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

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ABSTRACT

The top-down component of trophic cascades exerts a powerful effect between predator and 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 cues are powerful enough to control prey populations and indirectly protect primary producers. We evaluated the effects of cues associated with predation on the purple urchin, *Heliocidaris crassispina* and also examined the how starvation and season influenced the effects. Using time-lapse photography, we conducted a series of manipulative and *in situ* behavior experiments to determine foraging behavior patterns which demonstrate behavior modification. The results suggest that starved urchins were less sensitive to predation cues when compared to normally fed urchins. Field experiments indicated that a proportion of fed urchins fled when exposed to a predation cue (presence of a dead urchin), whereas all starved urchins remained regardless of the cue, which complemented the results from the laboratory. Sea urchin activity and feeding rates were lower in winter-spring than in summer-autumn. We suggest that starvation has a large influence over the decision-making

process of sea urchins, and their response is affected by season due to metabolic control. In general, the hunger response overrides predator avoidance behaviors and exposes prey species to higher risks of predation.

Keywords: top-down control, trophic cascade, behavior modification

1. INTRODUCTION

Predation and resource availability control food webs in the ecosystem (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. 2011). Within the canopy, the diversity of fish and invertebrate are high and they rely on the canopy for food and refuge (Lowry & Pearse 1973, Holbrook et al. 199, Kamimura & Shoji 2009). The sea urchin is a keystone species in marine forests, because they can overwhelm net benthic primary production (Tuya et al. 2004). In ecosystems where apex predator populations are intact, urchin populations are maintained through predation (Tegner & Levin 1983, Pearse & Hines 1987, Sala & Zabala 1996, Sievers & Nebelsick 2018). When predation pressure is removed, the urchin population leads to overgrazing, eventually converting them into barrens. In general, barren areas are characterized by low diversity and habitat complexity (Mangialajo et al. 2008). Large canopy forming macroalgae are replaced by grazing resistant turf forming macroalgae (Wright et al. 2005). However, 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) 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. 2013, Ogata et al. 2016), and installment of artificial reefs (Watanuki & Yamamoto 1990, Westermeier et al. 2013). Experimental evidence has shown that human intervention may succeed and promote seaweed forest recovery (Ling et al. 2010). However, maintaining restored algal forests is difficult due to the uncontrolled recruitment of urchin that eventually achieve population levels to establish dense feeding fronts (Lauzon-Guay & Scheibling 2007, Ling & Johnson 2009).

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). The direct reduction in 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 were observed in a variety of terrestrial and aquatic ecosystems (Shurin et al. 2002). In the aquatic ecosystem, the effects of top-down trophic cascade 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 TMII may be comparable in magnitude to that of DMII, because behavior change has population-wide effects, whereas direct predation only affects the individual (Peacor & Werner 2001).

Historically, algal forests composed of large brown algae created dense expansive belts around the coastline around 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 (Okuda 2008, Haraguchi & Sekida 2008, Fujita 2010). The loss of seaweed forests has led to the decline in coastal fisheries production (Kiyomoto et al. 2013). Efforts to revert the decline in seaweed forests has produced numerous guidelines and technology,

however success is limited (Terawaki et al. 2003, Fujita 2010, Kuwahara et al. 2010, Fujita 2015).

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 2019) and that negative responses from manipulative experiments ranged from strong (Campbell et al. 2001, Hagen et al. 2002) to weak (Harding & Scheibling 2015). Here we use dead conspecifics as a deterrent (Campbell et al 2001, Morishita & Barreto 2011) to explore the effects of other factors such as the prey species' nutritional condition and season on the urchin's decision-making process. The individual's nutritional condition (i.e. satiety vs. starvation) has been known to modulate an individual's perception of risk (Clark 1994) while the season is associated with reproductive phenology (Agatsuma et al. 2000, Yatsuya & Nakahara 2004a). In this study we examine prey behavior towards predation signals to better understand the role of predators in indirectly maintaining the integrity of the seaweed bed ecosystem.

The following questions were addressed in this study: 1) How does *H. crassispina* modify its foraging behavior when they encounter odor cues perceived to be a threat and a non-threat? 2) How does urchin nutritional state influence their tolerance to predation risk? and 3) How does season affect urchin response to predation risk?

