



Effects of dead conspecifics, hunger states, and seasons on the foraging behavior of the purple urchin *Heliocidaris crassispina*

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ABSTRACT: Trophic cascades are a powerful result of predator–prey relationships in an ecosystem. In aquatic environments, the signals associated with predators and predation are used by prey as a cue to avoid encountering predators when foraging for food. These behavioral cues can be powerful enough to control prey populations and indirectly protect primary producers. Here, we evaluated the effects of cues associated with predation on the purple urchin *Heliocidaris crassispina* and examined effects of hunger state and season, using time-lapse photography. A series of laboratory and *in situ* manipulative experiments were conducted to determine patterns of foraging behavior and behavioral modifications. We showed that starved urchins were less sensitive to predation cues compared to normally fed urchins. Field experiments indicated that 70 % of fed urchins fled when exposed to a predation cue (presence of a dead urchin) whereas starved urchins remained regardless of the cue, supporting results from the laboratory using dead urchin and algal cues. Sea urchin activity and feeding rates were lower in winter–spring than in summer–autumn. Results suggest that hunger state has a large influence over the behavioral response of sea urchins, while also being affected by season due to metabolic control. In general, starvation appears to override predator avoidance behaviors, which exposes prey species to higher risks of predation.

KEY WORDS: Top-down control · Trophic cascade · Behavior modification

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1. INTRODUCTION

Predation and resource availability control food webs (Nielsen & Navarrete 2004, Lynam et al. 2017). These interacting forces, together with variabilities in environmental stress, depend on the regulating effect (i.e. energy allocation, expenditure, and transfer) they exert on the community of producers and consumers (Menge & Sutherland 1987). Hairston et al. (1960) hypothesized that populations of herbivores and the level of herbivory were generally controlled by predation rather than by food supply (i.e.

‘green world’ hypothesis), and therefore the collapse of predator populations increased the likelihood of herbivore domination (Estes & Palmisano 1974).

In temperate regions, macroalgal forests are an important coastal ecosystem (Steneck et al. 2002, Smale et al. 2010, Langlois et al. 2012). Within the canopy, the high diversity of fish and invertebrates are dependent on the canopy for food and refuge (Lowry & Pearse 1973, Holbrook et al. 1990, Kamimura & Shoji 2009). Sea urchins are keystone species in marine forests because they can overwhelm benthic primary production (Tuya et al. 2004,

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Kriegisch et al. 2019b). In ecosystems where apex predator populations are intact, urchin populations are kept in check by predation (Tegner & Levin 1983, Pearse & Hines 1987, Sala & Zabala 1996, Sievers & Nebelsick 2018). When that predation pressure is removed, the urchin populations increase, leading to overgrazing that eventually converts seaweed beds into barrens (reviewed by Ling et al. 2015). In general, barren areas are characterized by low diversity and habitat complexity (Mangialajo et al. 2008, Ling 2008). Large canopy-forming macroalgae are replaced by grazing-resistant, turf-forming macroalgae (Wright et al. 2005), considered to be an intermediary stable state supported by strong feedback mechanisms (Filbee-Dexter & Wernberg 2018). As grazing pressure surpasses the thresholds of the remaining primary producers, the community state eventually transitions into a species-poor stable state (Steneck et al. 2002, Filbee-Dexter & Scheibling 2014, Ling et al. 2015) that is easily maintained by a few urchins (Tuya et al. 2004, Bonaviri et al. 2011). However, sustained human intervention or the recovery of predator populations can revert barrens into a macroalgal-dominated state (Blamey et al. 2013, Steneck et al. 2013).

Attempts to revert barrens into seaweed forests are not uncommon. Methods include manual removal or destruction of urchins and other herbivores to encourage natural recruitment of juvenile seaweeds (Yotsui & Maesako 1993, Watanuki et al. 2010, Nanri et al. 2011), small-to-medium scale transplantation of fertile seaweed thalli and mass dispersal of viable spores (Hernandez-Carmona et al. 2000, Yoon et al. 2014, Ogata et al. 2016), and installment of artificial reefs (Watanuki & Yamamoto 1990, Westermeier et al. 2014). Experimental evidence has shown that human intervention may succeed and promote seaweed forest recovery (Ling et al. 2010, Tracey et al. 2015, Verdura et al. 2018, Layton et al. 2020, Verges et al. 2020). However, maintaining restored algal forests becomes difficult when uncontrolled urchin population levels eventually establish dense feeding fronts (Lauzon-Guay & Scheibling 2007, Ling & Johnson 2009). Regardless of the situation, the decision to restore ecosystems must be evidence-based and scale- and context-specific (Johnson et al. 2017).

Harnessing the effect of natural predators on prey to indirectly maintain the population of primary producers may be a more practical solution (Schmitz et al. 2004). Direct reduction of the population of herbivores through consumption is called density-mediated indirect interaction (DMII), while the modification of prey behavior is called trait-mediated indirect

interaction (TMII) (Schmitz et al. 2004). These interactions have been observed in a variety of terrestrial and aquatic ecosystems (Shurin et al. 2002). In the aquatic ecosystem, trophic cascades seem to be more prominent than in terrestrial ecosystems (i.e. marine benthos > marine plankton > terrestrial food web) (Strong 1992, Halaj & Wise 2001, Shurin et al. 2002). The non-lethal effect of TMIs may be comparable in magnitude to that of DMIs because behavior change has population-wide effects, whereas direct predation only affects the individual (Peacor & Werner 2001, Pessarrodona et al. 2019).

Historically, algal forests composed of large brown algae created dense expansive habitat around the coastline of Japan and supported a large diversity of economically important fish and invertebrates (Uki et al. 1986, Kamimura & Shoji 2009). Presently, seaweed forests in Japan are undergoing a catastrophic decline ('isoyake') and the remaining seaweed forests are at high risk (Haraguchi & Sekida 2008, Okuda 2008, Fujita 2010). The loss of seaweed forests has led to a decline in coastal fisheries production (Kiyomoto et al. 2013). Efforts to revert the decline in seaweed forests has resulted in the production of numerous guidelines and methodologies, but success has been limited (Terawaki et al. 2003, Fujita 2010, 2015, Kuwahara et al. 2010).

In this study, we focused on determining the impact of a non-lethal perceived threat on the foraging behavior of the purple urchin *Heliocidaris crassispina* (Agassiz). Experimental studies have shown urchins to have complex foraging behaviors (Vanderklift & Kendrick 2005, Kriegisch et al. 2019a) and that escape responses from experiments manipulating predatory cues ranged from strong (Campbell et al. 2001, Hagen et al. 2002) to weak (Harding & Scheibling 2015). Here, we used dead conspecifics as a deterrent (Campbell et al. 2001, Morishita & Barreto 2011) to explore the effects of urchin hunger state and season on predatory risk aversion. Hunger state (i.e. satiation vs. starvation) has been known to modulate an individual's perception of risk (Clark 1994), while season is associated with reproductive phenology (Agatsuma et al. 2000, Yatsuya & Nakahara 2004a).

