POPULATION REGULATION OF SEA URCHINS IN A ROCKY SUBTIDAL ECOSYSTEM

by

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at

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To my grandmother, Teresa Feehan, who inspired me to pursue a PhD. She attended the University of Alberta from 1967 to 1975, while raising her seven sons, and graduated at the age of 45 with a Bachelor of Education with Distinction.
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ABSTRACT

Herbivory by sea urchins is an important control of seaweed biomass worldwide. For my doctoral thesis, I investigate biological processes that govern the dynamics of a kelp bed ecosystem on the Atlantic coast of Nova Scotia: increases in sea urchin (*Strongylocentrotus droebachiensis*) density that trigger a phase shift to urchin barrens, and an amoebic disease of sea urchins that triggers the reverse shift to kelp beds. I demonstrate experimentally that a phase shift from kelp beds to barrens can occur through formation of destructive grazing aggregations of sea urchins within kelp beds. However, recurrent outbreaks of disease are preventing the establishment of urchin grazing aggregations and stabilizing the kelp bed state. These epizootics have increased in frequency over the last 35 years in association with increasing strong storms and peak sea temperatures, conditions that may favour introduction of the pathogenic agent (*Paramoeba invadens*) from possible offshore source populations and its spread along the coast. *P. invadens* has remained functionally and physiologically stable over this period, suggesting that environmental change likely is responsible for an increase in disease. Predation on juvenile sea urchins by cancrid crabs may limit recovery of urchin populations within a kelp bed following disease outbreaks, and this is mediated in part by the availability of spatial refuges to juveniles, such as spaces within the holdfasts (anchoring structures) of kelp. Globally, a reduction in sea urchin grazing pressure due to epizootics has led to profound changes in the structure and functioning of coastal marine ecosystems, with shifts from sea urchin barrens to kelp beds in Nova Scotia and California, and shifts from coral- to macroalgal-dominance on reefs in the Caribbean and tropical western Atlantic. My research underscores the importance of longitudinal studies to monitor changes in the frequency and extent of sea urchin epizootics, environmental correlates that may explain these events, and the attendant impacts of sea urchin die-offs on the ecology of coastal ecosystems.
**LIST OF ABBREVIATIONS AND SYMBOLS USED**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AGD</td>
<td>Amoebic Gill Disease</td>
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<tr>
<td>AIRS</td>
<td>Atmospheric Infrared Sounder</td>
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<td>ANCOVA</td>
<td>Analysis of Covariance</td>
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<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>BC</td>
<td>Bear Cove</td>
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<td>Cranberry Cove</td>
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<td>CI</td>
<td>Croucher Island</td>
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<tr>
<td>CPUE</td>
<td>Catch Per Unit Effort</td>
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<td>CTS</td>
<td>Coastal Time Series</td>
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<td>GI</td>
<td>Gravel Island</td>
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<td>hour(s)</td>
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<td>HHB</td>
<td>Halifax Harbour Buoy</td>
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<td>Horse Island</td>
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<td>Mean Square</td>
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<td>MODIS</td>
<td>Moderate Resolution Imaging Spectroradiometer</td>
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<td>NASA</td>
<td>National Aeronautics and Space Administration</td>
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<td>Non-nutrient</td>
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<tr>
<td>NOAA</td>
<td>National Oceanographic and Atmospheric Administration</td>
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<td>NOMADS</td>
<td>National Operational Model Archive and Distribution System</td>
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<td>OH</td>
<td>Owl’s Head</td>
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<td>SI</td>
<td>Southwest Island/Sable Island</td>
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<td>SMB</td>
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<td>The Lodge</td>
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<td>U</td>
<td>Across-shore Component</td>
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<td>USA</td>
<td>United States of America</td>
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<td>V</td>
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<td>Week(s)</td>
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<td>Water-borne Transmission Experiment</td>
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<td>Proportion of sea urchin morbidity or mortality</td>
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<tr>
<td>$T_m$</td>
<td>Mean temperature at 8 m depth in the 2-week period following a storm</td>
</tr>
<tr>
<td>$T_{min}$</td>
<td>Mean annual minimum sea temperature (February through March)</td>
</tr>
<tr>
<td>$T_{TL}$</td>
<td>Temperature acquired from 8 m depth at The Lodge (TL)</td>
</tr>
<tr>
<td>$u_b$</td>
<td>Bottom orbital velocity</td>
</tr>
<tr>
<td>$V_{\text{haptera}}$</td>
<td>Volume occupied by haptera within a holdfast</td>
</tr>
<tr>
<td>$V_{\text{refuge}}$</td>
<td>Volume of refuge space within a holdfast</td>
</tr>
<tr>
<td>$W$</td>
<td>Maximum sustained wind speed of a hurricane</td>
</tr>
<tr>
<td>$w_i$</td>
<td>Proportion of sea urchins within size class $i$</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
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<td>--------</td>
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<tr>
<td>$x_i$</td>
<td>Median test diameter of size class $i$</td>
</tr>
<tr>
<td>$\bar{x}_w$</td>
<td>Weighted mean of the median test diameters of sea urchin size classes containing the largest 5% of the population</td>
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<td>$Y$</td>
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<td>$\alpha$</td>
<td>Significance level</td>
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<tr>
<td>$\chi^2$</td>
<td>Chi-square test statistic</td>
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CHAPTER 1

INTRODUCTION

Destructive grazing by sea urchins in temperate ecosystems worldwide has led to catastrophic phase shifts from ecosystems dominated by fleshy macroalgae to so-called sea urchin barrens (Ling et al. 2015). These phase shifts have resulted in dramatic losses to ecosystem productivity and structural complexity (Mann 1982). The first recorded phase shift to sea urchin barrens occurred in the Aleutian Islands in the Northwest Pacific, and resulted from overgrazing of the kelps *Alaria fistulos* and *Laminaria* spp. by sea urchins *Strongylocentrotus polyacanthus* (Filbee-Dexter & Scheibling 2014). This grazing event was preceded by an increase in sea urchin density due to release from predation, following overhunting of sea otters for the fur trade in the early 1800s (Simenstad et al. 1978). Similar shifts to barrens have since been observed in California in the Northeast Pacific, Tasmania in the Southwest Pacific, Norway in the Northeast Atlantic, and Maine and Eastern Canada in the Northwest Atlantic (Filbee-Dexter & Scheibling 2014, Ling et al. 2015).

In Nova Scotia, Canada, shifts to sea urchin barrens have occurred due to destructive grazing of the dominant kelps *Laminaria digitata* and *Saccharina latissima* by *Strongylocentrotus droebachiensis*. This was first recorded in the late 1960s, when dense aggregations of sea urchins developed within kelp beds in a large embayment (St. Margarets Bay) on the Atlantic coast of Nova Scotia (Breen & Mann 1976b, Mann 1977). By the late 1970s, sea urchin barrens dominated most of this coast (Wharton & Mann 1981). Dense aggregations of sea urchins within kelp beds are thought to have developed due to an increase in sea urchin density following release from predation through historical overfishing of finfish (Scheibling 1996) or by temperature-mediated pulses of sea urchin recruitment (Hart & Scheibling 1988). In the early 1980s, sea urchin barrens along the entire coast shifted back to kelp beds following outbreaks of an amoebic disease (paramoebiasis) that caused mass mortality of sea urchins in the barrens (Miller 1985, Scheibling 1986). Recurrent destructive grazing events were recorded in St.
Margarets Bay, and elsewhere along the coast of Nova Scotia, in the early 1990s and late
2000s. However, a shift to barrens has not been observed since the 1970s due to recurrent
outbreaks of disease (Scheibling et al. 1999), which appear to be correlated with
increasing strong storm activity and warm sea temperatures (Scheibling & Hennigar
1997, Scheibling & Lauzon-Guay 2010). Evidence for the increasing importance of
disease in regulating sea urchin populations indicates that processes that determine the
structure and dynamics of the kelp bed ecosystem have changed considerably since the
pioneering studies by K. H. Mann almost 50 years ago.

The overarching goal of my thesis is to examine biological processes that
currently regulate the alternative-state dynamics of this system on the Atlantic coast of
Nova Scotia. In Chapter 2, I examine the formation of grazing aggregations of
Strongylocentrotus droebachiensis within a kelp bed by experimentally increasing urchin
density to simulate conditions under which destructive grazing and a phase shift to
barrens was first observed in the 1960s. In Chapters 3 and 7, I use a multiyear field
experiment to examine a statistical link between outbreaks of disease and the occurrence
of strong storms and warm sea surface temperatures, and explore causal mechanisms that
may account for this relationship. In Chapter 4, I confirm the identity of Paramoeba
invadens as the pathogenic agent responsible for recurrent mass mortalities of sea urchins
over the past 35 years. In Chapter 5, I use a 44-year time series (1968 – 2012) to examine
the relative role of recurrent disease and predation in regulating the recovery of sea
urchin populations within kelp beds following mass mortality. In Chapter 6, I examine
the dynamics of predation by cancrid crabs on recruitment of juvenile sea urchins within
these kelp beds using field and laboratory experiments. In Chapter 8, I examine the
impacts of sea urchin epizootics on ecosystem structure and functioning globally, and
discuss patterns and processes occurring over large spatial and temporal scales. Finally in
Chapter 9, I summarize the current role of disease in the rocky subtidal ecosystem of
Nova Scotia based on the findings of my PhD research, and I propose broadening our
conceptual framework of community dynamics to include host-pathogen interactions that
can lead to disease-induced trophic cascades in marine and terrestrial ecosystems.
CHAPTER 2

AGGREGATIVE FEEDING BEHAVIOUR IN SEA URCHINS LEADS TO DESTRUCTIVE GRAZING IN A NOVA SCOTIAN KELP BED

2.1. ABSTRACT

Grazing aggregations of the sea urchin *Strongylocentrotus droebachiensis* drive the transition between alternative ecosystem states in Nova Scotia, from productive kelp beds to less productive barrens. This transition can be initiated by the formation of gaps within a kelp bed, containing dense aggregations of sea urchins. We examined the importance of local density of sea urchins and pre-existing gaps in a kelp canopy in mediating the formation of destructive grazing aggregations of sea urchins in a kelp bed. We transplanted 14000 adult sea urchins from a barrens on the Atlantic coast of Nova Scotia into ~ 4.5 m² plots within a nearby kelp bed, at densities above and below a predicted threshold value for destructive grazing, and simulated disturbance to the kelp bed by removing the kelp canopy in half of the plots. Sea urchin abundance and gap formation and expansion (as loss of kelp canopy cover) were monitored in and around plots weekly for 9 weeks. Grazer-mediated gap formation began 3 weeks after sea urchins were introduced, and increased for the remainder of the experiment. Our results indicate a direct linear relationship between sea urchin abundance and increase in gap area within undisturbed treatments. Gaps expanded in the kelp bed at sea urchin densities below the putative threshold for destructive grazing, indicating that the kelp bed was less resilient to grazing than predicted. Our findings provide insights into mechanisms controlling the stability of the kelp bed ecosystem state and mediating shifts from kelp beds to barrens in Nova Scotia.
2.2. INTRODUCTION

Transitions between alternative ecosystem states often are considered catastrophic events because they cause abrupt changes in ecosystem structure and function that can lead to loss of ecosystem services to humans (Scheffer et al. 2001). Understanding the mechanisms that drive shifts between contrasting community configurations is critical to judicious management and conservation of these ecosystems (Scheffer et al. 2001, Beisner et al. 2003). To restore or maintain an ecosystem state that is ecologically or economically desirable, we must first understand feedback mechanisms that stabilize a given state and the factors that reduce its resilience.

Population outbreaks of sea urchins have repeatedly led to destructive grazing of kelp beds in temperate coastal regions (North & Pearse 1970, Breen & Mann 1976b, Hagen 1983, Johnson et al. 2005), with dramatic implications for ecosystem productivity and services (Mann 1982). Kelps create 3-dimensional structure and provide food and habitat for a diverse fauna, including many ecologically or economically valuable species, such as fish, lobsters, and sea otters (Dayton 1985). Along the Atlantic coast of Nova Scotia, grazing by high-density aggregations (fronts) of sea urchins Strongylocentrotus droebachiensis at the offshore margin of kelp beds drives transitions between alternative community states, from kelp beds to sea urchin barrens, on a decadal scale (Johnson & Mann 1988, Scheibling et al. 1999, Brady & Scheibling 2005, Lauzon-Guay & Scheibling 2007a). Previous studies provide evidence of a threshold biomass of sea urchins (~ 2 kg m^{-2}) for destructive grazing of kelp beds in Nova Scotia (Breen & Mann 1976a,b, Scheibling et al. 1999, Lauzon-Guay & Scheibling 2007a). At this threshold, sea urchins undergo a shift in feeding behaviour, from passive feeding on drift algae and grazing coralline substrata to gregarious feeding that enables them to effectively weigh-down and consume kelp blades (Breen & Mann 1976b, Lauzon-Guay & Scheibling 2007a). Lauzon-Guay et al. (2008) expressed this threshold as a ratio of sea urchin to kelp biomass (1:2) in a model of the formation and propagation of grazing fronts that showed strong concordance between predicted and observed results.

In the late 1960s, Mann (1972a) observed gaps in the kelp bed, with high densities of sea urchins, in St. Margarets Bay, a large semi-protected embayment near Halifax,
Nova Scotia. These gaps gradually expanded and coalesced, resulting in a loss of 140 km² of kelp bed in the bay and a shift to the barrens state by 1973 (Breen & Mann 1976b, Mann 1977). Since those pioneering studies, destructive grazing by sea urchins has been recorded repeatedly within St. Margarets Bay (as it has elsewhere in Nova Scotia) at the deep margin of kelp beds (Scheibling et al. 1999, Lyons & Scheibling 2008), although the transition to the barrens state was interrupted in each case by outbreaks of disease that eliminated the sea urchins. To our knowledge, the initial formation of gaps within a kelp bed, attributed to sea urchin grazing, has not been recorded in the Northwest Atlantic.

To explore the possibility that sea urchin aggregations within a kelp bed could lead to a shift to the barrens state, Lauzon-Guay & Scheibling (2010) developed a coupled map lattice model to simulate the spatial dynamics of kelp and sea urchin abundance over time, under different sets of conditions relating to urchin movement, spatial variability in recruit density, localized sea urchin aggregation, and localized disturbance that creates gaps in the kelp bed. For example, their model shows that pre-existing gaps in a kelp bed can catalyze the shift to barrens by causing sea urchins to aggregate along the perimeter of the gap and graze outwards. This requires that sufficient numbers of sea urchins inhabit the kelp bed when gaps are formed, and that sea urchins migrate to the gap perimeter as they forage. The model also shows that a localized aggregation of sea urchins within a kelp bed can result in destructive grazing leading to gap formation. As these gaps expand, an influx of sea urchins from a background population within the kelp bed maintains sea urchin density along the gap margin.

The Lauzon-Guay & Scheibling (2010) model not only indicates that formation of sea urchin grazing aggregations and consequent canopy loss within a kelp bed are theoretically possible, but also yields predictions that can be used to inform manipulative field experiments to test causal mechanisms. The present study experimentally examines 2 factors that can potentially trigger destructive grazing within kelp beds and mediate the transition to a barrens state: local density of sea urchins and pre-existing gaps in a kelp canopy. Based on the results of previous grazing experiments, we predicted that gaps within a kelp bed would form in areas where sea urchin biomass exceeded the established threshold (Breen & Mann 1976a,b, Scheibling et al. 1999). Also, because sea urchins tend to aggregate along a kelp-barrens interface (Scheibling et al. 1999, Lauzon-Guay &
Scheibling 2007a), we predicted that manually clearing kelps to create artificial gaps within the kelp bed would catalyze the formation of grazing aggregations and expand these cleared patches. We examined potential interactive effects on destructive grazing of local sea urchin density and presence of pre-existing gaps in a kelp canopy by manipulating these factors concurrently in a factorial experiment at a site where Breen & Mann (1976a) first documented the phenomenon between 1968 and 1973. Our findings provide insights into mechanisms that reduce resilience of the kelp bed state and drive shifts to the alternative and less productive barrens state.

2.3. MATERIALS AND METHODS

2.3.1. Study Site and Experimental Design

Our study site at The Lodge (44° 33.491’ N, 64° 01.493’ W) is located on the western shore of St. Margarets Bay (Fig. 2.1). At the time of the experiment, the shallow subtidal zone was covered by a dense kelp canopy (mainly Saccharina latissima and scattered Agarum clathratum and Laminaria digitata) with a turf understory of coralline (Corallina officinalis), foliose (Chondrus crispus), and filamentous (Polysiphonia lanosa, Bonnemaisonia hamifera) red algae. The substratum is a gradually sloping field of granitic boulders and cobble, which grades to sand at ~ 18 m depth. A preliminary SCUBA-diving survey conducted in June 2009 indicated that adult sea urchins Strongylocentrotus droebachiensis (> 20 mm test diameter) were rare at this site.

To examine the effects of local sea urchin density and small-scale disturbance to the kelp bed on the formation of destructive grazing aggregations, we used SCUBA to collect 14000 adult (42 to 61 mm test diameter, n = 20) sea urchins from a feeding front at Splitnose Point (44° 28.609’ N, 63° 32.741’ W), 40 km east-southeast of The Lodge (Fig. 2.1), and transplant them into the kelp bed at The Lodge on 14 July 2009. Our experimental array consisted of 32 circular plots spaced 7 m apart and equally divided among 4 depth strata running parallel to shore from 7 to 10 m depth (chart datum). Each plot was marked with a central float anchored to the substratum with marine epoxy glue. SCUBA divers manually cleared all kelps by completely removing thalli at the holdfast...
within a 1.2 m radius of the centre of half of the plots, as a disturbance treatment (hereinafter referred to as ‘disturbed plots’). All cleared kelps were collected in mesh bags and subsequently discarded in deeper water, 100s of metres from our experimental site. Sea urchins were dispersed by divers within an ~ 1.2 m radius of the centre of plots at 4 levels of density (0, 50, 100, and 200 sea urchins m$^{-2}$, or 0, 250, 500, and 1000 sea urchins plot$^{-1}$). These densities were selected to encompass values both below and above the putative 1:2 threshold ratio of sea urchin to kelp biomass required for destructive grazing to occur. One replicate of each treatment combination of sea urchin density by disturbance was randomly allocated to each depth stratum. Depth was used as a blocking factor to account for variation in environmental conditions across a depth gradient (e.g. temperature, light, water motion) (Fig. 2.2a).

Kelp defoliation by physical or biological disturbance, such as extreme wave forces during hurricanes (K. Filbee-Dexter & R.E. Scheibling unpubl.) or outbreaks of an epiphytic bryozoan that causes extensive blade loss (Saunders & Metaxas 2008, Scheibling & Gagnon 2009), can create large gaps or cause major thinning in Nova Scotian kelp beds. The area that we cleared for the experiment was limited by logistical constraints of manipulation and monitoring, although this patch size is within the scale of disturbance resulting from storm events (Ebeling et al. 1985, R.E. Scheibling pers. obs.).

2.3.2. Environmental Conditions

Water temperature was recorded at 10 min intervals using a temperature logger (StowAway TidbiT Temp Logger, Onset Computer) at 8 and 12 m depth at The Lodge throughout the experiment. Significant wave height (SWH; average height of the highest one-third of waves in a wave field) was recorded at a meteorological buoy (www.meds-sdmm.dfo-mpo.gc.ca, buoy identification no. C44258) at the mouth of Halifax Harbour (44° 30’ N, 63° 24’ W).
2.3.3. Kelp and Sea Urchin Biomass

Kelp biomass at the experimental site was 3.4 ± 1.6 kg m\(^{-2}\) (mean ± 1 SD, n = 32) based on pooled samples of kelps harvested from a 1 m\(^2\) quadrat placed haphazardly 2 to 4 m from each end of the experimental array at each depth stratum on 14 July, 2 August, and 3 and 24 September 2009 (quadrat locations were staggered among sampling dates to preclude overlap), and weighed on shore with a spring scale. Wet weight of the experimental sea urchins was 68 ± 20 g (mean ± 1 SD, n = 40) based on haphazard collections of 20 sea urchins from the experimental population on 24 July and 17 September 2009 weighed in the laboratory on an analytical scale (0.001 g precision) within 24 h of collection. Sea urchin biomass (fresh weight) was calculated for each experimental plot as the mean sea urchin wet weight multiplied by sea urchin density.

2.3.4. Sea Urchin Abundance

The abundance of sea urchins within experimental plots was monitored weekly for 9 weeks beginning 17 July 2009. SCUBA divers counted all adult sea urchins in a 0.25 m\(^2\) quadrat initially placed at the centre of each plot and then flipped in 4 contiguous lines radiating at right angles from the centre, stopping when sea urchins were no longer observed in the quadrat. The 4 quadrat counts taken equidistant from the centre of a plot in each radial line were summed and extrapolated to the total area of a conceptualized concentric circular band (0.5 m wide). The total sea urchin count was calculated as the central quadrat (~ 0.25 m radius) plus the estimates from each concentric band (0.25 to 0.75 m, 0.75 to 1.25 m, etc.). The total radius surveyed within each plot was increased over the course of the experiment from 2.25 to 3.25 m (corresponding to 4 to 6 contiguous quadrat samples from the centre of a plot) to account for urchins that had migrated toward the periphery of plots. Naturally occurring juvenile sea urchins (< 20 mm test diameter), which were cryptic and at low density, and dead sea urchins and tests were recorded within the quadrat during urchin abundance surveys. Decapod predators of sea urchins (cancrid crabs *Cancer irroratus* and *C. borealis*, and lobster *Homarus americanus*) were also recorded when observed within plots. Sea urchin densities
decreased rapidly during the initial 2 weeks of the experiment. Therefore, on 2 August each experimental plot was supplemented with sea urchins taken from a surplus supply (from the same experimental source population) maintained in a mesh corral on the sand bottom near the offshore margin of the kelp bed (18 m depth), and fed kelp. We added an additional 10% of the respective initial sea urchin density to each plot. During the final survey of sea urchin abundance on 17 September, divers exhaustively searched each experimental plot, up to a 3.5 m radius, and counted all dead and live sea urchins. At this time, an amoebic disease associated with the passage of Hurricane Bill on 23 August 2009 had caused 35% morbidity of the remaining urchins (Scheibling et al. 2010), and the experiment was terminated. For statistical analyses, mean sea urchin density (urchins m⁻²) was calculated within each plot at weekly intervals as the total sea urchin count per m² within a 2.25 m radius of the centre of the plot (96% of all sea urchins were found within this radius), to coincide with the radius used to monitor gap formation and expansion (see next section). Overall mean sea urchin density (urchins m⁻²) was calculated as the time-averaged (grand mean) density for each plot from 17 July to 10 September. To maintain relevant mean values, only sea urchin abundance data collected prior to the disease outbreak (up to 10 September, when moribund sea urchins were rare) were included in the statistical analyses.

2.3.5. Gap Formation and Expansion

Kelp loss was monitored within experimental plots using 2 types of measures: gap area and patch area. For all plots, gap area is the planar surface area of bottom not covered by kelp blades when a plot is viewed from a set height above bottom. Gap area increases as gaps form (in undisturbed plots) or expand (in all plots) because of grazing by sea urchins. Other biotic and abiotic factors can contribute to increases in gap area, as evidenced by increases in control plots without sea urchins. Patch area is the planar surface area of bottom devoid of attached kelps and was only measured in disturbed plots, where initial patches were created by clearing kelps within a 1.2 m radius (4.5 m²). Patch area increases by removal of additional kelp thalli by sea urchin grazing at the patch perimeter. Patch area is expected to be larger than gap area because patches, when
viewed from above, may be partially occluded by kelp blades around the patch perimeter. Wave-driven movement of these blades can cause some variation in successive measurements of gap area, even when patch area remains constant. In undisturbed plots, small and irregular patches of bottom devoid of kelps were created as a result of sea urchin grazing, but these were too difficult to measure as they increased in number and size and changed shape over time. Also, these small patches could not be referred to a known baseline, as in the disturbed plots. Thus, patch area was not measured in the undisturbed plots.

To determine gap area, all plots were photographed (Canon Powershot G10) from 4 to 6 m above bottom, approximately weekly for 5 to 6 weeks, beginning at Week 3 (7 August) for the undisturbed plots (at the first appearance of gaps in these plots), and at Week 4 (14 August) for the disturbed plots. A 0.25 m² quadrat, or 2-m long crossed plastic poles with 0.5 m graduations, was placed at the centre of each plot as a scale reference for all photographs. During the final week of the experiment we observed small amounts of drift kelp within the plots where these were previously absent. Divers removed the drift kelp prior to taking the final set of photographs (20 September). Gap area was measured from photographs using ImageJ (National Institutes of Health, USA). Gaps were identified as areas devoid of kelp cover where underlying turf algae, granite rock, or sand was visible. The area of all gaps observed within a plot was summed to yield the total gap area (m²). For statistical analyses, gap area measurements up to 20 September (after the disease outbreak) were included, as sea urchins were present in the plots until this date (albeit in low numbers in the final week) and could potentially cause kelp loss.

Patch area was monitored in the disturbed plots approximately weekly for a 7-week period from 23 July (1 week after sea urchin introduction) to 10 September. Divers used a plastic measuring tape to measure the radius from the plot centre to the nearest kelp stipes at 8 equidistant locations along the circumference of the plot. The radial measurements (r) were averaged within each plot to get an estimate of the patch area ($\pi r^2$).
2.3.6. Statistical Analysis

To test the efficacy of our manipulation of sea urchin density over the initial 8 weeks of the experiment (prior to disease outbreak), we used repeated-measures ANOVA (RM-ANOVA) with initial sea urchin density (3 levels) and disturbance (2 levels) as fixed factors, depth stratum (4 levels) as a random blocking factor, and time as the repeated factor. Sea urchin density was square-root-transformed to meet the assumption of homoscedasticity, and control plots (0 sea urchins m\(^{-2}\)) were not included in the analysis to eliminate zero counts. We also used RM-ANOVA to test for the effect of initial sea urchin density (4 levels, fixed), depth stratum (4 levels, random), and time on: 1) gap area of undisturbed plots over a 6-week period (7 August to 20 September), 2) gap area of disturbed plots over a 5-week period (14 August to 20 September), and 3) patch area of disturbed plots over a 7-week period (23 July to 10 September). Because sea urchin density during the experiment was variable both within and among the initial urchin density treatments, we used linear regression with overall mean sea urchin density (time-averaged from 17 July to 10 September for each plot) as the predictor variable to test for the effect of sea urchin abundance throughout the experiment on: 1) final gap area of undisturbed plots (on 20 September), 2) final gap area of disturbed plots (on 20 September), and 3) final patch area of the disturbed plots (on 10 September, the last date patch area was measured).

Statistical tests were run with Statistica 8 (StatSoft). Assumptions of homoscedasticity were tested using Cochran’s C-test (\(\alpha = 0.05\)). For ANOVA, interactions with the random blocking factor (depth stratum) that were highly non-significant (\(p > 0.25\)) were removed from the analysis and the interaction mean square was pooled with residual mean square (Underwood 1997). The assumption of sphericity in RM-ANOVA was non-significant using Mauchly’s test (\(\alpha = 0.05\)). Tukey’s HSD test (\(\alpha = 0.05\)) was used to compare levels of factors that were significant in ANOVA.
Fig. 2.1. Map of Nova Scotia. Inset: St. Margarets Bay showing study sites at The Lodge (TL) and Splitnose Point (SP). Shaded area along the coast = spatial extent of destructive grazing between 1968 and 1973 (Breen & Mann 1976a).
Fig. 2.2. Spatial map of experimental layout with 32 plots within a kelp bed divided into 4 strata (1 to 4) extending parallel to the shoreline across a depth gradient (7 to 10 m, chart datum). (a) Initial (seeded) urchin density in the disturbed (grey circles) and undisturbed (black circles) treatments (14 July). (b) Overall mean sea urchin density (time-averaged from 17 July to 10 September) in the disturbed and undisturbed treatments. (c) Final gap area on 20 September in the disturbed and undisturbed treatments.
2.4. RESULTS

2.4.1. Environmental Conditions

Daily temperature (mean ± 1 SD) from 14 July to 20 September was 12.7 ± 1.8 °C within the experimental area at 8 m depth and 10.5 ± 2.7 °C at 12 m depth, i.e. 2 m below the deepest stratum (Fig. 2.3a). SWH generally ranged from 0.5 to 2 m throughout the experiment, except for 2 storm events: Hurricane Bill (maximum SWH: 9 m) on 23 August and Tropical Storm Danny (maximum SWH: 3.5 m) on 30 August (Fig. 2.3b).

2.4.2. Sea Urchin Abundance

Our initial measure of sea urchin abundance after 3 d (17 July) showed that densities were already well below the levels seeded, and lowest in Stratum 4 (Fig. 2.4). Although sea urchin density continued to decrease throughout the experiment (Table 2.1, Fig. 2.4), particularly in the high-initial-density (200 urchins m⁻²) plots (Fig. 2.5), significant differences in relative abundance among initial density levels were maintained throughout the experiment (Tukey’s test, p < 0.05) until the mass mortality on 17 September (Table 2.1, Fig. 2.2b, 2.4). The effect of the disturbance treatment on sea urchin abundance was non-significant (Table 2.1). Sea urchin density varied significantly among the 4 depth strata (Table 2.1), with the fewest sea urchins remaining in Stratum 4 (Fig. 2.4). This result is concordant with our observations of dead urchins in quadrat surveys, which were most abundant in Strata 2 and 4, with a total of 29, 76, 33, and 82 observations of dead urchins or tests in Strata 1 to 4, respectively, during the experiment. We observed crabs and lobsters preying on sea urchins within experimental plots, but their abundance within strata (6, 5, 1, and 2 crabs and 9, 17, 16, and 13 lobsters in total in Strata 1 to 4, respectively) did not correlate with the abundance of dead urchins. Cunners *Tautogolabrus adspersus* preyed on moribund sea urchins during the disease outbreak.

Generally, sea urchins formed small actively grazing aggregations scattered throughout the plots (Fig. 2.6a,b). However, in 2 of the high-density (200 ind. m⁻²) disturbed plots, sea urchins formed a single large sedentary aggregation in the centre of
the plot that persisted throughout the experiment (Strata 1 and 3; Fig. 2.5, 2.6c,d). In both of the plots, the large aggregation formed on a boulder at the centre of the plot, and divers periodically observed sea urchins feeding on drift kelp, or attached blades that fell into the plot from the edge of the cleared area (Fig. 2.6d).

2.4.3. Gap Formation and Expansion

Gaps first appeared in the undisturbed treatment after 3 weeks (7 August; Fig. 2.6a) and increased significantly over time during the next 6 weeks (Table 2.2, Fig. 2.7a). Although the effect of initial sea urchin density on gap area was marginally non-significant (p = 0.067) among undisturbed plots (low replication and high variability in density treatments limited the power of the analysis), the mean increase in gap area was greatest in the high-initial-density treatments (100 and 200 urchins m$^{-2}$) (Table 2.2, Fig. 2.7a). The effect of stratum on gap area varied across time in the undisturbed treatment (Table 2.2), with the rate of gap expansion decreasing from Stratum 1 to Stratum 4. This significant interaction of time and stratum is likely the result of differences in sea urchin density among strata (at all initial density levels, excluding the control), with density decreasing from Stratum 1 to Stratum 4 (Fig. 2.4).

In the disturbed treatment, gap area measured over a 5-week period from 14 August to 20 September did not depend on initial sea urchin density or depth stratum but increased significantly with time (Table 2.2, Fig. 2.7b). However, initial sea urchin density, depth stratum, and time all had significant effects on patch area over a 7-week period from 23 July to 10 September (Table 2.2). Patch area in the control treatment (initial density: 0 urchins m$^{-2}$) was significantly lower than at all other levels of initial sea urchin density over this period (Tukey’s test, p < 0.03; Fig. 2.7c). Patch area in the control treatment on 23 July (5.5 m$^2$; Fig. 2.7c) approximates initial patch area in disturbed plots at the start of the experiment (14 July). Initial patch area exceeded the cleared area (4.5 m$^2$) because some kelps along the patch perimeter were outside the 1.2 m radius that was experimentally cleared. Overall, patches were significantly smaller on 23 July than during any of the succeeding weeks, which did not differ significantly (Tukey’s test, p > 0.50; Fig. 2.7c). This result suggests that patches expanded at initial
density levels of 50, 100, and 200 urchins m\(^{-2}\) (as compared to the control with 0 urchins m\(^{-2}\)) within the first 1 to 2 weeks of the experiment, when mean sea urchin densities were the highest (Fig. 2.4).

Overall, sea urchin density was highly variable both within and among treatments during the experiment. When final gap area is analyzed in relation to the overall time-averaged density during the experiment (Fig. 2.2b,c), there is a significant positive linear relationship in the undisturbed treatment (Table 2.3, Fig. 2.7d). This relationship was not significant in the disturbed treatment (Table 2.3) in terms of both gap (Fig. 2.7e) and patch area (Fig. 2.7f), although there was a slight positive trend in patch area (Table 2.3).