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 one-ton tank with a constant flow of sand-filtered seawater and aeration. A Tidbit v2 (Onset Corp.) temperature logger monitored the ambient water temperature. Urchins were fed *ad libitum* with an assortment of fresh algae collected from Shihondo, Omura Bay, Nagasaki Prefecture, Japan. The feeding experiments used *Sargassum patens* C. Agardh and were also collected from Omura

Bay. Stock *S. patens* were kept in a separate outdoor tank which received water overflowing from the urchin stock tank. Urchins were acclimatized to laboratory conditions for one week prior to the experiments. Experiments involving Summer-Autumn and Winter-Spring seasons are hereafter referred to as S-A and W-S, respectively.

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

2.2. Laboratory experiment 1: Urchin grazing rate

To test the hypothesis that the ambient *H. crassispina* grazing rate was influenced by temperature and season, a flow-through rectangular tank (70 x 112 x 12 cm) was prepared. Ten numbered containers (2.96 L) were set in the tank, separated into two treatments. A feeding treatment that included urchins and algae and a control which contained only algae. A continuous water supply (11 L min^{-1}) was provided by an overhead perforated polyvinyl chloride (PVC) frame. A Tidbit v2

(Onset Corp.) temperature logger recorded ambient water temperature.

A total of 24 trials were conducted for both S-A (July-November 2018) and W-S (February-April 2019). There were 4 control treatments and 6 feeding treatments for each trial. 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 while their horizontal test width was measured using a firm-joint outside-caliper and a Vernier caliper to the nearest 0.01 cm. Cuttings and urchins were haphazardly assigned to containers. A mesh-net frame was placed over the tank to cover all of the containers to prevent urchins from escaping the container. After 24 hours, 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 day^{-1}.

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

The experiment was designed to test the hypothesis that urchins will modify

foraging behavior when exposed to cues coming from dead conspecifics compared to controls (no odor cues).

The experiment used a flow-through tank similar to that of the previous experiment. However, water was supplied at a steady rate of 2.5 L min^{-1} through a hose. The hose was placed so that water flowed through the floor, in the middle of the tank. 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) where urchin behavior was recorded with a time-lapse camera (GoPro, Hero 4). At the center of the ring and above the hose, a perforated PVC cap was placed.

The experiment was started by placing the test urchin 10 cm from the center. The camera was mounted 40 cm above the ROI and the field of view (FOV) included the entire tank. The images were recorded every 30 sec and experiment was conducted for 1 hr. Four treatments were defined, 1) a control (no dead urchin or algae; no chemosensory cue) 2) an algae treatment (positive chemosensory cue), 3) a dead

urchin treatment (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 and the dead urchin 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 nutritional conditions (i.e., starved and fed) and in W-S and S-A.

An opaque plastic sheet covered the entire experimental apparatus to remove all ambient light. However, below the sheet, a red LED lamp (ISL-150X150, CCS Inc.) provided enough light to record images while minimizing light disturbance of 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 S-A (August-November 2018) and W-S (April-May 2019). 111 individuals were used for the S-A experiment and 88 individuals

were used for the W-S 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 (ver.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 (Table 1). The x and y coordinates of the sea urchin was analyzed to determine the time spent by an urchin performing a particular behavior (minutes) as well as sea urchin movement speed (cm min^{-1}).

2.4. Cue dispersal rate

The chemical cue plume was visualized and quantified using a Fluorescein tracer 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 sec^{-1}. The tracer concentration was 2% Fluorescein. A one-hour video recorded the dispersal of tracer. 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 measurement

The spatial homogeneity of the red light provided by the LED lamp was also assessed. Light was measured using five light loggers (HOBO MX2202 Temp/Light, Onset Corp.) that were placed on each ring to form a line. After the initial measurement, the line was rotated 30 degrees, for a total of four times. At every rotation, light was measured for one hour.