We examined prey behavior in response to predation cues to better understand the role of predators in indirectly maintaining the integrity of the seaweed bed ecosystem. The following questions were addressed: (1) How does season affect urchin feeding rate and response to predation risk? (2) How does urchin hunger state modify foraging behavior in the presence of threatening and non-threatening chemo-

sensory cues? (3) How does the urchins' hunger state affect their predator avoidance behavior in the field?

2. MATERIALS AND METHODS

2.1. Collection and maintenance of urchins and algae

Purple urchins *Heliocidaris crassispina* (Agassiz, 1864) were collected from the coastal waters of Kashiyama Town, Nagasaki Prefecture, Japan. Urchins were brought to the Institute for East China Sea Research, Nagasaki University, approximately 3.7 km south of the collection site. Urchins were placed inside an outdoor tank (170 × 110 × 70 cm) with a constant flow of sand-filtered seawater and aeration. A Tidbit v.2 (Onset Computer Corporation) temperature logger monitored ambient water temperature. Urchins were fed ad libitum with an assortment of fresh algae collected from Omura Bay, Nagasaki, Japan. The feeding experiments used *Sargassum patens* C. Agardh also collected from Omura Bay. Stock *S. patens* was kept in a separate outdoor tank which received water overflowing from the urchin stock tank. Urchins were acclimated to ambient laboratory conditions for 1 wk prior to the experiments (mean ± SD ambient temperature range for summer: 22.7 ± 4.79°C; winter: 15.9 ± 3.52°C). Experiments involving summer–autumn and winter–spring seasons are hereafter referred to as Su–Au and Wi–Sp, respectively.

Urchins were starved by placing selected individuals in a separate container with no food for 1 wk prior to experiments. This allowed for standardization of their nutritional condition and elicitation of a stronger hunger response prior to experiments (Scheibling & Anthony 2001).

2.2. Laboratory Expt 1: Urchin grazing rates by temperature and season

To test the hypothesis that the ambient *H. crassispina* grazing rate is influenced by temperature and season, a flow-through rectangular tank (70 × 112 × 12 cm) was prepared. Ten numbered containers (2.96 l) were set in the tank, separated into 2 treatments: a feeding treatment (6 containers) that included urchins and algae and a control (4 containers) which contained only algae. A continuous water supply (11 l min⁻¹) was provided by an overhead perforated PVC frame. A Tidbit v.2 temperature logger recorded ambient water temperature.

A total of 24 trials (24 h trial⁻¹) was conducted for both Su–Au (July–November 2018) and Wi–Sp (February–April 2019). Urchins used in Su–Au and Wi–Sp had test sizes of (mean ± SD) 4.28 ± 0.30 and 4.33 ± 0.45 cm, respectively. A total of 144 urchins was used in both seasons. There were 4 control treatments and 6 feeding treatments for each trial. The purpose of the controls was to measure biogenic changes to the algae other than the effect of grazing. Whole *S. patens* thalli were removed of epiphytes and other debris and cut into portions. The cut portions were dried with paper towels and weighed to the nearest 0.1 g to obtain initial fresh weight. Urchins were weighed to the nearest 0.1 g, and their horizontal test diameter was measured using a firm-joint outside-caliper and a Vernier caliper to the nearest 0.01 cm. *S. patens* cuttings and urchins were haphazardly assigned to containers. A mesh-net frame was placed over the tank to cover all containers and prevent urchins from escaping. At the end of each trial, the remaining uneaten algae were collected, dried with paper towels and re-weighed to obtain final fresh weight. Urchin feeding rate was the difference between the final and initial weight with units g algae urchin⁻¹ d⁻¹.

2.3. Laboratory Expt 2: Effect of positive and negative chemosensory cues on urchin foraging behavior

This experiment was designed to test the hypothesis that urchins will modify foraging behavior when exposed to chemosensory cues coming from dead conspecifics, compared to behavior exhibited by controls (no odor cues).

The experiment used a flow-through tank similar to that in Expt 1. However, water was supplied at a steady rate of 2.5 l min⁻¹ through a hose fixed with its tip flush to the edge of a hole in the middle of the tank floor. Water exited the tank through a 6 cm diameter hole in the tank wall, located 3 cm above the tank floor. Five concentric rings, 5 cm apart were marked on the tank floor around the water supply. The outermost ring defined the edges of the region of interest (ROI), within which urchin behavior was recorded with a time-lapse camera (GoPro Hero 4). A perforated PVC endcap (diameter 12.5 cm, height 2.5 cm) was placed on the innermost ring of the ROI, directly above the hose supplying the water from the tank floor.

The experiment was started by placing one live urchin 10 cm from the center of the ROI. The camera

was mounted 40 cm above the ROI and the field of view (FOV) included the entire tank (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m664p133_supp.pdf). The images were recorded every 30 s and experiment was conducted for 1 h. Four treatments were defined: (1) a control (no dead urchin or algae; no chemosensory cue), (2) an algae treatment (algae only; positive chemosensory cue), (3) a dead urchin treatment (dead urchin only; negative chemosensory cue), and (4) a dead urchin and algae interaction treatment (combined chemosensory cues). To expose the test urchin to the treatment effect, algae were attached to the top of the PVC cap with clips while a recently crushed *H. crassispina* was placed within a mesh bag below the cap. Therefore, water flowing through the hose and through the cap ensured that chemosensory cues from the treatment would be dispersed outwards across the tank. The experiment was conducted on both urchin hunger states (i.e. starved and fed) and in Su–Au and Wi–Sp.

An opaque plastic sheet covered the entire experimental apparatus to remove all ambient light. However, below the sheet, a red LED lamp (ISL-150×150, CCS Inc.) provided enough light to record images while minimizing light disturbance to the urchins (Flukes et al. 2012). After every trial, the test urchins were removed, and the experiment chamber was rinsed thoroughly with freshwater and seawater to eliminate chemical cues from the previous experiment.

Experiments were conducted in the Su–Au (August–November 2018) and Wi–Sp (April–May 2019) seasons. A total of 111 individuals with test diameter 4.31 ± 0.32 cm were used for the Su–Au experiment and 88 individuals with test diameter 4.27 ± 0.58 were used for the Wi–Sp experiment.

For each trial, the time-lapse images were concatenated into an mpeg-4 video using FFmpeg (FFmpeg Developers 2018) at a frame rate of 10 frames per second (fps). Videos were analyzed with Tracker v.5.0.6 (Brown 2018) to determine the movement pattern of the urchins. Each video frame (i.e. image) was counted as one event of a particular behavior. The following behaviors were possible: (1) none, any immobile behavior within the ROI, whereby urchins do not move more than 3 cm from starting point; (2) movement, whereby urchins move freely inside the ROI (i.e. video frame field-of-view); (3) interaction, whereby urchins make contact with the center of the ROI which may or may not contain seaweed or dead urchin (the change in seaweed weight was not measured); and (4) outside, whereby

urchins went outside the ROI. The x and y coordinates of sea urchins were analyzed to determine the time an urchin spent (in minutes) displaying a particular behavioral type and to determine urchin movement speed (cm min^{-1}).