Table 2.1. Repeated-measures ANOVA of effect of initial sea urchin density (3 levels: 50, 100, 200 ind. m\(^{-2}\)), disturbance (2 levels: disturbed, undisturbed), depth stratum (4 levels), and time on sea urchin density (ind. m\(^{-2}\), square-root transformed) over 8 weeks. Bold values are significant at \(p \leq 0.05\). For within-subject effects, Time (T) × Density (Dens), T × Disturbance (Disturb), T × Dens × Disturb, and T × Dens × Stratum (Strat) are tested against pooled T × Disturb × Strat MS and residual MS. For between-subject effects, Disturb, Strat, Dens × Disturb, and Dens × Strat are tested against pooled Disturb × Strat MS and residual MS.

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Table 2.2. Repeated-measures ANOVA of effect of initial sea urchin density (4 levels: 0, 50, 100, 200 ind. m\(^{-2}\)), depth stratum (4 levels), and time on: gap area of undisturbed plots over 6 weeks from 7 August to 20 September; gap area of disturbed plots over 5 weeks from 14 August to 20 September; and patch area of the disturbed plots over 7 weeks from 23 July to 10 September. Bold values are significant at p ≤ 0.05.

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Table 2.3. Linear regression of effect of overall mean sea urchin density (ind. m$^{-2}$) time-averaged from 17 July to 10 September on: final gap area of undisturbed plots on 20 September; final gap area of disturbed plots on 20 September; and final patch area of disturbed plots on 10 September. Bold values are significant at $p \leq 0.05$.

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Fig. 2.3. (a) Temperature at 8 and 12 m depth at The Lodge and (b) significant wave height (measured off Halifax) during the experiment. Note abrupt fluctuations in temperature and significant wave height associated with the passage of Hurricane Bill on 23 August and Tropical Storm Danny on 30 August, 2009. ND: no data available.
Fig. 2.4. Sea urchin density (ind. m$^{-2}$, mean + SE) at 4 levels of initial urchin density (see key) within depth strata 1 to 4 (a–d) over a 9-week period. Means are pooled over 2 levels of disturbance. Vertical arrow: sea urchins replenished in the plots.
Fig. 2.5. Distribution of sea urchin density over 8 weeks measured in 0.5 m increments from the centre of the plots to a 3 m radius for the 200 ind. m$^{-2}$ initial density treatment at 2 levels of disturbance within 4 depth strata.
Fig. 2.6. (a,b) Small active grazing aggregations of sea urchins (indicated by arrows in b) initiate small gaps in the kelp canopy of undisturbed plots. (c,d) Large sedentary (non-grazing) aggregations in disturbed plots passively feed on drift algae or prostrate kelp blades. Scale: sea urchins are ~ 5 cm diameter and the quadrat in (a,c) is 50 x 50 cm. (Photographs by R.E. Scheibling).
Fig. 2.7. Gap area (+1 SE; n = 4) at 4 levels of initial sea urchin density (ind. m⁻²; see keys) in the (a) undisturbed treatment over 6 weeks and (b) disturbed treatment over 5 weeks. (c) Patch area (+1 SE; n = 4) in the disturbed treatment at 4 levels of initial sea urchin density over 6 weeks. Final gap area in the (d) undisturbed treatment and (e) disturbed treatment, and (f) final patch area in the disturbed treatment as a function of time-averaged overall sea urchin density and biomass.
2.5. DISCUSSION

Our study is the first to demonstrate that localized increases in sea urchin density can lead to the formation of destructive grazing aggregations and creation of gaps in a kelp bed in the Northwest Atlantic. We showed that gaps in the kelp canopy in undisturbed plots increased in size with increasing mean sea urchin density from 0 to 26 urchins m\(^{-2}\) (0 to 1.8 kg urchins m\(^{-2}\)) in a linear manner. The absence of a density threshold for destructive grazing is inconsistent with previous field observations (Breen & Mann 1976a,b, Scheibling et al. 1999, Lauzon-Guay & Scheibling 2007a) and mathematical models (Lauzon-Guay et al. 2008, 2009). Given the biomass of kelp at our experimental site (3.4 kg m\(^{-2}\)) and average individual weight of transplanted sea urchins (68 g), we would predict a threshold density of 25 urchins m\(^{-2}\) (biomass of 1.7 kg urchins m\(^{-2}\)) for destructive grazing, based on a 1:2 threshold ratio of sea urchin to kelp biomass (Lauzon-Guay et al. 2008).

Differences in wave exposure between our study site, within a semi-protected embayment, and studies conducted at more exposed sites, where extensive fronts of sea urchins form along the offshore margins of kelp beds (Scheibling et al. 1999, Lauzon-Guay & Scheibling 2007b), may in part explain this discrepancy. Strong wave action can prevent sea urchins from climbing kelp stipes or anchoring kelp blades to feed (Velimirov & Griffiths 1979, Lauzon-Guay & Scheibling 2007b), and can inhibit sea urchin aggregative behaviour (Lauzon-Guay & Scheibling 2007b), thus increasing the threshold biomass of sea urchins required to pin-down and consume kelp. In contrast, at more wave-protected sites, kelp blades lie prostrate on the seabed, allowing smaller groups or individual sea urchins to consume them.

Differences in hydrodynamic conditions inside a kelp bed, compared to the kelp bed-barrens interface, also may account for the lack of a grazing threshold in our study. Breen & Mann (1976a,b) reported a grazing threshold of 2 kg urchins m\(^{-2}\) at our experimental site and adjacent areas within St. Margarets Bay in the 1970s. However, they observed grazing by a sea urchin front at the offshore kelp bed margin, whereas in our study grazing occurred within the kelp bed. Similarly, Konar & Estes (2003) found that sea urchins *Strongylocentrotus polyacanthus* transplanted into a kelp bed in the
Aleutian Islands destructively grazed kelps and decreased canopy cover, while sea urchins at a nearby kelp bed-barrens interface did not. They concluded that wave-induced kelp movement prevented sea urchins from breaching the kelp bed-barrens boundary. Water movement and pummeling of sea urchins can be dampened by drag on adjacent kelp blades (Friedland & Denny 1995), and this may allow sea urchins to feed more easily within a kelp bed.

Seasonal variation in the rate of destructive grazing of kelp beds by *Strongylocentrotus droebachiensis* has been attributed to increased wave action during late fall and winter (Scheibling et al. 1999, Lauzon-Guay & Scheibling 2007b). Strong wave forces caused by Hurricane Bill, and to a lesser extent by Tropical Storm Danny, likely interrupted or slowed sea urchin grazing for 1 or 2 d, although this had no apparent effect on change in gap area measured at weekly intervals (Fig. 2.7a,b). Water temperature appears to have little effect on the rate of destructive grazing below a threshold of ~ 17 °C (Lauzon-Guay & Scheibling 2007b), when sea urchin foraging activity is arrested because of thermal stress (Percy 1973, Lyons & Scheibling 2007). Since temperatures at 8 m depth generally ranged between 10 and 14 °C (average: 12.7 °C), it is unlikely that small fluctuations in bottom temperature influenced sea urchin grazing rate in our experiment.

In the Northeast Pacific, destructive grazing of kelp can be mediated by changes in sea urchin behaviour, even without increases in sea urchin abundance. Behavioural shifts from passive detritivory of drift kelp to active herbivory of attached sporophytes are strongly dependent on availability of kelp detritus (Ebeling et al. 1985, Harrold & Reed 1985, Tegner & Dayton 1991). Tegner & Dayton (1991) attribute the loss of kelp forests in Southern California in the late 1950s to dramatic reductions in the subsidy of kelp detritus to sea urchins (*Strongylocentrotus purpuratus, S. franciscanus*) within the forests, which caused a shift to destructive grazing. In some regions along the coast of California, patchiness in the availability of kelp detritus to *S. franciscanus* within the kelp forest can result in grazed patches (Harrold & Reed 1985). However, in contrast to the patches observed by Mann (1972a) in St. Margarets Bay, these patches were ephemeral (due to seasonal changes in the abundance of detritus) and were re-colonized by kelps in the course of a 2-year study (Harrold & Reed 1985).
Interestingly, we observed both passive and active feeding behaviour within the high initial urchin density (200 ind. m\(^{-2}\)) and disturbed treatment combination in our experimental array. Passive detritivory in *Strongylocentrotus droebachiensis* is only observed when detrital food is available; when macroalgae are scarce, adult sea urchins actively forage in search of drift algae on which to feed (Dumont et al. 2004). We detected no effect of sea urchin density on final gap area or patch area in the disturbed treatment, which likely was due, at least in some plots, to passive feeding behaviour by sea urchins on drift or prostrate kelp blades from the surrounding kelp bed that were trapped by sea urchins in the plot. This suggests a paradoxical effect of low wave exposure on sea urchin feeding behaviour in the kelp bed: reduced water motion may lower the threshold for destructive grazing while increasing the availability of prostrate kelp blades to sea urchins in sedentary aggregations and thereby inhibit foraging movements that would lead to active grazing at the patch edge. Low water motion also can increase the amount of drift kelp that is retained in kelp beds or forests (Harrold & Reed 1985), although drift kelp was rarely observed in our experimental plots (aside from fragments trapped by sea urchins).

A decrease in sea urchin density at the kelp-barrens interface of cleared patches, which resulted from individuals migrating to the patch perimeter, also limited patch or gap expansion in the disturbed treatment. Lauzon-Guay & Scheibling (2010) predicted that sea urchins in a kelp bed will form a feeding aggregation around the perimeter of a patch, and that urchin density within the aggregation will decrease as the patch size (and specifically perimeter) increases. For a destructive grazing threshold to be maintained, according to their model, a background density of sea urchins within the kelp bed is required to supplement the declining density at the patch perimeter. Since a background population of adult sea urchins was effectively absent at our site, density decreased as the experimental animals moved outwards from the central seeded area of the plot, diminishing their capacity to destructively graze kelp as a front. However, we did detect a significant effect of initial sea urchin density on patch area after the first 2 weeks of the experiment, when each of the sea urchin-seeded treatments (50, 100, and 200 ind. m\(^{-2}\)) had a larger patch area than the control treatment with no added sea urchins. We propose that sea urchin density at the edge of experimental patches was sufficiently high early in
the experiment to cause destructive grazing and patch expansion. These observations also suggest that gaps in the undisturbed plots would likely have stopped expanding once they reached a critical size at which sea urchin density along the edge of the patch decreased below the level required for sea urchins to graze cooperatively. An effect of initial sea urchin density in the disturbed treatment was not detected as a change in gap area in our analysis because this was not measured until Week 4 of the experiment, when sea urchin densities had already decreased markedly.

Sea urchin densities in kelp beds in Nova Scotia are typically well below levels required for destructive grazing, and lower than densities in adjacent barrens (mean ± 1 SD = 14 ± 12 ind. m⁻² in healthy kelp beds vs. 71 ± 28 ind. m⁻² in post-transitional barrens; Meidel & Scheibling 2001). This difference can be explained in part by lower recruitment of sea urchins in kelp beds than in barrens (Balch & Scheibling 2000). Although a high prevalence of predators and low cover of coralline algae (that induce larval settlement; Pearce & Scheibling 1990) may limit overall rates of recruitment in kelp beds (Raymond & Scheibling 1987, Balch & Scheibling 2000), localized high-density aggregations of sea urchins could arise as a result of spatial or temporal variability in recruitment (Scheibling 1996, Lauzon-Guay & Scheibling 2010). Stochastic processes such as temperature anomalies that affect larval survival may trigger major settlement events (Hart & Scheibling 1988).

We observed large reductions in the seeded sea urchin population, particularly in the first 2 weeks of the experiment and within the deepest stratum, that likely were caused by predation. Sea urchins remained rare in control plots (0 urchins m⁻²), even those adjacent to high-initial-density plots, indicating limited migration from seeded plots. We often observed cancriid crabs and lobsters directly preying on experimental sea urchins throughout the experiment. Evidence from the Gulf of Maine indicates that Cancer borealis has become a voracious predator of juvenile and adult Strongylocentrotus droebachiensis in the Northwest Atlantic, due to the removal by overfishing of higher-level predators of crabs, such as cod Gadus morhua (Steneck et al. 2002). Leland (2002) found that S. droebachiensis transplanted into a kelp bed in the Gulf of Maine were heavily preyed on by C. borealis during August and September. Crabs and lobsters are particularly active in late summer and early fall when sea
temperatures are the warmest. Accumulations of cracked and punctured tests in our experimental plots provided ample evidence of predation.

There was no consistent pattern among strata in predator abundance or sea urchin remains that explains the progressive decline in sea urchin abundance with depth. However, our surveys took place only during daylight hours, and nocturnal predation may account for depth-related differences in sea urchin mortality. Also, the quality of spatial refuges for sea urchins may have differed among strata. Increased sedimentation associated with low wave action may have limited refuge space in the deep stratum by infilling crevices and spaces between boulders. The availability of spatial refuges has been shown to be an important factor mediating predation rates of small sea urchins by crabs and lobsters (Scheibling & Hamm 1991).

Disturbed plots with large sedentary aggregations of sea urchins showed the smallest decrease in urchin density. Previous research suggests that large, 2-dimensional aggregations can provide a ‘size refuge’ from predators such as crabs and lobsters by decreasing the vulnerability of individual sea urchins to handling and detachment (Garnick 1978, Bernstein et al. 1981, Scheibling 1996). The persistence of these aggregations in our experimental plots following extreme wave conditions associated with Hurricane Bill suggests that this behaviour also may be adaptive in limiting dislodgment during storm events.

In a few cases, increases in gap area occurred in undisturbed plots without sea urchins or at the low initial urchin-density level (Fig. 2.7a). During our experiment, canopy loss that was not attributed to destructive grazing was most likely related to seasonal increases in erosion or fragmentation of kelp blades encrusted with Membranipora membranacea that are exposed to heavy wave action during storms (Krumhansl & Scheibling 2011). Following Hurricane Bill, we observed piles of drift kelp on the shore adjacent to our site. The cover of M. membranacea on kelps increased in September, and a concurrent study at the same site (but outside of our experimental array) showed that kelp erosion was significantly related to cover of the bryozoan during our experiment (Krumhansl & Scheibling 2011). Apart from direct effects on the kelp canopy cover, seasonal kelp erosion could accelerate destructive grazing by sea urchins by decreasing the kelp biomass (Lauzon-Guay & Scheibling 2007b).
Sea urchin mortality in the final weeks of the experiment was caused by a disease outbreak (paramoebiasis) associated with the passage of Hurricane Bill (Scheibling et al. 2010). Historically, disease outbreaks have decimated sea urchin populations in Nova Scotia, releasing kelp beds from grazing pressure and causing the shift from barrens to kelp beds (Scheibling 1984a, Scheibling & Hennigar 1997). These outbreaks have increased in frequency over the past 3 decades, a pattern that appears to be linked to the frequency of severe storm events (Scheibling & Lauzon-Guay 2010). Interestingly, we found low-density populations of sea urchins in kelp beds elsewhere in St. Margarets Bay in June 2010 (C. Feehan et al. unpubl. data), indicating that sea urchins were not eliminated throughout the bay in fall 2009. This likely reflects the density-dependence of host-pathogen dynamics (Anderson & May 1986), which has been observed in previous outbreaks of sea urchin disease in Nova Scotia (Scheibling & Stephenson 1984) and in California (Lafferty 2004). The increased likelihood of a disease outbreak occurring among dense aggregations of sea urchins (as observed during our experiment) suggests an important feedback mechanism that limits the resilience of sea urchin populations in kelp beds, particularly given the predicted increase in hurricane intensity in the North Atlantic with global climate change (Bender et al. 2010, Scheibling et al. 2010).

We have demonstrated experimentally that localized increases in sea urchin density can lead to the formation of grazing aggregations and expansion of gaps in a kelp bed. We did not find evidence for a threshold density of sea urchins for destructive grazing, which suggests that kelp beds are less resilient to destructive grazing from within the bed than predicted by grazing dynamics at the deep margin of beds. Future research should compare the feeding behaviour of high-density aggregations of sea urchins within kelp beds and at the kelp bed-barrens interface, together with relevant biotic and abiotic variables such as kelp biomass and wave action, to elucidate the mechanisms that determine grazing thresholds. The importance of predation and disease in controlling an experimental sea urchin population in our study suggests a paradigm shift for the Nova Scotian system. With projected increases in the intensity of these top-down controls on sea urchins in the Northwest Atlantic (Steneck et al. 2002, Scheibling & Lauzon-Guay 2010), we may be observing an increase in the stability of the kelp bed state. Increased understanding of aggregation and feeding behaviour of sea urchin populations within
kelp beds, and of the roles of predation and disease in limiting these populations, will aid in predicting the dynamics of this alternative-state ecosystem.

2.6. ACKNOWLEDGEMENTS

We thank J. Lindley, M. Saunders, K. Krumhansl, A. McCurdy, and V. Burdett-Coutts for field assistance, and A. Metaxas and 3 anonymous reviewers for helpful comments on an earlier draft of the manuscript. This research was funded by a Discovery Grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada (R.E. Scheibling) and by the Centre for Aquatic Habitat Research, Department of Fisheries and Oceans Canada (J.-S. Lauzon-Guay). C.J.F. was supported by an NSERC Canada Graduate Scholarship and Dalhousie Research Scholarship.

CHAPTER 3

AN OUTBREAK OF SEA URCHIN DISEASE ASSOCIATED WITH A RECENT HURRICANE: SUPPORT FOR THE “KILLER STORM HYPOTHESIS” ON A LOCAL SCALE

3.1. ABSTRACT

The frequency of epizootics causing mass mortality of sea urchins (Strongylocentrotus droebachiensis) along the coast of Nova Scotia, Canada has increased over the past three decades. Laboratory and field studies show that outbreaks of disease are caused by the amoeba Paramoeba invadens, and are associated with hurricane activity and warm seawater temperatures. A statistical model indicates that the probability of a mass mortality event increases with the proximity of a hurricane to the coast and the maximum sustained wind speed, and is greater when post-storm seawater temperature is above a threshold for disease propagation. To assess the reliability of the model in predicting mass mortality events on an annual scale, and to examine spatial variability in mortality (in the event of a disease outbreak) on a local scale (metres to kilometres), we transplanted sea urchins into cages in kelp beds at 6 sites around St. Margarets Bay, Nova Scotia, where localized outbreaks of paramoebiasis have been observed following hurricanes in the past. On 4 September 2010 a Category 1 hurricane (Earl) made landfall 110 km south-southwest of the experimental area. Based on the parameters of the storm, the model forecasted a 43 % probability of a disease outbreak. Morbidity of caged animals was first documented on 6 September 2010, and morbidity and mortality in the cages was ~ 50 % by the end of September and ~ 85 % two months after the storm. Laboratory experiments indicated that the temperature-dependent transmission or induction of morbidity was consistent with paramoebiasis. Our findings provide support for the efficacy of the model to predict the occurrence of disease outbreaks, although the source population(s) of the pathogenic agent and oceanographic mechanisms affecting its introduction and spread along the coast of Nova Scotia remain poorly understood.
3.2. INTRODUCTION

Increases in the frequency and severity of epizootics in various marine taxa have been attributed to ocean warming and mounting anthropogenic stress on ecosystems (Harvell et al. 2002, Lafferty et al. 2004, Ross 2002), although establishing causal linkages is complicated by the interplay of multiple factors that can mediate disease outbreaks (Harvell et al. 1999). Recent mathematical models have explored complex relationships between environmental factors and host-pathogen associations such as bacterial infections of salmonids (Del-Pozo et al. 2010) and an amoebic pathogen of sea urchins (Scheibling & Lauzon-Guay 2010). Empirical data are now required to assess the validity of such models and advance our understanding of linkages between environmental change and disease outbreaks, which can have profound effects on ecosystem function and services to humans.

Recurrent mass mortalities of sea urchins *Strongylocentrotus droebachiensis* caused by outbreaks of an amoebic disease (paramoebiasis) have been documented along the Atlantic coast of Nova Scotia during the past 3 decades (Scheibling 1984a, Scheibling & Hennigar 1997, Scheibling et al. 2010). By decimating populations of the dominant herbivore, this disease can trigger a shift between alternative community states of this ecosystem, from sea urchin barrens to kelp beds (Miller 1985, Scheibling 1986). Following disease events and re-establishment of kelp beds, sea urchins can repopulate the shallow subtidal zone through migration of adults from thermal refuges in deeper water, or through the settlement of widely dispersing planktonic larvae (Balch & Scheibling 2000, Brady & Scheibling 2005). As their density increases, they form cooperative feeding aggregations that destructively graze kelp beds reinstating the barrens (Breen & Mann 1976b, Scheibling et al. 1999). Since the 1970s, transitions between these community states have occurred on approximately decadal scales in Nova Scotia. However there is new evidence that disease events may be increasing in frequency with changes in environmental conditions that influence the introduction and propagation of paramoebiasis (Scheibling & Lauzon-Guay 2010). If so, this would act to increase persistence of the kelp bed state. Because disease is the only natural mechanism known to cause the mass mortality of sea urchins, understanding the etiology and
epizoology of paramoebiasis is critical to assessing the stability of kelp beds in a changing ocean climate.

The pathogenic agent *Paramoeba invadens* (Jones 1985) is a host-specific facultative parasite, thought to be non-indigenous to Nova Scotia because its lower thermal tolerance limit (~ 2 °C) is above the minimum winter temperature in shallow subtidal Nova Scotia (0 to -1 °C) (Chapman et al. 2002, Jellett & Scheibling 1988a). Water-borne transmission of the disease in *Strongylocentrotus droebachiensis* is strongly temperature-dependent (Scheibling & Stephenson 1984). Above a threshold for disease propagation around 10 °C, the time to morbidity (based on overt signs of the disease) in healthy urchins exposed to infected conspecifics decreases with increasing temperatures (Scheibling 1988, Scheibling & Stephenson 1984). Disease outbreaks in sea urchins occur in late summer and early fall in Nova Scotia when sea temperature is warmest, and infected individuals can recover when temperatures fall below the threshold in the late fall and winter (Scheibling 1988). A correlative association between sea urchin mass mortality and tropical storms or hurricanes has been established through long-term field observations along this coast (Scheibling & Hennigar 1997, Scheibling et al. 2010). However, outbreaks of paramoebiasis have been patchy at scales of 10s to 100s of km (Miller 1985, Scheibling & Hennigar 1997, Scheibling & Stephenson 1984, Scheibling et al. 1999) and the cause(s) of this spatial variability remain unknown.

Two hypotheses have been proposed to explain the association of sea urchin disease outbreaks with hurricanes. Firstly, *Paramoeba invadens* may be transported to the coast of Nova Scotia by horizontal advection from distant source populations during tropical storms or hurricanes (Scheibling & Hennigar 1997). Alternatively, the amoeba may be free-living or reside in a cyst form in deep basins close to the coast and become vertically suspended into warmer surface water during a storm event (Scheibling & Lauzon-Guay 2010). Recent outbreaks of disease in sea urchins near Halifax, Nova Scotia have been highly localized to St. Margarets Bay (Scheibling et al. 2010), a large embayment with a deep basin where bottom temperatures rarely drop below the amoeba's physiological tolerance limit (Heath 1973), favouring the latter hypothesis.

Scheibling & Lauzon-Guay (2010) have produced a statistical model that shows that mass mortalities of sea urchins over the past three decades can be predicted by the
intensity of tropical storm activity and post-storm sea temperatures. Specifically, the probability of a mass mortality of sea urchins increases with the proximity of a tropical storm or hurricane to the coast and its maximum sustained wind speed, and is greater when the mean seawater temperature following the storm is above a threshold of 12 °C (Scheibling & Lauzon-Guay 2010). Predicted future ocean warming and increased hurricane intensity (Bender et al. 2010) is expected to result in increasingly severe outbreaks of disease (Scheibling & Lauzon-Guay 2010).

This study assesses the reliability of the Scheibling & Lauzon-Guay (2010) model, and investigates spatial variability of paramoebiasis, on the scale of metres to kilometres, in a field experiment. We transplanted groups of adult *Strongylocentrotus droebachiensis* into kelp beds throughout St. Margarets Bay, where sea urchin mass mortalities have been opportunistically recorded following hurricanes in 2 out of the previous 7 years, from an adjacent headland where diseased urchins or mass mortality was not detected at these times (Scheibling et al. 2010). We monitored sea temperature, hurricane activity, and sea urchin morbidity and mortality during the 2010 hurricane season in the North Atlantic. We predicted that a storm with a high probability of association with a disease outbreak based on the model would result in paramoebiasis in our experimental sea urchins.

### 3.3. Materials and Methods

#### 3.3.1. Predicting a Disease Outbreak

Hurricane track and wind speed data were obtained from the National Hurricane Centre (http://www.nhc.noaa.gov/), and water temperature at 8 m depth was measured in St. Margarets Bay (see Field experiment), from 7 August to 16 November 2010. These data were used to parameterize a logistic regression model (Scheibling & Lauzon-Guay 2010) to predict the probability of an outbreak of paramoebiasis in sea urchins ($P_m$), associated with each storm:

$$P_m = 1/(1 + e^z)$$
\[ z = -14.352 + 0.082W - 0.069D^2 + 4.966T \]

where \( W \) (km h\(^{-1}\)) is the maximum sustained wind speed as the hurricane passed through a study grid between 35°N and the Atlantic coast of Nova Scotia, and between 55 and 70°W; \( D \) (km) is the proximity of a hurricane to the coast; and \( T \) is a dummy variable for the temperature threshold based on the mean temperature \((T_m)\) at 8 m depth in the 2-week period following each storm \((T = 1 \text{ if } T_m > 12.2 \, ^\circ\text{C}, T = 0 \text{ if } T_m < 12.2 \, ^\circ\text{C})\).

To assess the likelihood of paramoebiasis as the cause of morbidity and mortality of sea urchins in field cages (see Field experiment), we calculated \( Pt_{50} \), the predicted time (d) to ≥ 50 % morbidity or mortality from paramoebiasis in laboratory experiments (Scheibling et al. 2010), assuming introduction of the pathogen at the time of the storm:

\[ Pt_{50} = 23492T_m^{-2.7476} \]

We compared the predicted \( Pt_{50} \) to the observed timing of morbidity and mortality of caged sea urchins following a storm.

### 3.3.2. Field Experiment

Adult *Strongylocentrotus droebachiensis* (40 – 65 mm test diameter) were manually collected by divers on 7 August 2010 from a barrens habitat at 8 m depth along the headland at Splitnose Point (Fig. 3.1). They were returned to the laboratory in chilled plastic bins and maintained in oxygenated ambient seawater in large flow-through tanks for 2 d to recover from handling before being transplanted to cages in St. Margarets Bay on 9 August. Recurrent mass mortalities had decimated the sea urchin population in shallow water (<20 m) in the bay (Scheibling et al. 2010), necessitating the transplantation of adult sea urchins from Splitnose Point, where sea urchins remained healthy and abundant. The containment of transplanted sea urchins in cages provided an added benefit of accurate estimation of rates of morbidity and mortality. Groups of 20 sea urchins were placed into each of 2 replicate cages at 8 m depth in kelp beds at 6 sites throughout the bay: Horse Island, The Lodge, Mill Cove, Croucher Island, Luke Island, and Paddy's Head (Fig. 3.1). The cages were constructed from plastic milk crates (30 × 30 × 30 cm) with a wire mesh lid (1 × 3 cm aperture) and weighted on the bottom with
iron plates. They were placed 2 – 5 m apart and lodged between boulders on the rocky seabed to secure them in the event of a storm. Twenty sea urchins also were placed in each of 2 cages at 18 m depth at The Lodge, on a sand bottom near the lower limit of the kelp bed, where bottom temperature was expected to be at or below the thermal threshold for disease propagation (~ 10 °C), and attached to lengths of heavy chain to secure them on the bottom. We hypothesized that in the event of an outbreak of paramoebiosis, sea urchins at 18 m depth would not exhibit symptoms of disease due to suppression of _P. invadens_ at low temperatures. As a procedural control for transplantation and caging effects, 20 sea urchins were placed into 2 replicate cages at 8 m depth in a boulder field at Splitnose Point. Kelp fronds (_Saccharina latissima_) from the surrounding area were placed in each cage as food for the sea urchins. A slotted section of the cage lid, secured with plastic cable ties, could be bent open to provide access for examination of the enclosed sea urchins and addition of kelp.

To monitor sea urchin morbidity and mortality in the experimental cages, divers counted healthy, and moribund or dead urchins at 1 – 2-week intervals (monthly at Splitnose Point) from 9 August to 16 November. Sea urchins were classified as moribund if they exhibited overt signs of paramoebiosis: loss of attachment to the substrate, dishevelled spines, shrivelled and non-functional tube feet, and a gaping peristome (Scheibling & Stephenson 1984). Dead animals were removed from cages, and kelp fronds replenished, at each sampling interval. Moribund sea urchins observed in cages at 8 m depth in St. Margarets Bay were collected from 6 September to 21 October and pooled over sites for use in laboratory experiments to test for paramoebiosis (see Waterborne transmission experiments). As bottom temperature at 8 m decreased to near the threshold for the disease (~ 10 °C) in late October, moribund sea urchins found after 21 October were left in these cages to determine whether they would recover. The field experiment was terminated on 16 November when temperature at 8 m depth fell below the threshold for disease propagation. At this time, all remaining sea urchins in cages at 8 m depth in St. Margarets Bay were collected to test for paramoebiosis (see Thermal induction experiments). Bottom temperature was recorded continuously during the experiment using data loggers (StowAway TidbiT Temp Logger, Onset Computer) at each site and depth.
On 21 October, when sea temperature at 8 m in St. Margarets Bay was at the thermal threshold for paramoebiasis (~10 °C) and a peak in sea urchin morbidity and mortality had been observed in the cages, 2-way contingency table analysis was used to test for differences in percent morbidity and mortality (pooled over 2 replicate cages, except at Splitnose Point and Paddy's Head where only 1 replicate remained) between sites, or between depths at The Lodge. Multiple comparison tests were conducted to test for differences between the control site at Splitnose Point and each of 7 other experimental sites (Dunnett's test analogue, q' statistic), or between the 6 sites at 8 m depth in St. Margarets Bay (Tukey's test analogue, q statistic), at α = 0.05 (Zar 1999). To test for small-scale patchiness in disease prevalence, separate contingency tables also were used to compare cages (where 2 remained) within each site at 8 m depth in St. Margarets Bay.

3.3.3. Laboratory Experiments

Sea urchins collected from cages or surrounding habitats were tested for paramoebiasis in 2 types of experiments in the laboratory: 1) thermal induction experiments, designed to induce overt symptoms of paramoebiasis that may have been suppressed at temperatures below 10 °C; and 2) water-borne transmission experiments, to determine whether the temperature-dependent rate of transmission and progression of disease from moribund urchins to healthy conspecifics was consistent with paramoebiasis (Fig. 3.2). All experiments were conducted in 47-l glass aquaria supplied with oxygenated temperature-controlled ambient flowing seawater (~3 l min⁻¹) in a quarantine laboratory within the Aquatron facility at Dalhousie University. Field-collected sea urchins were maintained in the same aquaria and flow conditions at ambient temperature prior to use in laboratory experiments.

*Thermal induction experiments.* On 19 – 21 October, when temperatures were near the threshold for disease propagation and no further morbidity in the field was anticipated, all remaining sea urchins in cages at 8 m depth at Splitnose Point (our control site where overt signs of paramoebiasis were not observed, either within cages or in the wild population) and at 18 m depth at The Lodge (where symptomatic sea urchins were
occasionally detected) were collected to test for paramoebiasis in thermal induction experiments (Table 3.1). Because there were no signs of paramoebiasis at Splitnose Point, we hypothesized that sea urchins from cages at this site would remain asymptomatic when temperature was increased in the laboratory. In contrast, because caged sea urchins at The Lodge exhibited signs of paramoebiasis at 8 m depth but not at 18 m depth, we hypothesized that *Paramoeba invadens* likely was suppressed by low temperature in deeper water and sea urchins from 18 m depth would become symptomatic when temperature was increased. All remaining sea urchins in cages at 8 m depth in St. Margarets Bay also were collected for induction when the field experiment was terminated on 16 November, as they were asymptomatic at this time (Table 3.1). We hypothesized that these sea urchins, which likely were previously exposed to *P. invadens* in the field but were asymptomatic due to low temperatures at the time of collection, would develop paramoebiasis when temperature was increased. An additional induction experiment was conducted using asymptomatic sea urchins collected in baited traps from a sedimentary basin at 60 m depth off Southwest Island near the mouth of St. Margarets Bay (Fig. 3.1, Table 3.1). These sea urchins are exposed to temperatures of ~1 – 5 °C year-round (Heath 1973), well below the thermal threshold for paramoebiasis. This experiment was conducted to determine whether these deep-living sea urchins harbour the pathogenic amoeba.

For induction experiments, 14 to 20 sea urchins from each site were placed in each of 2 aquaria. One aquarium (induction treatment) was supplied with heated (~16 °C) seawater, and the other aquarium (control) with seawater at ambient temperature for the Aquatron (9 – 13 °C). Because one of the replicate cages at Splitnose Point was lost during the experiment, sea urchins from the remaining cage were only used in the induction treatment, while sea urchins collected at the same time from the surrounding barrens were used in the control. Moribund sea urchins were recorded daily and removed from each aquarium (some were used in water-borne transmission experiments, below). Moribund sea urchins were defined as those exhibiting overt signs of paramoebiasis that had lost attachment to the aquarium walls and could not right themselves within 20 min of being inverted (Scheibling & Stephenson 1984). On 17 November, following 3 cases
of cannibalism in the induction treatment with sea urchins from Splitnose Point, kelp fronds were added to all of the aquaria as food for the sea urchins.