2.6. Field experiment: 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 (32.988014 °N, 129.118638 °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 plan area was deployed. A time-lapse camera (TLC200 PRO, Brinno, Taiwan) 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 hours and stored as a video with a frame rate of 15 fps. The experiment was during slack tide, when the tidal current was negligible. A velocity logger (Compact-EM, Alec Electronics Co.) and a water level logger (HOBO U20-001, Onset Corp.) 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 m^2 experimental plot. Drift algae (i.e. *Sargassum* spp., *Dictyopteris* spp.) common during the experiment period was collected and clipped outside of the plastic cage. Test sea urchins were placed in direct contact with the algae until they attached. This was considered as the control. In a separate treatment, a dead urchin was added inside the cage to determine whether urchins would be repelled by a dead conspecific. The experiments were conducted on both urchin nutritional conditions.

Video was analyzed with Tracker (ver. 5.0.6) (Brown 2018) to track the urchins, however the tripod attracted small fish, which occluded the field of view.

Additionally, during a number of days, the area experienced relatively high waves, which vibrated the tripod that resulted in poor quality images. Thus, only the initial and final position (stay or flee) of the urchin was noted after the 3 hr experiment period.

2.7. Data analyses

All statistical analyses were done using R version 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). Four Markov chains generated 2000 samples per chain. All posterior distributions were examined visually to evaluate the convergence of the Markov chains and posterior predictive ability.

The urchin behavior in the laboratory experiments were analyzed with a hurdle-negative binomial model (Eq. 1),

	$y = (1 - \pi)\mathrm{NegBin}(0, \theta) + \pi\mathrm{NegBin}(\mu, \theta)$	(1)

	$\mu = x\beta$ $log\,\frac{\boldsymbol{\pi}}{1-\boldsymbol{\pi}} = \boldsymbol{x\alpha}$	

where, y is a vector of observations, and in this case the number of occurrences for a behavior during the 1-hour 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. 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 nutritional state of the test urchin, season (i.e., S-A or W-S), and the type of behavior excluding the "none" behavior (Fletcher et al. 2005) (Table 1).

In the case of the sea urchin speed and time spent per behavior, 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 Ridout et al. 1998.

The β coefficients of the all hurdle models were given weakly informative Student's t-distributions as prior distributions, with 3 degrees of freedom, 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.

The field experiments were analyzed with a simpler binomial generalized linear model with a random intercept for the tidal state (Eq. 2).

	$y = Binom(n, \pi)$ $log \frac{\pi}{1-\pi} = x\gamma + \delta$	(2)

In Eq. 2, the 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 nutritional 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. The vector of coefficients is γ. The prior distributions for the coefficients and random intercept was a Student's t-distribution with 3 degrees of freedom, a location of 0, and a scale of 5.

3. RESULTS

3.1. Laboratory experiment 1: Urchin grazing rate

The results revealed differences in feeding rates between seasons. Sea urchins had higher mean feeding rates in the S-A (1.3 ± 0.1 g algae urchin^{-1} day^{-1}) (Table 2: A). Conversely, sea urchin feeding rates decreased by about 38% during the W-S (0.8 ± 0.1 g algae urchin^{-1} day^{-1}). There was a 23% difference between seasons in the mean maximum water temperature of the experiment conditions (Figure 1).

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

3.2.1. Frequency of behaviors

The time-lapse experiment showed that the counts of the four behavior types

varied widely across sea urchin condition, season, and treatment (Supp. Figure 1). The occurrence of the immobile behavior “none” occurred more in W-S (starved: 15 urchins, fed: 21 urchins) than in S-A (starved: 16 urchins, fed: 15 urchins). The occurrence of this behavior in W-S 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 the S-A.

Sea urchins were generally more active in S-A than in W-S season. For interaction events, this behavior was more frequent in S-A and in starved sea urchins, occurring in 6% and 5% of all experiment trials, respectively. For the W-S, interaction occurred only in 2% of trials while only occurring in 4% of fed urchins.

The model predictions on the effect of the dead urchin on interaction events can be observed when comparing algae only (Figure 2C, G) and combined chemosensory cues treatment (Figure 2D, H). Predictions show that when a dead urchin was present together with the algae, fed urchins had decreased interaction counts of up to 64.8% and 72.3% during S-A and W-S, respectively. For starved urchins, they had a 14% increase and a 49% decrease in interaction counts for S-A and W-S,

respectively. In W-S, starved urchins also had a higher proportion of immobile individuals. At the same time, the presence of the dead urchin also increased the number of events outside of the ROI across both seasons for fed (44.8% and 39.2% for S-A and W-S, respectively) and starved (93.2% and 54.1% for S-A and W-S, respectively). Overall, fed urchins seem to be sensitive to the chemical cues from dead urchins while starved urchins were more tolerant during the summer but were also less active in winter.

