

Spatial dynamics of the green sea urchin *Strongylocentrotus droebachiensis* in food-depleted habitats

Desta L. Frey, Patrick Gagnon*

*Corresponding author: pgagnon@mun.ca

Marine Ecology Progress Series 552: 223–240 (2016)

Supplement 1

Methods 1. Collection and maintenance of urchins

Urchins used in Experiment 1 and Experiment 2 were hand collected by divers at depths of 3 to 6 m in the barrens at Bread and Cheese Cove (BCC) in January, June, and July 2012. They were transported in large containers filled with seawater to the Ocean Sciences Centre (OSC) of Memorial University of Newfoundland. Upon arrival at the OSC (<5 hours after collection), urchins were transferred to 330-L holding tanks supplied with ambient flow-through seawater pumped in from a depth of ~5 m in the adjacent embayment, Logy Bay, and sorted by size. All individuals with a test diameter of 40 to 60 mm that clung or displaced readily in the tanks, indicating that the podia functioned normally, were kept for the experiments. This size class was chosen because individuals of this size are sexually mature (Himmelman 1986, Raymond & Scheibling 1987, Munk 1992) therefore eliminating potential behavioral differences between mature and non-mature individuals, and it was the most frequent size class at times of collection. Each holding tank contained 200 urchins. Urchins used in Experiment 1 were not fed because urchin feeding in eastern Canada at the time the experiment was conducted (January) is typically low (Scheibling & Hatcher 2007, P. Gagnon, unpublished data) and feeding them could have altered metabolic activity and behavior. Urchins used in Experiment 2 were fed every two days with 25 g (wet weight) of freshly collected *Alaria esculenta* blades cut into pieces of ~2.5 x 2.5 cm to standardize hunger levels at a time of year (June and July) when feeding in eastern Canada markedly increases (Scheibling et al. 1999, Gagnon et al. 2004, Lauzon-Guay & Scheibling 2007, Frey & Gagnon 2015). Urchin feces and unconsumed kelp were removed from the holding tanks every two days. Water temperature in the holding tanks prior to trials in Experiment 1 and Experiment 2 was measured with a temperature logger with a precision of ± 0.5 °C (HOBO Pendant; Onset Computer Corporation). It averaged 4.1 °C (± 0.2) and 10.0 °C (± 0.9), respectively.

Methods 2. Designation of microhabitats in Experiment 1

Urchins had access to six microhabitats: (1) flat; (2) protrusion; (3) depression; (4) ledge; (5) crevice; and (6) wall. The surface area of these microhabitats was respectively 0.64, 0.06, 0.13, 0.04, 0.14, and 0.73 m², yielding an experimental area of 1.74 m². The free surface of the 12 tiles formed the flat microhabitat. Topographical features were added to nine tiles to create the protrusion, depression, and ledge microhabitats, with three tiles per habitat. Protrusion tiles had one concrete brick (0.2 x 0.1 x 0.05 m [L, W, H]) in the centre. Depression tiles had one gently sloping depression (0.21 m in diameter, 0.04 m deep) in the centre surrounded by a flat, horizontal rim (0.03 m at the narrowest point). Ledge tiles had one rectangular (0.2 x 0.1 x 0.003 m [L, W, H]) piece of acrylic in the centre fastened at an angle of 45° relative to the tile. Bricks and acrylic pieces in the protrusion and ledge microhabitats were oriented perpendicularly to the longitudinal walls of the tank to create similar water flows among trials of the same wave velocity. Grooves (0.02 m wide, 0.05 m deep) between the 12 adjacent tiles, as well as between the peripheral tiles and the tank walls, formed the

crevice microhabitat. The longitudinal tank walls flanking the tiles formed the wall microhabitat. Urchins were in the depression or ledge microhabitats if >50% of the test overlapped with the habitat or in the protrusion microhabitat if touching the sides or top of a brick. They were in the crevice microhabitat if partially inserted in, or extending across, grooves between adjacent tiles or between the peripheral tiles and the tank walls. They were in the wall microhabitat if completely on a wall or if their aboral surface was distinctly tilted with two facing edges in contact with both a peripheral tile and a wall, as determined by zooming in on images. They were in the flat microhabitat if anywhere else.