*Water-borne transmission experiments.* We conducted 3 water-borne disease transmission experiments using the same methodology and apparatus as Scheibling & Stephenson (1984). In all experiments, we recorded time to \( \geq 50 \% \) morbidity (\( t_{50} \), d) of sea urchins after exposure to putatively infected conspecifics, for comparison with \( t_{50} \) values from previous water-borne transmission experiments at 16 °C using sea urchins infected by *Paramoeba invadens* (\( t_{50} = 10 \) d, Scheibling & Stephenson 1984).

Expt. 1 tested for paramoebiasis in moribund sea urchins collected on 6 September from cages at 8 m depth in St. Margarets Bay and pooled over the 6 sites (Table 3.1). Because these sea urchins exhibited symptoms of paramoebiasis in the field, we hypothesized that they would transmit the disease to healthy individuals in the laboratory. Groups of 20 healthy (asymptomatic) sea urchins (from the same source used in the field experiment and maintained in the laboratory since collection) were placed in 4 aquaria supplied with ambient seawater on 8 September. These urchins were visually inspected to ensure that they had no lesions or other abnormalities, and a subset were tested to ensure that they could right themselves within 5 min when inverted. Three moribund urchins were placed in each of 2 glass “source” tubes that were individually spliced into the incurrent water supply of 2 randomly selected aquaria as replicates of the putative disease-exposed treatment. Three healthy urchins were placed in 2 other tubes that supplied the 2 remaining aquaria as replicates of the control treatment. The aquaria were maintained at ambient temperature (< 10 °C) for 7 d and then heated above the threshold for paramoebiasis, to ~ 16 °C on 15 September. One control aquarium from Expt. 1 containing 20 healthy urchins was maintained at 16 °C in the laboratory until 13 December 2010, as a long-range control for a pathogen in the incoming ambient seawater.

Expt. 2 tested for paramoebiasis in sea urchins that developed signs of morbidity in thermal induction experiments using caged sea urchins from 18 m depth at The Lodge or 8 m depth at Splitnose Point (Table 3.1). The design was similar to Expt. 1. Because moribund sea urchins from 18 m at The Lodge exhibited high rates of morbidity with symptoms characteristic of paramoebiasis in the thermal induction experiment, we
they would transmit disease to healthy (asymptomatic) conspecifics. Asymptomatic sea urchins were collected from 8 m depth at Bear Cove near the mouth of Halifax Harbour, ~ 7 km N of Splitnose Point (Fig. 3.1), on 22 November. They were visually inspected and tested for the ability to right themselves, and placed in 3 aquaria (n = 18 per aquarium) supplied with heated (16 °C) seawater on 26 November. Two to three moribund sea urchins (depending on the number available) from The Lodge (18 m depth) or Splitnose Point (8 m depth) were placed in the source tube of 2 aquaria, as putative disease-exposed treatments for the respective source sites. Three asymptomatic sea urchins from Bear Cove were placed in the source tube of the remaining aquarium, as a control.

Expt. 3 was conducted to determine whether sea urchins at 60 m depth in St. Margarets Bay, which were asymptomatic following a thermal induction experiment, are susceptible to paramoebiasis (Table 3.1). This experiment was conducted to ensure that the asymptomatic sea urchins are not resistant carriers of *Paramoeba invadens*. These sea urchins were placed into 2 aquaria supplied with heated (16 °C) seawater on 30 November. Three moribund sea urchins from the thermal induction experiment with specimens from cages at 8 m depth in St. Margarets Bay were placed in the source tube of 1 aquarium containing 17 sea urchins as a putative disease-exposed treatment. Three asymptomatic sea urchins from the 60 m site were placed in the source tube of a second aquarium containing 12 sea urchins as a control.

Replication of aquaria within treatments was not possible in Expt. 2 and 3 because of space limitations within the quarantine laboratory. However, the low variation we observed between replicate aquaria in the disease-exposed treatment in Expt. 1, and the absence of morbidity in the control, were consistent with the results all previous water-borne transmission experiments (Jellett & Scheibling 1988b, Scheibling & Stephenson 1984, Scheibling et al. 2010).
Table 3.1. Thermal induction experiments (TIE) and water-borne transmission experiments (WTE) conducted to identify the presence or absence of paramoebiasis in *Strongylocentrotus droebachiensis*. ‘Dates’ is the time-period for each experiment. ‘T’ is the average temperature within each experimental treatment and control. Sea urchins were collected for TIE from cages or barrens at 8 m depth at Splitnose Point (SP), cages at 18 m depth at The Lodge (TL) in St. Margaret’s Bay (SMB), sediments at 60 m depth in SMB, or cages at 8 m depth at 6 sites (pooled) in SMB. In WTE ‘Source’ is the origin of putatively disease-infected sea urchins, and ‘Target’ is the origin of asymptomatic sea urchins. Source sea urchins in disease-exposed treatments for WTE were collected from cages at 8 m depth at 6 sites in SMB, cages at 8 m depth at SP, or cages at 18 m depth at TL. Target sea urchins for WTE were collected from barrens at 8 m depth at SP and Bear Cove (BC), or sediments at 60 m depth in SMB. Source sea urchins in the control treatments in WTE were from the same supply as the target urchins. Dates of collection of source and target sea urchins are shown in brackets.

<table>
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<tr>
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<td>2 Nov–13 Dec</td>
<td>Induction</td>
<td>SP 8 m cage (19 Oct)</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>SP 8 m cage (19 Oct)</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>2 Nov–13 Dec</td>
<td>Induction</td>
<td>TL 18 m cages (21 Oct)</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>TL 18 m cages (21 Oct)</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>2 Nov–27 Nov</td>
<td>Induction</td>
<td>SMB 60 m sediment (2 Nov)</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>SMB 60 m sediment (2 Nov)</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>16 Nov–13 Dec</td>
<td>Induction</td>
<td>SMB 8 m cages (16 Nov)</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
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<table>
<thead>
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<th>Source</th>
<th>Target</th>
<th>T (°C)</th>
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<td>SP 8 m barrens (7 Aug)</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>SP 8 m barrens (7 Aug)</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>25 Nov–14 Dec</td>
<td>Exposed</td>
<td>SP 8 m cage (19 Oct)</td>
<td>BC 8 m barrens (22 Nov)</td>
<td>16</td>
</tr>
<tr>
<td></td>
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<td>Exposed</td>
<td>TL 18 m cages (21 Oct)</td>
<td>BC 8 m barrens (22 Nov)</td>
<td>16</td>
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<tr>
<td></td>
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<td>Control</td>
<td>BC 8 m barrens (22 Nov)</td>
<td>BC 8 m barrens (22 Nov)</td>
<td>16</td>
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<tr>
<td>3</td>
<td>30 Nov–9 Dec</td>
<td>Exposed</td>
<td>SMB 8 m cages (16 Nov)</td>
<td>SMB 60 m sediment (2 Nov)</td>
<td>16</td>
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<tr>
<td></td>
<td></td>
<td>Control</td>
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<td>SMB 60 m sediment (2 Nov)</td>
<td>16</td>
</tr>
</tbody>
</table>
Fig. 3.1. Map of experimental sites and sea urchin collection sites along the coast of Nova Scotia, Canada. The inset shows 7 sites within St. Margarets Bay; Horse Island (HI), The Lodge (TL), Mill Cove (MC), Croucher Island (CI), Luke Island (LI), Paddy’s Head (PH), and Southwest Island (SI); and two additional sites along headlands near the mouth of Halifax Harbour, Splitnose Point (SP) and Bear Cove (BC).
Fig. 3.2. Schematic diagram of laboratory experiments conducted on sea urchins collected from field cages or surrounding habitats. If sea urchins appeared moribund upon collection (Y), a waterborne transmission experiment was conducted using these sea urchins as an infection source to confirm paramoebiasis. If sea urchins appeared asymptomatic upon collection (N), a thermal induction experiment was conducted to induce morbidity. If sea urchins appeared moribund following a thermal induction experiment, a waterborne transmission experiment was conducted using these sea urchins as an infection source to confirm paramoebiasis.
3.4. RESULTS

3.4.1. Predicting a Disease Outbreak

During the experimental period from 7 August to 16 November, 4 hurricanes passed through our study area (Table 3.2). Hurricane Earl tracked closest to Nova Scotia and had the greatest probability of association with a sea urchin mass mortality, as predicted by the Scheibling & Lauzon-Guay (2010) model \(P_m = 0.43\), Table 3.2). Hurricane Earl was preceded by a 2-week period of unusually high seawater temperatures, ranging from 18 to 21 °C at 8 m in St. Margarets Bay (averaged over 4 sites) and at Splitnose Point, and from 11 to 20 °C at 18 m at The Lodge (Fig. 3.3a). The hurricane was characterized by a small peak in temperature followed by a sharp decrease, evident at 8 and 18 m depth (Fig. 3.3a). Mean water temperature during the 2-week period following the hurricane was 14.0 and 12.4 °C at 8 m depth in St. Margarets Bay and Splitnose Point respectively, and \(Pt_{50}\) was estimated at 16.7 and 23.3 d (i.e. approximately half the experimental individuals were expected to show signs of paramoebiasis by 21 and 28 September at the respective locations, if infected). Mean water temperature during the same 2-week period was 8.7 °C at 18 m depth at The Lodge, below the thermal threshold for symptomatic paramoebiasis.

3.4.2. Field Experiment

Moribund sea urchins, exhibiting symptoms consistent with paramoebiasis, were first observed on 6 September in cages at 8 m depth at all 6 sites in St. Margarets Bay (Fig. 3.3b). The percentage of sea urchins that were moribund or dead (mean ± SD, pooled over the remaining 11 cages at 8 m in St. Margarets Bay; 1 cage at Paddy's Head was lost during Hurricane Earl) increased to 41 (± 8) % by 24 September and 53 (± 9) % by 30 September, and levelled off at 85 (± 9) % by 21 October, when sea temperature was ~ 10 °C, with no further increase to the end of the experiment on 16 November (Fig. 3.3a,b). In late October, we observed recovery of moribund sea urchins in 8 m cages at Mill Cove and Croucher Island (Fig. 3.3b). Morbidity and mortality in cages at 18 m at
The Lodge was 30 (± 5) % on 21 October (Fig. 3.3b). One cage was lost at 8 m depth at Splitnose Point between 8 September and 19 October. Mortality was 25 % in the remaining cage on 19 October (Fig. 3.3b). Symptoms of paramoebiasis were not observed in cages at Splitnose Point or in the surrounding barrens throughout the experiment and at Bear Cove on 22 November.

At peak levels of sea urchin morbidity and mortality on 21 October, there was a significant effect of site on percent morbidity and mortality in the cages ($\chi^2 = 91.9$, df = 7, $p < 0.001$; Fig. 3.3b). Morbidity and mortality at 8 m depth was significantly greater at each of 6 sites in St. Margarets Bay than in the control cage at Splitnose Point ($q' > 3.10$, $p < 0.01$), but there was no difference between cages at 18 m depth at The Lodge and the control cage ($q' = 0.35$, $p > 0.05$). The loss of a replicate control cage at Splitnose Point suggests these results should be interpreted with caution. However, the similarity in mortality rate between the remaining cage (where paramoebiasis was not evident in the surrounding population) and the 2 replicate cages at 18 m depth at The Lodge (where the disease was suppressed by low temperature) validates our estimate of mortality due to cage effects at the control site. On 21 October, the frequency of moribund or dead sea urchins in cages was significantly greater at 8 than 18 m depth at The Lodge ($\chi^2 = 43.1$, df = 1, $p < 0.001$). Morbidity and mortality among the 6 sites at 8 m depth in St. Margarets Bay differed on 21 October ($\chi^2 = 26.0$, df = 5, $p < 0.001$), and 2 distinct groups emerged from paired comparisons among sites: 1) The Lodge and Luke Island with complete morbidity or mortality, and 2) Horse Island, Mill Cover, Croucher Island, and Paddy's Head, which did not differ significantly ($q < 1.40$, $p > 0.50$), but were each significantly different from The Lodge and Luke Island ($q > 4.10$, $p < 0.05$). There was a significant difference in the frequency of morbidity and mortality between the 2 replicate cages at Mill Cove ($\chi^2 = 9.2$, df = 1, $p = 0.002$) and at Horse Island ($\chi^2 = 11.6$, df = 1, $p = 0.001$), but no difference between cages at Croucher Island ($\chi^2 = 2.5$, df = 1, $p = 0.114$).
3.4.3. Thermal Induction Experiments

All asymptomatic sea urchins collected on 21 October from cages at 18 m at The Lodge showed signs of morbidity after 24 d at 16 °C, whereas at ambient temperature (9 – 13 °C, Aquatron seawater), 64 % exhibited morbidity after 41 d (Fig. 3.4a,b). Forty-eight percent of asymptomatic sea urchins collected from cages at 8 m depth at Splitnose Point showed signs of morbidity after 19 d at 16 °C, but no additional sea urchins became symptomatic over the next 22 d, and no morbidity was observed at ambient temperature (Fig. 3.4a,c). Three of the sea urchins in the induction treatment (16 °C) from Splitnose Point were lost to cannibalism, resulting in a total of 71 % morbidity and mortality after 41 d. Asymptomatic sea urchins remaining in cages at 8 m depth in St. Margarets Bay at the end of the field experiment on 16 November showed 90 % morbidity after 27 d at 16 °C, whereas no morbidity was observed at ambient temperature (Fig. 3.4a,d). Asymptomatic sea urchins collected from 60 m depth in St. Margarets Bay on 2 November showed 5 % morbidity at 16 °C and no morbidity at ambient temperature after 25 d.

3.4.4. Water-borne Transmission Experiments

In all water-borne transmission experiments, no sea urchins died or showed signs of infection in control treatments (Table 3.3). In Expt. 1, all healthy sea urchins exposed to moribund conspecifics from cages at 8 m depth in St. Margarets Bay were moribund by Day 11 and $t_{50}$ was reached after 9 d (Table 3.3, Fig. 3.5). In Expt. 2, $t_{50}$ of sea urchins exposed to moribund conspecifics from cages at 18 m depth at The Lodge was 10 d (Table 3.3). Morbidity was not observed in sea urchins exposed to moribund conspecifics at 16 °C from the cage at 8 m depth at Splitnose Point after 19 d when the experiment was terminated (Table 3.3). In Expt. 3, $t_{50}$ of sea urchins from 60 m depth in St. Margarets Bay exposed to moribund conspecifics from the 8 m cages in St. Margarets Bay was 8 d (Table 3.3).

Sea urchins in a control aquarium maintained at 16 °C for 96 d (from 8 September to 13 December) did not exhibit signs of morbidity (Fig. 3.4b,c,d). However, an
equipment malfunction resulting in a temperature spike from 9 to 20 °C within 24 h on 27 October caused 60 % mortality of the sea urchins (loss of 12 out of 20 urchins) on 29 October. These sea urchins exhibited symptoms of heat shock, with an inability to right when inverted followed rapidly by spine loss and death (Percy 1973), and not the overt signs of paramoebiasis, such as a gaping peristome and dishevelled spines (Scheibling & Stephenson 1984).

Table 3.2. Tropical storms and hurricanes occurring between 35°N and the Atlantic coast of Nova Scotia, and between 55 and 70°W from 7 August to 16 November 2010. Date is when the storm was closest to the coast of Nova Scotia. Wind is the maximum sustained wind speed of a storm at a minimum distance from the coast (Dist); T is the mean temperature at 8 m depth in St. Margarets Bay in the 2-week period following the storm. \( Pt_{50} \) is the predicted time to \( \geq 50 \% \) morbidity of healthy \( Strongylocentrotus droebachiensis \) from paramoebiasis following a storm at a given T, using the formula \( Pt_{50} = 23492T^{-2.7476} \) (Scheibling et al. 2010). \( P_m \) is the probability of association of a storm with a disease outbreak in \( S. droebachiensis \) predicted by the Scheibling & Lauzon-Guay (2010) model. Period of \( \geq 50 \% \) morbidity is the time over which we observed \( \geq 50 \% \) morbidity and mortality of sea urchins in 8 m cages in St. Margarets Bay. The storm (Hurricane Earl) most likely associated with a disease outbreak in sea urchins based on \( Pt_{50} \) and the observed period of \( \geq 50 \% \) morbidity is shown in bold.

<table>
<thead>
<tr>
<th>Storm</th>
<th>Date</th>
<th>Lat (°N)</th>
<th>Long (°W)</th>
<th>Wind (km h(^{-1}))</th>
<th>Dist (km)</th>
<th>T (°C)</th>
<th>( Pt_{50} ) (d)</th>
<th>( P_m )</th>
<th>Period of ( \geq 50 % ) Morbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Danielle</td>
<td>30 Aug</td>
<td>40.0</td>
<td>52.8</td>
<td>138.9</td>
<td>1019</td>
<td>16.4</td>
<td>10.8</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Earl</td>
<td>4 Sept</td>
<td>43.0</td>
<td>65.7</td>
<td>111.0</td>
<td>29</td>
<td>14.0</td>
<td>16.7</td>
<td>0.43</td>
<td>24 Sept–21 Oct</td>
</tr>
<tr>
<td>Igor</td>
<td>21 Sept</td>
<td>41.3</td>
<td>56.8</td>
<td>120.4</td>
<td>532</td>
<td>14.4</td>
<td>15.4</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Shary</td>
<td>30 Oct</td>
<td>37.9</td>
<td>52.9</td>
<td>120.4</td>
<td>1169</td>
<td>10.0</td>
<td>42.0</td>
<td>&lt;0.001</td>
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</tbody>
</table>
Table 3.3. Water-borne transmission experiments. Time to morbidity (d) and % survival of asymptomatic *Strongylocentrotus droebachiensis* when exposed to putatively infected conspecifics collected from cages at 8 m depth at 6 sites (pooled) in St. Margarets Bay (SMB), at 18 m at The Lodge in St. Margarets Bay (TL) and at 8 m at Splitnose Point (SP) in water-borne transmission experiments 1, 2 and 3 conducted from 15 September to 14 December 2010. Predicted values are from water-borne transmission experiments conducted by Scheibling & Stephenson (1984).

<table>
<thead>
<tr>
<th>Expt #</th>
<th>Putative Disease Source</th>
<th>T (°C)</th>
<th>Treatment</th>
<th>Time to morbidity (d)</th>
<th>Survival (%)</th>
<th>Duration (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥ 5 %</td>
<td>≥ 50 %</td>
<td>≥ 95 %</td>
</tr>
<tr>
<td>1</td>
<td>SMB 8 m cage</td>
<td>15</td>
<td>Exposed</td>
<td>1 - 9</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>TL 18 m cage</td>
<td>16</td>
<td>Exposed</td>
<td>8</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>SP 8 m cage</td>
<td>16</td>
<td>Exposed</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>SMB 8 m cage</td>
<td>16</td>
<td>Exposed</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Predicted values</td>
<td>Exposed</td>
<td>16</td>
<td>8.5</td>
<td>10</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>16</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Fig. 3.3. (a) Daily averages of seawater temperature (°C) before and after Hurricane Earl (4 September, indicated by vertical line) at 3 groups of experimental sites with caged sea urchins: 8 m depth in St. Margarets Bay (SMB 8 m; averaged over 4 sites); 8 m depth at Splitnose Point (SP 8 m); and 18 m depth at The Lodge in St. Margarets Bay (TL 18 m). A 10 °C thermal threshold for paramoebiasis is indicated by a horizontal dashed line. (b) Mean cumulative (+ 1 SE) percent morbidity and mortality of sea urchins in cages before and after Hurricane Earl at 6 sites at 8 m depth in St. Margarets Bay (Horse Island, HI; Mill Cove, MC; Croucher Island, CI; Luke Island, LI; Paddy's Head, PH; and The Lodge, TL), The Lodge at 18 m depth, and Splitnose Point at 8 m depth.
Fig. 3.4. Thermal induction experiments. (a) Seawater temperature (°C) in the induction and control treatment. A 10 °C thermal threshold for paramoebiasis is indicated by a horizontal dashed line. Cumulative morbidity of sea urchins in the induction and control treatment using sea urchins from: (b) 18 m cages at The Lodge (TL 18 m), (c) an 8 m cage at Splitnose Point (SP 8 m), and (d) 8 m cages in St. Margarets Bay (SMB 8 m), as well as for a long-range control aquarium with healthy sea urchins at the temperature of the induction treatment.
Fig. 3.5. Water-borne transmission Expt. 1. (a) Seawater temperature in laboratory aquaria. A 10 °C thermal threshold for paramoebiasis is indicated by a horizontal dashed line. (b) Cumulative morbidity of sea urchins over an 11-d period, following an increase in temperature to above the thermal threshold for paramoebiasis on 15 September, in 2 treatments: exposed to putatively moribund conspecifics collected from 8 m cages in St. Margarets Bay, and an unexposed control. $t_{50}$ is the time to $\geq 50\%$ morbidity of healthy sea urchins exposed to moribund conspecifics above the thermal threshold for paramoebiasis.
3.5. DISCUSSION

3.5.1. Hurricane-induced Disease Outbreak

Following the passage of Hurricane Earl on 4 September 2010, a disease outbreak consistent with paramoebiasis occurred in *Strongylocentrotus droebachiensis* in cages at 8 m in St. Margarets Bay. Our observation of a high frequency of combined morbidity and mortality (max. 70 % at Luke Island) on 24 September, 20 d after the passage of the hurricane conforms to a predicted time of 16.7 d to ≥ 50 % morbidity (*Pt_{50}* of sea urchins based on previous laboratory experiments, with the caveat that some mortality could have been due to caging effects and not disease per se (up to 25 % based on the control cage at Splitnose Point).

Our results support the Scheibling & Lauzon-Guay (2010) model, which predicts that Hurricane Earl had a 43 % probability of association with a sea urchin epizootic (*P_m*). This value for Earl is not significantly different from the mean *P_m* of previous candidate storms (57 ± 28 % SD), based on this model (t = 1.75, df = 11, p = 0.11). Hurricane Igor (*P_m* = 0.19), which came within 532 km of Nova Scotia on 21 September 2010, may have contributed to the morbidity and mortality observed in early October. If so, the model provides a conservative estimate of the probability of a sea urchin mass mortality (Scheibling & Lauzon-Guay 2010). Other hurricanes during the experimental period had a very low probability of association with an epizootic (*P_m* < 0.001).

Unusually high water temperatures immediately preceding Hurricane Earl suggest *Paramoeba invadens* was not present in caged sea urchins in St. Margarets Bay prior to the hurricane. From 21 August to 4 September mean temperature at 8 m depth in St. Margarets Bay was ~ 19 °C. *Pt_{50}* for paramoebiasis at 19 °C is 7 d. Thus, if *P. invadens* were present at our experimental sites at the time of the warming event we would have seen ~ 50 % morbidity of sea urchins by 28 August. However, we did not observe symptoms of paramoebiasis in sea urchins in St. Margarets Bay until 6 September, more than 2 weeks after the start of the warming event, leading us to conclude that the pathogenic agent was either not present or not in contact with sea urchins in the shallow subtidal zone of the bay prior to Hurricane Earl.
3.5.2. *Paramoeba invadens* as the Infective Agent

Our water-borne transmission experiments indicated that the disease affecting sea urchins in St. Margarets Bay was most likely paramoebiasis. The average time to $\geq 50\%$ morbidity ($t_{50}$) at 16 °C was 9 d in Expt. 1 and 10 d in Expt. 2 (with sea urchins from The Lodge), which are directly comparable to results of similar laboratory experiments conducted following mass mortalities of sea urchins in the early 1980s ($t_{50} = 10$ d with exposure to 3 infected conspecifics at 16 °C, Scheibling & Stephenson 1984) when *Paramoeba invadens* was identified as the disease agent (Jones & Scheibling 1985). The absence of symptoms in sea urchins in all control treatments at 16 °C indicates that morbidity was due to disease and not thermal stress. The agreement of our $t_{50}$ results with those of previous water-borne transmission experiments (Jellett & Scheibling 1988b, Scheibling & Stephenson 1984, Scheibling et al. 2010), in combination with observations that moribund sea urchins exhibited the characteristic symptoms of paramoebiasis (Scheibling & Stephenson 1984), provides strong evidence that *P. invadens* was the causative agent of the disease outbreak in caged sea urchins in St. Margarets Bay.

Previous studies have found that sea urchins exhibit symptoms of paramoebiasis 2 to 3 weeks after the passage of a hurricane (Scheibling et al. 2010). We observed the first moribund and dead sea urchins in cages in St. Margarets Bay on 6 September, just 2 d following Hurricane Earl. However, these early indications of morbidity were likely the result of stress in the cages caused by warm temperatures prior to Hurricane Earl. Sea temperature at 8 m depth reached a maximum of ~ 20.4 °C on 31 August, which is near the maximum thermal tolerance of *Strongylocentrotus droebachiensis* of ~ 22 °C (Percy 1973). Hypoxia may have exacerbated thermal stress at this time, as kelp fronds supplied as food and drift algae that accumulated in the cages putrefied and turned the sediments anoxic.

As expected, few moribund sea urchins were found in cages at 18 m depth where the mean temperature (8.7 °C) in the 2-week period following Hurricane Earl was below the thermal threshold for paramoebiasis. Lack of morbidity in sea urchins at deeper, cooler depths has been recorded during previous disease outbreaks in Nova Scotia – e.g. below 24 m at an exposed headland at the mouth of Halifax Harbour in 1999 (Brady &
Scheibling 2005) and at 18 m depth at The Lodge in 2009 (Scheibling et al. 2010). The presence of a thermal refuge supports the notion that deep-living sea urchins in adjacent habitats act as sources of migrants or larvae repopulating shallow kelp beds following a mass mortality event (Brady & Scheibling 2005).

The temperature-dependant propagation of paramoebiasis is also reflected by the recovery of symptomatic urchins at 8 m depth in late October, when temperatures were near or below a 10 °C threshold for the disease. Scheibling & Stephenson (1984) found that symptomatic sea urchins collected from a barrens recovered from paramoebiasis when placed in 8 °C seawater in the laboratory. However, morbidity recurred when these sea urchins were heated to 18 °C 5 months later (Scheibling 1984a). Given that disease outbreaks in Nova Scotia do not occur around the annual peak in sea temperature in every year, sea urchins do not remain chronically infected over winter. Jellett & Scheibling (1988a) found that *Paramoeba invadens* in monoxenic culture showed negative growth at 2 °C, which is above the winter minimum (0 to -1 °C) along the Atlantic coast of Nova Scotia. This suggests that amoebae either die within their hosts or are eliminated by the sea urchin’s immune system during the coldest part of the year (February/March), when temperatures can remain below 2 °C for several weeks (Scheibling & Hennigar 1997).

Contrary to expectation, a high rate of morbidity occurred in the thermal induction experiment with sea urchins from the cage at 8 m at Splitnose Point, where paramoebiasis was not present. A high incidence of cannibalism (23 %) in the thermal induction treatment suggests that starvation was a confounding factor. Previous experiments have shown that cannibalism in *Strongylocentrotus droebachiensis* occurs in response to food limitation (Himmelman & Steele 1971). Sea urchins in a cage at Splitnose Point were likely food-limited when collected for the laboratory experiments because this cage was monitored and supplied with kelp less frequently than cages in St. Margarets Bay. Sea urchins can survive long periods in food-limited habitats (Lang & Mann 1976); however as poikilotherms their metabolic requirements are directly dependent on temperature (Percy 1973). Starvation may have been incurred when sea urchins were heated from ambient temperature (~ 11 °C) to 16 °C in the induction treatment. To substantiate these results, we conducted an *ad hoc* thermal induction experiment using sea urchins collected from 8 m depth in the barrens at Splitnose Point

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on 19 October 2010. These sea urchins did not exhibit morbidity and there was no mortality after 23 d at 16 °C, confirming the absence of paramoebiasis at Splitnose Point.

3.5.3. Spatial Distribution of Paramoebiasis

There was high variability in sea urchin morbidity or mortality both within and among sites at 8 m depth in St. Margarets Bay, indicating that the disease outbreak was patchy on the scale of metres to kilometres. We observed lower levels of morbidity and mortality at Mill Cove, Croucher Island, Horse Island, and Paddy's Head, than at Luke Island and The Lodge. Similarly, Johnson & Mann (1993) found that disease decimated sea urchins at a site near Luke Island (Paul Point), while sea urchins at Mill Cove were unaffected during the first recorded epizootic in Nova Scotia in fall 1980. High variability in morbidity and mortality among cages within sites in St. Margarets Bay suggests the pathogen may have been patchily distributed in the water column on the scale of metres. Water circulation and localized mixing could be important determinants of the distribution and abundance of *Paramoeba invadens* in the water column in St. Margarets Bay. This, in turn, would influence spatial patterns in occurrence and severity of disease outbreaks in sea urchins, as water-borne transmission of paramoebiasis is strongly dosage-dependant in *Strongylocentrotus droebachiensis* (Scheibling & Stephenson 1984).

The absence of disease outbreaks at Splitnose Point and Bear Cove, along the headlands adjacent to St. Margarets Bay, suggests that this disease outbreak was a localized event. The sparse distribution of naturally occurring sea urchin populations in shallow water across this region (St. Margarets Bay to Splitnose Point) in 2010 and 2011 (K. Filbee-Dexter & R.E. Scheibling, unpubl. data) may explain why the disease outbreak did not propagate beyond St. Margarets Bay. Disease outbreaks often are positively correlated with host abundance (Anderson & May 1986, Lafferty 2004) and patchiness in sea urchin morbidity has been associated with sea urchin density in previous outbreaks of paramoebiasis in Nova Scotia (Scheibling & Stephenson 1984).

Sea urchins from 60 m depth off Southwest Island at the mouth of St. Margarets Bay did not exhibit symptoms of paramoebiasis in a thermal induction experiment, indicating they were not affected by disease. When exposed to moribund conspecifics in
a water-borne transmission experiment however, these individuals succumbed to paramoebiasis. We conclude that the deep-living sea urchins in and around St. Margarets Bay are not a likely source of *Paramoeba invadens* nor are they resistant to the disease. Jellett et al. (1989) were unsuccessful in finding free-living *P. invadens* in sediment and water samples in Halifax Harbour. However, given the limited area sampled in both studies, we cannot preclude the possibility that the amoeba may reside in deep sediments or in sea urchin populations elsewhere along the Scotian shelf.

Based on our results, we can conclude that *Paramoeba invadens* was either locally introduced (or resident) in St. Margarets Bay, or broadly introduced along the coast but only caused paramoebiasis within the bay. The latter hypothesis would suggest that St. Margarets Bay is a site of incubation for *P. invadens*, perhaps due to its warmer temperatures and long seawater residence time (10 – 30 d, Heath 1973), as compared to the exposed shoreline. Given that the seawater circulation in St. Margarets Bay is anti-clockwise (Heath 1973), future studies should investigate the occurrence of disease at headlands immediately on either side of the mouth of bay during an outbreak of paramoebiasis, to determine if the embayment is a sink or a source for virulent amoebae.

3.5.4. Ecological Implications of Recurrent Disease Outbreaks and Directions for Future Research

Recurrent outbreaks of paramoebiasis in sea urchins (*Strongylocentrotus droebachiensis*) in Nova Scotia in the early 1980s led to the re-establishment of kelp beds in St. Margarets Bay, following a decade of persistent sea urchin barrens (Scheibling 1984a). Sea urchins had formed grazing fronts at Mill Cove and adjacent areas in St. Margarets Bay by 1992 (Scheibling et al. 1999), but disease outbreaks in the mid to late 1990s once again decimated these populations (Brady & Scheibling 2005, Miller & Nolan 2000, Scheibling & Hennigar 1997). Since then, kelp beds have persisted and adult sea urchins remain sparsely distributed in the shallow subtidal zone, likely due to recurrent, localized outbreaks of disease (Miller & Nolan 2000, Scheibling et al. 2010, this study).

The observed morbidity and mortality of *Strongylocentrotus droebachiensis* due to paramoebiasis, following a hurricane with a high probability of association with a mass
mortality, supports the model of Scheibling & Lauzon-Guay (2010). Based on observed (Emanuel 2005) and predicted (Bender et al. 2010) increases in hurricane intensity, their model predicts an increased frequency of severe outbreaks of sea urchin disease that will favour the persistence of kelp beds along the Atlantic coast of Nova Scotia. Our results show that disease outbreaks can be patchy along this coast, making it difficult to predict the extent of disease-mediated mortality events. The Scheibling & Lauzon-Guay (2010) model is unable to predict the spatial variability of paramoebiasis observed in this study, indicating that additional factors, such as sea urchin density and ocean currents are required to predict the extent of disease outbreaks along the coast of Nova Scotia.

Further research is needed to monitor disease outbreaks across a range of habitats and spatial scales to identify other potential disease hotspots and increase our understanding of the introduction and spread of paramoebiasis. For example, more extensive sampling for *Paramoeba invadens* in deep-living sea urchins, could establish whether local sources of amoeba are present along the coast of Nova Scotia. Genetic barcoding of *P. invadens*, isolated from naturally infected sea urchins could be used to effectively monitor the amoeba in water and sediment samples before, during and after a disease outbreak to gain insights into host-pathogen dynamics, factors affecting the spread of disease (e.g. sea urchin density, temperature, salinity, currents), and the fate of amoebae as temperatures drop. It also will allow us to search broadly for source populations and explore dispersal mechanisms, such as advection and turbulent mixing by hurricanes, which potentially introduce *P. invadens* to the coast of Nova Scotia. Finally, a multiyear record of hurricane activity and occurrence of paramoebiasis in Nova Scotia, based on controlled field experiments such as the one we present here is needed to critically test the Scheibling & Lauzon-Guay (2010) model.