3.2.2. Time spent per behavior

The time-lapse experiment demonstrated the ability of the treatments, condition and the season to influence the sea urchin's allocated time performing a specific behavior (Supp. Figure 2). Overall, 34% of sea urchins spent the entire 1-hr experiment period immobile. Of all the immobile urchins, 42% were immobile during the winter-spring season while 27.9% represented the summer-spring season experiments.

The model predictions on the effects of a dead urchin chemosensory cues on time spent per behavior are shown in Figure 3. The predictions show that compared to

algae treatment (Figure 3C, G), the presence of a dead urchin (Figure 3D, H) reduced mean interaction times for fed urchins by about 5.57 and 4.07 minutes during S-A and W-S, respectively. For starved urchins, there was a mean increase by about 1.7 min and a decrease of about 5.14 min for S-A and W-S, respectively. The time spent outside the ROI also increased across both seasons for fed (10.2 and 5.2 minutes for S-A and W-S, respectively) and starved (5.7 and 5.07 minutes for S-A and W-S, respectively) urchins. The decrease in time spent for interaction by fed urchins and the relative increase in the proportion of time spent outside indicates that urchins were repelled by the presence of the dead urchin together with the algal food. Conversely, the 11.4% increase in time spent by the starved urchins interacting with the algae despite the presence of the dead urchin indicates that hunger was able to increase its tolerance to signals of predation.

3.2.3. Urchin movement speed

The time-lapse experiment revealed urchin movement speeds varied across the treatments depending on their condition and season. It was noted that even when the urchins were exhibiting the behavior “none”, minute movement speeds were

recorded as urchins were shuffling in place to within the 3cm limit (Table 1). The urchins exhibited greater speeds when they were interacting with the center of the ROI in the presence of a dead urchin while overall, fed urchins had higher movement speeds relative to the starved urchins (Supp Figure 3).

The model predictions on urchin speed (cm min^{-1}) indicates that urchins tend to move at a different pace depending on the presence or absence of a dead urchin (Figure 4D, H) than with algae only (Figure 4C, G). When a dead urchin was present, interaction speeds for fed urchins typically decreased by 1.48 and 0.95 cm min^{-2} during S-A and W-S, respectively. The opposite was found for starved urchins because their speeds while interacting with the algae increased by 1.25 and 5.13 cm min^{-1} for S-A and W-S, respectively when a dead urchin was present. However, when outside the ROI, fed urchins had increased speeds by up to 2.39 cm min^{-2} during S-A, while showing decreased speeds by up to 1.39 cm min^{-2} during W-S. Starved urchins still show the same pattern of high speeds outside the ROI by up to 9.18 and 5.26 cm min^{-2} for S-A and W-S, respectively. Examining urchin speeds show that signals of predation may cause considerable stress to *H. crassispina* as indicated by the relatively high movement speeds even when outside the ROI.

3.2.4. Cue dispersal rate

The results from the experiment on the rate of spread of the Fluorescein tracer dye showed high variation among the trials (Table 2: B2). The time it took for the tracer to reach the 10 cm mark was modelled 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 and above the treatment container, took the least amount of time at 13.5±5.4 s (mean ± standard deviation). The treatments could be ranked from those that took the least amount of time to the greatest amount of time and resulted in an order of control, algae effect, dead urchin and algae interaction effect, and dead urchin effect. The high standard deviations for all treatments was due to a low sample size (3 trials per treatment). However, high variations between trials among the algae, dead urchin and dead urchin and algae treatments suggested that the variation was associated with the size of the dead urchin or the density of the algae used as treatment for the experiment.