The number of urchins in each microhabitat was corrected for differences in surface area among microhabitats by multiplying the number of urchins in the microhabitat by the ratio of the surface area of the largest microhabitat (wall, 0.73 m²) to the surface area of the microhabitat. Ratios were: 1.1 (flat), 12.2 (protrusion), 5.5 (depression), 18.3 (ledge), 5.2 (crevice), and 1.0 (wall). The standardized proportion of urchins in each microhabitat was then obtained by dividing the average of corrected numbers of urchins at 15, 30, and 45 min by the sum of averages of corrected numbers of urchins in the six microhabitats. Standardization inevitably magnified observations for those microhabitats with the smallest surface areas (protrusion, depression, ledge, and crevice). However, it was necessary to enable comparisons among microhabitats and unlikely to affect the analysis and interpretation given the clear data trends (see results).

Methods 3. Acclimation of urchins to waves prior to the onset of trials

Experiments 1 and 2: In trials with waves, the motor was turned on to create an initial wave velocity of 0.1 m s⁻¹. The velocity was gradually increased over the following two and five minutes in the 0.2 and 0.3 m s⁻¹ treatments, respectively. This gradual increase was necessary to allow urchins to adapt to the increasing hydrodynamic forces and avoid dislodgement. However, it yielded different acclimation times among wave velocities, with 1 min at 0.1 m s⁻¹, 3 min at 0.2 m s⁻¹, and 6 min at 0.3 m s⁻¹.

Experiment 1: A one-way MANOVA (applied to the logit-transformed data to correct for heterogeneity of the residuals in the analysis on the raw data, n=240) with the factor Waves showed that the standardized proportions of urchins in each of the six microhabitats at the onset of trials (i.e. at the conclusion of the acclimation time) did not differ among velocity treatments ($F_{18,72}=1.206$; $p=0.280$). This was because urchins moved more rapidly at low than high wave velocities but acclimation time increased with wave velocity. Patterns of urchin-microhabitat associations beyond acclimation were therefore unaffected by the different acclimation times among wave velocities.

Experiment 2: A one-way ANOVA (applied to the square-root transformed data to correct for non-normality of the residuals in the analysis on the raw data, n=120) with the factor Waves showed that the nearest-neighbor R-ratio (R) at the onset of trials did not differ among velocity treatments ($F_{3,116}=1.32$, $p=0.272$). Patterns of urchin distribution (R) at the end of trials were therefore unaffected by acclimation time.

Methods 4. R-ratio calculations

R-ratio was calculated with the equation $R = (r_a / r_e)$, where r_a is the mean nearest neighbor distance (NND; the linear distance between the centre of each individual and the centre of its closest neighbor) in the observed population, and r_e is the mean NND expected under a random distribution for a given population density, ρ , obtained from the equation $r_e = 0.5\rho^{-0.5}$. The R-ratio for an area with no boundary strip can artificially yield an uniform distribution because organisms near the edges of the area tend to have higher NNDs than those near the centre (Sinclair 1985, Krebs 1999). To avoid this potential bias, NNDs of individuals with >50% of the test inside of a 5-cm boundary strip bordering the four sides of the 3 x 3 grid of tiles were omitted. This strip was sufficiently large to contain entire urchins and it minimized area loss. Omitting urchins in the strip also ensured that R-

ratios applied to only those urchins that were not physically constrained by, or in contact with, the tank walls or nettings, and hence free to remain solitary or seek conspecifics. Urchins in the strip that were the nearest neighbors of urchins in the inner area (0.64 m^2) were nevertheless used to calculate NNDs for the latter individuals (Krebs 1999). As noted by Clark & Evans (1954), if the area sampled is relatively small there can be individuals as close to each other as their physical size permits and that simultaneously have uniformly distributed body centres. To avoid potential bias towards uniform distribution, every NND was corrected for the minimum spatial requirement of urchins. This was done by subtracting the mean test diameter of 10 haphazardly chosen urchins from every NND in each trial. Urchins that were smaller than the mean test diameter and in contact with another urchin had negative NNDs. Negative NNDs were impractical, and hence substituted a value of zero. Urchins on the tank walls were not included in calculations of R, primarily because the factors that affect the distribution of urchins may differ between vertical and horizontal surfaces. Therefore, urchin densities (ρ) used in R calculations varied among trials, reflecting numbers of urchins in the inner (horizontal) surface at the end of trials.

Methods 5. Statistical analysis (additional details)

Experiment 1:

Analysis 1. No additional details.