2.4. Cue dispersal rate

The chemical cue plume was visualized and quantified using a 2% Fluorescein tracer-seawater solution as a proxy. A 3 mm diameter hose was attached to the tank floor so that the tracer was injected below the PVC cap and perpendicular to the water flow. The 50 ml of tracer was injected at a rate of 1.6 ml s^{-1} . Dispersal of the tracer was recorded on video for 1 h. Three trials were conducted per treatment, including control. The time for the tracer to reach the 10 and 20 cm ring was recorded and analyzed to determine if there were any differences among treatments.

2.5. Light measurements

The spatial homogeneity of the red light provided by the LED lamp was also assessed. Light was measured using 5 light loggers (HOBO MX2202 Temp/Light, Onset Computer Corporation) that were placed on each ring to form a line. After the initial measurement, the line was rotated 30° , for a total of 4 times. At every rotation, light was measured for 1 h.

2.6. Effect of positive and negative chemosensory cues on urchin foraging behavior in the field

To examine the effects of chemosensory cues by food and dead conspecifics on sea urchin behavior under natural conditions, we prepared a site that was located at a depth of 4–5 m in a barren rocky area adjacent to natural stands of *Sargassum macrocarpum* in Arikawa Bay (32.9880°N , 129.1186°E), Nakadorijima Island, Nagasaki, Japan. A 2 m^2 plot of flat rocky substrate was selected. For each experimental trial, a 2 m tall, slotted angle-bar tripod frame with an approximately 1 m^2 FOV was deployed. A time-lapse camera (TLC200 PRO, Brinno) enclosed in a custom acrylic housing was secured to the top of the frame. Images were taken every 30 s for a total of 3 h and stored as a video with a frame rate of 15 fps. The experiment was conducted during slack tide, when the tidal current was negligible. A velocity logger (Compact-EM,

Alec Electronics) and a water level logger (HOBO U20-001, Onset Computer Corporation) were deployed 1 m from the experimental plot to record hydrodynamic conditions during the experiment.

A weighted plastic cage was placed in the middle of the 1 m² experimental plot. Drift algae (i.e. *Sargassum* spp., *Dictyopteris* spp.) common during the experiment period was collected and clipped outside of the plastic cage. For each treatment, 5 trials were conducted. For the control, a single urchin was placed in direct contact with the algae until they attached. For the dead urchin and algae treatment, a recently killed *H. crassispina* was added inside the cage with the algae to determine whether urchins would be repelled. Urchins were killed just before the experiment started, by crushing their test. The experiments were conducted first on the fed and then on the starved urchins. A total of 20 individuals with test sizes of 4.72 ± 0.68 cm were used for the field experiment.

Video was analyzed with Tracker v.5.0.6 (Brown 2018) to track the urchins; however, the tripod attracted small fish, which occluded the FOV. Additionally, during a number of days the area experienced relatively high waves, which vibrated the tripod and resulted in poor quality images. Thus, only the initial and final position (stay or flee) of the urchin was noted after the 3 h experiment period.

2.7. Data analyses

2.7.1. Laboratory Expt 1: Urchin grazing rate

Urchin grazing rate data was analyzed using a Bayesian generalized linear model where the mean grazing rate (g algae urchin⁻¹ d⁻¹) was the response variable and the explanatory variable was the season. Weakly informative priors were used for the intercept and coefficients. A Gaussian distribution with a location of 1.1 and a scale of 2.5 was the prior for the intercept, a Gaussian distribution with a location of 0 and scale of 2.5 was the prior for the coefficients, and an exponential prior with a rate of 1 was the prior for the error term.

2.7.2. Laboratory Expt 2: Effect of positive and negative chemosensory cues on urchin foraging behavior

Initial inspection of the urchin movement behavior data revealed an over-abundance of zeroes, as not all

behaviors were represented equally for every trial. Meanwhile, some behaviors had more occurrences compared to others. Both observations generally cause issues such as zero-inflation and over-dispersion. To overcome this problem, hurdle-models were used for model fitting which consist of a 2-step procedure beginning with a Bernoulli probability, which evaluates whether a count is non-zero. If a positive, non-zero value is found, this 'hurdle' is crossed, and the process continues with a truncated-at-zero count distribution model for the non-zero state (Lewin et al. 2010, Kassahun et al. 2014). This is similar to a decision-making process because the outcome of an individual's behavior can depend on existing environmental conditions (i.e. treatment).

Specifically, urchin behaviors in the laboratory experiments were analyzed with a hurdle-negative binomial model:

$$\begin{aligned} y &= (1 - \pi)\text{NegBin}(0, \theta) + \pi\text{NegBin}(\mu, \theta) \\ \mu &= x\beta \\ \log \frac{\pi}{1 - \pi} &= x\alpha \end{aligned} \quad (1)$$

where y is a vector of observations, in this case the number of occurrences for a behavior during the 1 h observation period; π is a vector of probabilities for non-zero values, α and β are vectors of coefficients for a model including all treatment interactions; and x is a matrix of factors that include all treatment interactions. The number of occurrences is assumed to follow a negative binomial distribution, with a vector of locations μ and a scale θ . The main treatments are the presence or absence of algae and the dead urchin, the hunger state of the test urchin, season (i.e. Su–Au or Wi–Sp), and the type of behavior, excluding the behavior 'none' (see Fletcher et al. 2005, Zuur et al. 2009).

In the case of sea urchin speed and time spent per behavior, where the response was a continuous variable, a hurdle-gamma model was applied. The structure of the model is similar to Eq. (1); however, rather than a negative binomial distribution, a gamma distribution is assumed. In this case, $y = (1 - \pi)\Gamma(0, \theta) + \pi\Gamma(\mu, \theta)$. For more details on the merits of the hurdle model, see Lewin et al. (2010).

The β coefficients of the hurdle-negative binomial model and the hurdle-gamma model were given weakly informative Student's t -distributions as prior distributions, with 3 df, a location of 0, and a scale of 1. The α coefficients were given logistic distributions as priors with a location of 0 and a scale of 1. The prior for θ was a Γ distribution with a shape and scale of 0.01.

2.7.3. Cue dispersal rate

The data on the Fluorescein tracer dispersal experiment was analyzed using a Bayesian generalized linear model, where the time it took for the tracer to reach the 10 cm mark was the response variable and the explanatory variables were the experimental treatments. The prior distributions were similar to that of the urchin grazing rate analysis, but the location for the prior intercept was 18.

2.7.4. Light measurements

For the light experiment, a Bayesian generalized linear model was fitted to the data, where the response variable was the light level and the explanatory variables were the positions of the light loggers. The prior distributions were similar to that of the urchin grazing rate analysis, except that the location for the prior intercept was 1.7.