The geographical range of *Strongylocentrotus droebachiensis* in the Northwest Atlantic extends from the Canadian Arctic Archipelago to Cape Cod, USA (Scheibling & Hatcher 2007), however outbreaks of paramoebiasis in sea urchins appear to be highly limited to Nova Scotia. To our knowledge there has been only a single published account of paramoebiasis in Gulf of Maine (Caraguel et al. 2007), and no recorded outbreaks in Gulf of St. Lawrence, where sea urchin populations have been well studied over the past 3 decades (Gagnon et al. 2004, Steneck et al. 2004). Caraguel et al. (2007) isolated
Neoparamoeba pemaquidensis from moribund sea urchins in the Gulf of Maine in fall 2002. Amoebae of the genera Paramoeba and Neoparamoeba have caused crab and lobster mortalities in the northeastern USA (Mullen et al. 2005, Newman & Ward 1973). Interestingly, an outbreak of paramoebiasis in lobsters in Long Island Sound in fall 1999 (Mullen et al. 2004) caused by N. pemaquidensis (Mullen et al. 2005) coincided with reports of crab and sea urchin mortalities in that area (Mullen et al. 2004) and an outbreak of paramoebiasis in sea urchins in Nova Scotia (Brady & Scheibling 2005, Miller & Nolan 2000) following a strong hurricane (Scheibling & Lauzon-Guay 2010). The concurrence of these disease outbreaks suggests a common source for pathogenic amoeba along the Northeast coast of North America (Scheibling & Lauzon-Guay 2010). Mullen et al. (2005) suggest that P. invadens could be a synonym of N. pemaquidensis, although this has not been confirmed by genetic analysis. Local oceanographic processes may limit introduction of the pathogen to the Gulf of Maine, while cool temperatures could prevent propagation of the disease in Newfoundland and the northern Gulf of St. Lawrence. Based on observed (Levitus et al. 2000) and predicted (IPCC 2007) ocean warming with global climate change, we may observe a northern expansion of the range of P. invadens. In the Northwest Atlantic, the greatest increases in surface temperature have occurred in winter (Hayhoe et al. 2007), which could lead to amoebae overwintering in new regions and habitats. To substantiate these predictions, further information regarding the source of infective populations of P. invadens is required. Establishing a mechanistic link between hurricanes and introduction and spread of paramoebiasis in Nova Scotia could provide insight to a more general understanding of the etiology of this disease, which can so profoundly affect the ecology of subtidal communities.
3.6. ACKNOWLEDGEMENTS

We thank J. Lindley, K. Krumhansl, K. Filbee-Dexter, F. Francis and J. Bryk for field assistance, and J. Bryk for assistance with laboratory experiments. A. Metaxas and three anonymous reviewers provided helpful comments on an earlier draft of the manuscript. This research was funded by a Discovery Grant to R.E. Scheibling from the Natural Sciences and Engineering Research Council (NSERC) of Canada. C.J.F. was supported by an NSERC Canada Graduate Scholarship and Dalhousie Research Scholarship.

CHAPTER 4

VALIDATING THE IDENTITY OF *PARAMOEBAA INVADENS*, THE CAUSATIVE AGENT OF RECURRENT MASS MORTALITY OF SEA UCHINS IN NOVA SCOTIA, CANADA

4.1. ABSTRACT

Sea urchins (*Strongylocentrotus droebachiensis*) along the coast of Nova Scotia, Canada suffer mass mortalities due to infection by a pathogenic amoeba, *Paramoeba invadens* JONES 1985. Due to a lack of genetic information, it has been speculated that *P. invadens* could actually be a form of *Neoparamoeba pemaquidensis*, a species associated with disease in *S. droebachiensis* and lobsters *Homarus americanus* in the northeastern USA. During a disease outbreak in fall 2011, we isolated amoebae from moribund sea urchins collected from 4 locations along ~ 200 km of the Nova Scotian coastline. We experimentally infected healthy sea urchins by injection with cultured amoebae and water-borne exposure to diseased conspecifics, and found that the timing and rate of morbidity corresponded to that of similar laboratory experiments conducted in the early 1980s. These results provide strong evidence that the pathogen infecting sea urchins in 2011 is indeed *P. invadens*. A concurrent study, analyzing nuclear and parasome SSU rDNA (Johnson-Mackinnon 2012), showed that our isolates of *P. invadens* represent a distinct species that is most closely related to *N. branchiphila*, a suspected pathogen of sea urchins *Diadema aff. antillarum* in the Canary Islands, Spain.
4.2. INTRODUCTION

In marine ecosystems, the identity and source of the causative agent(s) of epizootics are often unresolved, due in part to difficulties in detecting potential pathogens in host tissues or the surrounding environment. For example, the pathogenic agent that decimated the sea urchin *Diadema antillarum* throughout its geographic range in the Caribbean in 1983, causing a phase shift from corals to fleshy macroalgae on many reefs, remains unidentified (Lessios 1988a). Similarly, causal agents have only been identified for 6 of 19 common coral diseases, which also have had important ecosystem-level effects (Harvell et al. 2007). Even when agents are identified, difficulties with reliably identifying pathogens to the ‘species’ level can result in incorrect assumptions about the host-specificity or generality of the agent (Young et al. 2008, Crosbie et al. 2012). In recent years, genetic tools have become a reliable means of precisely identifying pathogens associated with diseases of marine organisms (e.g. Senapin et al. 2007, Young et al. 2007, Dyková et al. 2011).

Along the Atlantic Coast of Nova Scotia, Canada, recurrent mass mortalities of the green sea urchin *Strongylocentrotus droebachiensis* drive transitions between the alternative community states in the rocky subtidal zone, viz. kelp beds and sea urchin barrens (Miller 1985, Scheibling 1986). These disease outbreaks are associated with tropical storm and hurricane activity and warm sea temperatures, and have increased in frequency over the past 3 decades (Scheibling & Hennigar 1997, Scheibling & Lauzon-Guay 2010). The pathogenic agent, an amoeba isolated from moribund urchins following a disease outbreak in 1983, was described as a new species, *Paramoeba invadens* (Jones 1985). The pathology of paramoebiasis in sea urchins has been characterized and Koch’s postulates confirmed (Jones et al. 1985, Jones & Scheibling 1985). Paramoebiasis is highly host-specific and temperature-dependent (Scheibling & Stephenson 1984, Jellett et al. 1988, Jellett & Scheibling 1988a), and only affects sea urchins in shallow water (< 25 m depth) in the summer or early fall when sea temperatures are above ~ 10 °C, the thermal threshold for disease propagation (Scheibling 1984, 1988, Brady & Scheibling 2005, Feehan et al. 2012a). However, the source population(s) of the pathogenic amoeba and mechanism of spread along the coast of Nova Scotia remain poorly understood.
Paramoeba and Neoparamoeba are ubiquitous in temperate marine environments, and most species are free-living (Page 1970, Jones 1985, Dyková et al. 2005, Kudryavtsev et al. 2011). Nonetheless, they also act as facultative parasites in a variety of marine species aside from sea urchins, including Atlantic salmon Salmo salar in aquaculture, and decapod crustaceans along the eastern seaboard of the USA (Table 4.1). Analysis of small-subunit (SSU) ribosomal DNA (rDNA) is an effective method used to unambiguously discriminate between closely related species of Paramoeba and Neoparamoeba (Table 4.1). No genetic information has been available on P. invadens, however, and in its absence it has been suggested that this species could be a form of N. pemaquidensis (basionym P. pemaquidensis), a morphologically similar amoeba associated with diseases of both lobsters Homarus americanus and sea urchins Strongylocentrotus droebachiensis in the northeastern USA (Mullen et al. 2005).

The issue of whether Paramoeba invadens exists as a distinct species has obvious implications for understanding the transmission of this disease within the environment, its geographic extent and the nature of sources for new outbreaks. The objective of our study was to determine whether amoebae infecting sea urchins in Nova Scotia in fall 2011 are the same as a pathogen (P. invadens) isolated from diseased sea urchins since the early 1980s. To do this, we conducted 2 types of laboratory experiments examining the dynamics of paramoebiasis caused by amoebae isolated in fall 2011, and compared our results to those of similar experiments conducted since the early 1980s. Our work compliments the findings of a concurrent study by Johnson-Mackinnon (2012), who used SSU rDNA to show that our 2011 isolates represent a distinct species (and not, for example, a variant of Neoparamoeba pemaquidensis) (Feehan et al. 2013).
Table 4.1. *Paramoeba/Neoparamoeba* spp. identified from marine invertebrate and vertebrate hosts. ‘Disease’ refers to whether *Paramoeba/Neoparamoeba* spp. were associated with disease (morbidity or mortality) in the host (Yes) or not (No). ‘Mode of ID’ refers to the identification of the amoeba species from each host.

<table>
<thead>
<tr>
<th>Amoeba sp.</th>
<th>Host</th>
<th>Disease</th>
<th>Location</th>
<th>Mode of ID</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sea urchin <em>Strongylocentrotus droebachiensis</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Yes</td>
<td>Nova Scotia, Canada</td>
<td>SSU rDNA</td>
<td>Feehan et al. (2013)</td>
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<tr>
<td><em>Paramoeba/Neoparamoeba branchiphila</em></td>
<td>Atlantic salmon <em>Salmo salar</em> (aquaculture)</td>
<td>Yes&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Tasmania, Australia</td>
<td>SSU rDNA</td>
<td>Dyková et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Blue crab <em>Callinectes sapidus</em></td>
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<td>Gulf of Mexico, USA</td>
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<td>Dyková et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Sea urchin <em>Diadema aff. antillarum</em></td>
<td>Yes</td>
<td>Canary Islands, Spain</td>
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<td>Dyková et al. (2011)</td>
</tr>
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<td>Sea urchin <em>Heliocidaris erythrogramma</em></td>
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<td>SSU rDNA</td>
<td>Dyková et al. (2007)</td>
</tr>
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<td>Sea urchin <em>Paracentrotus lividus</em></td>
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<td>Dyková et al. (2007)</td>
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<td>Southern bluefin tuna <em>Thunnus maccoyii</em> (carcass)</td>
<td>No</td>
<td>Port Lincoln, Australia</td>
<td>SSU rDNA</td>
<td>Dyková et al. (2007)</td>
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<td><em>Paramoeba/Neoparamoeba pemaquidensis</em></td>
<td>American lobster <em>Homarus americanus</em></td>
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<td>Mullen et al. (2005)</td>
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<td>Amoeba sp.</td>
<td>Host</td>
<td>Disease</td>
<td>Location</td>
<td>Mode of ID</td>
<td>Source</td>
</tr>
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<td>Morphology</td>
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<td>Wong et al. (2004), Dyková et al. (2007)</td>
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<td>Wong et al. (2004)</td>
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<td>Washington State and California, USA</td>
<td>SSU rDNA</td>
<td>Wong et al. (2004)</td>
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<td><em>Strongylocentrotus droebachiensis</em></td>
<td>Yes</td>
<td>Gulf of Maine, USA</td>
<td>SSU rDNA</td>
<td>Caraguel et al. (2007)</td>
</tr>
<tr>
<td>Southern bluefin tuna</td>
<td><em>Thunnus maccoyii</em> (carcass)</td>
<td>No</td>
<td>Port Lincoln, Australia</td>
<td>SSU rDNA</td>
<td>Dyková et al. (2007)</td>
</tr>
<tr>
<td>Turbot</td>
<td><em>Scophthalmus maximus</em> (aquaculture)</td>
<td>Yes</td>
<td>Northwestern Spain</td>
<td>Morphology</td>
<td>Dyková et al. (1998)</td>
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<td>Fiala &amp; Dyková (2003)</td>
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<td>Host</td>
<td>Disease</td>
<td>Location</td>
<td>Mode of ID</td>
<td>Source</td>
</tr>
<tr>
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<td>Young et al. (2008)</td>
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<td>Yes</td>
<td>West coast of Norway</td>
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<td>Steinum et al. (2008)</td>
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<td>SSU rDNA</td>
<td>Young et al. (2008)</td>
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<td>Atlantic salmon <em>Salmo salar</em> (aquaculture)<em>a</em></td>
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<td>Young et al. (2007, 2008), Bridle et al. (2010), Crosbie et al. (2012)</td>
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<td>Young et al. (2008), Nowak et al. (2010)</td>
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<td>Ayu <em>Plecoglossus altivelis</em> (aquaculture)</td>
<td>Yes</td>
<td>Japan</td>
<td>SSU rDNA</td>
<td>Crosbie et al. (2010b)</td>
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<td>Chinook salmon <em>Oncorhynchus tshawytscha</em></td>
<td>Yes</td>
<td>Queen Charlotte Sound, New Zealand</td>
<td>SSU rDNA</td>
<td>Young et al. (2008)</td>
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<td></td>
<td>Rainbow trout <em>Oncorhynchus mykiss</em> (aquaculture)</td>
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<td>SSU rDNA</td>
<td>Young et al. (2008)</td>
</tr>
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<td></td>
<td>Turbot <em>Scophthalmus maximus</em> (aquaculture)</td>
<td>Yes</td>
<td>Northwestern Spain</td>
<td>SSU rDNA</td>
<td>Young et al. (2008)</td>
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<tr>
<td><em>Paramoeba/ Neoparamoeba sp.</em></td>
<td>Atlantic salmon <em>Salmo salar</em> (aquaculture)</td>
<td>Yes</td>
<td>Galway and Cork, Ireland</td>
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<td>Rodger et al. (2011)</td>
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<td>Host</td>
<td>Disease</td>
<td>Location</td>
<td>Mode of ID</td>
<td>Source</td>
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<td>Morphology</td>
<td>Adams et al. (2008)</td>
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<td>Sea bass <em>Dicentrarchus labrax</em> (larvae, aquaculture)</td>
<td>Yes</td>
<td>Yes</td>
<td>Mediterranean Sea</td>
<td>Morphology</td>
<td>Dyková et al. (2000)</td>
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<td>Turbot <em>Scophthalmus maximus</em> (aquaculture)</td>
<td>Yes</td>
<td>Yes</td>
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<td>Morphology</td>
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<tr>
<td>Rainbow trout <em>Oncorhynchus mykiss</em> (aquaculture)</td>
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<td>Yes</td>
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<td>Morphology</td>
<td>Munday et al. (1990)</td>
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<td>Atlantic salmon <em>Salmo salar</em> (aquaculture)</td>
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<td>Morphology</td>
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<td>Seabream <em>Diplodus puntazzo</em> (aquaculture)</td>
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<td>Morphology</td>
<td>Dyková &amp; Novoa (2001)</td>
</tr>
<tr>
<td>Sea bass <em>Dicentrarchus labrax</em> (aquaculture)</td>
<td>Yes</td>
<td>Yes</td>
<td>Northwestern Spain and Mediterranean Sea</td>
<td>Morphology</td>
<td>Dyková &amp; Novoa (2001)</td>
</tr>
<tr>
<td><em>Paramoeba</em> sp. (probably <em>P./N. pemaquidensis</em>)</td>
<td>Yes</td>
<td>Yes</td>
<td>Long Island Sound, USA</td>
<td>Morphology</td>
<td>Mullen et al. (2004)</td>
</tr>
</tbody>
</table>

*aKoch’s postulates fulfilled*  
*bParamoeba/Neoparamoeba* sp. likely misidentified as causative agent of disease (Young et al. 2008).
4.3. MATERIALS AND METHODS

4.3.1. Collection of Infected Sea Urchins

Following the passage of Hurricane Katia on 9 September 2011, SCUBA divers observed a mass mortality of sea urchins at 8 m depth in barrens at Splitnose Point, Nova Scotia, and in experimental cages in kelp beds within and immediately outside St. Margarets Bay, 40 km west-southwest of Splitnose Point (Fig. 4.1). Caged sea urchins had been transplanted to St. Margarets Bay from Splitnose Point on 6 August 2011 as part of an ongoing experiment investigating the association between hurricanes and disease outbreaks (Feehan et al. 2012a). Moribund sea urchins, with overt signs of paramoebiasis, including loss of attachment to the substrate, dishevelled spines, shrivelled and non-functional tube feet and a gaping peristome (Scheibling & Stephenson 1984), were collected by divers from cages in St. Margarets Bay and from barrens at Splitnose Point on 29 September and 9 November 2011, respectively.

To determine the extent of the disease outbreak, and to obtain additional isolates of amoebae, we acquired sea urchins from 2 other sites along the coast of Nova Scotia (Fig. 4.1). On 19 October 2011, we collected sea urchins in baited traps from a sedimentary basin at 60 m depth off Owl’s Head, near the mouth of St. Margarets Bay. On 22 November 2011, we acquired sea urchins that had been collected by divers from barrens at Sandy Point, Shelburne County, ~ 200 km southwest of St. Margarets Bay (purchased from C. Hopkins, Barrington Passage, Nova Scotia). At both sites, bottom temperature at the time of collection was below the thermal threshold for paramoebiasis (~ 5 °C at 60 m in St. Margarets Bay, K. Filbee-Dexter & R.E. Scheibling unpubl. data; ~ 9 °C at 8 m depth at Sandy Point, C. Hopkins pers. comm.). Both groups of sea urchins developed signs of paramoebiasis after they were maintained in flowing seawater aquaria at 16 °C in the laboratory, suggesting that the pathogenic agent was present at these sites but suppressed by low temperatures in the field. Observations made by local fishers of moribund urchins at Sandy Point, and mass mortality of sea urchins at nearby sites (Barrington Bay and Lockport, Shelburne County) in October and November 2011 (C.
Hopkins pers. comm.) provide corroborating evidence that paramoebiasis was present at this site.

All sea urchins were transported in chilled plastic bins to the Aquatron at Dalhousie University, Halifax, Nova Scotia, within 6 h of collection. Sea urchins collected from Splitnose Point on 5 August 2011 (prior to disease outbreak), and maintained at 16 °C in the laboratory from 19 September to 24 November did not exhibit signs of morbidity, indicating that a pathogen was not present in the seawater system of the Aquatron.

4.3.2. Polyxenic and Monoxenic Culturing of Amoebae

Amoebae were isolated from the radial nerve of moribund sea urchins obtained either directly from the field (Splitnose Point at 8 m depth: isolate SP; St. Margarets Bay cages at 8 m: isolate SMB-8) or from thermal induction experiments (St. Margarets Bay at 60 m: isolate SMB-60; Sandy Point, Shelburne at 8 m: isolate SPS), using the methods of Jones & Scheibling (1985). Briefly, strips of radial nerve with associated radial water-vascular canal (0.5 cm length) were excised from the oral region of sea urchins and placed on 0.6 % non-nutrient (NN) marine agar (salinity 35). After 1 week, amoebae had moved out of the tissues and into the semi-solid agar. Amoebae were subcultured by removing a 1 cm² piece of 0.6 % agar onto 1.2 % NN agar with a 200 µl liquid overlay containing bacterial prey (*Escherichia coli* in sterile seawater). The liquid overlay was prepared by inoculating 3 ml of lysogeny broth (LB) with *E. coli* at 37 °C for 24 h, centrifuging the liquid culture (3000 × g, 5 min), and replacing the supernatant and resuspending the pellet in 3 ml of seawater.

Monoxenic cultures of amoebae were obtained using the methods of Jellett & Scheibling (1988b). A 1 cm² piece of polyxenic culture of amoebae was removed to 1 ml of antibiotic solution (10000 UI penicillin, 10000 µg streptomycin) for 5 h, and then plated onto 1.2 % NN agar with liquid overlay (as described above). Amoebae in both monoxenic and polyxenic culture were maintained at 18 °C and subcultured onto 1.2 % NN agar with liquid overlay at 1 to 2 week intervals.
4.3.3. Laboratory Experiments

We conducted 2 types of experiments (below) to test whether the dynamics of paramoebiasis caused by amoebae isolated from moribund sea urchins in fall 2011 were consistent with those observed in similar experiments since the early 1980s and attributed to *Paramoeba invadens* (Scheibling & Stephenson 1984, Jones & Scheibling 1985, Jellett & Scheibling 1988b, Scheibling et al. 2010, Feehan et al. 2012a). All experiments were conducted in 47 l glass aquaria supplied with oxygenated temperature-controlled flowing seawater (~ 3 l min⁻¹) in a quarantine laboratory of the Aquatron at Dalhousie University.

**Water-borne transmission experiment.** To determine whether the temperature-dependent rate of transmission and progression of disease from moribund sea urchins to healthy conspecifics was consistent with paramoebiasis, we conducted a water-borne transmission experiment from 30 September to 16 October 2011, using the same methodology and apparatus as Scheibling & Stephenson (1984). We recorded time to ≥ 50 % morbidity (*t*₅₀, d) of sea urchins after exposure to infected conspecifics, for comparison with values from previous water-borne transmission experiments at 16 °C using sea urchins infected with *Paramoeba invadens* (Scheibling & Stephenson 1984, Jellett & Scheibling 1988b, Scheibling et al. 2010, Feehan et al. 2012a). Morbidity was measured as time to loss of attachment, when sea urchins had detached from the sides or bottom of aquaria and could not right themselves within 20 min. Moribund sea urchins were recorded daily and removed from each aquarium. Groups of 20 healthy sea urchins (collected from Splitnose Point on 5 August 2011, prior to the disease outbreak and maintained in flow-through seawater tanks in the laboratory until use) were placed in 2 aquaria supplied with 16 °C seawater. Sea urchins were visually inspected to ensure that they had no lesions or other abnormalities, and a subset was tested to ensure that they could right themselves within 5 min when inverted. Three moribund sea urchins collected from 8 m cages in St. Margarets Bay on 29 September (the same source as isolate SMB-8) were placed in a glass ‘source’ tube that was spliced into the incurrent seawater supply of one aquarium as the disease-exposed treatment. Three healthy sea urchins (from the same Splitnose Point collection as above) were placed in another tube that supplied the remaining aquarium as the control treatment.
**Injection experiments.** To determine whether the temperature-dependent rate of progression of disease in healthy sea urchins injected with cultured amoebae was consistent with paramoebiasis, we conducted an injection experiment using the methods of Jones & Scheibling (1985) and Jellett & Scheibling (1988b) (Table 4.2). As in the water-borne transmission experiment, moribund sea urchins were recorded daily and removed from each aquarium. We measured $t_{50}$ of sea urchins following injection with amoebae for comparison with similar experiments conducted in the 1980s (Jones & Scheibling 1985, Jellett & Scheibling 1988b, Table 4.2). Groups of 3 to 4 sea urchins (from the sample collected from Splitnose Point on 5 August) were placed in 6 replicate aquaria with seawater at 16 °C. Sea urchins were visually inspected and a subset was tested to ensure that they could right themselves when inverted. Sea urchins in 3 randomly selected aquaria were injected with 1 ml of monoxenic culture of amoebae (isolate SMB-8) that was treated with antibiotics (10 000 IU penicillin, 10 000 µg streptomycin) 2 h prior to injection, as the infection treatment. The inoculum was diluted to a concentration of ~ 600 amoebae ml$^{-1}$, as measured using a haemocytometer. Sea urchins in the remaining 3 aquaria were injected with 1 ml of antibiotic-treated *Escherichia coli* as a control. The control inoculum was diluted to the same concentration of bacterial cells as the inoculum of the infection treatment. Streaks with the injection treatment and control inoculum on marine nutrient agar showed no growth after 24 h and individual colonies after 1 week at 18 °C.

To determine whether there are different infective strains of the amoeba along the coast of Nova Scotia, we conducted a second injection experiment to compare the virulence of the 4 isolates: SMB-8, SMB-60, SP and SPS (Table 4.2). We measured rates of sea urchin morbidity ($t_{50}$) following injection with amoebae from the respective sites using an experimental design similar to the first injection experiment. Because all healthy sea urchins from laboratory reserves (sea urchins collected from Splitnose Point on 5 August 2011) had been used in previous experiments, healthy sea urchins for the second injection experiment were obtained from the St. Lawrence estuary, where paramoebiasis has not been observed. Sea urchins were collected on 13 December 2011 by divers at Fisheries and Oceans Canada, Institut Maurice-Lamontagne, Mont Joli, Quebec, and ground-transported in chilled plastic bins to Dalhousie University within 24 h of
collection. Groups of 10 sea urchins were placed in 10 replicate aquaria with 16 °C seawater. Sea urchins within 2 randomly selected aquaria for each of the 4 sites were injected with 1 ml of antibiotic-treated monoxenic culture of amoebae isolated from moribund urchins from a given site, to generate 4 infection treatments based on site-specific amoeba cultures. Each inoculum was diluted to a concentration of ~ 300 amoebae ml⁻¹. Sea urchins in the remaining 2 aquaria were injected with 1 ml of antibiotic-treated *Escherichia coli* as a control. The control inoculum was diluted to the same concentration of bacterial cells as the infection treatment (isolate SMB-8) with the greatest concentration (smallest dilution factor), as a conservative measure. Streaks of inocula from the 4 infection treatments and the control on marine nutrient agar showed no growth after 24 h at 18 °C. The effect of amoeba source (fixed factor, 4 levels: SMB-8, SMB-60, SP, SPS) on *t*₅₀ of sea urchins was analyzed using 1-way analysis of variance (ANOVA).

To determine whether the virulence of the amoeba is maintained in culture over time, we conducted a third injection experiment reusing 2 of the amoeba cultures used in the second experiment (SP, SMB-8; Table 4.2). The experimental design was identical to the second experiment, except that the cultures were ~ 6 weeks older. The effect of amoeba source (fixed factor, 2 levels: SMB-8, SP) and culture age (fixed factor, 2 levels: ~ 15 weeks, ~ 21 weeks) on *t*₅₀ of sea urchins was analyzed using 2-way ANOVA.

For each injection experiment, we cultured the radial nerve of 3 to 5 moribund sea urchins in each infection treatment within 24 h of the onset of signs of paramoebiasis, and in controls at the end of the experiment, to determine whether amoebae were present, using the methods described above.
Table 4.2. Injection experiments to measure rate of morbidity of sea urchins injected with *Paramoeba invadens*. ‘Urchin source’ is the collection site for healthy experimental sea urchins (SP: Splitnose Point, Nova Scotia; GSL: Gulf of St. Lawrence, Quebec; NS: unknown sites along the coast of Nova Scotia). ‘Amoeba source’ is the collection site (and depth in m) of moribund sea urchins from which amoebae were isolated (SMB: St. Margarets Bay, Nova Scotia; SPS: Sandy Point, Shelburne, Nova Scotia). ‘Culture age’ refers to the start of the experiment. Dose is the estimated mean (± SE) number of amoebae ml\(^{-1}\) injected into sea urchins. \(n\) is the number of urchins per aquarium. ND: no data available.

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Period</th>
<th>Urchin source</th>
<th>Amoeba source</th>
<th>Culture age (wk)</th>
<th>Dose (amoebae ml(^{-1}))</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24 Nov–12 Dec</td>
<td>SP</td>
<td>SMB (8 m)</td>
<td>5</td>
<td>600 ± 90</td>
<td>3–4</td>
</tr>
<tr>
<td>2</td>
<td>12 Feb–14 Mar</td>
<td>GSL</td>
<td>SMB (8 m)</td>
<td>16.5</td>
<td>300 ± 82</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SMB (60 m)</td>
<td>12.5</td>
<td>300 ± 63</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SP (8 m)</td>
<td>13.5</td>
<td>300 ± 45</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SPS (8 m)</td>
<td>11.5</td>
<td>300 ± 51</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>26 Mar–15 May</td>
<td>GSL</td>
<td>SMB (8 m)</td>
<td>22.5</td>
<td>333 ± 49</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SP (8 m)</td>
<td>19.5</td>
<td>333 ± 20</td>
<td></td>
</tr>
<tr>
<td>Jones &amp; Scheibling (1985)</td>
<td>NS</td>
<td>NS</td>
<td>ND</td>
<td>100–300</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Jellett &amp; Scheibling (1988)</td>
<td>NS</td>
<td>NS</td>
<td>5</td>
<td>300</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 4.1. Collection sites of *Strongylocentrotus droebachiensis* along the coast of Nova Scotia, Canada. The insets show 1 site near Shelburne, Nova Scotia (SPS: Sandy Point, Shelburne), 5 sea urchin caging sites within and immediately outside of St. Margarets Bay (MC: Mill Cove; TL: The Lodge; GI: Gravel Island; CC: Cranberry Cove; LI: Luke Island), 1 site at the mouth of St. Margarets Bay (OH: Owls Head) and 1 site along a headland near the mouth of Halifax Harbour (SP: Splitnose Point).
4.4. RESULTS

In the water-borne transmission experiment, healthy sea urchins exposed to moribund conspecifics from cages at 8 m depth in St. Margarets Bay were all moribund by Day 12, and \( t_{50} \) was reached after 9 d (Table 4.3, Fig. 4.2). This \( t_{50} \) was within the ranges observed in previous water-borne transmission experiments conducted since the early 1980s (Table 4.3).

In the first injection experiment, which examined whether the temperature-dependent rate of progression of disease in healthy sea urchins injected with cultured amoebae was consistent with paramoebiasis caused by *Paramoeba invadens*, all but one of the healthy sea urchins injected with amoebae isolated from moribund conspecifics in cages in St. Margarets Bay (isolate SMB-8) were also moribund by Day 15, with a mean \( t_{50} \) of 12 d (Table 4.3, Fig. 4.3). Mean \( t_{50} \) conformed closely to that observed in similar experiments conducted in the early to mid-1980s (Table 4.3, Fig. 4.3).

In the second injection experiment, which compared the virulence of 4 isolates of amoebae (cultures of *Paramoeba invadens* from moribund sea urchins collected at different sites: SMB-8, SMB-60, SP and SPS), 1-way ANOVA showed significant differences in \( t_{50} \) among the 4 isolate treatments (\( F_{3,4} = 7.73, p = 0.039 \); Fig. 4.4a). In one replicate of the SP treatment, sea urchins only reached 40 % morbidity after 31 d, which was used as a conservative estimate of \( t_{50} \) for that replicate in the analysis. The estimated mean \( t_{50} \) of sea urchins in the SP treatment (30 d) was greater than the mean \( t_{50} \) in SMB-8, SMB-60 and SPS treatments (17 to 19 d), meaning that SP was less virulent (Tukey’s HSD test, \( \alpha = 0.05 \)).

In the third injection experiment, sea urchins were injected with amoebae from 2 of the source treatments used in the second experiment (SMB-8, SP), but when these cultures were 6 weeks older. In both cases, \( t_{50} \) was about 1.5 times greater than in the second experiment, but the difference between source treatments was consistent between experiments, with SP again producing a larger \( t_{50} \) than SMB-8 (Fig. 4.4a,b). The estimated mean \( t_{50} \) in the SP treatment was 44 d in the third experiment, while mean \( t_{50} \) in the SMB-8 treatment was 28 d (Fig. 4.4b; note that in 1 replicate of the SP treatment, sea urchins only reached 20 % morbidity after 50 d, which we used as a highly conservative
estimate of $t_{50}$ for that replicate). A 2-way ANOVA showed significant effects of amoeba source/isolate ($F_{1,4} = 8.37$, $p = 0.044$) and culture age ($F_{1,4} = 11.32$, $p = 0.028$), but no interaction between these factors ($F_{1,4} = 0.09$, $p = 0.783$).

*Paramoeba*-like amoebae (Jones 1985) were observed in all cultures of radial nerves of moribund sea urchins taken from the infection treatments of injection experiments. No sea urchins died or showed signs of morbidity in any of the controls of both types of experiments, and amoebae were not observed in cultures of radial nerves of control sea urchins from injection experiments.

Table 4.3. Waterborne transmission and injection experiments conducted following disease outbreaks since 1980. $t_{50}$ is the time to $\geq 50\%$ morbidity (exhibiting signs of paramoebiasis, d) following exposure to 3 to 5 moribund conspecifics. $N$ is the number of experiments conducted in each year. For all experiments, survival was 100% in controls. In disease-exposed treatments, survival was 0% in waterborne transmission experiments and 10% in injection experiments. For all experiments, water temperature was 16 °C.