3.2.5. Light measurement

The results of the light experiment showed that the highest light intensity was at

the center of the experiment chamber, directly over the treatment container, at 3.0 ± 0.1 lux (Table 2: B1). The light intensity slowly decreased outwards from the ROI, with the 10 cm area receiving approximately 2.1 ± 0.3 lux, and the outer edge receiving only 0.4 ± 0.3 lux. Despite the uneven light distribution across the experiment chamber, the camera was able to record the sea urchin behavior in enough detail to allow later analysis using the tracking software. More importantly, sea urchin activity was not discernibly affected by the light intensity throughout the experiment.

3.3. Field experiment: 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 which fled, 4 sea urchins

(40%) were from the treatment which contained the dead urchin. The binomial model predictions suggested a strong link between urchin condition and outcome of behavior (Figure 5).

3.4. Field experiment: Ambient environment conditions

The data recorded by the temperature, current speed, and depth loggers indicate the specific environmental conditions during field experiments (Supp. Figure 4). Of the 20 trials conducted, 11 experiments were conducted during low slack tide while 9 were conducted during high slack tide. In general, mean temperatures and mean current speeds were higher during low tide relative to high tides.

4. DISCUSSION

4.1. Factors affecting sea urchin behavior patterns

Time-lapse photography revealed high variations in the frequency of behavior occurrences and movement patterns among individual *Heliocidaris crassispina*

across the different treatments. This lack of distinct directionality was also reported for other urchin species (Flukes et al. 2012, Kriegisch et al. 2019). Despite these observations, we found discrete behavior patterns between starved and fed *H. crassispina*, which suggests that the tolerance to a perceived risk increased when sea urchins were deprived of food. For starved urchins, there was a greater proportion of urchin interaction events and increased interaction time with the seaweed 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 placed together with the seaweed.

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 impacted 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* showed that gut clearance was achieved in 3 days for *H. crassispina* and 6 days for *H. pulcherrimus*. Additionally, *H. pulcherrimus* survived a maximum of 49 days without food albeit negatively impacting gut and gonad indices (Kaneko et al. 1981 in Agatsuma et al 2013). In our study, the one-week 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). It should be noted that the microcosm experiment utilized a relatively small chamber which may have allowed the saturation of sea urchin effluents faster and thus increase the urchin responses artificially. 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) and 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 *Corallina* 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). Other signs of food limitation manifested themselves as differences in morphometric changes such as smaller than average test size and longer jaw lengths to maximize grazing efficiency (Pedersen & Johnson 2008).

A recent study utilizing starved and satiated *Strongylocentrotus droebachiensis* showed that both sea urchin groups did not react 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 did not reduce urchin foraging behavior in the laboratory and in the field. In contrast, studies on chemical alarm cues showed dead conspecifics and chemically-labelled predators had a strong adverse effect on urchin behavior (Campbell et al. 2001, Morishita & Barreto 2011). Specifically, the urchins distinctly avoided waters conditioned with gut, coelomic, and gonad homogenates, which were 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 had distinct importance for prey species because this functions as a learning tool to enable naive prey to associate predators with danger (Magurran & Girling 1986). 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. 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 little chance in encountering chemical cues relating to predation.

When comparing sea urchin behavior patterns across seasons, we found that there

was a discrete pattern observed between the S-A and W-S experiments. In general, sea urchins were more active and had higher movement speeds during S-A compared to sea urchins used in W-S. The greater decrease in the interaction frequency and interaction time in W-S for starved urchins was attributed to lesser urchins interacting with the treatment as well as more urchins moving outside. Interestingly, urchin speed was predicted to be highest in W-S when starved individuals were exposed to dead urchins together with food. This was 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 fed urchins (Figure 3). Their level of activity was also reflected in their feeding rates as urchins in August had the highest average feeding rates while urchins in February had the lowest rates. This was a similar pattern found among cold-water urchin species where temperature was one of the main drivers of metabolic activity (Agatsuma et al. 2000, Brockinton & Clarke 2001).