2.7.5. Field experiment: Effect of positive and negative chemosensory cues on urchin foraging behavior in the field

The field experiments were analyzed with a Bayesian binomial generalized linear model with a random intercept for the tidal state:

$$y = \text{Binom}(n, \pi) \quad (2)$$

$$\log \frac{\pi}{1 - \pi} = x\gamma + \delta$$

In Eq. (2), y is the vector of observations, n is the vector of total trials and π is the vector of probabilities; x is the matrix of treatments, which in this case is a linear combination of the hunger state of the test urchin (i.e. starved or fed) and the presence or absence of the dead urchin; δ is a random intercept for tidal state, and γ is the vector of coefficients. The prior distributions for the coefficients and random intercept was a Student's t -distribution with 3 df, a location of 0, and a scale of 5.

All statistical analyses were done using R v.3.6.1 (R Development Core Team 2019) and all Bayesian inference was done with Stan (Stan Development Core Team 2019) through the 'brms' (Bürkner 2017) and 'RStanarm' packages (Goodrich et al. 2018). Stan

primarily uses a Hamiltonian Monte Carlo sampler to construct the posterior distributions of the parameters. For all models, a total of 4 chains were evaluated to generate 2000 samples chain⁻¹. All chains of all models were assessed for convergence.

3. RESULTS

3.1. Laboratory Expt 1: Urchin grazing rate

The results revealed differences in feeding rates between seasons. Sea urchins had higher expected mean feeding rates in Su–Au of about 1.3 g algae urchin⁻¹ d⁻¹ (1.2–1.5 95% highest density intervals [HDI]) (Table 1A). Conversely, sea urchin feeding rates decreased to 0.8 g algae urchin⁻¹ d⁻¹ (0.6–1.0 95% HDI) in Wi–Sp. There was a 23% difference in the mean maximum ambient water temperature between seasons (Fig. 1).

3.2. Laboratory Expt 2: Effect of positive and negative chemosensory cues on urchin foraging behavior

3.2.1. Behavior counts

The light intensity during the experiment was low (Table S1 in the Supplement) but allowed us to observe the movement of urchins with the time-lapse camera. The time-lapse experiment showed that the counts of the 4 behavior types varied widely across sea urchin condition, season, and treatment. The occurrence of the immobile behavior 'none' occurred more in Wi–Sp (starved: 15 urchins; fed: 21 urchins) than in Su–Au (starved: 16 urchins; fed: 15 urchins) (Fig. 2). The occurrence of this behavior in Wi–Sp

Table 1. Results of the Bayesian generalized linear models on (A) Expt 1: feeding rate and season and (B) cue dispersal time to reach the 10 cm mark. The table shows the estimates, expected value, and lower and upper limits of the 95% highest density interval (HDI) of the expected value

Estimates	Expected value	2.5%	97.5%
(A) Lab Expt 1: Feeding rate (g algae urchin⁻¹ d⁻¹)			
Summer–Autumn	1.3	1.2	1.5
Winter–Spring	0.8	0.6	1.0
(B) Cue dispersal time (seconds)			
Control	12.2	–1.4	24.9
Algae	17.5	4.3	31.1
Dead urchin	23.0	9.8	36.5
Algae + dead urchin	19.7	7.8	33.4

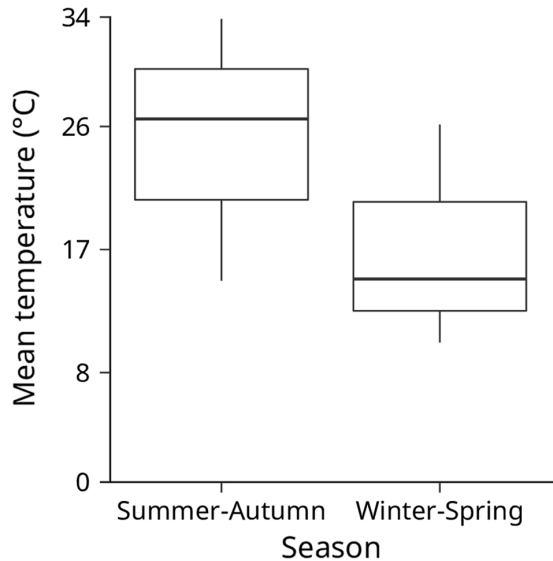


Fig. 1. Mean temperature difference between Summer-Autumn and Winter-Spring seasons in the feeding rate experiment

represents 40.9% of the sea urchins used in that season while this behavior represented about 27.9% of the total sea urchins used in Su-Au.

Sea urchins were generally more active in Su-Au than in Wi-Sp. For interaction, this behavior was more frequent among trials in Su-Au (6%) than in Wi-Sp (2%). Interaction was also more frequent among starved sea urchins (5%) than fed urchins (4%).

Comparing the effects of the algae-only and combined chemosensory cues treatments showed differing responses across nutritional states. The model showed that when only algae were present, fed urchins had a mean interaction count of up to 33.8 (2–71 95% HDI) in Su-Au and 26.6 (1–61 95% HDI) in Wi-Sp. When a dead urchin was present together with the algae (Fig. 2L,P), this led to a mean decrease in their interaction counts to 12.9 (1–32 95% HDI) and 8.3 (1–21 95% HDI) in Su-Au and Wi-Sp, representing about 61.8 and 68.7% decrease, respectively. For starved urchins, their hunger state led to high interaction counts relative to fed urchins of 66.7 (1–161 95% HDI) in Su-Au and 66.0 (1–163 95% HDI) in Wi-Sp when only algae were present. Under the combined chemosensory cues treatment, starved urchins had interaction counts of 51.1 (5–116 95% HDI) in Su-Au and 24.5 (2–60 95% HDI) in Wi-Sp. This shows a 23.4 and 62.9% decrease between seasons, respectively. In Wi-Sp, starved urchins also had a higher proportion of immobile individuals

across both hunger states. The presence of the dead urchin with the algae also increased the number of 'outside' behaviors across both seasons for fed urchins (8.7 and 25.2% for Su-Au and Wi-Sp, respectively), but not for starved urchins. They show decreased 'outside' behaviors of up to 33.2 and 6.11% for Su-Au and Wi-Sp, respectively. Overall, both hunger states seem to be sensitive to the chemical cues from dead urchins, but starved urchins appeared to interact more with the algae despite the predation cues. The expected value and prediction intervals for behavior counts are shown in Table S2 while those for the probability of behaviors achieving zero counts are shown in Table S3.

3.2.2. Time spent per behavior

The time-lapse experiment demonstrated that condition and season influenced the sea urchins' allocated time performing a specific behavior (Fig. 2). Overall, 34% of sea urchins spent the entire 1 h experiment period immobile. Of that number, 42 and 27.9% occurred in the Wi-Sp and Su-Au experiments, respectively.