Analysis 2. Prior to running the one-way MANOVA, a two-way MANOVA with the factors Waves (null, low, intermediate, and high wave velocity) and Block (each daily block of one replicate of each treatment) was run to determine if results differed among blocks. There was no significant effect of the factor Block ($F_{54,162}=1.078$; $p=0.353$) at $\alpha=0.25$, the recommended significance level to make decisions about the removal or retention of block variables or block-by-factor interactions in general linear models (Quinn & Keough 2002, Sokal & Rohlf 2012). The one-way MANOVA was therefore run on data pooled from all blocks.

Experiment 2:

Analysis 3. No additional details.

Analysis 4. Because the factor Block in the three-way ANOVA was significant (see Results), the mean squares (MS) values of the Waves x Block and Density x Block terms were used as denominators to calculate the F-value for the factors Waves and Density, respectively. This procedure is recommended for factorial randomized complete block designs with sufficient degrees of freedom to include factor-by-block interactions in the model, and when such interactions are significant at $\alpha=0.25$ (Quinn & Keough 2002, Sokal & Rohlf 2012).

Analysis 5. Prior to running this analysis, a three-way ANOVA with the factors Waves (null, low, intermediate, and high wave velocity), Density (low, intermediate, and high urchin density), and Block (each block of four days during which one replicate of each treatment was done) was run to determine if results differed among blocks. There were no significant factor-by-block interactions (Waves x Block: $F_{27,52}=0.98$, $p=0.504$; Density x Block: $F_{18,52}=0.46$, $p=0.965$). The two-way ANOVA was therefore run on data pooled from all blocks.

Analysis 6. The factor Block was not significant at $\alpha=0.25$ in three of the twelve two-way ANOVAs (see Results). It was nevertheless retained in all models for consistency.

Field surveys:

Analysis 7. Each data point in the regressions for the inner bedrock platform was the mean proportion of urchins from the 10 plots for a given sampling event and corresponding mean sea temperature and significant wave height (SWH). The standardized proportion of urchins on flat bedrock was the

difference between 100% and the standardized proportion of urchins in crevices. Accordingly, the latter two models yielded reciprocal results, which are nevertheless presented to discuss different perspectives. Three out of the 10 concrete bricks used to create the protrusion microhabitat had disappeared in early September. Accordingly, each data point in the model for the protrusion microhabitat was the mean proportion of urchins from the 10 protrusions for the first 15 sampling events and from seven protrusions for the last seven sampling events, and corresponding mean sea temperature and SWH. Mean sea temperature and SWH were calculated over the 48 h preceding each sampling event because preliminary analysis showed stabilization of variation beyond 48 h. Each data point in the regressions for the outer bedrock platform was the R-ratio or mean urchin density from 10 to 15 plots for a given sampling event and corresponding mean sea temperature and SWH. The absence or presence of interactive effects between the two explanatory variables (temperature and SWH) was not known a priori. All analyses were therefore conducted using the multiplicative error model approach, whereby explanatory variables are tested for both individual and interactive effects (Kleinbaum et al. 2008). If interactive effects were not significant, models with individual effects of only those explanatory variables that were significant in the truncated models were presented.