Studies on the reproductive biology of *H. crassispina* showed that this species had a

distinct seasonal cycle in terms of gonadal development and maturation. In Nagasaki, a study on the reproductive patterns of *H. crassispina* (Yamasaki & Kiyomoto 1993 in Agatsuma et al 2013) has found that this species spawns during the months of July to August while recovery period was from September to January. The rest of the following months were dedicated to growth and maturation of the gonads. This pattern was also similar with 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 to 8 month spawning period was recorded. This relatively long spawning period was represented by two 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). This 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 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 from 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 results provide evidence of urchin condition affecting the strength of behavior modification in the field. Compared to fed sea urchins, all starved urchins stayed regardless of the presence or absence of a dead urchin. The result of our experiment was in contrast with the field experiment using live cancrid 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 of controlling for local flow conditions. The data recorded from the field shows high variability in flow speeds as well as temperature 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 was greatly dependent on dilution and degree of shear-induced mixing of odor signals (Webster & Weissberg 2001). This was particularly true for small benthic invertebrates because sampling the water for odor molecules occurs at a relatively faster 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 may be plausible 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 appear to be a behavior related to minimizing predation risk in lieu of feeding opportunity. However, with only a short 3-hr experiment period, we were not able to observe how the starved urchins would behave once they have adequately fed

on the seaweed or how long the dead urchin effluents remained effective. Hence, for future studies, we propose a longer observation time for urchin behaviors and identification of the components responsible for urchin alarm response and their maximum length of efficacy.

5. CONCLUSIONS

The behavioral responses of *Heliocidaris crassispina* in our experiments appear to encompass all classifications in the prey behavior types proposed by Fraser & Huntingford (1986). Our results showed that *H. crassispina* foraging behavior was flexible and was able to assess and adjust accordingly to the presence of chemical cues associated with predation. To some extent, our results support the idea that the type of season and phenology appear to modulate urchin behavior and foraging activity (Luttberg 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, length of interaction time and increases movement speeds, indicating escape behaviors. Furthermore, starved urchins seemed to be more 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 animal condition was a key determinant in its decision-making process and that their level of hunger may override behaviors associated with predator avoidance. This puts them at a disadvantage as starved animals feed more boldly, exposing themselves further to the dangers of predation.

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8. TABLES

Table 1. List of sea urchin behavior defined in this study.

Behavior type	Definition
None	Any immobile behavior within the region of interest. The urchin does not move 3 cm away from its starting point.
Movement	The urchin is freely moving inside the region of interest.
Interaction	The urchin makes contact with the center of the region of interest which may or may not contain seaweed or dead urchin.
Outside	When the urchin is outside the region of interest.

1 Table 2. Results of the Bayesian linear models on A) Experiment 1: feeding rate and

2 season, B) Experiment 2: temperature conditions, B1) light distribution and B2)

3 cue dispersal time to reach the 10 cm mark. The table shows the estimates, mean

4 and the lower and upper limits of the 95% confidence interval.

5

Estimates	Mean	2.5%	97.5%
A.) Lab experiment 1: Feeding rate (g algae day^{-1})			
Summer-Autumn	1.3	1.2	1.5
Winter-Spring	0.8	0.6	1.0
B.) Lab experiment 2: Temperature conditions (°C)			
Stock tank	18.6	18.6	18.7
Control	18.7	18.4	19.0
Algae	19.2	18.9	19.5
Dead urchin	18.8	18.4	19.3
Algae + dead urchin	19.1	18.7	19.5
B1.) Light distribution (Lux)			
Center	3.0	2.8	3.3

Middle	2.1	2.0	2.3
Outer	0.5	0.3	0.70
B2.) Cue dispersal time (sec)			
Control	13.5	2.0	25.3
Algae	17.3	5.0	30.2
Dead urchin	22.6	9.8	35.3
Algae + dead urchin	19.5	6.4	31.8

9. FIGURE LEGENDS

Fig.1. Mean temperature difference between seasons in the feeding rate experiment.

Fig. 2. Model predictions on mean count of urchin behaviors. The left and right columns are represented by S-A and W-S, respectively. Rows are represented by the treatments. Beginning from the top are control (A, E), dead urchin (B, F), algae (C, G), and dead urchin + algae (D, H). On the x-axis, behaviors are abbreviated as M (movement), I (Interaction), and (Outside).

Fig. 3. Model prediction on mean urchin time spent per behavior (min). The left and right columns are represented by the S-A and W-S, respectively. Rows are represented by the treatments. Beginning from the top are control (A, E), dead urchin (B, F), algae (C, G), and dead urchin + algae (D, H). On the x-axis, behaviors are abbreviated as M (movement), I (Interaction), and (Outside).