The model predictions showed that the presence of a dead urchin had an influence over the time urchins spent performing a particular behavior. Under the algae treatment, fed urchins had an average interaction time of about 17.7 min (0.988–40.5 95% HDI) in Su-Au and 13.6 min (1.24–33.3 95% HDI) in Wi-Sp. Under the combined chemosensory cues treatment, fed urchins had a mean interaction time of 6.5 min (0.180–14.7 95% HDI) in Su-Au and 4.3 min (0.180–10.6 95% HDI) in Wi-Sp. This shows an 11.2 and 9.3 min difference in interaction time across seasons, respectively. Starved urchins were predicted to have relatively higher mean interaction times relative to fed urchins when only algae were present (Su-Au: 33.4 min, 1.61–82.1 95% HDI; Wi-Sp: 33.2 min, 0.982–81.1 95% HDI). Under the combined chemosensory cues treatment, urchins in Su-Au had a mean interaction time of 25.3 min (0.929–59.2 95% HDI) while urchins in Wi-Sp had a mean interaction time of 12.9 min (0.552–31.5 95% HDI). This shows a decrease of 8.1 and 20.3 min for Su-Au and Wi-Sp, respectively. The time spent outside the ROI also increased across both seasons for fed urchins (4.1 and 7.6 min for Su-Au and Wi-Sp, respectively). The starved urchins showed decreased time outside the ROI by about 8.4 and 0.9 min for Su-Au and Wi-Sp, respectively. As expected, when a dead urchin was present, the fed urchins interacted less with the

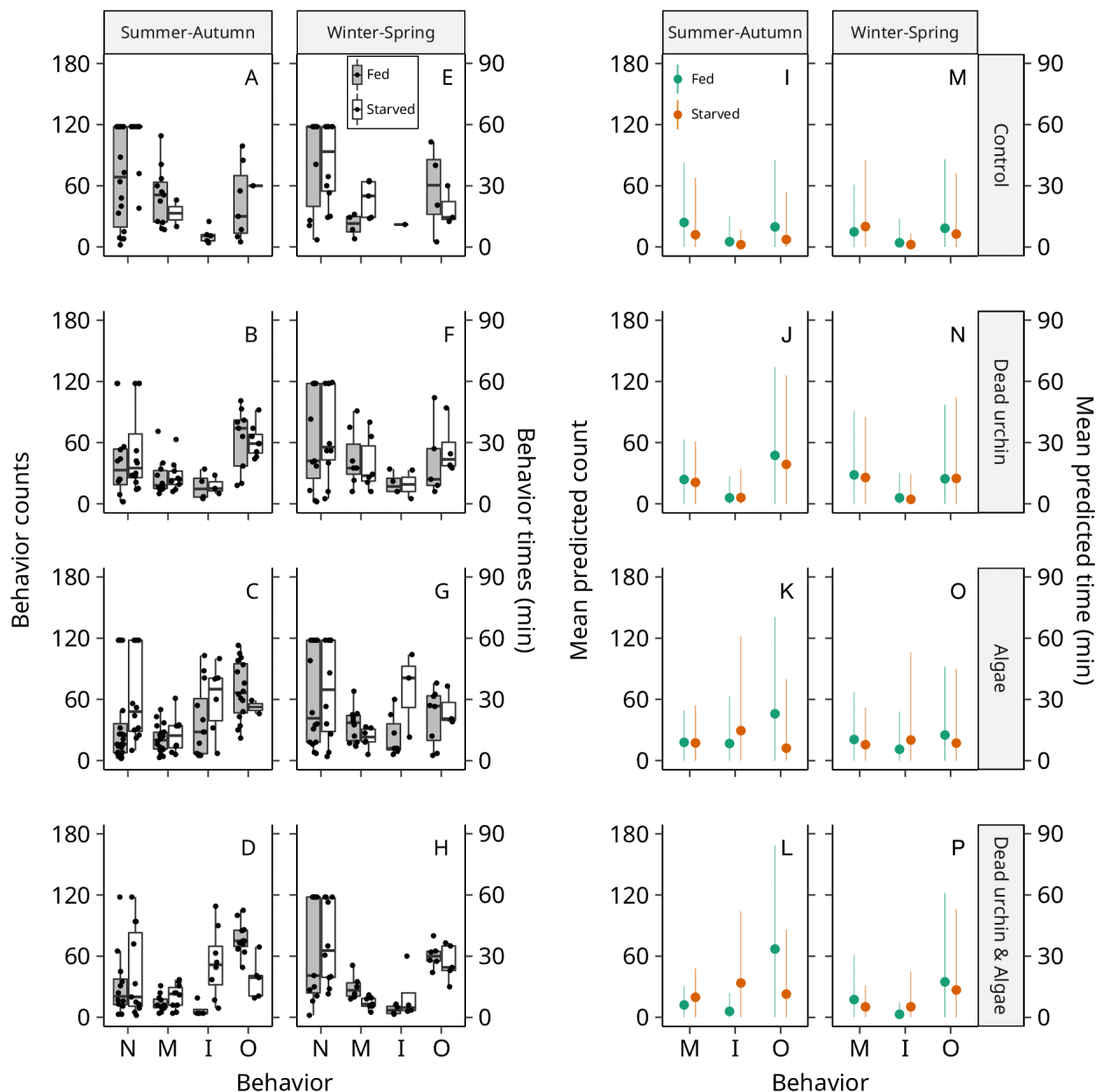


Fig. 2. Expt 2 (A–H) empirical data and (I–P) model prediction of the urchin behavior counts (left y-axes) and urchin behavior time (right y-axes). The scale for behavior time is half that of the behavior counts because time-lapse was taken at 30 s intervals, with 2 frames comprising every 1 min observation. For behavior counts, each frame was counted as one observation. Columns: seasons; rows: treatments. Behavior abbreviations: N: none; M: movement; I: interaction; O: outside. Boxplot extents: 25 and 75 % percentiles; horizontal line: median; vertical lines: whiskers extending 1.5 times the inter-quartile range; overlaid points: observations for each behavior. In (I–P), the points indicate the predicted mean while bars are the 95 % highest density intervals of the predictions

algae and increased their time spent outside the camera FOV, indicating that the urchins were repelled by the presence of the dead urchin chemical cues. Similarly, starved urchins showed a decrease in interaction time but by a slightly lesser rate. The decrease in time spent outside, despite the presence

of the dead urchin, suggests that the hunger state was able to influence urchin behavior. Estimates and prediction intervals for time spent per behavior are shown in Table S4 while those for the probability of the behavior time being zero minutes are shown in Table S5.

3.2.3. Urchin movement speed

The time-lapse experiment revealed urchin movement speeds to vary across treatments depending on their condition and the season. It was noted that even when the urchins were exhibiting the behavior 'none', small movement speeds were recorded as urchins shuffled in place within the 3 cm limit. The urchins exhibited greater speeds when a dead urchin was present. Overall, fed urchins had higher movement speeds relative to the starved urchins (Fig. 3).