<table>
<thead>
<tr>
<th>Type of Experiment</th>
<th>Year(s) of Outbreak</th>
<th>$t_{50}$ (d)</th>
<th>$N$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Waterborne</em></td>
<td>2011</td>
<td>9</td>
<td>1</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>2010</td>
<td>8–10</td>
<td>3</td>
<td>Feehan et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>11</td>
<td>1</td>
<td>Scheibling et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>1982</td>
<td>12–13.5</td>
<td>7</td>
<td>Jellett &amp; Scheibling (1988b)</td>
</tr>
<tr>
<td></td>
<td>1982–83</td>
<td>8.5–11.5</td>
<td>7</td>
<td>Scheibling &amp; Stephenson (1984)</td>
</tr>
<tr>
<td><em>Injection</em></td>
<td>2011</td>
<td>12</td>
<td>1</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>1982</td>
<td>13.5</td>
<td>1</td>
<td>Jellett &amp; Scheibling (1988b)</td>
</tr>
</tbody>
</table>
Fig. 4.2. Cumulative frequency of morbidity of sea urchins exposed to moribund conspecifics (from cages in St. Margarets Bay) in a waterborne transmission experiment. Dashed line indicates 50 % morbidity. There was no morbidity in controls (not shown). Temperature = 16 °C.
Fig. 4.3. Comparison of cumulative frequency of morbidity of sea urchins injected with amoebae isolated from moribund conspecifics collected at 8 m depth in St. Margarets Bay in 2011 and in a similar experiment conducted by Jones & Scheibling (1985). Dashed line indicates 50 % morbidity. There was no mortality in controls (not shown) in either experiment. Temperature = 16 °C. Data are mean ± SE for 3 replicate aquaria. Data from 1985 are offset by + 0.25 d to avoid overlap of data points between years.
Fig. 4.4. Cumulative frequency of moribidity of sea urchins injected with amoebae isolated from (a) moribund conspecifics from 4 sites along the coast of Nova Scotia: St. Margarets Bay at 8 m (SMB-8) and 60 m depth (SMB-60), Sandy Point, Shelburne (SPS) and Splitnose Point (SP); and (b) the same cultures for SMB-8 and SP that were 6 weeks older. Dashed line indicates 50 % morbidity. There was no morbidity in controls (not shown) in either experiment. Temperature = 16 °C. Data are mean ± SE for 2 replicate aquaria. Data for SMB-8 are offset by ± 0.25 d for clarity.
4.5. DISCUSSION

4.5.1. Validating the Identity of *Paramoeba invadens* as the Pathogen of Sea Urchins in Nova Scotia

Water-borne disease transmission and injection experiments conducted from 1980 to 2011, culminating in the studies reported here, show a consistent rate of transmission and propagation of paramoebiasis in sea urchins *Strongylocentrotus droebachiensis*. This represents very strong evidence that the pathogen infecting sea urchins in 2011 is indeed *Paramoeba invadens* Jones 1985. A concurrent study of nuclear and parasome SSU rDNA (Johnson-Mackinnon 2012) confirmed that our 2011 isolates of *P. invadens* represent a distinct species. These results indicate that *P. invadens* is most closely related to *Neoparamoeba branchiphila*, a suspected pathogen of sea urchins *Diadema* aff. *antillarum* in the Canary Islands, Spain (Johnson-Mackinnon 2012, Feehan et al. 2013). *N. branchiphila* also has been isolated from tissues of crabs in the USA, sea urchins in Australia and Greece, and finfish in aquaculture throughout the world (Table 4.1).

Outbreaks of paramoebiasis in sea urchins along the Atlantic coast of Nova Scotia are associated with hurricanes and tropical storms, and warm sea temperatures (Scheibling & Lauzon-Guay 2010, Feehan et al. 2012a); however, the source of the infective population of *Paramoeba invadens* remains unknown. The minimum thermal tolerance of *P. invadens* (~ 2 °C) is above the winter minimum in ocean temperature (0 to -1°C) along this coast (Jellett & Scheibling 1988a). Therefore, it has been considered unlikely that *P. invadens* overwinters in sea urchins or in the surrounding environment (Jellett et al. 1989). Two mechanisms have been postulated to explain recurrent introductions of the pathogen to shallow coastal waters during severe storm events: 1) horizontal advection from distant source populations (Scheibling & Hennigar 1997) and 2) vertical mixing of amoebae residing in deep sedimentary basins along the Scotian shelf (Scheibling & Lauzon-Guay 2010), where temperatures rarely fall below ~ 5 °C (K. Filbee-Dexter & R.E. Scheibling unpubl. data).

Our injection experiments indicated differences in virulence among cultures of amoebae from moribund sea urchins at 4 sites spanning ~ 200 km of coastline (linear
distance) and across a depth range of ~ 50 m, with amoebae from one site (SP) having lower virulence than amoebae from the other 3 sites (SMB-8, SMB-60 and SPS). However, a concurrent analysis of nuclear SSU rDNA showed no fixed genetic differences among the Paramoeba invadens isolates (Johnson-Mackinnon 2012). If there were indeed spatial variance in virulence, this would suggest that there is more than one infective subtype of P. invadens along the Nova Scotian coast and that the pathogen is introduced at local scales (10s of km), favouring the hypothesis that amoebae reside in nearshore sedimentary basins.

4.5.2. Global Trends in Paramoeba/Neoparamoeba-associated Disease Events

Amoebae assigned to the genera Paramoeba and Neoparamoeba have been associated with disease in various marine organisms worldwide (Table 4.1). However, to our knowledge, Koch’s postulates have only been fulfilled for 2 species: P. invadens in sea urchins in Nova Scotia (Jones & Scheibling 1985, this study), and N. perurans in Atlantic salmon in aquaculture (Crosbie et al. 2012, Table 4.1). This may reflect the difficulty of culturing these amoebae in the first place (Johnson 1977, Mullen et al. 2004, 2005, Crosbie et al. 2012) or the loss of virulence in culture over time (i.e. reduced infectivity or severity of disease; Jellett & Scheibling 1988b, Kent et al. 1988, Zilberg et al. 2001).

The results of our successive injection experiments confirm that Paramoeba invadens loses virulence in culture over the scale of weeks. We found a ca. 1.5-fold increase in \( t_{50} \) of infected Strongylocentrotus droebachiensis between ~ 15- and 21-week-old cultures. Jellett & Scheibling (1988b) also found that virulence of monoxenic cultures of P. invadens decreased in sea urchins after 15 weeks. Other studies have documented changes in metabolism of other Paramoeba and Neoparamoeba spp. that are associated with a loss of virulence (Dyková et al. 2000, Kent et al. 1988, Dyková et al. 2000).

There is evidence that some Paramoeba and Neoparamoeba spp. are opportunistic pathogens, infecting only stressed or diseased individuals. For example, an outbreak of N. pemaquidensis in lobster Homarus americanus in Long Island Sound, USA, in 1999 was associated with thermal stress and crowding (Mullen et al. 2004). In
the Canary Islands, *N. branchiphila* was isolated from sea urchins *Diadema* aff. *antillarum* infected with ‘bald sea urchin disease’ caused by the bacterium *Vibrio* sp. (Dyková et al. 2011). In amoebic gill disease (AGD)-infected Atlantic salmon in aquaculture, co-infections with sea lice, bacteria, gelatinous zooplankton, *Ichthyobodo* (marine costia) and other amoeba species have been observed (Bermingham & Mulcahy 2006, Nowak et al. 2010, Bustos et al. 2011, Rodger et al. 2011). In our laboratory infection experiments, there was no indication that sea urchins were physiologically stressed: water temperature (16 °C) was well below the maximum thermal tolerance of *Strongylocentrotus droebachiensis* (22°C), and no mortality occurred in control sea urchins maintained under the same conditions for 9.5 weeks. We found no evidence to indicate that a co-occurring pathogen is involved in disease outbreaks in sea urchins in Nova Scotia; however, we cannot disregard the possibility that an undetected and consistently co-occurring microbe(s) was present in inocula of *P. invadens* used in injection experiments in our study or in previous ones (Jones & Scheibling 1985, Jellett & Scheibling 1988b).

It has been suggested that *Neoparamoeba pemaquidensis* and *N. branchiphila* were misidentified as the causative agent of AGD in Atlantic salmon *Salmo salar* (Young et al. 2008). Retrospective analyses of Atlantic salmon gill tissue using *in situ* probes that hybridize with the SSU rDNA indicate that several cases of AGD previously attributed to *N. pemaquidensis* or *N. branchiphila* were actually caused by *N. perurans*, the proven causative agent of AGD (Young et al. 2008). Crosbie et al. (2012) suggested that current culturing procedures select for non-virulent amoebae species (*N. branchiphila* or *N. pemaquidensis*) instead of *N. perurans* when both are present, leading to misidentification of the actual disease agent and the artificial appearance of rapid loss of virulence. For example, investigators have found that naïve Atlantic salmon develop AGD following water-borne exposure to *N. pemaquidensis* or *N. branchiphila* freshly isolated from the gills of infected conspecifics (Douglas-Helders et al. 2003, Morrison et al. 2005, Vincent et al. 2007); however, AGD is not induced by exposure to cultured *N. pemaquidensis* or *N. branchiphila* (Douglas-Helders et al. 2003, Mullen et al. 2004, 2005, Crosbie et al. 2007, 2010a, Vincent et al. 2007). Similarly, Morrison et al. (2005) found
that only fresh cultures of *N. pemaquidensis* (< 72 h old) induced AGD in naïve Atlantic salmon.

Infections with *Paramoeba* and *Neoparamoeba* spp. typically exhibit strong temperature dependence with threshold dynamics. Paramoebiasis in sea urchins off Nova Scotia occurs only above a threshold temperature of ~ 10 °C, and rates of morbidity of sea urchins increase up to ~ 20 °C, corresponding to the thermal optimum for growth of *P. invadens* in culture (Scheibling & Stephenson 1984, Jellett & Scheibling 1988a). Similarly, infection by *Paramoeba*/*Neoparamoeba* of farmed salmonids in Tasmania, Australia (Munday et al. 1990, Clark & Nowak 1999, Douglas-Helders et al. 2001), and blue crab *Callinectes sapidus* in the northeastern USA (Johnson 1977), occurs only above threshold temperatures of ~ 10 and 13 °C, respectively. Furthermore, the only documented outbreak of AGD in Atlantic salmon in aquaculture in Norway occurred above a threshold of ~ 11 °C and was associated with sea temperatures ~ 3.5 °C above the seasonal average (Steinum et al. 2008). Given the temperature dependence of *Paramoeba*/*Neoparamoeba*-associated disease outbreaks, continued ocean warming could have important implications for the resilience of susceptible host populations. Indeed, increases in the frequency and severity of outbreaks of sea urchin paramoebiasis and range of AGD are associated with increasing or unusually high sea temperatures in the affected regions (Steinum et al. 2008, Scheibling & Lauzon-Guay 2010).
4.6. ACKNOWLEDGEMENTS

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CHAPTER 5

DISEASE AS A CONTROL OF SEA URCHIN POPULATIONS IN NOVA SCOTIAN KELP BEDS

5.1. ABSTRACT

In Nova Scotia, Canada, periodic outbreaks of amoebic disease (paramoebiasis) cause mass mortality of sea urchins *Strongylocentrotus droebachiensis* in subtidal barrens. However, in kelp beds, where sea urchins are cryptic and generally less dense than in barrens, disease outbreaks are not readily observed and the importance of disease in regulating these populations is unknown. To determine whether sea urchin populations in kelp beds are controlled by disease, we analyzed population data from kelp beds at a single location (St. Margarets Bay) across a span of 44 years (1968 to 2012) to compare changes in size structure and density in relation to the timing of disease outbreaks in adjacent sea urchin aggregations and barrens. We found that sea urchin density, maximum test diameter and percentage of adults decreased following disease outbreaks and increased during intervening periods without disease, indicating that disease regulates the population in kelp beds by limiting survival to adulthood. Our results suggest that disease has replaced predation as a major agent controlling sea urchin populations in Nova Scotian kelp beds.

5.2. INTRODUCTION

Sea urchins are dominant grazers in kelp forests worldwide and can destructively graze kelps to the extent of causing a phase shift to barrens – which is generally considered an alternative state of a collapsed kelp ecosystem (Filbee-Dexter & Scheibling 2014). Shifts to barrens are associated with a marked loss of species diversity, habitat complexity, and community productivity (Mann 1982). Therefore, understanding factors that control sea urchin populations and maintain resilience of the kelp state is of urgent
concern for conservation and management of these valued ecosystems. Historically, predation is thought to have been the major controlling agent of sea urchin abundance in kelp-dominated ecosystems (reviewed by Estes & Duggins 1995, Scheibling 1996, Sala et al. 1998). Overfishing or overhunting of urchin predators, such as sea otters, demersal fish or large crustaceans, has had cascading trophic-level effects leading to the formation of urchin barrens in cold temperate regions throughout the world (reviewed by Tegner & Dayton 2000, Jackson et al. 2001, Steneck et al. 2002). Disease also can play an important role in controlling sea urchins, and accounts for the boom-bust population dynamics (Uthicke et al. 2009) observed in some kelp systems (Scheibling 1984a, Lafferty 2004). Overfishing of predators can have indirect negative effects on sea urchins if the incidence of disease increases with the density of the host population (Tegner & Dayton 2000, Behrens & Lafferty 2004, Lafferty 2004). However, the extent to which disease can replace the functional role of predation as the major agent of population control of sea urchins in kelp ecosystems remains equivocal (Lafferty 2004).

Along the Atlantic coast of Nova Scotia, population outbreaks of green sea urchins *Strongylocentrotus droebachiensis* drive phase shifts from kelp beds to barrens (Johnson & Mann 1988, Scheibling et al. 1999, Brady & Scheibling 2005, Lauzon-Guay & Scheibling 2007a), whereas outbreaks of amoebic disease (paramoebiasis) cause mass mortality of sea urchins in barrens and aid the reverse shift back to kelp beds (Miller 1985, Scheibling 1986). Population outbreaks of *S. droebachiensis* were first documented in Nova Scotia in the late 1960s and early 1970s in St. Margarets Bay, a large semi-protected embayment near Halifax, and were attributed to a release from predation as a result of overfishing of sea urchin predators such as finfish (reviewed by Scheibling 1996). During the 1970s, kelp beds in St. Margarets Bay transitioned to barrens due to destructive grazing by dense aggregations of adult sea urchins within and along the margins of the beds (Breen & Mann 1976b). Prior to these sea urchin outbreaks, kelp beds in the bay were thought to represent a stable ecosystem (Mann 1972a,b). Barrens persisted as the dominant state of the rocky subtidal ecosystem until the early 1980s, when outbreaks of paramoebiasis decimated sea urchins in barrens within the bay and along the entire Atlantic coast of Nova Scotia (Scheibling 1984a). Outbreaks of paramoebiasis have since increased in frequency along this coast in association with
severe storm events and warming temperatures, which may play a role in the introduction and spread of the pathogenic agent (Scheibling & Lauzon-Guay 2010, Scheibling et al. 2013). Since the early 1980s, kelp beds have persisted in St. Margarets Bay, and barrens have not been observed (except on 2 occasions). In the summer/fall of 1992 and 2003, aggregations of sea urchins migrating from offshore sand bottoms, where temperatures are below a threshold for disease (~ 10 °C, Brady & Scheibling 2005), began destructively grazing kelps along the western shore of the bay (Scheibling et al. 1999, Lyons & Scheibling 2008). However, these advancing grazing fronts were eliminated by outbreaks of paramoebiasis (in the fall of 1995 and 2003) before the transition to barrens was complete.

In kelp beds, where sea urchins are cryptic and generally less dense than in grazing fronts or barrens (Meidel & Scheibling 1998), disease outbreaks are not readily observed and the importance of disease in regulating urchin populations is unknown. To determine whether the populations within kelp beds have been controlled by outbreaks of disease, we analyzed sea urchin population data collected over 44 years (1968 to 2012) from kelp beds in St. Margarets Bay. We compared changes in population size structure and density in relation to the timing of outbreaks of paramoebiasis in adjacent sea urchin aggregations and barrens, and our results suggest that disease has replaced predation as a major agent controlling sea urchin populations in Nova Scotian kelp beds.

5.3. MATERIALS AND METHODS

To characterize sea urchin populations in kelp beds in St. Margarets Bay, we compiled records of sea urchin population size structure and density in kelp beds at Mill Cove (44° 36’ N; 64° 04’ W) or adjacent sites (within a 10 km radius) within the bay, from 17 years of published and unpublished data collected over a span of 44 years between 1968 and 2012 (Table 5.1). Sampling protocols varied slightly among studies. In surveys conducted in 1968, 1973, and 1977, 1992 to 1995, and 2009 to 2012, divers destructively sampled 0.25, 0.5 or 1.0 m² quadrats placed randomly along transect lines or haphazardly on the seafloor within the kelp bed, and counted and measured (test diameter, mm) all sea urchins within each quadrat. In surveys from 1982 to 1990 divers
haphazardly sampled the kelp bed by overturning boulders over an area of ~ 1000 m² and counted and measured (test diameter, mm) all sea urchins encountered in 2 to 5 person-hours of searching. Sampling from 1982 onwards was conducted in kelp beds that were re-established following mass mortality of sea urchins in former sea urchin barrens in 1980 and 1981 (Miller 1985). In all studies, divers searched for small and cryptic sea urchins under boulders and cobbles, in crevices and amongst turf algae. Kelp biomass varied from 1.1 to 5.2 kg m⁻² (Table 5.1) with two exceptions: in 1968 when biomass was unusually high (20.1 kg m⁻²; Breen & Mann 1976b) and in 2012 when it was unusually low (~ 0.1 kg m⁻²; J. O’Brien & R.E. Scheibling unpubl. data).

To examine whether disease regulates population size structure of sea urchins within kelp beds, we examined 14 years of available data (between 1982 and 2012) for changes in 1) the mean test diameter of the largest 5 % of the population and 2) the percentage of the population composed of adults (> 20 mm test diameter, Meidel & Scheibling 2001) in relation to the timing of outbreaks of paramoebiasis recorded in adjacent barrens or among experimentally transplanted sea urchins in kelp beds in St. Margarets Bay. These metrics of population size structure indicate whether recruits are surviving to adulthood and adults are increasing in size (age). Since only size-frequency distributions (rather than individual urchin test diameters) are available for some years, the mean test diameter of the largest 5 % of the population was calculated as a weighted mean of the median test diameters of the sea urchin size classes containing the largest 5 % of the population (\( \bar{x}_w \)):

\[
\bar{x}_w = \frac{\sum_{i=1}^{N} (w_i x_i)}{\sum_{i=1}^{N} w_i}
\]

where \( x_i \) is the median test diameter of size class \( i \), \( w_i \) is the proportion of sea urchins within that size class, and \( N \) is the total number of sea urchin size classes. For each weighted mean, we calculated a weighted standard deviation (\( sd_w \)):
The package ‘SDMTools’ for R (Institute for Statistics and Mathematics of the Wirtschaftsuniversität (WU) Wien) was used for both calculations.

To determine whether disease regulates sea urchin abundance within kelp beds, we also examined changes in sea urchin density within the kelp bed in relation to the timing of disease outbreaks for 7 years of available data between 1992 and 2012.
Table 5.1. Sampling protocols of studies used to construct a time series (1968 to 2012) of sea urchin population data in kelp beds in St. Margarets Bay (SMB). ‘Data’ is the type of data available from each study: population density (D) and/or size frequency (S). TD = sea urchin test diameter. ND = no data. A single 0.5 m² quadrat was also sampled within a barren patch in the kelp bed in 1973.

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Depth (m)</th>
<th>Sampling method</th>
<th>Urchin size sampled (TD, mm)</th>
<th>Data</th>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td>1968</td>
<td>Within SMB</td>
<td>0–20</td>
<td>0.25 m² quadrats (n = 165)</td>
<td>&gt; 9</td>
<td>D</td>
<td>Miller &amp; Mann (1973)</td>
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<tr>
<td>1973</td>
<td>Western shore SMB</td>
<td>4–12</td>
<td>0.5 m² quadrats (n = 6)</td>
<td>&gt; 5</td>
<td>D, S</td>
<td>Breen &amp; Mann (1976b)</td>
</tr>
<tr>
<td>1977</td>
<td>Boutilier Point, SMB</td>
<td>0–10</td>
<td>0.25 m² quadrats (n = 10)</td>
<td>ND</td>
<td>D</td>
<td>Chapman (1981)</td>
</tr>
<tr>
<td>Dec 1982</td>
<td>Mill Cove, SMB</td>
<td>8–10</td>
<td>Haphazard search (2–4 person-hours)</td>
<td>&gt; 1–2</td>
<td>S</td>
<td>Raymond &amp; Scheibling (1987)</td>
</tr>
<tr>
<td>Jun, Sept 1983</td>
<td>Mill Cove, SMB</td>
<td>5–6–9</td>
<td>Haphazard search (4–5 person-hours)</td>
<td>&gt; 1–2</td>
<td>S</td>
<td>R.E. Scheibling unpublished data</td>
</tr>
<tr>
<td>Jun 1992</td>
<td>Mill Cove, SMB</td>
<td>6–10</td>
<td>1 m² quadrats (n = 4–10)</td>
<td>&gt; 2</td>
<td>D, S</td>
<td>Scheibling et al. (1999)</td>
</tr>
<tr>
<td>Aug 1994</td>
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<td>Aug 1995</td>
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<tr>
<td>Jun 2009</td>
<td>Birchy Head, SMB</td>
<td>8–10</td>
<td>1 m² quadrats (n = 8–15)</td>
<td>&gt; 2</td>
<td>D, S</td>
<td>This study</td>
</tr>
<tr>
<td>Jun 2010</td>
<td>Birchy Head &amp; Mill Cove, SMB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Jun 2012</td>
<td>Mill Cove, SMB</td>
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</tr>
</tbody>
</table>

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5.4. RESULTS AND DISCUSSION

The population size structure of *Strongylocentrotus droebachiensis* in kelp beds in St. Margarets Bay varied in relation to outbreaks of paramoebiasis between 1980 and 2012. Outbreaks of disease were observed in sea urchin aggregations or barrens in the bay in fall 1980 and 1981 (Miller 1985), 1995 (Scheibling & Hennigar 1997) and 2003 (Lyons & Scheibling 2008), and at the site of sea urchin transplantation experiments (that measured spatial patterns of disease) in fall 2009 (Scheibling et al. 2010), 2010 (Feehan et al. 2012a), 2011 (Feehan et al. 2013) and 2012 (Scheibling et al. 2013) (Fig. 5.1). The mean test diameter of the largest 5% of the sea urchin population in the re-established kelp bed at Mill Cove increased significantly from December 1982 to August 1995, when there was no sign of disease in St. Margarets Bay (Fig. 5.1a, 5.2). This is consistent with a significant increase in the percentage of adults in the population, from 0 to ~30%, over the same period (Fig. 5.1b, 5.2). The mean test diameter of the largest 5% of the population in 1984 (20 mm) approximates the size at maturity of *S. droebachiensis*, indicating that the population was composed almost exclusively of juveniles at this time. Growth experiments by Raymond & Scheibling (1987) indicate that *S. droebachiensis* takes ~2.5 years to reach maturity. The sea urchin population recovered from the 1981 disease outbreak after ~4 years, as indicated by the test diameter of the largest 5% of the population and percentage of adults from 1985 to 1995 (31.9 ± 2.0 mm and 21.7 ± 3.3%, mean ± SE), which are comparable to baseline data from 1973 (28.5 mm and 25%, in a grazed patch within the kelp bed) before paramoebiasis was recorded in Nova Scotia (Fig. 5.1a,b). Recurrent disease outbreaks from 2009 to 2012 were followed by a decrease in both the mean test diameter of the largest 5% of the population and the percentage of adult sea urchins (Fig. 5.1a,b). Collectively, these temporal patterns in the size structure of the population suggest that disease has been limiting the survival of sea urchins to adulthood in kelp beds in St. Margarets Bay.

Changes in sea urchin density within kelp beds in relation to the timing of outbreaks of paramoebiasis provides a second line of evidence that disease is controlling sea urchin populations in St. Margarets Bay. Annual records of sea urchin density in kelp beds in the bay are available from 1992 to 1995 and 2009 to 2012 (excluding 2011). In
the absence of disease outbreaks between June 1992 and August 1995, sea urchin population density increased significantly within the kelp bed (ANOVA, $F_{3,23} = 9.05$, $p < 0.001$), from ~16 to 100 urchins m$^{-2}$, with adult density increasing 15-fold (Fig. 5.1c). Sea urchin density decreased significantly ($F_{2,29} = 21.49$, $p < 0.001$), from ~60 to 3 urchins m$^{-2}$, following recurrent outbreaks of paramoebiasis from 2009 to 2011. By June 2012, adult sea urchins were rare within the kelp bed (0.25 urchins m$^{-2}$). The density of adult sea urchins observed in the kelp bed in 1995 (~29 urchins m$^{-2}$) is below that observed in the early 1970s, during a sea urchin population outbreak that led to destructive grazing of the kelp bed (38 urchins m$^{-2}$ in 1973). These results, combined with the observed mass mortality events in 1995 and 2003, which arrested destructive grazing by an advancing sea urchin front at the deep margin of the kelp bed (Scheibling et al. 1999, Lyons & Scheibling 2008), suggest that disease exerts an important control on sea urchin populations within kelp beds in St. Margarets Bay. In consequence, there is a reduced potential for a phase shift from kelp forest to barrens.

Although this study covers a large temporal scale, it is limited spatially to a single site. To investigate potential variability in sea urchin density on the scale of kilometres, we sampled 10 sites throughout St. Margarets Bay in June 2010 (including Mill Cove), separated by 1.5 to 12.5 km (linear distance). These results indicate that mean sea urchin density (sampled in 1 m$^2$ quadrats, $n = 8$ per site) ranged from 1.1 to 11.2 urchins m$^{-2}$, with a grand mean ($\pm$ SD) of $5.0 \pm 3.3$ urchins m$^{-2}$ for the 10 sites (C. J. Feehan unpubl. data). Mean densities in June 2010 at Mill Cove ($6.9 \pm 7.7$ urchins m$^{-2}$) and Birchy Head ($5.0 \pm 1.4$ urchins m$^{-2}$), the sites used in our analysis (Table 5.1), approximate the grand mean for all 10 sites.

Along the coasts of Norway and Maine (USA) the major factors thought to control populations of *Strongylocentrotus droebachiensis* are settlement failure due to temperature-related mortality of sea urchin larvae (Fagerli et al. 2013) and predation by large decapods (Steneck et al. 2013), respectively. Peak settlement of sea urchins on artificial (plastic turf) collectors at Mill Cove in 2010 (mean $\pm$ SD, $n = 4$ collectors: $100 \pm 68$ settlers m$^{-2}$; C.J. Feehan unpubl. data) was similar to that recorded on similar collectors from 1992 to 1994 (mean $\pm$ SD, $n = 4$ years: $110 \pm 143$ settlers m$^{-2}$; Balch & Scheibling 2000), indicating little change in peak settlement over ~2 decades. Despite
mass mortalities of shallow populations of sea urchins in St. Margarets Bay, reproductive populations in deeper waters (where sea urchins have a thermal refuge from disease) may provide a relatively constant source of larvae (Filbee-Dexter & Scheibling 2012 and unpubl. data). The only predator abundance data available for St. Margarets Bay over the period of our study is catch per unit effort (CPUE) for lobster *Homarus americanus* (Lobster Fishing Area 33 East, Department of Fisheries and Oceans Canada (DFO) 2011). Although CPUE increased from 1990 (~ 0.15 kg trap$^{-1}$) to 2010 (~ 0.35 kg trap$^{-1}$), there was no change in CPUE between 1992 and 1995 (~ 0.15 kg trap$^{-1}$) when sea urchin density increased significantly in St. Margarets Bay (Fig. 5.1c). These data provide no evidence for a link between lobster abundance and sea urchin density in St. Margarets Bay, which is consistent with results of an earlier study (Scheibling 1984b).

Outbreaks of paramoebiasis are increasing in frequency along the coast of Nova Scotia due to changing oceanographic conditions associated with a changing ocean climate (Scheibling & Lauzon-Guay 2010, Scheibling et al. 2013). Large embayments such as St. Margarets Bay may be hotspots for paramoebiasis due to warmer peak temperatures and longer residence times of seawater, relative to the exposed coast (Feehan et al. 2012a). Recurrent disease outbreaks between 2009 and 2012 have nearly eliminated sea urchins in St. Margarets Bay. Based on the observed trend in disease outbreaks, we predict that populations of *Strongylocentrotus droebachiensis* will likely not recover in the shallow subtidal zone of the bay. In the absence of destructive grazing aggregations of sea urchins, kelp beds along the coast of Nova Scotia should persist for the foreseeable future. However, these kelp beds may be undergoing a new phase shift to turf algae dominance (e.g. filamentous red algae *Polysiphonia lanosa* and *Bonnemaisonia hamifera*), as evidenced by unusually low kelp density in St. Margarets Bay in 2012 (J. O’Brien & R.E. Scheibling unpubl. data). In recent years, large-scale shifts of perennial macrophytes to ephemeral filamentous algae have occurred in temperate regions throughout the world, in association with eutrophication, climate change, and changes in grazing pressure, epibionts and sedimentation (Eriksson et al. 2002, Connell et al. 2008, Anderson et al. 2011, Moy & Christie 2012, Wernberg et al. 2013). It remains to be seen whether large-scale shifts to turf algae also are occurring along the coast of Nova Scotia, and how this may alter the dynamics of kelp beds, sea urchins and disease.
Transmission of paramoebiasis is dosage dependent (Scheibling & Stephenson 1984); therefore, the extent and severity of a disease outbreak in barrens will likely influence the rate of mortality of sea urchins in adjacent kelp beds. In 1980 and 1981, widespread disease outbreaks caused near complete mortality of sea urchins in shallow barrens (< 25 m depth) across 100s of kilometres (linear distance) of Nova Scotian coastline (Scheibling 1986). The broad spatial extent of the host population in barrens at this time likely facilitated the propagation of the amoebic pathogen, accounting for the absence of adult sea urchins within the kelp bed in St. Margarets Bay in December 1982 (Fig. 5.1a,b, 5.2). In contrast, there were some surviving adult sea urchins in the kelp bed following disease outbreaks in 2009 to 2011 (Fig. 5.1a,b, 5.2). In recent years sea urchin barrens have been discontinuous along the coast, and absent within St. Margarets Bay. This lower density of the host sea urchin population may account for the survival of some sea urchins following recent disease outbreaks.

Predation has long been considered the major controlling agent of sea urchin abundance in kelp beds (Scheibling 1996). Although the settlement rate of *Strongylocentrotus droebachiensis* is similar in kelp beds and barrens in Nova Scotia, populations are generally less dense in kelp beds (Balch & Scheibling 2000). This pattern has been attributed to higher post-settlement mortality of sea urchins in kelp beds due to a higher abundance of predators. Sea urchin population outbreaks in St. Margarets Bay in the late 1960s and early 1970s were associated with a long-term reduction in predation pressure due to overfishing (reviewed by Scheibling 1996), and this may have contributed to major recruitment pulses during this period (Hart & Scheibling 1988, Meidel & Scheibling 2001). Despite the impact of historical overfishing, predation likely remains an important source of sea urchin post-settlement mortality in Nova Scotian kelp beds.

*Strongylocentrotus droebachiensis* is most susceptible to predation during the late juvenile to early adult life stage, since small juveniles can effectively utilize spatial refuges from predators (e.g. small-mouthed fish, decapod crustaceans, sea stars) and larger adults have a size refuge from most predators (Scheibling & Hamm 1991). This can create a bottleneck in the development of an adult population that results in a bimodal size frequency distribution (Scheibling 1996). Therefore, in a situation where predation is
the primary control of population growth, we expect that the size of the largest adults in a
developing population would progressively increase as some individuals escape
predation, but there would be a lag in the increase in the proportion of adults due to the
bottleneck. We did not observe a bimodal size distribution (Fig. 5.2), providing evidence
that predation is not the major control of sea urchin populations in kelp beds in St.
Margarets Bay. There does appear to be a slight lag in the increase in the percentage of
adults from 1982 to 1984, which may indicate that predation was impeding the initial
establishment of the sea urchin population. However, recruitment in the early 1990s
resulted in an exponential increase in the adult density (Fig. 5.1c), suggesting that
predation alone cannot prevent the establishment of sea urchin populations within these
kelp beds. In contrast, sea urchins were decimated by recurrent disease outbreaks in 2009
to 2011 (Fig. 5.1c). The non-occurrence of sea urchin population outbreaks and the
attendant formation of barrens in St. Margarets Bay in recent decades (since disease was
first recorded in 1980) suggests that disease has replaced predation as the major agent
controlling sea urchin populations.

Lafferty (2004) investigated the effects of fishing for sea urchin predators on sea
urchin density and incidence of disease. Using a 20-year dataset of kelp forest
communities at Channel Islands National Park, California, he found that outbreaks of
bacterial disease in sea urchins (*Strongylocentrotus purpuratus*, *S. franciscanus* and
*Lytechinus anameus*) were more frequent outside of a marine reserve than inside the
reserve (where protected populations of spiny lobster *Panulirus interruptus* limited the
population abundance of sea urchins). In Nova Scotia, the first documented outbreaks of
paramoebiasis also were associated with high sea urchin densities in barrens. However, in
contrast to the situation in Nova Scotia, bacterial disease in California did not fully
replace predation in controlling sea urchin populations, as evidenced by sustained high
densities of urchins and overgrazing of algae outside of the reserve.