Fig. 4. Model prediction on mean urchin speed (cm min^{-1}). The left and right

columns are represented by the S-A and W-S, respectively. Rows are represented by the treatments. Beginning from the top are control (A, E), dead urchin (B, F), algae (C, G), and dead urchin + algae (D, H). On the x-axis, behaviors are abbreviated as M (movement), I (Interaction), and (Outside).

Fig. 5. Binomial model predictions on urchin behavior outcome in the field. Starvation makes urchins less likely to flee regardless of the presence or absence of a dead urchin.

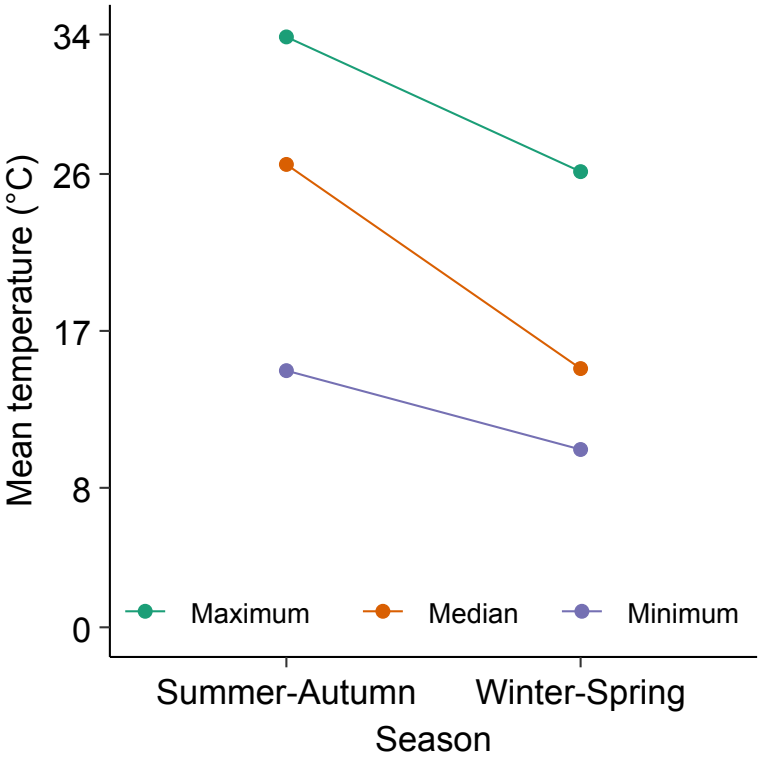
34
26
17
8
0
Mean temperature (°C)
Maximum
Median
Minimum
Summer-Autumn
Winter-Spring
Season

Figure 1

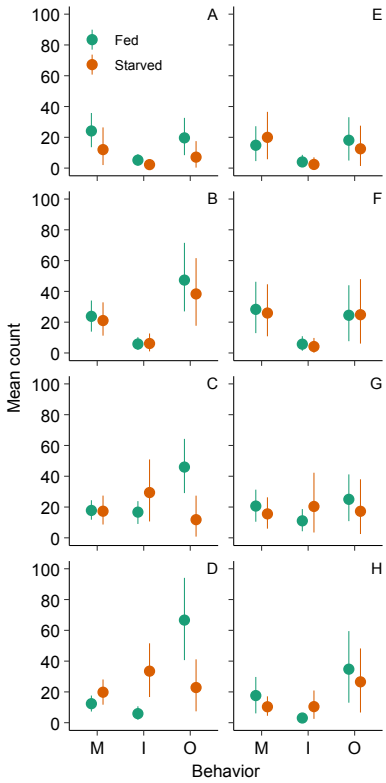
Fed
Starved
A
B
C
D
E
F
G
H
Mean count
Behavior
M
I
O
0
20
40
60
80
100