The model predictions indicated that urchins tended to move at a different pace depending on the treatment (Fig. 3). When only algae were present, fed urchins had mean interaction speeds of 16.6 cm min^{-1} (0.243–46.5 95 % HDI) in Su–Au and 12.5 cm min^{-1} (0.164–34.3 95 % HDI) in Wi–Sp. Under the combined chemosensory cues treatment, fed urchins had mean interaction speeds of 24 cm min^{-1} (0.141–65.9 95 % HDI) in Su–Au and 21.1 cm min^{-1} (0.375–64.4 95 % HDI) in Wi–Sp. Between treatments, fed urchin speed while interacting with the algae increased by

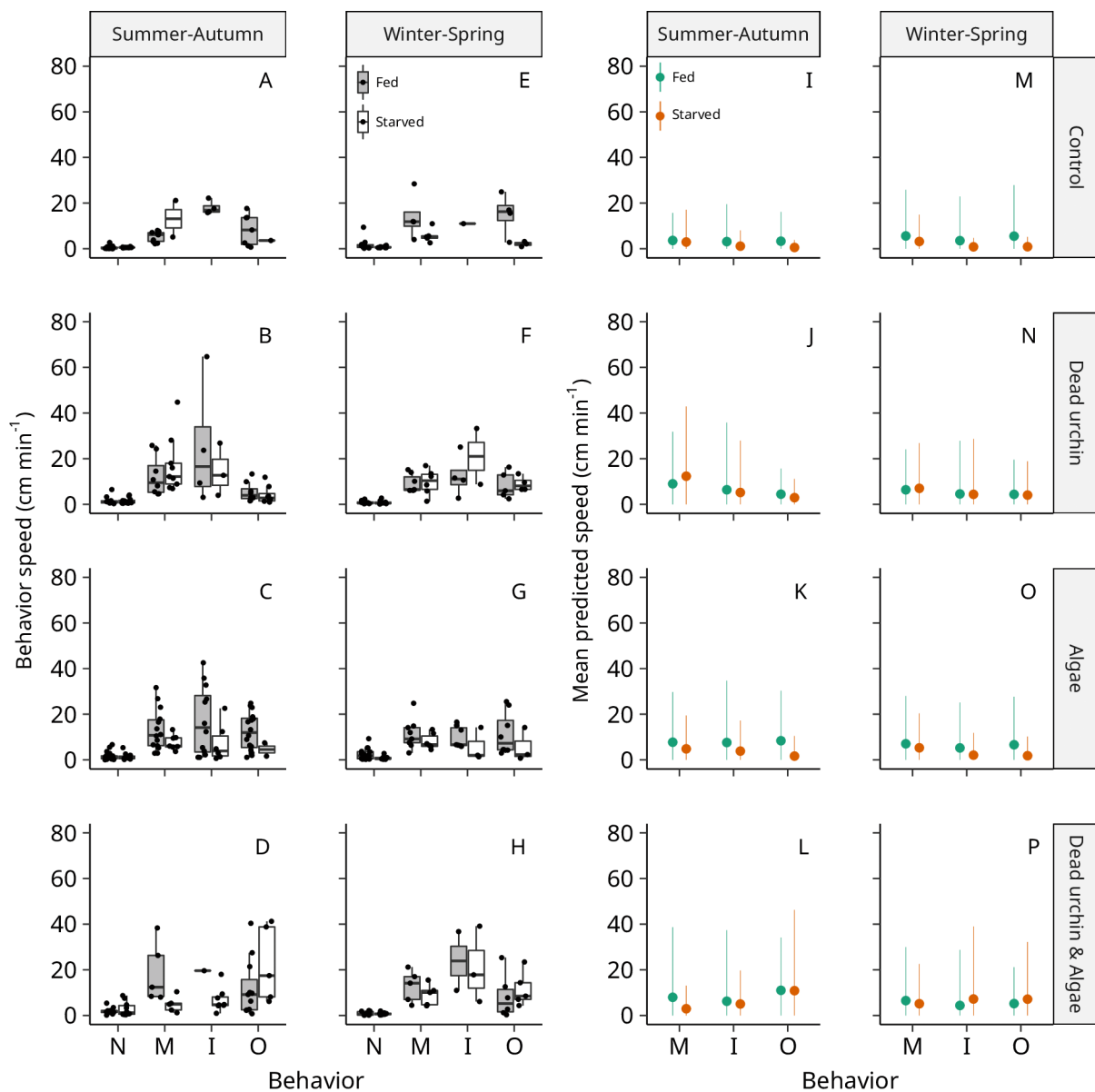


Fig. 3. Expt 2 (A–H) empirical data and (I–P) model prediction on urchin speed (cm min^{-1}) for each behavior. Columns: seasons; rows: treatments. Boxplot parameters and abbreviations as in Fig. 2

7.4 and 8.6 cm min⁻¹ in Su–Au and Wi–Sp, respectively, when a dead urchin was present. For starved urchins, under the algae-only treatment, predicted interaction speed was 7.9 cm min⁻¹ (0.030–21.7 95 % HDI) in Su–Au and 6.8 cm min⁻¹ (0.073–18.7 95 % HDI) in Wi–Sp. In the combined chemosensory cues treatment, interaction speeds were 8.3 cm min⁻¹ (0.043–23.0 95 % HDI) in Su–Au and 21.4 cm min⁻¹ (0.302–60.9 95 % HDI) in Wi–Sp. There appeared to be a slight increase in urchin speed in Su–Au of about 0.4 cm min⁻¹, but a large rate of increase of about 14.6 cm min⁻¹ for urchins in Wi–Sp. Movement rates within the ROI also increased for fed urchins while outside speeds increased for starved urchins. Examining urchin speeds showed that signals of predation may cause stress to *Heliocidaris crassispina*, as indicated by the relatively high movement speeds across both nutritional states and seasons even when outside the ROI. Estimates and prediction intervals for urchin speeds per behavior are shown in Table S6 while those for the probability of behavior speeds being zero cm min⁻¹ are shown in Table S7.

3.2.4. Cue dispersal rate

The results from the experiment examining the rate of spread of the Fluorescein tracer dye showed high variation among trials (Table 1B). The time it took for the tracer to reach the 10 cm mark was modeled since it represented the area where the urchin would first encounter the chemosensory cues coming from the center of the ROI. The control, with nothing beneath or above the treatment container, took the least amount of time and had an expected mean time of 12.2 s (–1.4–24.9 95 % CI). Ranking the treatments from those that took the least to the greatest amount of time resulted in the order: control, algae effect, dead urchin and algae interaction effect, and dead urchin effect. The wide range for all treatments was due to the low sample size (3 trials treatment⁻¹). However, high variations between trials among the algae, dead urchin and dead urchin and algae treatments suggests that the variation was associated with the size of the dead urchin or the density of the algae used as treatment for the experiment.