Literature cited

- Clark PJ, Evans FC (1954) Distance to nearest neighbor as a measure of spatial relationships in populations. *Ecology* 35:445-453
- Frey DL, Gagnon P (2015) Thermal and hydrodynamic environments mediate individual and aggregative feeding of a functionally important omnivore in reef communities. *Plos One* 10:e0118583
- Gagnon P, Himmelman JH, Johnson LE (2004) Temporal variation in community interfaces: kelp-bed boundary dynamics adjacent to persistent urchin barrens. *Mar Biol* 144:1191-1203
- Himmelman JH (1986) Population biology of green sea urchins on rocky barrens. *Mar Ecol Prog Ser* 33:295-306
- Kleinbaum DG, Kupper LL, Nizam A, Muller KE (2008) *Applied Regression Analysis and Other Multivariable Methods*. Thomson Brooks/Cole, Belmont, CA
- Krebs CJ (1999) *Ecological Methodology*. Benjamin Cummings, San Francisco, CA
- Lauzon-Guay JS, Scheibling RE (2007) Seasonal variation in movement, aggregation and destructive grazing of the green sea urchin (*Strongylocentrotus droebachiensis*) in relation to wave action and sea temperature. *Mar Biol* 151:2109-2118
- Munk JE (1992) Reproduction and growth of green sea urchins *Strongylocentrotus droebachiensis* (Müller) near Kodiak, Alaska. *J Shellfish Res* 11:245-254
- Quinn GP, Keough MJ (2002) *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge
- Raymond BG, Scheibling RE (1987) Recruitment and growth of the sea urchin *Strongylocentrotus droebachiensis* (Müller) following mass mortalities off Nova Scotia, Canada. *J Exp Mar Biol Ecol* 108:31-54
- Scheibling RE, Hatcher BG (2007) Ecology of *Strongylocentrotus droebachiensis*. In: Lawrence JM (ed) *Edible Sea Urchins: Biology and Ecology*. Elsevier Science, Amsterdam, p 353-392
- Scheibling RE, Hennigar AW, Balch T (1999) Destructive grazing, epiphytism, and disease: the dynamics of sea urchin - kelp interactions in Nova Scotia. *Can J Fish Aquat Sci* 56:2300-2314
- Sinclair DF (1985) On tests of spatial randomness using mean nearest neighbor distance. *Ecology* 66:1084-1085
- Sokal RR, Rohlf FJ (2012) *Biometry: The Principles and Practice of Statistics in Biological Research*. W.H. Freeman and Co., New York, NY

Supplement 2

Table S1. Mean (SE) peak longitudinal water velocity (m s^{-1}) in each microhabitat in Experiment 1 and Experiment 2 for the low (0.1 m s^{-1}), intermediate (0.2 m s^{-1}), and high (0.3 m s^{-1}) wave velocity treatments.

Experiment	Microhabitat	Wave velocity treatment		
		Low	Intermediate	High
1, 2	flat	0.102 (0.001)	0.230 (0.005)	0.325 (0.003)
1	protrusion	0.099 (0.001)	0.226 (0.002)	0.323 (0.003)
1	depression	0.083 (0.001)	0.191 (0.002)	0.277 (0.002)
1	ledge	0.097 (0.001)	0.202 (0.005)	0.292 (0.007)
1	crevice	0.106 (0.001)	0.215 (0.001)	0.299 (0.003)
1, 2	wall	0.131 (0.001)	0.254 (0.002)	0.326 (0.002)

Table S2. Surface area of crevice and flat microhabitats and corresponding conversion factors for each of the 10 plots of $0.5 \times 0.5 \text{ m}$ surveyed at Bread and Cheese Cove between 30 April and 25 October 2012. Each conversion factor is the ratio of the surface area of the largest microhabitat across plots (flat, plot 9, 0.22 m^2) to the surface area of the corresponding microhabitat in the plot.

Microhabitat	Plot	Surface area (m^2)	Conversion factor
Crevice	1	0.047	4.68
	2	0.085	2.59
	3	0.037	5.95
	4	0.045	4.89
	5	0.070	3.14
	6	0.087	2.53
	7	0.080	2.75
	8	0.065	3.38
	9	0.030	7.33
	10	0.062	3.55
Flat	1	0.203	1.08
	2	0.165	1.33
	3	0.213	1.03
	4	0.205	1.07
	5	0.180	1.22
	6	0.163	1.35
	7	0.170	1.29
	8	0.185	1.19
	9	0.220	1.00
	10	0.188	1.17

Table S3. Summary of two-way ANOVA (applied to raw data) examining the effect of Waves (null, low, intermediate, and high wave velocity) and Block (each daily block of one replicate of each treatment) on the displacement of green sea urchins (*Strongylocentrotus droebachiensis*) in Experiment 1.

Source of Variation	df	MS	F-value	p
Waves	3	1074.2	5.44	0.005
Block	9	452.9	2.29	0.046
Error	27	197.5		
Corrected Total	39			

Table S4. Summary of one-way ANOVAs (applied to logit-transformed data) examining the effect of Waves (null, low, intermediate, and high wave velocity) on proportions of green sea urchins (*Strongylocentrotus droebachiensis*) in the six microhabitats in Experiment 1.