Figure 2

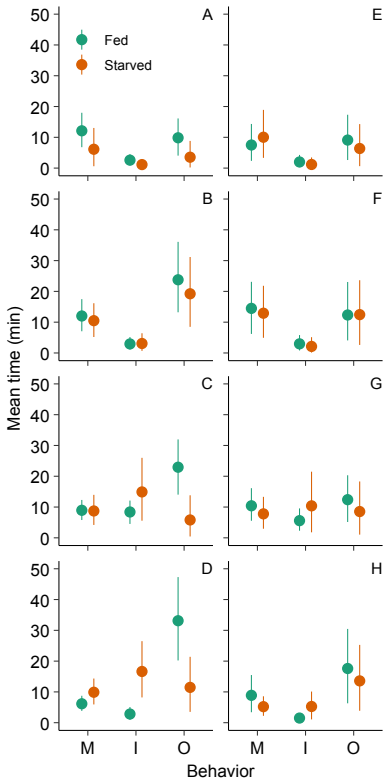
Fed
Starved
A
B
C
D
E
F
G
H
Mean time (min)
0
10
20
30
40
50
M
I
O
Behavior

Figure 3

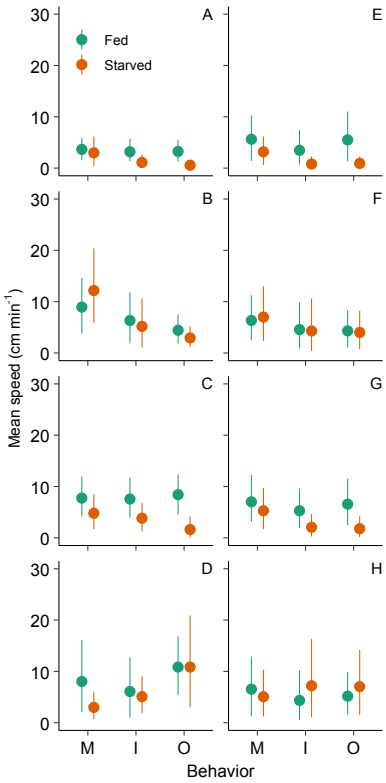
Fed
Starved
A
B
C
D
E
F
G
H
30
20
10
0
Mean speed (cm min^{-1})
M
I
O
Behavior

Figure 4

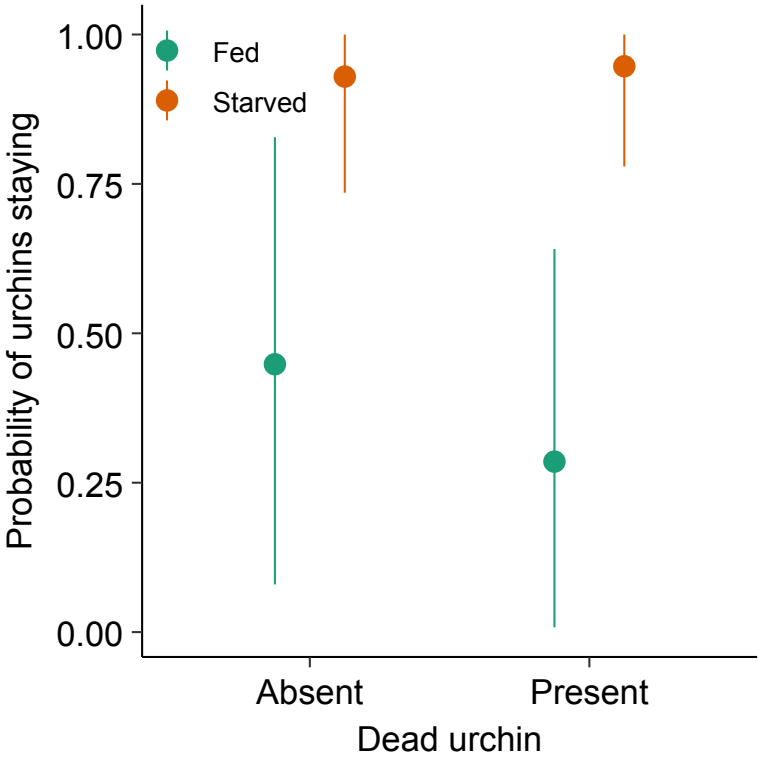
Fed
Starved
1.00
0.75
0.50
0.25
0.00
Probability of urchins staying
Absent
Present
Dead urchin

Figure 5