3.3. Effect of positive and negative chemosensory cues on urchin foraging behavior in the field

The field experiment showed that sea urchin condition produced discrete responses between starved

and fed urchins to the presence of dead conspecifics adjacent to an available food source. Of the 20 sea urchins used in the experiment, all 10 starved sea urchins (100 % of the starved condition) stayed and remained in contact with the treatment cage. For the fed sea urchins, only 3 stayed (30 %) while 7 fled from the treatment cage (70 %). Of the 7 sea urchins that fled, 4 (40 %) were from the treatment which contained the dead urchin. The binomial model predictions suggest a strong link between urchin condition and outcome of behavior (Fig. 4).

Of the 20 trials conducted, 11 experiments were conducted during low slack tide and 9 during high slack tide. In general, mean temperatures and current speeds were higher during low tide relative to high tides (Fig. S2).

4. DISCUSSION

4.1. Factors affecting sea urchin behavior patterns

The results of our study provide evidence that TMIs are an effective component of top-down trophic cascades (Schmitz et al. 2004). We found distinct behavioral patterns between starved and fed *Heliocidaris crassispina*, indicating that hunger-state determines an individual's propensity to accept a certain degree of risk to acquire critically needed resources. For starved urchins, there was a greater proportion of urchin interaction and increased interaction time with the algae despite the presence of a dead urchin. Fed urchins exhibited predator avoidance behaviors, observed as decreased interaction and increased occurrences of behaviors spent outside the ROI when a dead urchin was present with the algae.

Studies on predator–prey relationships highlight the 'Hobson's choice' (i.e. face the risk of predation or starve) dilemma all prey species face upon venturing out from the safety of their refuge when they forage for food (Clark 1994). Ultimately, the decisions prey species make lean towards optimizing the trade-off to their advantage by minimizing risk while maximizing benefits. However, intrinsic (i.e. reproductive condition and hunger level) and extrinsic (i.e. temperature, light, and salinity) factors also play an important role in affecting decision-making processes for aquatic organisms. For example, the effects of starvation in urchins not only impact their energy reserves but also their gut and gonad indices (Lawrence 1970). A study on the effects of starvation on *H. crassispina* and *Hemicentrotus pulcherrimus*

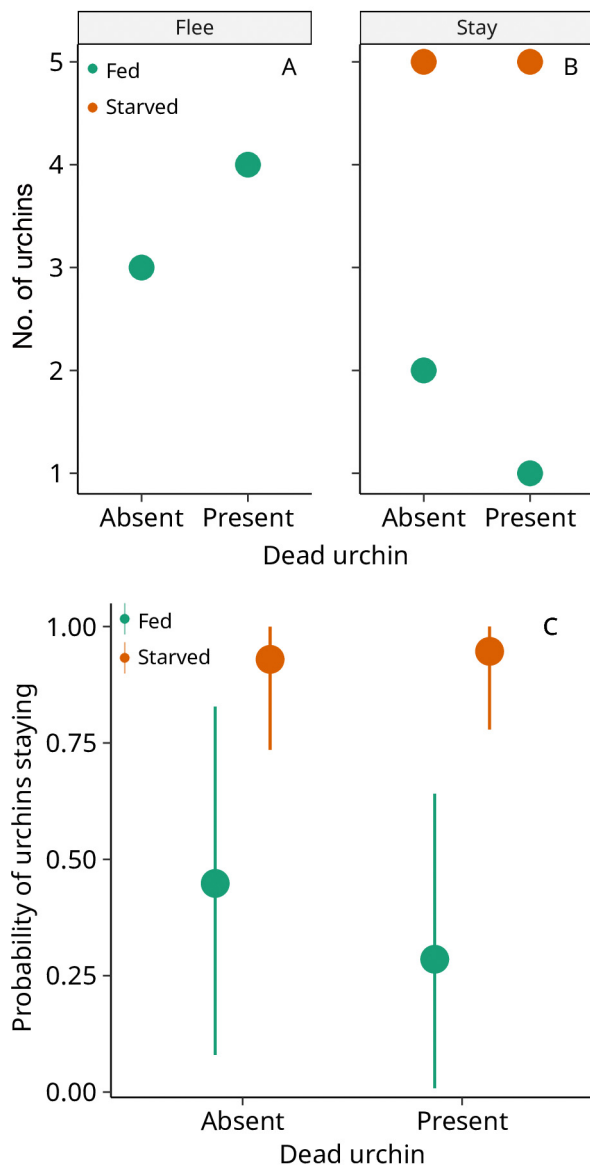


Fig. 4. Field experiment (A,B) empirical data and (C) binomial model prediction of urchin behavior outcome in the field. For (A) and (B), y-axis: number of urchin counts; x-axis: either presence or absence of a dead urchin with the algae as treatment; columns: urchin response. Note: (A) refers to 'flee' and since all starved urchins (orange dots) stayed, they are only reflected in (B). For the binomial model, y-axis: the binomial probability of urchin behavior (i.e. flee: 0, stay: 1); x-axis: presence or absence of a dead urchin together with the algae; points: expected means; vertical lines: 95% highest density intervals of the expected values. Results show that starvation makes urchins less likely to flee regardless of the presence or absence of a dead urchin

showed that gut clearance was achieved in 3 d for *H. crassispina* and 6 d for *H. pulcherrimus*. Additionally, *H. pulcherrimus* survived a maximum of 49 d without food, albeit negatively impacting gut and gonad

indices (Kaneko et al. 1981 as cited in Agatsuma 2013). In our study, the 1 wk starvation period induced a hunger response which appeared to override predator avoidance behaviors. The starved urchins, which had an energy deficit, were willing to accept greater risks by feeding longer and more frequently in the presence of a dead urchin to increase energy reserves, hence supporting the asset protection principle (Clark 1994). Note that the microcosm experiment utilized a relatively small chamber which may have allowed a faster saturation of sea urchin effluents and thus increased the urchin responses artificially. Our experiment did not provide refuge for urchins, which may explain their rapid movements inside and outside the ROI when dead urchin cues were present. Furthermore, the manipulation of sea urchin condition by starvation as done in our study does not fully mimic conditions in the field. Recall that sea urchins are generalist algal feeders (Vadas 1977), have flexible dietary preferences, and are omnivorous (Rodriguez-Barreras et al. 2015). *H. crassispina* in seaweed bed habitats generally had higher gonad indices and were larger in size compared to urchins collected from a habitat dominated by *Coralina* spp. (Yatsuya & Nakahara 2004a). In the barrens, they were more cryptic and switched to feeding on a mixed diet composed of drift *Sargassum* and calcareous algae (Yatsuya & Nakahara 2004b).

A recent study utilizing starved and satiated *Strongylocentrotus droebachiensis* showed that neither sea urchin group reacted adversely to the presence of a live nearby predator (*Cancer borealis*) (Harding & Scheibling 2015). Their findings indicate that the olfactory cues coming from live predatory crabs do not reduce urchin foraging behavior in the laboratory and in the field. In contrast, studies on chemical alarm cues showed that dead conspecifics and chemically labelled predators had strong adverse effects on urchin behavior (Campbell et al. 2001, Morishita & Barreto 2011). Specifically, the urchins distinctly avoided waters conditioned with urchin gut, coelomic, and gonad homogenates, which are the materials most likely to be exposed when a predator breaks an urchin's test (Campbell et al. 2001).