Herbivorous sea urchins have been impacted by disease in other coastal
ecosystems worldwide. However, apart from Nova Scotia, the only documented case of
widespread (100s of km) mass mortality was an outbreak of an unidentified pathogen that
decimated *Diadema antillarum* on coral reefs throughout its geographic range in the
Caribbean in 1983 (Lessios 1988a). In Norway, *Strongylocentrotus droebachiensis* is
infected by an endoparasitic nematode, *Echinomermella matsi*, which decreases reproductive output and survival of adult sea urchins (Hagen 1992, Stien et al. 1998, Stien 1999) but does not cause mass mortality or phase shifts from barrens to kelp beds (Stien et al. 1995, reviewed by Norderhaug & Christie 2009). Bald sea urchin disease, a bacterial infection of sea urchins, has caused localized mass mortalities of sea urchins *Diadema aff. antillarum* in the Canary Islands, Spain (Dyková et al. 2011), *Paracentrotus lividus* in the Canary Islands and northwestern Mediterranean (Boudouresque & Verlaque 2007, Girard et al. 2012), and *Strongylocentrotus franciscanus* and *S. purpuratus* in the North Pacific (Rogers-Bennett 2007). While these systems are characterized by localized transitions from sea urchin barrens to macroalgal beds, the role of bacterial or macroparasitic disease in mediating these phase shifts is yet to be determined. This may be due in part to the interplay of multiple factors affecting sea urchin populations, such as recruitment variability and harvesting (Sala et al. 1998, Boudouresque & Verlaque 2007). Longitudinal studies, as we have shown here, can be particularly useful in elucidating the role of disease in controlling sea urchin populations in complex and dynamic marine ecosystems.
Fig. 5.1. (a) Mean test diameter (mm) of the largest 5% of the sea urchin population in kelp beds in St. Margarets Bay (SMB). The horizontal dashed line indicates average size at maturity (20 mm). Error bars are standard deviation of mean test diameter of the largest 5% of the population. (b) Percent of the population composed of adults (> 20 mm test diameter) in 15 years for which data were available between 1973 and 2012. For the years 1982 to 1995 (n = 11 for each regression), linear regression shows a significant positive relationship between mean test diameter of the largest 5% of the population and year (a), and percent of the population composed of adults and year (b). (c) Mean sea urchin density in kelp beds in SMB (urchins m⁻², total bar) divided into 3 size groups of sea urchins (early juvenile, < 10 mm; late juvenile, 10 – 20 mm; adult, > 20 mm) in 10 years for which data were available (1968 – 2012). Bars with no shading are total density when size-class data were not available. Error bars are standard error (SE) of mean total population density. Data in 1973 are for sea urchins in grazed patches within the kelp bed (P) and in the surrounding kelp bed (KB). The dominant ecosystem state in SMB is shown for the timeframe of the study; dashed sections indicate the initiation of urchin barrens due to destructive grazing by sea urchins. Black arrows (1980 – 2003) indicate outbreaks of paramoebiosis in barrens in SMB. Grey arrows (2009 – 2012) indicate outbreaks of paramoebiosis in experimentally transplanted sea urchins in the kelp bed. ND = No data. Data sources and sampling protocols are given in Table 5.1. a Data in 1973 were strongly influenced by a heavy sea urchin recruitment event within the kelp bed (Meidel & Scheibling 2001). b The outlier is based on a sample collected in May 1986, prior to the annual peak in settlement of *S. droebachiensis* in Nova Scotia (June/July; Balch & Scheibling 2000). Because sampling was conducted prior to a peak in settlement, early juveniles were rare in the kelp bed and the percent of the population composed of adults was consequently high (see also Fig. 5.2).
Fig. 5.2. Size-frequency distribution of sea urchin test diameters (mm) in kelp beds in St. Margarets Bay (SMB), Nova Scotia for 14 years for which data were available between 1982 and 2012, and in barren patches grazed by sea urchins within the kelp bed in 1973. Red arrows indicate a disease outbreak in adjacent sea urchin aggregations and barrens. Vertical lines indicate size-groups of sea urchins (early juvenile, < 10 mm; late juvenile, 10 – 20 mm; adult, > 20 mm). The percent of the population composed of adults is shown for each year. Horizontal dashed lines indicate breaks in the data record. n = number of individuals sampled. Data sources and sampling protocols are given in Table 5.1.
5.5. ACKNOWLEDGEMENTS

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CHAPTER 6

HARBOURING THE ENEMY: KELP HOLDFASTS PROTECT JUVENILE SEA URCHINS FROM PREDATORY CRABS

6.1. ABSTRACT

Predation is an important agent of post-settlement mortality of sea urchins that is mediated by the availability and suitability of spatial refuges, particularly during the vulnerable juvenile stage. In laboratory and field caging experiments, we show that holdfasts of a dominant kelp, *Saccharina latissima*, provide a spatial refuge for juvenile sea urchins *Strongylocentrotus droebachiensis* (< 20 mm, test diameter) from crabs *Cancer borealis* and *C. irroratus*, considered to be the dominant predators of sea urchins in kelp bed ecosystems in the Northwest Atlantic. In treatments with individual crabs of either species, the presence of holdfasts reduced predation on juvenile sea urchins by ~20 to 30% compared to treatments with no refuge. Crabs consumed juveniles (from 5 to 19 mm) in each of three 5 mm size classes in proportion to their abundance, regardless of treatment. In kelp beds in St. Margarets Bay, Nova Scotia, Canada, the number of juvenile sea urchins per holdfast ranged from 0.3 to 0.9, with juveniles in holdfasts accounting for two-thirds of the total urchin population density at one site. Up to 4 juveniles occurred within a single holdfast, and there was a significant positive relationship between juvenile size (but not number) and holdfast volume. Small adult sea urchins were not found within holdfasts in kelp beds and rarely occupied holdfasts presented to them in laboratory cages. Our findings indicate an ontogenetic shift in sea urchin-kelp interactions, whereby kelp facilitates recruitment of its major grazer.
6.2. INTRODUCTION

Ecosystem-level phase shifts from luxuriant kelp beds to an alternative sea urchin barrens state, characterized by a reduction in productivity, habitat complexity, and biodiversity, have been documented on temperate rocky reefs worldwide (Steneck et al. 2002, Filbee-Dexter & Scheibling 2014). The ecological and economic consequences of this ‘collapse’ to a barrens state, generally brought about through destructive grazing of kelp beds by sea urchins (North & Pearse 1970, Breen & Mann 1976b, Hagen 1983, Johnson et al. 2005), underscores the importance of understanding the mechanisms that determine sea urchin abundance and the resilience of the kelp state (Filbee-Dexter & Scheibling 2014). Population outbreaks of sea urchins resulting in a shift to the barrens state have been attributed to release from predation due to overfishing, which results in a trophic cascade (reviewed by Scheibling 1996, Steneck et al. 2004, Estes et al. 2010). Alternatively, high settlement rates associated with environmental anomalies (e.g. warm sea temperature) may lead to recruitment pulses of sea urchins that overwhelm predatory controls (Hart & Scheibling 1988, Hernández et al. 2010), leading to the eventual formation of destructive grazing aggregations (Lauzon-Guay & Scheibling 2010).

Sea urchins, like most benthic marine invertebrates, are subject to high rates of mortality at early life-history stages, as evidenced by order of magnitude declines in abundance following settlement of planktonic larvae (Rowley 1989, Scheibling & Raymond 1990, Hunt & Scheibling 1997). Predation is thought to be an important source of post-settlement mortality of sea urchins (Scheibling & Hamm 1991, McNaught 1999, Hereu et al. 2005, Scheibling & Robinson 2008, Jennings & Hunt 2010, Bonaviri et al. 2012, Clemente et al. 2013), although it is inherently difficult to study in the field, and these dynamics remain poorly resolved. Juvenile sea urchins are prey to a variety of benthic invertebrates and demersal fish (Keats et al. 1985, Scheibling & Hamm 1991, McNaught 1999, Scheibling & Robinson 2008, Jennings & Hunt 2010), while adults generally are vulnerable only to large-bodied predators such as sea otters, large fish and decapod crustaceans (Duggins 1980, Tegner & Dayton 1981, Hagen & Mann 1992, Shears & Babcock 2002). Post-settlement predation rate is mediated by the availability of spatial refuges, including biogenic (e.g. macroalgal turfs, mussel beds, adult spine

Along the Atlantic coast of Nova Scotia, Canada, destructive grazing by green sea urchins *Strongylocentrotus droebachiensis* drives a phase shift from a kelp bed to a barrens state that potentially is stable on a decadal scale (Mann 1977, Scheibling et al. 1999). A reverse shift back to kelp beds occurs when outbreaks of disease cause mass mortality of sea urchins, enabling kelps and other seaweeds to recolonize the rocky subtidal zone (Scheibling 1986, Scheibling et al. 2013). The reestablishment of sea urchin populations within emergent kelp beds following these mass mortality events occurs mainly through recruitment via the planktonic larval stage (Balch & Scheibling 2000). Predation on juvenile sea urchins is broadly considered to be a major determinant of recruitment success and the expansion of sea urchin populations within these kelp beds (reviewed by Scheibling 1996, Scheibling & Hatcher 2013; but see also Feehan & Scheibling 2014a).

Predation of *Strongylocentrotus droebachiensis* in the northwestern Atlantic is mediated by the availability and suitability of spatial refuges from a variety of predators, including small-mouthed fish (e.g. sculpin *Myoxocephalus octodecemspinosus*, cunner *Tautogolabrus adspersus*), decapod crustaceans (e.g. crabs *Cancer borealis*, *C. irroratus*) and sea stars (e.g. *Asterias vulgaris*) (Scheibling 1996, Scheibling & Hatcher 2013). Mortality due to predation is thought to be particularly high during the late juvenile and early adult phase of the benthic life history, when sea urchins outgrow small spatial refuges, such as crevices and interstices of cobbles, and move onto exposed rock surfaces to graze kelp (Himmelman 1986, Scheibling & Raymond 1990, Scheibling & Hamm 1991). Larger adult urchins reach a size refuge from most predators (Scheibling 1996). Bimodal size distributions observed for populations of *S. droebachiensis*, and other sea urchin species in temperate regions (e.g. *S. franciscanus* in California), have been attributed to high levels of predation on intermediate-sized sea urchins (Tegner & Dayton 1981, Tegner & Levin 1983, Scheibling & Hamm 1991). The ontogenetic transition
between juvenile and adult habitats, with high associated mortality, can create a bottleneck that limits the growth rate of the sea urchin population and confers resilience to the kelp-bed state (Feehan & Scheibling 2014a). Recent evidence indicates that large decapods, specifically cancriid crabs, have become the apex predators of sea urchins in the northwestern Atlantic because of overfishing of higher trophic level predators, such as large demersal fish (Steneck et al. 2004, 2013).

During a diving survey of populations of *Strongylocentrotus droebachiensis* in kelp beds in St. Margarets Bay, Nova Scotia, in June 2010, we observed juvenile sea urchins (< 20 mm test diameter, Meidel & Scheibling 2001) inhabiting holdfasts (anchoring structures) of the dominant kelp *Saccharina latissima*. Previous studies at sites with more wave exposure off adjacent headlands showed that kelp (*Laminaria digitata* and *S. latissima*) holdfasts provide a microhabitat for a variety of epifaunal and cryptofaunal invertebrates, including bivalves, sea stars, brittle stars and polychaetes (Schmidt & Scheibling 2006, Knip & Scheibling 2007, Scheibling & Lauzon-Guay 2007). Although juvenile *Strongylocentrotus droebachiensis* were not recorded within kelp holdfasts in these studies, young post-settlers (2 to 6 mm test diameter) have been observed on branched and undercut crustose coralline algae *Lithothamnion glaciale* (Keats et al. 1985, Scheibling & Raymond 1990) or turfs of finely branched arborescent coralline algae *Corallina officinalis* (R.E. Scheibling pers. obs.) in sea urchin barrens. It has been suggested that these macroalgal microhabitats provide juvenile sea urchins with a spatial refuge from predators (Keats et al. 1985, Scheibling & Robinson 2008). Here, we examine the hypothesis that holdfasts of the dominant kelp (*Saccharina latissima*) are a spatial refuge for juvenile *S. droebachiensis* from cancriid crabs (*Cancer borealis* and *C. irroratus*) using caging experiments in both field and laboratory settings. These crabs are abundant in Nova Scotian kelp beds and other macroalgal habitats (Schmidt & Scheibling 2007, Kelly et al. 2011) and have long been considered important predators of sea urchins (Bernstein et al. 1981, Scheibling & Hamm 1991). We also document the abundance and size distribution of sea urchins within kelp holdfasts in St. Margarets Bay and examine the relationship between holdfast size (volume of available space) and the number and size of resident sea urchins. Our findings indicate that holdfasts are indeed an important
microhabitat and spatial refuge for juvenile sea urchins and indicate an ontogenetic shift in sea urchin-kelp interactions, whereby kelp facilitates recruitment of its major grazer.

6.3. MATERIALS AND METHODS

6.3.1. Sampling of Sea Urchins in Kelp Holdfasts

To measure the abundance and size distribution of sea urchins Strongylocentrotus droebachiensis within kelp holdfasts, we used SCUBA to haphazardly sample adult sporophytes of the dominant species Saccharina latissima (> 1 m blade length) in kelp beds at 8 to 12 m depth from 2 sites located ~ 2 km apart (linear distance) in St. Margarets Bay, Nova Scotia (The Lodge: 44° 33.552’ N, 64° 01.869’ W; Birchy Head: 44° 34.473’ N, 64° 02.491’ W) in July 2010 and June and August 2011 (Table 6.1). Holdfasts were carefully loosened from the substratum using a dive knife, and the kelp blade and stipe were excised ~ 4 cm above the junction with the holdfast. Holdfasts were placed in separate plastic bags and transported to the laboratory, where they were dissected, and the associated sea urchins were counted and measured (test diameter, 0.1 mm accuracy) using vernier calipers.

We examined the relationship between the refuge space within a kelp holdfast and the number and size of associated sea urchins at both sites in June 2011. We estimated the available refuge space (\( V_{\text{refuge}} \), ml) by subtracting the volume occupied by the haptera (\( V_{\text{haptera}} \), ml) from the volume of a cone that approximated the shape of a holdfast (simplified from Jones 1971):

\[
V_{\text{refuge}} = \frac{1}{3} \pi r^2 h - V_{\text{haptera}}
\]

where \( r \) (mm) is the radius of the holdfast (average of the minimum and maximum diameter at the base divided by 2) and \( h \) (mm) is the height of the holdfast (measured parallel to the stipe). \( V_{\text{haptera}} \) is measured as the volume of water displaced by the holdfast (excluding the stipe). Simple linear regression was used to examine a relationship between size or number of associated sea urchins and holdfast volume.
To estimate the proportion of the sea urchin population inhabiting holdfasts within a kelp bed, we compared the estimated density within holdfasts to the total density of sea urchins measured in a haphazard sample of 1-m² quadrats at The Lodge in June 2010 (n = 8). Density in holdfasts was calculated by multiplying the mean number of individuals per holdfast, based on our sample in July 2010, by the average density of mature sporophytes of *Saccharina latissima* measured in a sample of 1-m² quadrats in June 2010 (n = 4).

The Kolmogorov-Smirnov 2-sample test was used to examine whether the size-frequency distribution of sea urchins in kelp holdfasts (pooled over samples collected in 2010 and 2011) differed from that of the total sea urchin population in the kelp bed (in all microhabitats, including holdfasts) at The Lodge in 2010.

### 6.3.2. Laboratory Experiments

To determine whether kelp holdfasts provide a spatial refuge to sea urchins from predatory crabs, we conducted laboratory experiments testing the survival of juvenile sea urchins (<20 mm) enclosed with a single crab (Jonah crab *Cancer borealis* or Atlantic rock crab *C. irroratus*) in 2.3 l hemispherical (25 cm diameter) plastic cages with or without holdfasts of *Saccharina latissima* as 2 levels of a refuge treatment (Fig. 6.1a). The cages were constructed from kitchen colanders that were slotted and perforated to provide 2 mm wide openings to permit water flow. For each unit, a second (top) colander served as a weighted lid for the cage (Fig. 6.1b). The cages were placed in seawater tables such that the water line (~12 cm depth at centre) was level with the top of the bottom colander, preventing sea urchins from fleeing onto the lid during the experiment. This hemispherical cage design eliminated refuge space for sea urchins in corners while enabling the crab to access the entire curved bottom area.

Divers collected juvenile sea urchins from urchin barrens and collected crabs (males, 75 to 120 mm carapace width) and holdfasts of *Saccharina latissima* (>1 m blade length) from kelp beds at sites between Halifax Harbour and St. Margarets Bay between May and August 2013. Crabs and sea urchins were maintained in laboratory aquaria with flowing oxygenated ambient seawater prior to use in experiments and fed ad
libitum on kelp and crushed adult sea urchins, respectively. Sea urchins and other associated fauna (e.g. brittle stars, polychaetes, bivalves) were removed from holdfasts before holdfasts were used in experiments.

For each experiment with a given species of crab, we conducted 4 or 5 trials blocked in time in a replicated block design (Table 6.2). For each trial, 12 cages, each containing 5 juvenile sea urchins from 3 size classes (1 urchin, 5 – 9 mm; 3 urchins, 10 – 14 mm; 1 urchin, 15 – 19 mm), were placed in ~ 140 l seawater tables with flowing (~ 3 l min⁻¹) ambient seawater. For the refuge treatment, we attached 5 holdfasts of Saccharina latissima (stipe excised ~ 4 cm above junction with holdfast) in each of 4 cages by pinning individual haptera to the bottom hemisphere of the cage with 10 cm (length) plastic cable ties to mimic attachment to a rocky substrate (Fig. 6.1a). This represents a density of 50 thalli m⁻² (based on surface area of the hemispherical cage bottom), which approximates the upper range of mean kelp density at Mill Cove in St. Margarets Bay (12 to 42 thalli m⁻²) and the midpoint of this range at Little Duck Island in neighbouring Mahone Bay (40 to 60 thalli m⁻²), recorded during intervals between defoliation events caused by an invasive bryozoan from 1992 to 2002 (Scheibling & Gagnon 2009).

After an acclimation period of 8 to 24 h (we observed that sea urchins took at least 8 h to enter and remain within holdfasts in the absence of a predator), we randomly assigned a single crab to each of 4 replicate cages in both the refuge (with holdfasts) and the no-refuge (no holdfasts) treatment. An additional 4 cages with sea urchins but without a crab or holdfasts acted as a control for other sources of sea urchin mortality (e.g. disease or stress). To reduce variability due to recent feeding history, crabs were starved for 48 h before use in each trial. Sea urchin mortality (proportion out of 5 urchins) in each cage was measured 48 h after crabs were added to the treatment cages. Two-way ANOVA was used to test for an effect of refuge (fixed factor, 2 levels: holdfasts, no refuge) and trial (random factor, 4 to 5 levels) on the proportion of sea urchins consumed by crabs (in separate tests for each crab species). At termination of each trial, the carapace width of crabs and test diameter of the surviving sea urchins were measured using a plastic measuring tape (1 mm accuracy). A chi-squared goodness-of-fit test was used to examine differences between observed and expected frequencies of 3 size classes of sea urchin (5 – 9, 10 – 14, 15 – 19 mm test diameter) surviving in cages with crabs.
Cancer borealis or C. irroratus) at 2 levels of a refuge treatment (holdfast refuge, no refuge). Expected frequencies were based on the null hypothesis of no size-selective predation. Data were pooled over trials in separate analyses for each crab species.

Individual crabs were used in a maximum of 2 trials and were allowed to acclimate for at least 1 week in the laboratory before each trial. Reusing crabs in different trials could potentially introduce a bias if larger and/or more voracious crabs were consistently used within a particular treatment. To minimize this risk, crabs were randomly assigned to cages within each trial. Inspection of the mean carapace width of crabs within treatments suggests no bias in crab size among treatments within trials (Table 6.2).

For the experiment with Cancer irroratus, seawater temperature was continuously recorded (1 h intervals) with a temperature logger (StowAway TidbiT Temperature Logger, Onset Computer) placed within one of the seawater tables. For the experiment with C. borealis, we used temperature records provided by the Aquatron, Dalhousie University (laboratory seawater source), for the May trial. For the trials in June and July, we obtained temperatures recorded at 7 to 8 m depth in Bedford Basin (~ 5 km from the Aquatron intake and at the same depth; J. Hackett pers. comm.), which we adjusted for warming (+ 1 °C) during transfer to our laboratory. The experimental array was illuminated by natural light from a large northwest-facing window.

To examine size-specific utilization of kelp holdfasts as refuge/habitat by sea urchins, we measured the tendency of sea urchins in 5 size classes (juveniles: 5 – 9, 10 – 14, 15 – 19 mm test diameter; small adults: 20 – 24, 25 – 29 mm) to enter and reside within holdfasts of Saccharina latissima in our experimental cages. In each of 3 trials blocked in time, we introduced 1 sea urchin from each of the 5 size classes to each of 4 cages with 5 attached holdfasts (1 holdfast per sea urchin, 1 urchin per size class per cage) in an unreplicated block design. Cages were maintained in seawater tables with flowing (~ 3 l min⁻¹) ambient seawater. After 24 h, we measured the proportion of sea urchins (out of 4) within each size class that were within a kelp holdfast. One-way ANOVA was used to examine differences among size classes (fixed factor, 5 levels) in the proportion of sea urchins residing within holdfasts. Tukey’s HSD test (α = 0.05) was used to compare means among levels of size class.
6.3.3. Field experiment

To examine whether kelp holdfasts provide a spatial refuge to sea urchins from predatory crabs under ambient conditions in the field, we conducted an experiment testing the survival of juvenile sea urchins (< 20 mm) enclosed with a single crab (*Cancer borealis*) in 200 l cylindrical cages (5 mm aperture nylon mesh) with or without holdfasts of *Saccharina latissima* as 2 levels of a refuge treatment (Fig. 6.1c,d). A cylindrical cage design (diameter = 50 cm, height = 100 cm) was used to minimize refuge space for sea urchins in corners. The bottom of each cage was reinforced with plastic-coated steel mesh to allow for attachment of holdfasts and bolted to a round plastic base (diameter = 75 cm) that was anchored to the seafloor with an iron weight (Fig. 6.1c). The mesh was attached to a hollow plastic ring (‘hula hoop’) at the top of the cage and suspended with small floats (Fig. 6.1c). The lid of the cage was fashioned from another plastic ring covered with mesh and attached to the cage top with plastic cable ties to allow divers access for observation (Fig. 6.1c). Crabs, sea urchins and kelp for the field experiment were collected from the same sites and over the same period as for the laboratory experiments (see above). Water temperature was recorded at 10 min intervals using a temperature logger (StowAway TidbiT Temperature Logger, Onset Computer) at 8 m depth at The Lodge (< 2 km south-southwest of Birchy Head).

We conducted 3 trials blocked over time in a replicated block design (Table 6.2). For each trial, 13 cages, each containing 10 juvenile sea urchins from 3 size classes (2 urchins, 5 – 9 mm test diameter; 6 urchins, 10 – 14 mm; 2 urchins, 15 – 19 mm), were deployed in a linear array at 8 m depth on a level sand patch within a kelp bed at Birchy Head. In each of 6 cages, we attached 10 kelp holdfasts (stipe excised ~ 4 cm above junction with holdfast) to the cage bottom (using the same method of attachment as in the laboratory experiments) for the holdfast refuge treatment (Fig. 6.1d). This represents a density of 50 thalli m\(^{-2}\) (based on surface area of the circular cage bottom), like that of our laboratory experiments. We manually placed sea urchins inside of holdfasts in the refuge treatment. We then added a single *Cancer borealis* (males, 70 – 120 mm carapace width) randomly assigned to each of the 6 replicate cages in both the refuge (with holdfasts) and the no-refuge (no holdfasts) treatment. A single cage with sea urchins but
no holdfasts or crab acted as a control for other sources of sea urchin mortality. Crabs were starved for 48 h before use in the field experiment to standardize recent feeding history. Individual crabs were used in a maximum of 2 trials and were allowed to acclimate in the laboratory for at least 1 week before each trial.

Video cameras (GoPro Hero2, Woodman Labs), with an extra battery pack (GoPro BacPac) to extend the battery life of the camera to 4.5 h, were mounted to the inside lid of cages of the refuge treatment to monitor sea urchin and crab behaviour at 30 s intervals (time-lapse recording). Predation was not observed during the first 4.5 h of the field experiment in time-lapse video of the holdfast refuge treatment, likely because crabs were still acclimating to the cages. Juvenile sea urchins were frequently observed moving into and out of holdfasts, suggesting that they also were acclimating to their surroundings during this period. Attempts by larger juveniles to move into holdfasts were sometimes unsuccessful. At the end of each trial, carapace width of crabs (mm) and test diameter (mm) of the surviving sea urchins were measured using a plastic measuring tape. Sea urchin mortality (proportion out of 10 urchins) in each treatment and control cage was measured 48 h after crabs were added to the treatment cages. Two-way ANOVA was used to test for an effect of refuge (fixed factor, 2 levels: holdfasts, no refuge) and trial (random factor, 3 levels) on the proportion of sea urchins consumed. A chi-squared goodness-of-fit test was used to examine differences between observed and expected frequencies of 3 size classes of sea urchin (5 – 9, 10 – 14, 15 – 19 mm test diameter) surviving in cages with crabs at 2 levels of a refuge treatment (holdfast refuge, no refuge). Expected frequencies were based on the null hypothesis of no size-selective predation. Data were pooled over trials for the analysis.

All statistical tests were run with Statistica 8 (StatSoft). Assumptions of homoscedasticity for ANOVA were tested using Cochran’s C-test ($\alpha = 0.05$).
Table 6.1. Dates and sites of sampling of holdfasts of *Saccharina latissima* (> 1 m blade length), indicating depth (m) of sample, sample size (n, number of holdfasts), total number of sea urchins *Strongylocentrotus droebachiensis* within holdfasts, number of sea urchins per holdfast (pooled or as a range for each site and sampling date), mean (± SD) test diameter (TD, mm) of sea urchins within holdfasts and mean (± SD) holdfast volume (ml). ND: no data.

<table>
<thead>
<tr>
<th>Date</th>
<th>Site</th>
<th>Depth (m)</th>
<th>Sample size (n)</th>
<th>Total no. urchins</th>
<th>No. urchins per holdfast</th>
<th>Urchin TD (mm)</th>
<th>Holdfast volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 Jul 2010</td>
<td>The Lodge</td>
<td>8–12</td>
<td>110</td>
<td>58</td>
<td>0.53</td>
<td>9.8 ± 3.5</td>
<td>ND</td>
</tr>
<tr>
<td>23 Jun 2011</td>
<td>The Lodge</td>
<td>8–12</td>
<td>35</td>
<td>16</td>
<td>0.46</td>
<td>6.0 ± 4.8</td>
<td>90 ± 92</td>
</tr>
<tr>
<td>30 Jun 2011</td>
<td>Birchy Head</td>
<td>12</td>
<td>10</td>
<td>9</td>
<td>0.90</td>
<td>5.8 ± 1.6</td>
<td>48 ± 26</td>
</tr>
<tr>
<td>24 Aug 2011</td>
<td>The Lodge</td>
<td>8–12</td>
<td>59</td>
<td>17</td>
<td>0.29</td>
<td>9.6 ± 4.8</td>
<td>ND</td>
</tr>
</tbody>
</table>
Table 6.2. Summary of field and laboratory experiments investigating kelp holdfasts as a refuge for juvenile sea urchins *Strongylocentrotus droebachiensis* from predatory crabs *Cancer borealis* and *C. irroratus*, indicating the number of experimental trials, start date of trials in 2013, mean (± SD) seawater temperature (°C), mean (± SE) carapace width of crabs (CW, mm) and mean (± SE) predation rate of crabs (urchins crab$^{-1}$ d$^{-1}$) in cages at 2 levels of a refuge treatment (holdfasts, no refuge) (n = 4).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Trial #</th>
<th>Start</th>
<th>Water temperature (°C)</th>
<th>CW of crabs (mm)</th>
<th>Predation rate (urchin crab$^{-1}$ d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Holdfasts</td>
<td>No Refuge</td>
</tr>
<tr>
<td><strong>Laboratory</strong></td>
<td></td>
<td></td>
<td></td>
<td>Holdfasts</td>
<td>No Refuge</td>
</tr>
<tr>
<td><em>Cancer</em></td>
<td>1</td>
<td>24 May</td>
<td>8.1 ± 0.4</td>
<td>86 ± 3</td>
<td>85 ± 4</td>
</tr>
<tr>
<td><em>borealis</em></td>
<td>2</td>
<td>11 June</td>
<td>6.2 ± 0.1</td>
<td>99 ± 2</td>
<td>84 ± 5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>26 June</td>
<td>8.1 ± 0.1</td>
<td>91 ± 6</td>
<td>101 ± 2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>17 July</td>
<td>10.2 ± 0.3</td>
<td>101 ± 3</td>
<td>105 ± 3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>25 July</td>
<td>10.4 ± 0.3</td>
<td>103 ± 3</td>
<td>104 ± 6</td>
</tr>
<tr>
<td><em>Cancer</em></td>
<td>1</td>
<td>29 July</td>
<td>9.7 ± 0.4</td>
<td>83 ± 1</td>
<td>83 ± 3</td>
</tr>
<tr>
<td><em>irroratus</em></td>
<td>2</td>
<td>10 August</td>
<td>8.8 ± 0.3</td>
<td>85 ± 4</td>
<td>80 ± 2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>24 August</td>
<td>8.9 ± 0.4</td>
<td>79 ± 3</td>
<td>86 ± 1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>27 August</td>
<td>10.3 ± 0.2</td>
<td>78 ± 3</td>
<td>81 ± 1</td>
</tr>
<tr>
<td><strong>Field</strong></td>
<td></td>
<td></td>
<td></td>
<td>Holdfasts</td>
<td>No Refuge</td>
</tr>
<tr>
<td><em>Cancer</em></td>
<td>1</td>
<td>31 July</td>
<td>11.4 ± 0.9</td>
<td>103 ± 2</td>
<td>103 ± 3</td>
</tr>
<tr>
<td><em>borealis</em></td>
<td>2</td>
<td>13 August</td>
<td>8.6 ± 0.7</td>
<td>99 ± 4</td>
<td>100 ± 2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>24 August</td>
<td>7.3 ± 0.8</td>
<td>97 ± 8</td>
<td>90 ± 6</td>
</tr>
</tbody>
</table>
Fig. 6.1. Refuge treatment of the laboratory (a) and field (d) experiment showing juvenile sea urchins *Strongylocentrotus droebachiensis* and a crab *Cancer irroratus* (a) or *C. borealis* (d) amid attached holdfasts of the kelp *Saccharina latissima*. (b) Laboratory caged constructed from two 25 cm diameter plastic colanders, one stacked on top of the other and held in place by a lead weight, used to enclose juvenile sea urchins with a predatory crab and with or without holdfasts as a spatial refuge. The bottom colander was immersed (~12 cm depth at the centre) in a flow-through seawater table, providing a barrier to sea urchins at the air-water interface. (c) Field cage (50 cm diameter, 100 cm height, 5 mm mesh) used to enclose sea urchins with a predatory crab and with or without holdfasts as a spatial refuge. Cages were placed in a linear array on a level sand patch within a kelp bed at Birchy Head, St. Margarets Bay. (Photographs: (a,b,d) C.J. Feehan, (c) A. Pinder)
6.4. RESULTS

6.4.1. Field Observations

The number of *Strongylocentrotus droebachiensis* per holdfast of *Saccharina latissima* ranged from 0.29 to 0.90 in samples from kelp beds in St. Margarets Bay in summer 2010 and 2011 (Table 6.1). Up to 4 sea urchins occurred within a single holdfast. The mean test diameter of *S. droebachiensis* in these samples ranged from 5.8 to 9.8 mm (Table 6.1) about a grand mean of 8.8 mm (Fig. 6.2a). Sea urchins > 20 mm (approximate size at sexual maturity; Meidel & Scheibling 2001) were not observed in holdfasts (Fig. 6.2a). There was a significant positive relationship between the mean test diameter of sea urchins within a holdfast and holdfast volume (Fig. 6.3) but no relationship between the number of sea urchins within a holdfast and holdfast volume (Table 6.1). The mean ± SD density of adults of *S. latissima* (> 1 m blade length) at The Lodge in summer 2010 was 6.8 ± 4.0 sporophytes m\(^{-2}\), giving an estimated mean density of sea urchins within holdfasts of 3.6 ± 2.1 urchins m\(^{-2}\), two-thirds of the total population density in the kelp bed (5.5 ± 6.8 urchins m\(^{-2}\)). The majority (~ 98 %) of the sea urchin population within the kelp bed in summer 2010 was composed of juveniles (< 20 mm) (Fig. 6.2b). The size-frequency distribution of sea urchins in kelp holdfasts (pooled over samples collected in 2010 and 2011) did not differ from that of the total sea urchin population in the kelp bed (in all microhabitats, including holdfasts) at The Lodge in 2010 (Kolmogorov-Smirnov 2-sample test: \(D_{44,100} = 0.071, p > 0.10\)) (Fig. 6.2). Sea urchin density at The Lodge in 2010 approximated the grand mean (5.0 ± 3.3 urchins m\(^{-2}\)) for kelp beds at 10 sites (including The Lodge) sampled throughout St. Margarets Bay at that time (Feehan & Scheibling 2014a).

