl. Acad. Sci. USA **85:** 4387–4391.

Atema, J. 1995. Chemical signals in the marine environment: Dispersal, detection, and temporal signal analysis. Proc. Natl. Acad. Sci. USA **92:** 62–66.

Cameron, R. R., and S. C. Schroeter. 1980. Sea urchin recruitment: Effect of substrate selection on juvenile distribution. Mar. Ecol. Prog. Ser. **2:** 243–247.

Campbell, A., S. Coppard, C. D'Abreo, and R. Tudor-Thomas. 2001. Escape and aggregation responses of three Echinoderms to conspecific stimuli. Biol. Bull. **201:** 175–185.

Carr, M. H. 1994. Effects of macroalgal dynamics on recruitment of a temperate reef fish. Ecology **75:** 1320–1333.

Chivers, D. P., and R. F. Smith. 1998. Chemical alarm signalling in aquatic predator-prey systems: A review and prospectus. Ecoscience **5:** 338–352.

Dean, T. A., J. L. Bodkin, S. C. Jewett, D. H. Monson, and D. Jung. 2000. Changes in sea urchins and kelp following a reduction in sea otter density as a result of the Exxon Valdez oil spill. Mar. Ecol. Prog. Ser. **199:** 281–291.

Delany, J., A. A. Myers, D. McGrath, R. M. O'Riordan, and A. M. Power. 2003. Role of post-settlement mortality and 'supply-side' ecology in setting patterns of intertidal distribution in the chthamalid barnacles *Chthamalus montagui* and *C. stellatus.* Mar. Ecol. Prog. Ser. **249:** 207–214.

Eckman, J. E., D. O. Duggins, and A. T. Sewell. 1989. Ecology of understory kelp environments. I. Effects of kelps on flow and particle transport near the bottom. J. Exp. Mar. Biol. Ecol. **129:** 173–187.

Gagnon, P., G. Wagner, and J. H. Himmelman. 2003. Use of a wave tank to study the effects of water motion and algal movement on the displacement of the sea star *Asterias vulgaris* towards its prey. Mar. Ecol. Prog. Ser. **258:** 125–132.

Gaines, S., and J. Roughgarden. 1985. Larval settlement rate: A leading determinant of structure in an ecological community of the marine intertidal zone. Proc. Natl. Acad. Sci. USA **82:** 3707–3711.

Gaylord, B. 1999. Detailing agents of physical disturbance: Wave-induced velocities and accelerations on a rocky shore. J. Exp. Mar. Biol. Ecol. **239:** 85–124.

Gosselin, L. A., and P. Y. Qian. 1997. Juvenile mortality in benthic marine invertebrates. Mar. Ecol. Prog. Ser. **146:** 265–282.

Hagen, N. T., A. Andersen, O. B. Stabell. 2002. Alarm responses of the green sea urchin, *Strongylocentrotus droebachiensis*, induced by chemically labelled durophagous predators and simulated acts of predation. Mar. Biol. **140:** 365–374.

Hazlett, B. A. 1999. Responses to multiple chemical cues by the crayfish *Orconectes virilis.* Behaviour **136:** 161–177.

Heck, K. L., L. D. Coen, and S. G. Morgan. 2001. Pre- and post-settlement factors as determinants of juvenile blue crab *Callinectes sapidus* abundance: Results from the north-central Gulf of Mexico. Mar. Ecol. Prog. Ser. **222:** 163–175.

Hunt, H. L., and R. E. Scheibling. 1997. The role of early post-settlement mortality in recruitment of benthic marine invertebrates: A review. Mar. Ecol. Prog. Ser. **155:** 269–301.

Johnson, B. R., P. F. Borroni, and J. Atema. 1985. Mixture effects in primary olfactory and gustatory receptor cells from the lobster. Chem. Senses **10:** 367–373.

Levitan, D. R., M. A. Sewell, and F.-S. Chia. 1992. How distribution and abundance influence fertilization success in the sea urchin *Strongylocentrotus franciscanus.* Ecology **73:** 248–254.

Low, C. G. 1975. The effect of grouping of *Strongylocentrotus franciscanus,* the giant red sea urchin, on its population biology. Ph.D. thesis, Univ. of British Columbia.

Mattison, J. E., J. D. Trent, A. L. Shanks, T. B. Akin, and J. S. Pearse. 1977. Movement and feeding activity of red sea urchins (*Strongylocentrotus franciscanus*) adjacent to a kelp forest. Mar. Biol. **39:** 25–30.

Mauck, R. A., and K. C. Harkless. 2001. The effect of group membership on hiding behaviour in the northern rock barnacle, *Semibalanus balanoides.* Anim. Behav. **62:** 743–748.