Microhabitat	Source of variation	df	MS	F-value	p
Flat	Waves	3	0.23	17.57	<0.001
	Error	36	0.01		
	Corrected total	39			
Protrusion	Waves	3	0.06	1.74	0.177
	Error	36	0.04		
	Corrected total	39			
Depression	Waves	3	0.03	0.90	0.450
	Error	36	0.03		
	Corrected total	39			
Ledge	Waves	3	0.05	1.32	0.283
	Error	36	0.04		
	Corrected total	39			
Crevice	Waves	3	0.53	49.63	<0.001
	Error	36	0.01		
	Corrected total	39			
Wall	Waves	3	0.86	15.80	<0.001
	Error	36	0.05		
	Corrected total	39			

Table S5. Summary of three-way ANOVA (applied to square root-transformed data) examining the effect of Waves (null, low, intermediate, and high wave velocity), Density (low, intermediate, and high urchin density), and Block (each block of four days during which one replicate of each treatment was done) on the nearest neighbor R-ratio (R) of green sea urchins (*Strongylocentrotus droebachiensis*) with a clumped distribution (R significantly lower than 1) at the end of trials in Experiment 2.

Source of variation	df	MS	F-value	p
Waves	3	0.061	4.17	0.011
Density	2	0.068	5.44	0.007
Block	9	0.024	2.80	0.010
Waves × Density	6	0.022	2.56	0.031
Waves × Block	27	0.015	1.72	0.050
Density × Block	18	0.013	1.47	0.143
Error	48	0.010		
Corrected Total	113			

Table S6. Summary of two-way ANOVA (applied to logit-transformed data) examining the effect of Waves (null, low, intermediate, and high wave velocity), and Density (low, intermediate, and high urchin density) on the proportion of bounded aggregations of green sea urchins (*Strongylocentrotus droebachiensis*) at the end of trials in Experiment 2.

Source of Variation	df	MS	F-value	p
Waves	3	0.773	7.32	<0.001
Density	2	0.163	1.54	0.218
Waves × Density	6	0.375	3.54	0.003
Error	106	0.106		
Corrected Total	117			

Table S7. Summary of two-way ANOVAs (applied to $\log[x+1]$ -transformed^[1] and raw^[2] data) examining the effect of Waves (null, low, intermediate, and high wave velocity) and Block (each block of four days during which one replicate of each treatment was done) on the number of green sea urchins (*Strongylocentrotus droebachiensis*) per bounded and unbounded aggregation and number of solitary urchins on the tiles and tank walls under three densities at the end of trials in Experiment 2.

Configuration	Density	Source of variation	df	MS	F-value	p
Bounded aggregation	Low ¹	Waves	3	0.177	17.15	<0.001
		Block	9	0.017	1.64	0.153
		Error	27	0.010		
		Corrected Total	39			
	Intermediate ¹	Waves	3	0.581	45.32	<0.001
		Block	9	0.020	1.56	0.182
		Error	25	0.013		
		Corrected Total	37			
	High ¹	Waves	3	1.261	67.66	<0.001
		Block	9	0.038	2.02	0.077
		Error	27	0.019		

Configuration	Density	Source of variation	df	MS	F-value	p
		Corrected Total	39			
Unbounded aggregation	Low ²	Waves	2	0.198	2.23	0.310
		Block	6	0.849	9.55	0.098
		Error	2	0.089		
		Corrected Total	10			
	Intermediate ¹	Waves	3	0.019	0.64	0.614
		Block	9	0.022	0.73	0.679
		Error	7	0.030		
		Corrected Total	19			
	High ¹	Waves	3	0.070	2.99	0.096
		Block	9	0.069	2.95	0.071
		Error	8	0.023		
		Corrected Total	20			
Solitary on tiles	Low ²	Waves	3	39.09	2.53	0.078
		Block	9	25.30	1.64	0.154
		Error	27	15.44		
		Corrected Total	39			
	Intermediate ²	Waves	3	184.70	8.07	0.001
		Block	9	26.88	1.17	0.350
		Error	27	22.89		
		Corrected Total	39			
	High ²	Waves	3	1035.69	35.96	0.001
		Block	9	41.11	1.43	0.226
		Error	27	28.80		
		Corrected Total	39			
Solitary on walls	Low ²	Waves	3	1176.29	104.52	<0.001
		Block	9	10.45	0.93	0.517
		Error	27	11.25		
		Corrected Total	39			
	Intermediate ²	Waves	3	7267.83	254.19	<0.001
		Block	9	91.51	3.20	0.009
		Error	27	28.59		
		Corrected Total	39			
	High ²	Waves	3	14378.62	102.80	<0.001
		Block	9	251.70	1.80	0.115
		Error	27	139.90		
		Corrected Total	39			