6.4.2. Experimental Results

In laboratory experiments with predatory crabs, we found significantly lower mortality of juvenile sea urchins in treatments with kelp holdfasts than in those with no refuge, for both *Cancer irroratus* and *C. borealis* (Table 6.3, Fig. 6.4). Similarly, in a
field experiment with *C. borealis*, we found significantly lower mortality of juvenile sea urchins in cages with kelp holdfasts compared to cages with no refuge (Table 6.3, Fig. 6.5). Most of the surviving sea urchins were found within holdfasts at the end of each experimental trial: available data indicate that > 99 and 86 ± 13 % (SD) of surviving sea urchins were within holdfasts at the end of trials 1 to 3 in laboratory and field experiments with *C. borealis*, respectively. Predation by both species of crab resulted in broken sea urchin test fragments within cages. We observed no mortality of sea urchins in control treatments without holdfasts or a crab in both the laboratory and field experiments. Predation rate on sea urchins tended to be higher for *C. borealis* than for *C. irroratus* in the holdfast refuge treatment in the laboratory experiments (grand mean ± SE of trials: 1.0 ± 0.2 vs. 0.5 ± 0.2 urchins crab⁻¹ d⁻¹, respectively; t₇ = 2.35, p = 0.051) but did not differ among species in the no-refuge treatment (1.6 ± 0.2 vs. 1.3 ± 0.2 urchins crab⁻¹ d⁻¹, respectively; t₇ = 1.07, p = 0.32) (Table 6.2). The mean size (carapace width) of *C. borealis* was greater than that of *C. irroratus* in both treatments (holdfast refuge: t₇ = 3.75, p < 0.01; no refuge: t₇ = 2.46, p = 0.044) (Table 6.2). Predation rates by *C. borealis* were significantly higher in the field than in the laboratory in both treatments (holdfast refuge: 2.0 ± 0.3 vs. 1.0 ± 0.2 urchins crab⁻¹ d⁻¹, respectively; t₆ = 3.26, p = 0.017; no refuge: 3.0 ± 0.4 vs. 1.6 ± 0.2 urchins crab⁻¹ d⁻¹, respectively; t₆ = 3.70, p = 0.010) (Table 6.2). There was no difference in the size of *C. borealis* in field and laboratory experiments (t₁₄ = 0.727, p = 0.48) (Table 6.2). Predation rates of crabs do not appear to be related to seawater temperature during each experiment (Table 6.2). In both the laboratory and field experiment, the size-frequency distribution of surviving sea urchins (in three 5 cm size classes, pooled across all trials) in cages with crabs (*C. irroratus* or *C. borealis*) did not differ from the expected size-frequency based on the null hypothesis of no size-selective predation (i.e. consumption by crabs was proportional to initial abundance in a size class) in either treatment (holdfast refuge, no refuge) (Table 6.4).

There was a significant difference among size classes in the proportion of sea urchins residing within holdfasts after 24 h of enclosure in laboratory cages (1-way ANOVA: F₄,10 = 11.455, p < 0.001) (Fig. 6.6). Two separate groups emerged, with a significantly higher proportion of sea urchins from smaller, juvenile size classes (< 20
mm) within holdfasts compared to sea urchins from larger, adult size classes (20 – 29 mm) (Tukey’s test, p < 0.001, Fig. 6.6).

Table 6.3. Two-way ANOVA of the effect of refuge (fixed factor, 2 levels: holdfasts, no refuge) and trial (random factor, 3 to 5 levels) on the proportion of sea urchins Strongylocentrotus droebachiensis consumed by a crab after 48 h in laboratory cages (Cancer borealis or C. irroratus) or field cages (C. borealis). Refuge and Trial were tested against the pooled interaction (Refuge × Trial) and error MS. Bold values are significant at α = 0.05. Data for all tests conform to the assumptions of normality and homoscedasticity (Cochran’s C-test, α = 0.05).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Laboratory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer borealis</td>
<td>Refuge</td>
<td>1</td>
<td>0.576</td>
<td>4.604</td>
<td><strong>0.039</strong></td>
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<tr>
<td></td>
<td>Trial</td>
<td>4</td>
<td>0.149</td>
<td>1.187</td>
<td>0.334</td>
</tr>
<tr>
<td></td>
<td>Refuge x Trial</td>
<td>4</td>
<td>0.009</td>
<td>0.060</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>30</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cancer irroratus</td>
<td>Refuge</td>
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<td>0.845</td>
<td>5.465</td>
<td><strong>0.027</strong></td>
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<tr>
<td></td>
<td>Trial</td>
<td>3</td>
<td>0.113</td>
<td>0.733</td>
<td>0.541</td>
</tr>
<tr>
<td></td>
<td>Refuge x Trial</td>
<td>3</td>
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<td>0.520</td>
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<td>Error</td>
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<td>0.163</td>
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<tr>
<td>Cancer borealis</td>
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<td></td>
<td>Trial</td>
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<td>0.177</td>
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<tr>
<td></td>
<td>Refuge x Trial</td>
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<td>0.020</td>
<td>0.314</td>
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<tr>
<td></td>
<td>Error</td>
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<td>0.065</td>
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Table 6.4. Chi-squared goodness-of-fit ($\chi^2$) test for difference between observed and expected frequencies of 3 size classes of sea urchins *Strongylocentrotus droebachiensis* (5 – 9, 10 – 14, 15 – 19 mm test diameter) surviving in cages with crabs *Cancer irroratus* or *C. borealis* at 2 levels of a refuge treatment (holdfast refuge, no refuge). Expected frequencies were based on the null hypothesis of no size-selective predation. Data are pooled over 2 to 5 trials for each analysis; $N =$ total number of surviving urchins.

<table>
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<tr>
<th>Experiment</th>
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<th>df</th>
<th>$\chi^2$</th>
<th>p</th>
</tr>
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<td><em>Cancer borealis</em></td>
<td>Holdfasts</td>
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<td>0.926</td>
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<td></td>
<td>No refuge</td>
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<tr>
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<td>Holdfasts</td>
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<td>2</td>
<td>5.333</td>
<td>0.069</td>
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<tr>
<td></td>
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<td>2</td>
<td>0.607</td>
<td>0.738</td>
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Fig. 6.2. Size-frequency (test diameter, mm) distributions of sea urchins *Strongylocentrotus droebachiensis* sampled in (a) holdfasts of *Saccharina latissima* in kelp beds in St. Margarets Bay, Nova Scotia, in summer 2010 and 2011 (see Table 6.1 for sample sites and dates); and (b) all microhabitats in a kelp bed at The Lodge, St. Margarets Bay, in June 2010.
Fig. 6.3. Relationship between size of sea urchins *Strongylocentrotus droebachiensis* (test diameter, mm) and kelp *Saccharina latissima* holdfast volume (ml). Data are from a sample of 15 holdfasts from kelp beds in St. Margarets Bay in June 2011 (Table 6.1). Error bars are ± SD of mean test diameter for holdfasts with 2 to 4 sea urchins.
Fig. 6.4. Proportion of mortality of sea urchin *Strongylocentrotus droebachiensis* juveniles (out of 5) exposed for 48 h to a single crab, (a) *Cancer borealis* or (b) *C. irroratus*, in laboratory cages at 2 levels of a refuge treatment (5 kelp holdfasts, no holdfasts). Data are mean (+ SE) for 4 replicate cages for each of 4 or 5 experimental trials and grand mean (+ SE) for all trials.
Fig. 6.5. Proportion of mortality of sea urchin *Strongylocentrotus droebachiensis* juveniles (out of 10) exposed for 48 h to a single crab, *Cancer borealis*, in field cages at 2 levels of a refuge treatment (10 kelp holdfasts, no holdfasts). Data are mean proportion of urchin mortality (+ SE) for 6 replicate cages for each of 3 experimental trials and a grand mean (+ SE) for all trials.
Fig. 6.6. Proportion of sea urchins *Strongylocentrotus droebachiensis* (out of 4) within each of 5 size classes (test diameter, mm) found within kelp holdfasts after 24 h in laboratory cages. The vertical line indicates approximate size at maturity of sea urchins (20 mm). Error bars are ± SE for n = 3 experimental trials.

6.5. DISCUSSION

6.5.1. Kelp Holdfasts as Spatial Refugia

We found that holdfasts of the dominant kelp *Saccharina latissima* act as an important refuge habitat to juvenile sea urchins *Strongylocentrotus droebachiensis* in Nova Scotian kelp beds. Kelp holdfasts reduced the vulnerability of juvenile sea urchins (< 20 mm test diameter) to predation by cancrid crabs (*Cancer borealis* and *C. irroratus*) by ~ 20 to 30 % in field and laboratory caging experiments. Most surviving sea urchins exposed to a crab in the holdfast refuge treatment were found within holdfasts at the end of experimental trials in both the laboratory and field experiment, indicating that the holdfasts provided a spatial refuge from predation. These crabs currently are considered the dominant predators of *S. droebachiensis* in kelp beds and other macroalgal habitats in
the Northwest Atlantic (Scheibling 1996, Steneck et al. 2002) and can reach densities of 0.5 to 2.3 crabs m\(^{-2}\) in the summer and fall in Nova Scotia (Schmidt & Scheibling 2007, Kelly et al. 2011). Any reduction in vulnerability to crab predation afforded by kelp holdfasts (this study), or other biogenic or abiotic refuges (Keats et al. 1985, Witman 1985, Scheibling & Raymond 1990, Scheibling & Hamm 1991), is likely to greatly influence sea urchin recruitment rates.

The maximum size threshold of sea urchins that can effectively use kelp holdfasts as a spatial refuge occurs at ~ 20 mm test diameter. We did not observe adult sea urchins within holdfasts in the kelp beds that we sampled in summer 2010 and 2011. Similarly, we found that adult sea urchins (>20 mm) were less likely to move into and remain within holdfasts in our laboratory experiment that examined size-specific utilization of holdfasts. Time-lapse video of our field experiment showed that some large juvenile sea urchins (15 to 19 mm) that attempted to move into holdfasts were unsuccessful during the first 4.5 h in cages with crabs (Cancer borealis). However, there was no evidence of size-selective predation among the 3 experimental size classes of juvenile sea urchins in field or laboratory cages with crabs (C. irroratus or C. borealis), indicating that holdfasts likely provided protection from crab predation across the entire juvenile size range.

In our laboratory experiments, Cancer irroratus and C. borealis exhibited similar predation rates on juvenile sea urchins in the no-refuge treatment; however, C. borealis was a more effective predator in the refuge treatment with holdfasts. We occasionally observed individuals of C. borealis severing the haptera of holdfasts using their claws, suggesting that the larger claw size of C. borealis may allow it to more easily extract sea urchins from holdfasts than C. irroratus of similar body size. It should be noted that this behaviour could be an artifact of laboratory containment, given that crabs were not offered alternative prey that may have involved less handling effort (Wong & Barbeau 2006). Moody & Steneck (1993) also found that C. borealis exhibits different foraging tactics than C. irroratus on blue mussel Mytilus edulis (e.g. C. borealis utilized only crushing tactics, while C. irroratus was more dexterous and utilized a greater variety of tactics). We found that predation rate on sea urchins by C. borealis was 2-fold greater in field than in laboratory cages (Table 6.2). Given that crab size and water temperature
were similar in field and laboratory experiments, we attribute this difference in predation rate to cage design or some other artifact of laboratory containment.

Kelp holdfasts have been shown to harbour a broad range of algal, invertebrate and fish species in kelp beds worldwide (Ghelardi 1971, Ojeda & Santelices 1984, Anderson et al. 1997, Christie et al. 2003, Schmidt & Scheibling 2006, Knip & Scheibling 2007, Blight & Thompson 2008, Schaal et al. 2012). Studies in the northeastern and southeastern Pacific indicate that juvenile sea urchins frequently shelter within kelp holdfasts (Dayton 1975, Vasquez et al. 1984, Pearse & Hines 1987, Tegner et al. 1995). Pearse & Hines (1987) showed that juvenile *Strongylocentrotus* spp. in California, USA, move into kelp holdfasts once they have outgrown other spatial refuges, such as crevices. Dayton (1975) observed juvenile *Strongylocentrotus* sp. in holdfasts of *Laminaria* spp. in Alaska, USA, where sea urchin populations are strongly controlled by sea otter predation. He noted that sea urchins disappeared from a study site when the kelp canopy cover was removed, suggesting that kelp provides a refuge from predation by sea otters. Along the southwestern coast of South Africa, holdfasts of kelp *Ecklonia maxima* facilitate recruitment of juvenile conspecifics by providing a refuge from grazers such as sea urchins, abalone, limpets and gastropods (Anderson et al. 1997).

Christie et al. (2003) found a significant positive relationship between the total number of individuals within holdfasts of *Laminaria hyperborea* in Norway (incorporating up to 77 faunal species, including sea urchins *Echinus esculentus* and *Psammechinus miliaris*) and holdfast volume. Although we did not observe a direct relationship between sea urchin abundance and holdfast volume of *Saccharina latissima*, there were numerous other invertebrates within the holdfast that were not considered. Knip & Scheibling (2007) recorded 15 taxa (family, genus or species level) from 6 phyla in holdfasts of *L. digitata* in a kelp bed along a headland (Splitnose Point) ~ 40 km east-southeast of St. Margarets Bay. *Strongylocentrotus droebachiensis* was not recorded, possibly because the haptera of *L. digitata* in wave-exposed habitats are tightly applied to the rock substratum, leaving less refuge space for juvenile sea urchins. These holdfasts are filled by small bivalves and by brittle stars and sea stars that can conform to small and irregular gaps among haptera.
We found that the size of sea urchins within holdfasts was directly related to the volume of space within a holdfast. Larger holdfasts can provide a spatial refuge to sea urchins in late juvenile or early adult stages that may be most vulnerable to predation, as they have outgrown smaller physical refuges (e.g. narrow crevices, interstices of cobbles) but have not yet reached a size refuge from small-mouthed fish and decapod predators (Scheibling & Hamm 1991). Mesopredators of juvenile sea urchins, such as sea stars and polychaetes, were rare within kelp holdfasts in our study, with only single individuals of *Asterias* sp., *Nereis* sp., or Polynoidae observed in a total of 45 holdfasts sampled in June 2011. We observed no predatory decapods (e.g. juvenile canid crabs) within holdfasts. This suggests that predation pressure on juvenile sea urchins within holdfasts may be minimal.

Tegner et al. (1995) found that juveniles of *Strongylocentrotus purpuratus* and *S. franciscanus* graze holdfasts of giant kelp *Macrocystis pyrifera* in California, forming cavities that increase susceptibility of kelp to breakage during storms, thereby increasing kelp mortality. We found no evidence that juvenile *S. droebachiensis* cause appreciable damage to holdfasts of *Saccharina latissima*, likely because they are sustained by particulate algal detritus actively or passively trapped within the holdfast microhabitat (Bernstein et al. 1981, Rowley 1990, Scheibling & Hamm 1991, Dumont et al. 2004).

**6.5.2. Facilitation of Sea Urchin Recruitment by Kelp: Consequences for Kelp Bed Resilience**

Positive feedback mechanisms within both kelp beds and barrens increase the resilience of each state to phase shifts, and these often involve facilitation or inhibition of sea urchin recruitment (reviewed by Filbee-Dexter & Scheibling 2014). For example, a higher cover of coralline algae in barrens compared to kelp beds may result in increased settlement of sea urchin larvae in barrens due to induction by coralline algae (Pearce & Scheibling 1990, Baskett & Salomon 2010). Conversely, the lower cover of coralline algae in kelp beds promotes persistence of kelp beds by reducing settlement rates of sea urchins (Baskett & Salomon 2010). Other feedback mechanisms stabilizing the kelp bed state include increased habitat available for benthic macroinvertebrates and demersal fish that prey on sea urchins; the whiplash effect of wave-driven kelp fronds, which impedes
sea urchin grazing; and the production of detrital algae within the kelp bed, which promotes passive detritivory rather than destructive grazing by resident sea urchins (Filbee-Dexter & Scheibling 2014).

Adult sea urchins were rare in the kelp bed sampled in St. Margarets Bay in summer 2010, following an epizootic that caused a mass mortality of sea urchins the previous fall (Feehan & Scheibling 2014a). Our results suggest that kelp holdfasts can act to facilitate the reestablishment of sea urchin populations in kelp beds after disease outbreaks by providing refuge to juvenile urchins from predators such as cancrid crabs. These crabs are now considered apex predators in kelp beds in the northwestern Atlantic as a consequence of historical overfishing of higher trophic level predators, such as large demersal fish (Steneck et al. 2004). In a recent review, Filbee-Dexter & Scheibling (2014) conclude that while positive feedbacks that strengthen resilience of kelp bed ecosystems are relatively well known, examples of negative feedbacks that could destabilize kelp ecosystems are generally lacking. Given that dense populations of *S. droebachiensis* destructively graze kelp beds in the northwestern Atlantic, our findings suggest a potentially important negative feedback mechanism whereby a dominant kelp facilitates recruitment of its major grazer. This also underscores the importance of considering ontogenetic shifts in predator-prey interactions that can govern ecosystem dynamics.
6.6. ACKNOWLEDGEMENTS

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CHAPTER 7

OCEANOGRAPHIC AND METEOROLOGICAL PROCESSES MEDIATING SEA URCHIN DISEASE OUTBREAKS IN NOVA SCOTIA

7.1. ABSTRACT

Along the coast of Nova Scotia disease outbreaks in sea urchins are linked to North Atlantic hurricanes and warm sea temperatures. The pathogen (*Paramoeba invadens*) is unable to withstand minimum sea temperatures along this coast, and is reintroduced periodically with storms during periods of peak temperatures. However, a mechanistic understanding of this process is lacking and the nature of source populations of *P. invadens* remains unknown. We conducted a 5-year field experiment (2010 – 2014) monitoring disease outbreaks in and around St. Margarets Bay, Nova Scotia, and analyzed these data in combination with reports of hurricane activity and available oceanographic (sea temperature, waves) and meteorological (winds) data to 1) evaluate the reliability of a logistic regression model linking disease outbreaks to hurricanes and warm sea temperatures, and 2) test hypotheses for a mechanism of introduction of *P. invadens* with storms. Disease outbreaks were observed in 4 years, with the onset of mass mortality (≥ 50 % morbidity or mortality) ranging from early August to mid October. We found strong support for the logistic regression model to predict a disease outbreak based on hurricane activity and sea temperature in 2010 and 2011. In 2012 a disease outbreak occurred in the absence of a storm and was preceded by a strong positive anomaly in winter sea temperature, suggesting survival of the pathogen from the previous year. In 2014 a disease outbreak occurred in association with a strong fall storm that was not categorized as a hurricane. Available physical data favour the hypothesis that *P. invadens* originates in warm offshore surface waters that are horizontally transported to the coast during a storm. However, these inferences remain equivocal, indicating the need for high-resolution dynamical modelling of ocean circulation and rapid identification of *P. invadens* in the water column or sediments using genetic tools.
7.2. INTRODUCTION

Identification of physical mechanisms involved in episodic biological events and the transport of biological material in the marine environment is of growing interest in studies of biological invasions (Byers & Pringle 2006), population connectivity (Cowen & Sponaugle 2009), and infectious disease (Burge et al. 2014). Regarding the latter, correlative evidence exists for an association between increasing disease outbreaks in marine organisms and changing environmental factors, including increasing sea temperatures and frequency or intensity of storms, and shifts in ocean currents (Burge et al. 2014). Given that disease outbreaks can have important implications for ecosystem structure and functioning, there is need for interdisciplinary studies that examine physical-biological coupling in the dynamics of marine disease. This is particularly critical given ongoing changes in the marine environment as a result of anthropogenic stresses such as climate change.

Recurrent disease outbreaks in herbivorous sea urchins *Strongylocentrotus droebachiensis* along the Atlantic coast of Nova Scotia, Canada have resulted in the near-collapse of a once thriving sea urchin fishery, and the stabilization of a macroalgal-dominated ecosystem (Scheibling et al. 2013). A logistic regression model based on 30 years of field data (1980 – 2009) indicates that mass mortalities of sea urchins are associated with hurricanes and warm sea temperatures (Scheibling & Lauzon-Guay 2010). Specifically, mass mortalities due to disease tend to occur following storms with high maximum sustained wind speeds and that track close to the coast of Nova Scotia, and the probability of a mass mortality increases when sea surface temperatures (0 – 10 m depth) following a hurricane are above a threshold of 12 ºC (Scheibling & Lauzon-Guay 2010). This is consistent with field and laboratory observations that the disease only propagates at sea temperatures exceeding 10 – 12 ºC (Scheibling & Stephenson 1984, Feehan et al. 2012a).

An amoebic pathogen (*Paramoeba invadens*) has been identified as the causative agent of this disease, termed paramoebiasis (Jones & Scheibling 1985, Feehan et al. 2013). *P. invadens* has a lower thermal tolerance limit (~ 2 ºC) that is above the typical annual minimum sea temperature in the shallow subtidal zone of Nova Scotia (-1 to 0
°C), suggesting that it is an exotic pathogen periodically introduced during periods of annual maximum temperatures and eradicated over the winter months (Jellett & Scheibling 1988a; R. Buchwald, C.J. Feehan, R.E. Scheibling, A.G.B Simpson, in review). Laboratory culturing of *P. invadens* (Feehan et al. 2013) and field sampling of seabed sediments (Jellett et al. 1989, R. Buchwald unpubl. data) indicate that *P. invadens* is a facultative parasite that can survive in the marine environment outside of its sea urchin host. Three non-mutually exclusive hypotheses have been proposed to explain the association of sea urchin disease outbreaks with hurricanes: 1) *P. invadens* is transported to the coast of Nova Scotia from source populations in warm offshore surface waters by horizontal advection during a storm event (Scheibling & Hennigar 1997), 2) *P. invadens* is free-living in deep sedimentary basins near the coast that remain above its lower temperature threshold for survival year-round, and is vertically resuspended into shallow coastal waters during a storm event (Scheibling & Lauzon-Guay 2010), and 3) disease can recur in the absence of a storm when an outbreak in the previous summer/fall is followed by winter sea temperatures in the shallow subtidal zone that remain above the lower temperature threshold (Scheibling et al. 2013).

A previous field experiment based on a single year of data provides support for the reliability of the logistic regression model (Scheibling & Lauzon-Guay 2010) to predict a localized disease outbreak based on hurricane activity and sea temperature (Feehan et al. 2012a). However, multiple years of data are required to critically assess the model (Feehan et al. 2012a) and to examine the above hypotheses for the introduction of *Paramoeba invadens* with storms. Here, we conduct a 5-year field experiment (2010 – 2014) monitoring sea urchin disease outbreaks in and around St. Margarets Bay, Nova Scotia. We analyze these data, along with those from another experiment in the bay in 2009 and records of disease outbreaks in other areas of the coast within this timeframe, in relation to hurricane activity and oceanographic (sea temperature, significant wave height) and meteorological (wind, atmospheric pressure and temperature) data acquired from local oceanographic buoys, temperature loggers, or weather stations.
7.3. MATERIALS AND METHODS

7.3.1. Field Experiment

Outbreaks of disease in *Strongylocentrotus droebachiensis* were monitored at sites in and around St. Margarets Bay, Nova Scotia (Fig. 7.1) in a field experiment from 2010 to 2014, as per the methods of Feehan et al. (2012a). In mid July or early August, following a seasonal increase in sea temperature in the shallow subtidal zone to > 10 °C (approximate lower threshold for infective paramoebiosis), adult sea urchins (> 30 mm test diameter) were transplanted from a barrens habitat at 8 m depth at a headland (Splitnose Point), where sea urchins have persisted for over a decade, into kelp beds at St. Margarets Bay, where sea urchins have experienced periodic recurrent mass mortalities due to paramoebiosis (Fig. 7.1). Sea urchins were transplanted into 2 to 4 replicate cages (20 per cage) at 8 m depth in kelp beds at 3 to 6 sites within and immediately outside of St. Margarets Bay (Table A.1, Fig. 7.1). Cages were constructed from plastic milk crates (30 × 30 × 30 cm) with wire mesh lids (1 × 3 cm aperture), and weighted with iron plates. Cages were placed 2 to 5 m apart and secured between boulders on the rocky seabed. Sea urchins also were placed in 2 to 4 replicate cages at 8 m depth at Splitnose Point, to monitor disease in the source population, and at 18 m depth at one of the sites in St. Margarets Bay (The Lodge) on a sand bottom near the lower limit of the kelp bed, where bottom temperature is expected to be at or below 10 °C during summer/fall.

Divers monitored sea urchin survival and recorded dead and moribund sea urchins at weekly to biweekly intervals until the end of October, when sea temperatures dropped below 10 – 12 °C. Sea urchins were classified as moribund if they exhibited overt signs of paramoebiosis: loss of attachment to the substrate, dishevelled spines, shrivelled and non-functional tube feet, and a gaping peristome (Scheibling & Stephenson 1984). At each sampling interval, kelp fronds were added to cages as food for the sea urchins, and dead and moribund urchins were removed. Moribund sea urchins were transported to the laboratory, where *Paramoeba invadens* was identified as the causative agent of disease using waterborne transmission experiments (Scheibling et al. 2010, Feehan et al. 2012a) or culturing, injection experiments, and genetic analysis (Feehan et al. 2013, R.
Buchwald unpubl. data). Following evidence of disease at Splitnose Point in 2011, thermal induction experiments (Feehan et al. 2012a) were conducted at 16 °C (an optimal temperature for infective paramoebiasis) on sea urchins collected for the field experiment from 2012 to 2014. These experiments confirmed that a pathogen was not present in the tissues of sea urchins at the onset of the field experiment in July in any of these years.

7.3.2. Additional Sea Urchin Mortality Data

Following reports of large numbers of dead and dying sea urchins washing onshore at Point Pleasant Park in Halifax Harbour (Fig. 7.1) on 12 August 2012, and prior to any observations of mortality in the field experiment, we conducted diving and towed-video surveys over 2 weeks to monitor disease in the sea urchin population at Point Pleasant Park as well as Splitnose Point and other nearby headlands at Duncan’s Cove and Bear Cove, 4 km north and 7 km north-northwest (linear distance) of Splitnose Point, respectively (Scheibling et al. 2013). A mass mortality at Point Pleasant Park was attributed to infection by *Paramoeba invadens* through culturing and genetic analysis (R. Buchwald unpubl. data).

To extend our analysis of physical correlates with disease outbreaks across 6 successive years (2009 – 2014), we also acquired data from a previous field experiment in 2009 (Scheibling et al. 2010), the final year of data included in the Scheibling & Lauzon-Guay (2010) model. The 2009 study monitored disease in *Strongylocentrotus droebachiensis* transplanted to an array of 4.5-m² circular plots (n = 32) at 7 to 10 m depth in a kelp bed at The Lodge, which had stabilized at a mean density of ~ 4 urchins m⁻² on 21 August, prior to a strong hurricane (Bill). Sea urchin density was monitored at The Lodge at weekly intervals from 21 August to 17 September, when urchins began exhibiting symptoms of paramoebiasis. Sampling was repeated on 22 October, following mass mortality of sea urchins (Scheibling et al. 2010).
**7.3.3. Hurricane Activity and Model Evaluation**

Tropical storms and hurricanes occurring during the period of our study (2009 – 2014) and tracking within a study grid between 35°N and the Atlantic coast of Nova Scotia and between 55 and 70°W (Scheibling & Lauzon-Guay 2010) were identified using the Unisys weather database (http://weather.unisys.com/hurricane/atlantic/index.html). Hurricane track and wind speed data from the Unisys database, and sea temperature from a thermograph (StowAway TidbiT Temp Logger, Onset Computer) at 8 m depth at The Lodge, were used to parameterize a logistic regression model (Scheibling & Lauzon-Guay 2010) to predict the probability of an outbreak of paramoebiasis in sea urchins ($P_m$) associated with each storm:

$$P_m = 1/(1 + e^{-z})$$

$$z = -14.352 + 0.082W - 0.069D^2 + 4.966T$$

where $W$ (km h$^{-1}$) is the maximum sustained wind speed of the storm when closest to the coast, $D$ (km) is the closest distance of the centre of the storm to the coast, and $T$ is a dummy variable for a temperature threshold based on the mean temperature $T_m$ at 8 m depth in St. Margarets Bay in the 2-week period following a storm ($T = 1$ if $T_m > 12.2 \, ^\circ C$, $T = 0$ if $T_m < 12.2 \, ^\circ C$).

In years when disease was observed in the field experiment, the storm most likely associated with a disease outbreak (termed ‘candidate storm’) was identified by calculating $Pt_{50}$, the predicted time (d) to $\geq 50\%$ morbidity or mortality of sea urchins for each storm based on previous laboratory infection experiments (Scheibling et al. 2010), assuming introduction of a pathogen at the time when the storm was closest to the coast:

$$Pt_{50} = 23492T_m^{-2.7476}$$

A candidate storm was selected as the storm with a $Pt_{50}$ that best matched the observed timing of mass mortality ($\geq 50\%$ morbidity or mortality) of sea urchins in the field experiment ($t_{50}$). To evaluate the reliability of the logistic regression model, the candidate storm selected in each year based on $Pt_{50}$ was compared to the candidate storm selected by the model based on $P_m$. An unusually strong fall storm on 22 September 2014, characterized as a nor’easter and not a hurricane or tropical storm, was followed by
an outbreak of disease. $Pt_{50}$ also was calculated for this storm and compared to $t_{50}$ to determine its qualification as a candidate storm.

In 2011, mortality of caged sea urchins occurred within the first 3 weeks of the field experiment, and stabilized at 4 to 20% well before a storm (mortality rates averaged among replicate cages within sites). We attributed this early mortality to stress related to transplantation, as sea urchins were acclimated in the laboratory for < 24 hrs following collection in 2011 compared to at least 48 hrs in all other years. To avoid a potential bias in calculations of $t_{50}$ due to transplantation-related mortality, sea urchins that died within the first 3 weeks of the experiment were omitted from the analysis.

7.3.4. Sea Temperatures Associated with Disease Outbreaks

Given that sea temperature following a hurricane is expected to mediate the occurrence of a disease outbreak (Scheibling & Lauzon-Guay 2010), we examined the role of post-storm temperature in the progression of disease. To do this, we calculated a thermal integral above 10 °C (threshold for infective paramoebiasis) following the passage of a candidate storm, identified based on $Pt_{50}$ and $P_m$ ($TI_{10, post-storm}$, °D). $TI_{10, post-storm}$ was calculated beginning on the date that a candidate storm was closest to the coast of Nova Scotia and terminated once mean daily sea temperature was < 10 °C, or the proportion of sea urchin morbidity or mortality ($M_{prop}$) in the field experiment was ≥ 90%. $TI_{10, post-storm}$ was calculated as the sum of the mean daily temperature at 8 m depth in St. Margarets Bay minus 10 °C over this period. $M_{prop}$ in the field experiment, averaged across replicate cages (in 2010 – 2014) or plots (in 2009) within each site, was plotted against $TI_{10, post-storm}$ at each sampling interval in each year with a storm-associated sea urchin mass mortality to examine whether the progression of disease following a storm was related to time-integrated temperature above a threshold for infective paramoebiasis.

Storm intensity and sea temperature are inherently confounded because warm sea temperatures provide energy to passing hurricanes and thereby intensify them. Therefore, it is necessary to test an alternative hypothesis that the onset and progression of disease is related to the annual increase in sea temperature, regardless of storm activity. To do this, a time-integrated thermal integral above 10 °C ($TI_{10}$, °D) was calculated as per $TI_{10, post-storm}$.
storm but beginning on the first year-day with a mean daily sea temperature $\geq 10$ °C and for all years (including those without a storm-associated mass mortality). The start date of $TI_{10}$ varied among years from 31 May (2012) to 30 June (2013). $M_{prop}$ was plotted against $TI_{10}$ to examine whether the onset and progression of disease is simply related to the time-integrated temperature above a threshold for infective paramoebiasis. Calculations of $M_{prop}$ were corrected for transplantation-related mortality in 2011 in both the $TI_{10}$, post-storm and $TI_{10}$ analysis, as described above (see Hurricane Activity and Model Evaluation).

Since the lower temperature tolerance of $Paramoeba invadens$ (2 °C) is near the annual minimum sea temperature generally observed along the coast of Nova Scotia (-1 to 0 °C), an increase in minimum temperature is expected to have implications for overwinter survival of $P. invadens$. To examine the long-term trend in minimum sea temperatures, and the potential for $P. invadens$ to overwinter in the shallow subtidal zone of Nova Scotia, available historical mean daily sea temperatures (1980 – 2009) were acquired for the period of the winter minimum (February through March) at 0 – 10 m depth, along 70 km (linear distance) of coast (from Halifax to Lunenburg) and 5 km offshore, from the Coastal Time Series (CTS, www.mar.dfompo.gc.ca/science/ocean/database/Doc2003/cts2003app.html). Mean daily sea temperatures also were acquired for February through March of 2005 through 2014 from a thermograph at 8 m depth at The Lodge. Linear regression analysis indicated a strong concordance between temperatures acquired from the CTS ($T_{CTS}$) and the thermograph at The Lodge ($T_{TL}$) during a period of coincident measures from 2005 to 2009 ($T_{TL} = 0.89T_{CTS} + 0.08$, $r^2 = 0.92$). Therefore, data were combined from these sources to extend the historical record from 1980 to 2014. Deviations from the long-term average (1980 – 2014) were calculated for each year since 1980 to examine annual anomalies in minimum sea temperature.