MENGE, B. A. 2000. Recruitment vs. post-recruitment processes as determinants of barnacle population abundance. Ecol. Monogr. **70:** 265–288.

MILLER, B. A., AND R. B. EMLET. 1997. Influence of nearshore hydrodynamics on larval abundance and settlement of sea urchins *Strongylocentrotus franciscanus* and *S. purpuratus* in the Oregon upwelling zone. Mar. Ecol. Prog. Ser. **148:** 83–94.

MOBERG, P. E., AND R. S. BURTON. 2000. Genetic heterogeneity among adult and recruit red sea urchins, *Strongylocentrotus franciscanus.* Mar. Biol. **136:** 773–784.

NISHIZAKI, M. T., AND J. D. ACKERMAN. 2004. Juvenile-adult associations in sea urchins *Strongylocentrotus franciscanus* and *S. droebachiensis:* Is nutrition involved? Mar. Ecol. Prog. Ser. **268:** 93–103.

NOWELL, A. R., AND P. A. JUMARS. 1984. Flow environments of aquatic benthos. Annu. Rev. Ecol. Syst. **15:** 303–328.

NYSTROEM, P., AND K. ABJOERNSSON. 2000. Effects of fish chemical cues on the interactions between tadpoles and crayfish. Oikos **88:** 181–190.

OKUBO, A., J. D. ACKERMAN, AND D. P. SWANEY. 2002. Passive diffusion in ecosystems, p. 31–106. *In* A. Okubo and S. A. Levin [eds.], Diffusion and ecological problems: New perspectives, 2nd ed. Springer.

PAINE, R. T. 2002. Trophic control of production in a rocky intertidal community. Science **296:** 736–739.

QUIRT, J., AND D. LASENBY. 2002. Cannibalism and ontogenetic changes in the response of the freshwater shrimp *Mysis relicta* to chemical cues from conspecific predators. Can. J. Zool. **80:** 1022–1025.

RELYEA, R. A. 2003. How prey respond to combined predators: A review and an empirical test. Ecology **84:** 1827–1839.

ROGERS-BENNETT, L., W. BENNETT, H. FASTENAU, AND C. DEWEES. 1995. Spatial variation in red sea urchin reproduction and morphology: Implications for harvest refugia. Ecol. Appl. **5:** 1171–1180.

SCHNEIDER, R. A., AND P. A. MOORE. 2000. Urine as a source of conspecific disturbance signals in the crayfish *Procambarus clarkii.* J. Exp. Biol. **203:** 765–777.

SIH, A. 1980. Optimal behaviour: Can foragers balance two conflicting demands? Science **210:** 1041–1043.

SHERMAN, P. 1977. Nepotism and the evolution of alarm calls. Science **197:** 1246–1253.

SIMON, T. W., AND C. D. DERBY. 1995. Mixture suppression without inhibition for binary mixtures from whole cell patch clamp studies of in situ olfactory receptor neurons of the spiny lobster. Brain Res. **678:** 213–224.

STONER, D. S. 1990. Recruitment of a tropical colonial ascidian: Relative importance of pre-settlement vs. post-settlement processes. Ecology **71:** 1682–1690.

TEGNER, M. J., AND P. K. DAYTON. 1977. Sea urchin recruitment patterns and implications of commercial fishing. Science **196:** 324–326.

———, ———, P. B. EDWARDS, AND K. L. RISER. 1995. Sea urchin cavitation of giant kelp (*Macrocystis pyrifera* C. Agardh) holdfasts and its effects on kelp mortality across a large California forest. J. Exp. Mar. Biol. Ecol. **191:** 83–99.

TOTH, G. B., AND H. PAVIA. 2000. Water-borne cues induce chemical defense in a marine alga (*Ascophyllum nodosum*). Proc. Natl. Acad. Sci. USA **97:** 14418–14420.

VADAS, R. L., R. W. ELNER, P. E. GARWOOD, AND I. G. BABB. 1986. Experimental evaluation of aggregation behavior in the sea urchin, *Strongylocentrotus droebachiensis:* A reinterpretation. Mar. Biol. **90:** 433–448.

WISENDEN, B. D. 2000. Olfactory assessment of predation risk. Philos. Trans. R. Soc. Lond. B **355:** 1205–1208.

WITMAN, J. D. 1985. Refuges, biological disturbance, and rocky subtidal community structure in New England. Ecol. Monogr. **55:** 421–445.

ZIMMER, R. K., AND C. A. BUTMAN. 2000. Chemical signaling processes in the marine environment. Biol. Bull. **198:** 168–187.

ZIMMER-FAUST, R. K. 1993. ATP: A potent prey attractant evoking carnivory. Limnol. Oceanogr. **38:** 1271–1275.

Received: 11 March 2004
Accepted: 15 August 2004
Amended: 24 August 2004