In our study, instead of completely avoiding the source of the chemical cues coming from the dead urchin treatment, some urchins actually approached the dead urchin treatment. In small prey species of fish, this behavior is known as predator 'inspection', and has distinct importance for prey species because it functions as a learning tool to enable naive prey to associate predators with danger (Magurran & Girling 1986). As individuals of prey species grow and reach

sizes which provide refuge from direct predation, their fear of predators remains and continues to affirm the effects of top-down control (Pessarrodona et al. 2019). For urchins, since olfaction occurs when odor molecules reach receptors in their tube-feet, predator inspection may need to occur at close range as odor molecules increase in concentration (Magurran & Girling 1986). The next time they encounter familiar chemical cues relating to risk of predation, they may better assess the motivation of the predator and the relative risk of an impending predation event (Clark 1994). Furthermore, for urchins living in urchin barrens, it may be possible that these urchins had reached large population sizes due to the absence of their natural predators. The absence of predators meant that it was likely that they were naive and had had little chance to encounter chemical cues relating to predation.

When comparing sea urchin behavior patterns across seasons, we found that there was a discrete pattern observed between the Su–Au and Wi–Sp experiments. In general, sea urchins were more active and exhibited higher speeds in Su–Au compared to sea urchins used in Wi–Sp. The greater decrease in the interaction frequency and interaction time in Wi–Sp for starved urchins was attributed to fewer urchins interacting with the treatment as well as more urchins moving outside. Interestingly, urchin speed was predicted to be highest in Wi–Sp when starved individuals were exposed to dead urchins together with food. This is likely to be an evasive behavior in response to the scent of the dead urchin since the proportion of outside behaviors and movement speeds also increased. At the same time, when only food was present, starved urchins interacted with the algae longer and moved slower, indicating a stronger intent to feed compared to behavior exhibited by the fed urchins (Fig. 2). Their level of activity was also reflected in their feeding rates, as urchins in Su–Au had the highest average feeding rates while urchins in Wi–Sp had the lowest rates (Table 1A). A similar pattern was found among cold-water urchin species, for which temperature is one of the main drivers of metabolic activity (Agatsuma et al. 2000, Brockington & Clarke 2001).

Studies on the reproductive biology of *H. crassispina* showed that this species has a distinct seasonal cycle in terms of gonadal development and maturation. In Nagasaki, Japan, a study on the reproductive patterns of *H. crassispina* (Yamasaki & Kiyomoto 1993 as cited in Agatsuma 2013) found that this species spawns during the months of July–August while their recovery period is from Septem-

ber–January. The remaining months are dedicated to growth and maturation of the gonads. This pattern was also found in similar studies elsewhere in Japan (Kyoto: Yatsuya & Nakahara 2004a; Oga Peninsula: Feng et al. 2019) and in Korea (Yoo et al. 1982). In Hong Kong, a 7–8 mo spawning period was recorded. This relatively long spawning period was represented by 2 distinct spawning events in May–June and September–October (Urriago et al. 2016). After every spawning event, urchins experienced an abrupt decrease in gonad indices as well as lipid and fatty acid profiles (Martinez-Pita et al. 2010, Diaz de Vivar et al. 2019). The lipid- and nutrient-deficient state indicated that the urchins were in a low nutritional condition (Lawrence 1970). Urchins compensated by increasing their feeding rates beginning from the end of summer until the next spring, coinciding with winter macroalgal blooms (Kaehler & Kennish 1996). Increasing feeding rates from summer ensured the accumulation of energy to support gonadal growth and maturation, as reflected in the biochemical composition and other intrinsic gonad properties (Rocha et al. 2019). Hence, the rise in summer metabolic activity in urchins was only partially explained by temperature but was likely predominantly driven by feeding, growth, and reproduction (Brockington & Clarke 2001).

4.2. Field experiment

The field experiment results provide evidence of urchin condition affecting the strength of behavior modification in nature. Compared to fed sea urchins, all starved urchins stayed regardless of the presence or absence of a dead urchin. The results of our experiment contrast with a field experiment using live crab predators, where only a 6% flee response rate was recorded (Harding & Scheibling 2015). Few studies have previously investigated effects of predation risk cues on prey species in the field because of the inherent difficulty in controlling for local flow conditions. The data recorded from the field showed high variability in flow speeds and temperatures between low and high tides. A laboratory study simulating the flow of chemical odor plumes in turbulent conditions suggests that the success of odor-guided navigation is greatly dependent on dilution and the degree of shear-induced mixing of odor signals (Webster & Weissberg 2001). This is particularly true for small benthic invertebrates because sampling the water for odor molecules occurs at a relatively fast rate (Zimmer & Butman 2000), but at a lower height relative

to the substrate (Smee & Weissberg 2006). Furthermore, organisms attempting to orient themselves relative to the direction of the odor plume would find it challenging because odor dispersal occurs as intermittent odor packets interspersed with clean water (Finelli et al. 1999). In the present study, the sea urchins would have had no problem detecting the odor from the dead urchin and seaweed because they were placed in direct contact with the treatment cage at the start of the experiment, unlike in the laboratory experiment. Although concentrations of urchin effluent were not tested, it is likely that the immediate area surrounding the treatment cage would have been saturated with the dead urchin effluent. The fleeing response of some of the fed sea urchins appears to be a behavior related to minimizing predation risk in lieu of feeding. However, with only a short 3 h experimental period, we were not able to observe how the starved urchins would have behaved once they had adequately fed on the seaweed or how long the dead urchin effluents would have remained effective. Hence, for future studies, we propose a longer observation time for urchin behaviors as well as identification of the components responsible for the urchin alarm response and their maximum length of efficacy (i.e. Spyksma et al. 2020) as affected by dilution.

5. CONCLUSIONS

Our results showed that the foraging behavior of *Heliocidaris crassispina* was flexible and that individuals were able to assess and adjust accordingly to the presence of chemical cues associated with predation. To some extent, our results support the idea that season and phenology appear to modulate urchin behavior and foraging activity (Luttbeg et al. 2003). Our experiments also demonstrate that the presence of a dead urchin does not prevent live urchins from interacting with the seaweed but instead decreases the interaction frequency and length of interaction time while increasing movement speeds, indicating escape behaviors. All these changes in urchin behavior decrease feeding opportunities and therefore reduce the grazing pressure on algal biomass. Furthermore, starved urchins seemed to be insensitive and indifferent to predation cues. The 100% stay response from starved urchins despite the presence of a dead urchin in the field experiment further reinforced our hypothesis. Our findings suggest that the urchin's hunger state is a key determinant in its decision-making

process and that level of hunger may override behaviors associated with predator avoidance. This puts them at a disadvantage as starved urchins feed more boldly, further exposing themselves to the dangers of predation.

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