7.3.5. Oceanographic and Meteorological Variables Associated with Disease Outbreaks

To explore physical mechanisms for the correlation between sea urchin disease outbreaks and storm activity, a time series was constructed using available oceanographic and meteorological data across 6 successive years (2009 – 2014) encompassing our current field experiment and the previous one in 2009 (Scheibling et al. 2010). To
examine a potential role of wind forcing in the transport of a disease agent, hourly winds, atmospheric pressure and air temperature were acquired for Sable Island meteorological station (station no. 8204700) and Halifax Harbour Buoy (buoy no. C44258) (Fig. 7.1, www.meds-sdmm.dfo-mpo.gc.ca). Winds measured at Sable Island are considered to be representative of conditions over most of the Scotian Shelf (Petrie & Lively 1979, Sandstrom 1980). Winds were isolated into positive and negative U (alongshore) and V (across-shore) components, resolved relative to 60 °T, to examine evidence for wind-induced upwelling and downwelling, and alongshore currents, respectively. To examine a cumulative effect of wind over time, a time-integrated sum for the positive and negative U and V components of wind at Sable Island was calculated. Wind speed cubed ((m s\(^{-1}\))^3) was used for this analysis to reflect the energy content of the wind.

To examine a local response in sea state to regional changes in meteorological variables, hourly sea temperature was obtained from thermographs (StowAway TidbiT Temp Logger, Onset Computer) at 4, 8, 12, and 18 m depth at The Lodge, and hourly significant wave height (SWH) was acquired from Halifax Harbour Buoy. To examine evidence for resuspension of seabed sediments by storms as a possible mechanism of introduction of *Paramoeba invadens*, bottom orbital velocities (\(u_b\), m s\(^{-1}\)) were calculated at 8 m depth for the field experiment and at 60 m depth, typical of inshore sedimentary basins (K. Filbee-Dexter unpubl. data), using the MATLAB function ‘ubspecfun’ (Pat Wiberg, University of Virginia) parameterized with SWH and wave period measured at hourly intervals at Halifax Harbour Buoy. To examine evidence for potential horizontal transport of surface waters by storms as a mechanism of introduction of *P. invadens*, we examined the intensity and spatial extent of offshore surface winds (m s\(^{-1}\)) associated with candidate storms using the National Oceanographic and Atmospheric Administration’s (NOAA) National Operational Model Archive and Distribution System (NOMADS, Narr-A model).
Fig. 7.1. (a) Map of Nova Scotia showing Sable Island (SI) and tracks of hurricanes identified as candidate storms in 2009 – 2011 (for storm details see Table 7.1). The grey line is the 1000 m bathymetric contour. (b) Inset of (a) showing study sites within (HI, Horse Island; TL, The Lodge; MC, Mill Cove; CI, Croucher Island; LI, Luke Island; Paddy’s Head, PH) and immediately outside of (GI; Gravel Island, CC; Cranberry Cove) St. Margarets Bay, at an adjacent headland (SP; Splitnose Point), and in Halifax Harbour (PP; Point Pleasant Park), and the location of the Halifax Harbour Buoy (HHB).
7.4. RESULTS

7.4.1. Sea Urchin Disease Outbreaks, Hurricane Activity, and Model Evaluation

Disease outbreaks were observed in 4 of 5 years of our field experiment (2010 – 2012, and 2014, but not 2013). The onset of mass mortality (≥ 50 % morbidity or mortality) of sea urchins in the cages ranged from 16 August to 14 October (Table 7.1). The timing of mass mortality of sea urchins in 2010 and 2011 showed relatively strong concordance among sites at 8 m depth within or immediately outside of St. Margarets Bay (Fig. 7.2a,b). In contrast, disease outbreaks in 2012 and 2014 showed high variation among sites. In 2012 mass mortality occurred at the 2 sites at the mouth of St. Margarets Bay in late August, and at 1 (Luke Island) of 3 sites within the bay and at the site of the source population (Splitnose Point) in late September (Fig. 7.2c). Divers also noted dead and dying sea urchins on the seabed surrounding cages at Splitnose Point, and scattered pockets of diseased and dying sea urchins and their tests at shallower depths (3 – 4 m) in late September. Observations of disease in the field experiment in 2012 were preceded by a mass mortality of sea urchins in early August at Point Pleasant Park in Halifax Harbour, with > 65 % mortality of sea urchins recorded across a depth range of 0 to 10 m and up to 700 m offshore on 12 August. Disease was not present in the urchin population at Splitnose Point and two nearby sites (Duncan’s Cove and Bear Cove) during surveys on 27 August 2012. In 2014, the same pattern of site-specific mass mortality was observed in the field experiment in St. Margarets Bay and at Splitnose Point as in 2012, but in mid and late October, respectively (Fig. 7.2d).

Mass mortality of caged sea urchins was not observed at 18 m at The Lodge in St. Margarets Bay in any year, or at Splitnose Point in 2009 – 2011 and 2013. However, laboratory culturing and thermal induction experiments at 16 °C indicated that the pathogen was present in tissues of asymptomatic sea urchins at 18 m at The Lodge in 2010, 2011, and 2014, at 8 m at Splitnose Point in 2011, and at 8 m at The Lodge in 2014, at termination of the field experiment in each year (Feehan et al. 2012a, C.J. Feehan unpubl. data). Observed variability in the timing of disease outbreaks among sites in the field experiment (Fig. 7.2) suggests that the pathogen was introduced late in the
season (once temperatures at 8 m depth had dropped near or below a threshold for infective paramoebiasis) at Splitnose Point in 2011 and at The Lodge in 2014, resulting in a low incidence of disease. Infected sea urchins at 18 m at The Lodge likely remained asymptomatic due to consistently lower sea temperatures at this depth, often below the 10 °C threshold (Fig. 7.3).

The number of hurricanes or tropical storms tracking within our study grid in each year ranged from 1 to 8 (Table 7.1). Two hurricanes were identified as candidate storms based on a strong concordance ($\leq 3$ d) between the predicted ($P_{t50}$) and observed ($t_{50}$) time to 50 % morbidity or mortality of sea urchins, assuming introduction of a pathogen at the time of the storm: Hurricane Earl in 2010 and Hurricane Maria in 2011 (Table 7.1). Results of the logistic regression model gave a mean probability of mass mortality of sea urchins ($P_m$) associated with these storms (45 ± 2 % SD, Table 7.1) that was not significantly different from the mean of 12 previous candidate storms ($P_m = 57 \pm 28 \%$; Scheibling & Lauzon-Guay 2010) identified by the model ($t_{12} = 0.62$, $p = 0.55$). In contrast, the $P_m$ all other hurricanes or tropical storms during the field experiment (14 ± 27 %, $n = 17$) was significantly lower than that of all previous candidate storms identified by the model ($t_{27} = 4.12$, $p < 0.001$). In 2013, when a disease outbreak did not occur, only a single storm was observed and that storm had a $P_m < 0.1$ %. In 2014 a strong storm (nor’easter), that was not a tropical storm or hurricane, also was identified as a candidate storm based on a reasonable concordance (7 d) between the predicted ($P_{t50}$) and observed ($t_{50}$) time to 50 % morbidity and mortality of sea urchins, assuming introduction of a pathogen at the time of the storm. A disease outbreak in 2012 was not associated with a storm (Table 7.1), indicating that other oceanographic conditions can mediate outbreaks of disease.

7.4.2. Sea Temperatures Associated with Disease Outbreaks

The thermal integral above 10 °C following the passage of a candidate storm ($TI_{10, \ post-storm}$, °D) was a strong predictor of the progression of disease, with a significantly positive linear relationship between the proportion of morbidity or mortality of sea urchins ($M_{prop}$) and $TI_{10, \ post-storm}$ ($M_{prop} = 0.005 \ TI_{10, \ post-storm} + 0.054$, $r^2 = 0.753$, $p <$
post-storm at 50 % morbidity or mortality of sea urchins ranged from ~ 50 to 150 °D. By comparison, the annual thermal integral above 10 °C (TI10, °D) ranged from ~ 200 to 400 °D at 50 % morbidity or mortality of sea urchins, with no morbidity or mortality observed at 200 °D in 2013 (Fig. 7.4a).

The mean annual minimum sea temperature along the coast of Nova Scotia from February through March (Tmin), calculated from daily means over this period, increased significantly over the last 35 years from 1980 to 2014 (Tmin = 0.042Y - 83.3, r² = 0.220, p = 0.004) (Fig. 7.5a, see also R. Buchwald, C.J. Feehan, R.E. Scheibling, A.G.B Simpson, in review). The mean minimum temperature was at or below a 2 °C lower temperature threshold for survival of Paramoeba invadens in each year of the field experiment except 2012, when Tmin was 2.8 ± 0.4 °C (mean ± SD, n = 59) (Fig. 7.5b). Each year of the field experiment was preceded by a positive anomaly in winter sea temperature based on the 35-year record (Fig. 7.5a,b).

7.4.3. Oceanographic and Meteorological Variables Associated with Disease Outbreaks

Candidate storms had a strong signature in the time series of atmospheric temperature and pressure at Sable Island and Halifax Harbour, with a sharp drop in these variables when a storm was closest to the coast of Nova Scotia (Fig. A.1). Strong winds and large significant wave heights were associated with candidate storms in 2009, 2010 and 2014 (Fig. A.1). In 2011, when a candidate storm (Maria) tracked far offshore, strong winds were not observed on the date the storm was closest to the coast of Nova Scotia (Fig. 7.1, A.1). A peak in significant wave height (~ 3 m) the day after Maria had passed may indicate a lagged effect (Fig. A.1). There was no consistent pattern in positive and negative U- (across-shore) and V- (alongshore) component winds during the passage of a candidate storm to suggest conditions favourable for wind-induced upwelling/downwelling or alongshore currents (Fig. A.1). Likewise, the onset of mass mortality in 2009 – 2012 and 2014 was not consistently associated with periods of strong alongshore or across-shore winds (Fig. A.1).
Sea temperature measured at 4 depths in St. Margarets Bay (4, 8, 12 18 m) provides evidence for rapid, short-term warming (to $\geq 15 \, ^\circ C$) of the upper 12 to 18 m of the water column associated with the passage of candidate storms in 2009 – 2011 (Fig. 7.3a,b,c). In 2014 the water column was uniformly warm (~ 16 °C) to 18 m depth prior to the passage of a candidate storm (nor’easter), and no change in water column structure was observed immediately following the storm (Fig. 7.3f). Significant wave height was a strong predictor of bottom orbital velocity (Fig. A.1). Orbital velocities at 8 m depth during candidate storms ranged from 1.7 to 3.5 m s$^{-1}$ in 2009, 2010 and 2014, but were < 1 m s$^{-1}$ in 2011 (Fig. 7.3). Orbital velocities at 60 m depth during candidate storms in 2009 – 2011 and 2014 were consistently low (0.1 – 0.7 m s$^{-1}$) (Fig. 7.3).

There was large variation in the tracks of candidate storms off the coast of Nova Scotia (Table 7.1, Fig. 7.1a; see also Scheibling & Lauzon-Guay 2010). However, imagery of surface winds associated with each of these storms indicates less variation in the distance of regions of maximum winds (16 – 24 m s$^{-1}$) from the coast (Fig. 7.6). This is due mainly to high winds to the west (in the onshore direction) of the centre of Hurricane Maria (16 September 2011), which tracked furthest offshore (Fig. 7.1a, 7.6c). Infrared imagery of Hurricane Maria from NASA's Atmospheric Infrared Sounder (AIRS) indicates that clouds associated with Maria stretched to the coast of Nova Scotia (http://www.nasa.gov/mission_pages/hurricanes/archives/index.html, data not shown). In general, the onset of mass mortality occurred approximately 2 to 3 weeks following the passage of a candidate storm, and coincided with periods when the upper 12 to 18 m of the water column in St. Margarets Bay was warm, and mixed as indicated by uniform temperatures down to these depths (Fig. 7.3). Disease outbreaks in each year were arrested once sea temperature at 8 m depth dropped below 10 – 12 °C (Fig. 7.3).
Table 7.1. Tropical storms and hurricanes passing between 35°N and the Atlantic coast of Nova Scotia, and between 55 and 70°W, from 2009 to 2014. ‘Date’ is when a storm was closest to coast. ‘Wind’ is maximum sustained wind speed of a storm at the closest distance (‘Dist’); \( T \) is mean temperature at 8 m depth in St. Margarets Bay in the 2-week period following a storm. \( P_m \) is probability of a disease outbreak in \textit{Strongylocentrotus droebachiensis} following a storm predicted by the Scheibling \& Lauzon-Guay (2010) model. \( Pt_{50} \) is predicted time to \( \geq 50\% \) morbidity of \textit{S. droebachiensis} caused by paramoebiasis following a storm at a given \( T \), using the formula \( Pt_{50} = 23492T^{2.7476} \) (Scheibling et al. 2010). \( t_{50} \) is the observed time to \( \geq 50\% \) morbidity or mortality of sea urchins in cages following a storm. Period of mass mortality is the time over which \( \geq 50\% \) morbidity or mortality of sea urchins was observed in the field. Data for the storm most likely associated with a disease outbreak (‘candidate storm’), based on concordance of \( Pt_{50} \) and \( t_{50} \), are shown in bold.

<table>
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<th>Year</th>
<th>Storm</th>
<th>Date</th>
<th>Lat (°N)</th>
<th>Long (°W)</th>
<th>Wind (km h(^{-1}))</th>
<th>Dist (km)</th>
<th>( T ) (°C)</th>
<th>( P_m )</th>
<th>( Pt_{50} ) (d)</th>
<th>( t_{50} ) (d)</th>
<th>Period of mass mortality</th>
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<td>24</td>
<td>16 Sep–22 Oct</td>
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<td>14</td>
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</tbody>
</table>
Fig. 7.2. Mean cumulative (+ 1 SD) proportion of dead or moribund sea urchins at 8 m depth in cages over time in the field experiment in (a) 2010, (b) 2011, (c) 2012, and (d) 2014 at 3 or 6 sites within St. Margarets Bay (red lines), at 2 sites at headlands on either side of the bay (black lines) and at 1 site (Splitnose Point) where sea urchins were collected for all cages (green lines), and at 18 m depth at 1 site (TL) within the bay (blue lines) (for site names and details see Table A.1, Fig. 7.1). Vertical dashed line indicates date when a candidate storm was closest to the coast of Nova Scotia. Data are offset by ± 1 d for visual clarity. Data in (a) through (c) are modified from Feehan et al. (2012) and Scheibling et al. (2013).
Fig. 7.3. (following page): Time series of bottom orbital velocity \( (u_b, \text{ m s}^{-1}) \) at 8 (black lines) and 60 (blue lines) m depth, and sea temperature \( (^\circ \text{C}) \) at 4 depths at The Lodge, St. Margarets Bay (4 m, red lines; 8 m, green lines; 12 m, blue lines; 18 m, black lines) from early July to late October in (a) 2009, (b) 2010, (c) 2011, (d) 2012, (e) 2013, and (f) 2014. Vertical dashed line indicates date when a candidate storm was closest to the coast of Nova Scotia. In (a–c) and (e–f) solid gray bands indicate period of mass mortality of sea urchins in cages within and immediately outside St. Margarets Bay, defined as the date when ≥ 50 % morbidity or mortality of sea urchins was first observed to the date when mortality reached ≥ 90 % or began to plateau. In (d) the finely hatched band indicates period of mass mortality of a sea urchin population at Point Pleasant Park, the solid gray band indicates period of mass mortality of sea urchins in cages at 2 sites immediately outside of the bay, and the loosely hatched band indicates period of mass mortality of sea urchins in cages at a single site within the bay and at Splitnose Point (Fig. 7.1) (for detailed site-specific results see Fig. 7.2).
Fig. 7.4. Relationship between proportion of sea urchin morbidity or mortality ($M_{prop}$), averaged across replicate cages at 3 to 6 sites in and around St. Margarets Bay (for site names and details see Table A.1, Fig. 7.1) in a 5-year field experiment (2010 – 2014) and across plots at a single site in a similar study in 2009 (Scheibling et al. 2010), and the thermal integral above 10 °C calculated based on temperature at 8 m depth at The Lodge following: (a) an annual increase in the mean daily sea temperature to ≥ 10 °C ($TI_{10, °D}$) or (b) the passage of a late summer/fall candidate storm ($TI_{10, post-storm, °D}$). The dashed horizontal line indicates 50 % morbidity or mortality of sea urchins. Data in (b) are described by the linear relationship: $M_{prop} = 0.005 TI_{10, post-storm} + 0.054$, $r^2 = 0.753$, $p < 0.001$. Data for TL and MC in 2014 were excluded from the regression analysis, as mass mortality of sea urchins was not observed at these sites.
Fig. 7.5. Mean annual minimum sea temperature (°C) (February – March, $T_{min}$) over (a) a 35-year period (1980 – 2014) and (b) the past decade (indicated by a box in a). Data for 1980 – 2005 are based on records at 0 – 10 m depth over 70 km (linear distance) of coast (from Halifax to Lunenburg) and 5 km offshore from the Coastal Time Series (CTS) (as defined by Scheibling & Lauzon-Guay 2010). Records for 2005 – 2014 are from 8 m depth at The Lodge, St. Margarets Bay. Positive and negative anomaly temperatures over this period, based on the 35-year record, are shown in red and blue, respectively. The horizontal dashed lines indicate the lower temperature tolerance level of *Paramoeba invadens*. A linear regression of average temperature in each year ($Y$) in (a) indicates a significant increasing trend: $T_{min} = 0.042Y - 83.3$, $r^2 = 0.220$, $p = 0.004$ (see also R. Buchwald, C.J. Feehan, R.E. Scheibling, A.G.B Simpson, in review). Error bars in (b) are 95 % confidence intervals (daily averages, $n = 59$).
Fig. 7.6. Surface winds (m s$^{-1}$) from National Oceanographic and Atmospheric Administration’s (NOAA) National Operational Model Archive and Distribution System (NOMADS, Narr-A model) between 25 and 47°N and 55 and 80°W on the date when a candidate storm was closest to the coast of Nova Scotia: (a) Hurricane Bill, 23 Aug 2009; (b) Hurricane Earl, 4 Sept 2010; (c) Hurricane Maria, 16 Sept 2011; and (d) a strong nor’easter, 22 Sept 2014. Data source: http://nomads.nceo.noaa.gov/.
7.5. DISCUSSION

7.5.1. Support for the Killer Storm Hypothesis

Disease outbreaks in sea urchins *Strongylocentrotus droebachiensis* were preceded by a candidate storm in 3 years (2010, 2011, 2014) of our 5-year field experiment (2010 – 2014), as indicated by a strong concordance between a predicted ($Pt_{50}$) and observed ($t_{50}$) time to $\geq 50\%$ morbidity or mortality of sea urchins associated with a storm in these years. A logistic regression model (Scheibling & Lauzon-Guay 2010) successfully identified hurricanes as candidate storms in 2 years (2010, 2011), as indicated by a high probability of a disease outbreak associated with candidate storms based on the model ($P_m$). In 2014 a nor’easter, and not a hurricane or tropical storm, was categorized as a candidate storm. The strong signal in significant wave height (SWH) and atmospheric pressure at Halifax Harbour Buoy and Sable Island (2009 – 2014) indicates that this storm was a rare and intense event, with a signature similar to that of a strong hurricane. In 2013, when no disease outbreak occurred, only a single storm, with minimal $P_m$ ($< 0.1\%$), was observed. In combination, these results provide support for the reliability of the logistic regression model to predict a disease outbreak based on hurricane activity and sea temperature at 8 m depth over a 2-week post-storm period.

The proportion of sea urchin morbidity or mortality ($M_{prop}$) at sites in and around St. Margarets Bay in 2009 through 2011 and in 2014 was strongly related to $TI_{10, post-storm}$, the thermal integral above 10 °C following the date of passage of a candidate storm. The y-intercept of the regression of $M_{prop}$ versus $TI_{10, post-storm}$ was approximately zero, suggesting that *Paramoeba invadens* is introduced and has an effect shortly after the passage of a storm. The strong concordance between the predicted ($Pt_{50}$) and observed ($t_{50}$) time to $\geq 50\%$ morbidity or mortality of sea urchins in the field experiment, for all candidate storms, suggests that the pathogen is introduced within days following the passage of a storm. These results also support the Killer Storm Hypothesis, and confirm that progression of paramoebiosis following a storm is strongly temperature-dependant (Scheibling & Stephenson 1984, Scheibling et al. 1999). In contrast, the annual thermal integral above 10 °C ($TI_{10}$) was not a good predictor of the onset of a disease outbreak, as
indicated by the absence of sea urchin morbidity or mortality after 200 °D in 2013 compared to ~ 50 and 100 % morbidity or mortality after 200 °D in 2009 and 2012 respectively. A protracted period of warm temperature at 8 m depth from late September to late October 2013 (~ 1 month at 15 °C; near the optimal temperature for paramoeobiasis), with no associated disease outbreak, further confirms that temperature alone does not mediate occurrence of the disease.

7.5.2. Evidence for Hypotheses 1 and 2: Horizontal Transport vs. Vertical Mixing

Storms are important episodic events that can resuspend and transport sediments (Cacchione et al. 1987, Warner et al. 2008), and advect and restructure water masses on continental shelves (Xu et al. 2011, Miles 2014). Two hypotheses have been developed to explain the introduction of a sea urchin pathogen (*Paramoeba invadens*) with hurricanes in the context of these physical phenomena. Specifically, these hypotheses relate to the nature of putative source populations of *P. invadens*: 1) that the pathogen is transported to the coast of Nova Scotia from sources in warm offshore surface waters by horizontal advection during a storm event (Scheibling & Hennigar 1997), and 2) that the pathogen is free-living in deep sedimentary basins near the coast and is vertically suspended and horizontally advected to shallow coastal waters during a storm event (Scheibling & Lauzon-Guay 2010).

A modelling study of sediment transport in southern New Jersey during Hurricane Sandy (October 2012) shows that fine and medium grain-size sediments (particle sizes of 0.1 and 0.4 mm, respectively) were resuspended only at orbital velocities exceeding 1 – 2 m s\(^{-1}\) (Miles 2014). Given that orbital velocities at 60 m depth during the passage of candidate storms in 2009 through 2011 and in 2014 were well below 1 m s\(^{-1}\), Hypothesis 2 is not supported by these data. Our inability to detect *Paramoeba invadens* in sea urchins collected from a sedimentary basin at 60 m depth in St. Margarets Bay, during a disease outbreak in shallower water in fall 2010, also is inconsistent with Hypothesis 2 (Feehan et al. 2012a). Orbital velocities at 8 m depth during candidate storms in 2009, 2010 and 2014 ranged from 1.7 to 3.5 m s\(^{-1}\), suggesting resuspension of sediments at shallower depths in most years. However, low orbital velocities at 8 m (< 1 m s\(^{-1}\)) during
a candidate storm (Maria) in 2011, and observations of high orbital velocities at 8 m depth (> 1 – 2 m s\(^{-1}\)) that were not associated with candidate storms and disease outbreaks (e.g. 29 August 2011, 6 July 2014; Fig. 7.3), provide further indications that sediment resuspension is an unlikely mechanism of introduction of the pathogen. Given that wave data (used to calculate orbital velocities) were available only from an inshore buoy at Halifax Harbour, it is difficult to assess whether sediments were resuspended at depths further offshore (e.g. along the Scotian Shelf) during candidate storms. However, Miles (2014) found that minimal resuspension of sediments occurs at depths > 100 m during the passage of a strong hurricane.

Based on estimated maximum surface current speeds of 1 – 1.2 m s\(^{-1}\) for 4 to 6 h associated with hurricanes of a similar magnitude to our candidate storms (Miles 2014), maximum horizontal transport of surface waters by a storm is expected to be on the order of 10s of km over this period. Given that high winds associated with candidate storms were consistently within 10s of km of the coast (Fig. 7.6), it is possible that horizontal transport is a mechanism of introduction of *Paramoeba invadens* according to Hypothesis 1. The occurrence of consistently warm surface waters (> 15 °C) offshore at the time of a candidate storm (Fig. 7.7) may provide a reservoir of *P. invadens* (Scheibling & Hennigar 1997). Due to interactions with the Gulf Stream these offshore surface waters remain well above a 2 °C threshold for survival of *P. invadens* year-round (http://oceancolor.gsfc.nasa.gov/; data not shown). A lack of consistency in patterns of variables that act as a proxy for mixing (winds, waves) in our time series, during the passage of candidate storms, also suggests that horizontal advection of surface waters, rather than local vertical mixing, is important in the introduction of a pathogen. A rapid, short-term increase in temperature at 4 to 12 or 18 m depth at The Lodge in St. Margarets Bay to ≥ 15 °C during candidate storms also is consistent with shoreward horizontal advection of warm offshore surface waters.

The occurrence of subtropical and tropical fish species in coastal waters of Nova Scotia in late summer and fall provide circumstantial evidence for a mechanistic link between cross-shelf advection and the transport a pathogen. For example, in 1995 there were reports of tropical fish and sea turtles in the shallow waters along the coast immediately preceding a sea urchin disease outbreak (Scheibling & Hennigar 1997), and
we observed grey triggerfish (*Balistes capriscus*) at Paddy’s Head near the mouth of St. Margarets Bay on 22 July and 22 August 2012 (Scheibling et al. 2013).

7.5.3. Evidence for Hypothesis 3: Increasing Minimum Sea Temperatures

Records of hurricane activity and time series of wind and wave data suggest that a strong storm did not precede the onset of a disease outbreak in 2012. Positive anomalies were observed in minimum annual (February through March) sea temperatures in all winters preceding the field experiment. However, winter temperatures were significantly above a lower threshold for survival of *Paramoeba invadens* (2 °C; R. Buchwald, C.J. Feehan, R.E. Scheibling, A.G.B Simpson, in review) only in 2012. These results provide support for Hypothesis 3, suggesting that *P. invadens* may have overwintered in the shallow subtidal zone following introduction by a storm (Maria) in fall 2011. The early occurrence of a disease outbreak in the annual temperature cycle in 2012 (early August at Point Pleasant Park, mid August at sites immediately outside of St. Margarets Bay) provides further support that *P. invadens* overwintered and was present at the beginning of the seasonal cycle of coastal warming. Accordingly, the 2012 mass mortality began during a period of very warm sea surface temperatures (~ 19 °C for 2 weeks in mid August at Halifax Harbour Buoy). A trend of increasing minimum annual sea temperatures over the past 35 years suggests that warm winter temperatures (> 2 °C) are increasing in frequency, with potentially important implications for interannual survival and the development of a resident population of *Paramoeba invadens* in the shallow subtidal zone of Nova Scotia (R. Buchwald, C.J. Feehan, R.E. Scheibling, A.G.B Simpson, in review).

7.5.4. Conclusions and Directions for Future Research

We found strong support for the logistic regression model (Scheibling & Lauzon-Guay 2010) to predict outbreaks of sea urchin disease based on hurricane activity and sea temperatures. However, an outbreak of paramoebiosis in 2012 that occurred in the absence of a candidate storm indicates that winter sea temperatures following a disease
event in the preceding year must be considered when applying this model. A disease outbreak in 2014 that occurred in association with a strong storm that was not categorized as a hurricane indicates that the model also should be expanded to consider any strong fall storms (nor’easters), which are known to cause sediment resuspension and transport, and large-scale advection (Styles & Glenn 2005, Xu et al. 2011, Miles 2014).

Hurricanes with a high $P_m$ can occur after a candidate storm (e.g. Ophelia 2011, Gonzalo 2014); however, the implications of subsequent storms and their potential role in spreading Paramoeba invadens along the coast remains untested. A lag in the onset of a disease outbreak at some sites in 2012 and 2014 indicates the need for future work to examine the spread of the disease following introduction. Interestingly, an outbreak that occurred at Splitnose Point (sea urchin source) and a site (Luke Island) within St. Margarets Bay in late September 2012, following an initial outbreak in Halifax Harbour and at sites immediately outside the mouth of the bay in August, had a strong association with a storm that occurred on 10 September 2012 (Leslie: $P_m = 0.33$, which is within the range of all previous candidate storms). However, Leslie was not considered a candidate storm since a disease outbreak was already underway at the time.

Our analysis of oceanographic and meteorological data suggests that the most likely mode of introduction of Paramoeba invadens to the coast of Nova Scotia is from source populations in warm offshore surface waters that are horizontally transported to the coast during a storm event. There is need for in situ measurements of local and regional surface currents before, during and after a storm, combined with high-resolution dynamical modelling of ocean circulation, to further explore the role of horizontal advection of surface waters in the introduction of P. invadens. A genetic probe currently in development to detect P. invadens in seawater and seabed sediments (R. Buchwald unpubl. data) will be necessary to effectively determine the presence or absence of source populations in warm offshore surface waters or inshore basins. There also is need for additional physical data of water column structure and dynamical modelling to more carefully assess the role of vertical mixing and sediment resuspension and transport in the introduction of P. invadens. Despite the importance of these data, they are difficult to acquire given the logistical constraints of sampling during strong storm events. The use of novel ocean observation technology, such as autonomous underwater vehicles or
‘gliders’, in combination with adaptive sampling strategies to target storms, may provide a means of resolving ocean shelf-scale processes during storms (Miles 2014). These approaches, in combination with the application of a genetic probe, likely will be necessary to ultimately confirm the mode(s) of introduction of *P. invadens*.

![SST images](image1.png)

**Fig. 7.7.** Sea surface temperature (SST, °C) within a study grid between 35°N and the Atlantic coast of Nova Scotia and between 55 and 70°W averaged for the week that a candidate storm passed by Nova Scotia: (a) 21 – 28 Aug 2009; (b) 29 Aug – 5 Sept 2010; (c) 14 – 21 Sept 2011; and (d) 22 – 29 Sept 2014. Images are generated from global Level-3 standard mapped images (SMIs) of 4 x 4 km spatial resolution MODIS/Aqua 11 μm daytime SST. SMIs were obtained from the NASA Goddard Space Flight Centre Ocean Color Web (Feldman & McClain 2012).
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CHAPTER 8

EFFECTS OF SEA URCHIN DISEASE ON COASTAL MARINE ECOSYSTEMS

8.1. ABSTRACT

Outbreaks of disease in herbivorous sea urchins have led to ecosystem phase shifts from urchin barrens to kelp beds (forests) on temperate rocky reefs, and from coral to macroalgal-dominated reefs in the tropics. We analyzed temporal patterns in epizootics that cause mass mortality of sea urchins, and consequent phase shifts, based on published records over a 42-year period (1970 – 2012). We found no evidence for a general increase in disease outbreaks among 7 species of ecologically important and intensively studied sea urchins. Periodic waves of recurrent amoebic disease of Strongylocentrotus droebachiensis in Nova Scotia coincide with periods when the system was in a barrens state and appear to have increased in frequency. In contrast, following a major epizootic that decimated Diadema antillarum throughout the Caribbean in 1983, subsequent outbreaks of disease were highly localized and none have been reported since 1991. Epizootics of Strongylocentrotus in the Northwest Atlantic and Northeast Pacific, and Paracentrotus and Diadema in the eastern Atlantic, have been linked to climate change and overfishing of sea urchin predators. The spatial extent of recurrent disease outbreaks in these species, and the frequency of phase shifts associated with these epizootics, has decreased over time due to the expansion of the macroalgal state and its stabilization through positive feedback mechanisms. Longitudinal studies to monitor disease outbreaks in sea urchin populations and improved techniques to identify causative agents are needed to assess changes in the frequency and extent of epizootics, which can profoundly affect the structure and functioning of coastal marine ecosystems.
8.2. Introduction

An increase in the frequency of disease outbreaks has been documented across a range of marine taxa, including sea urchins, molluscs, turtles, corals, and mammals (Ward & Lafferty 2004), a trend that generally is linked to climate change (Harvell et al. 1999, Burge et al. 2014). More specifically, scientists predict that with increasing sea temperatures, marine parasites and pathogens will undergo latitudinal range expansions, and outbreaks of infectious disease will increase in frequency and severity (Harvell et al. 1999). However, our understanding of the dynamics of disease in the marine realm is still rudimentary and predictions about climate-mediated changes in disease frequency remain largely untested. For example, some parasites and pathogens could decline with ocean warming due to thermal stress (Harvell et al. 2002, Burge et al. 2014). Alternatively, an initial increase in epizootics could lead to a longer-term decline in the frequency of disease, as host populations are reduced below density thresholds for disease propagation (Lafferty et al. 2004). A lack of baseline data for most marine diseases limits generalization about long-term changes in the frequency and severity of disease outbreaks, and their potential impacts on marine ecosystems (Harvell et al. 1999, Ward & Lafferty 2004).

The effects of disease on marine ecosystems can be profound, as evidenced by the decimation of ecologically important consumers or foundation species that have caused large-scale (100s – 1,000s of km) shifts to potentially undesirable, alternative states (Harvell et al. 1999). An early and compelling example is the wasting disease of eelgrass (Zostera marina), which destroyed 90% of seagrass beds along both sides of the North Atlantic in the 1930s, causing dramatic changes in benthic community structure and function (Muehlstein 1989). A recurrence of wasting disease resulted in localized diebacks of eelgrass along the east coast of North America in the 1980s (Short et al. 1987). Epizootics with the potential to cause community-level effects also have been observed in other seagrass species (Robblee et al. 1991), temperate and tropical gorgonians and hermatypic corals (Harvell et al. 2007, Bally & Garrabou 2007, Maynard et al. 2011), molluscs (Miner et al. 2006, Ford & Smolowitz 2007), sea stars (Zann et al. 1990, Bates et al. 2009, Eckert et al. 2000) and sea urchins (Lessios 1988a, Lafferty
2004, Girard et al. 2012, Scheibling et al. 2013), and marine mammals (Ross 2002). An unprecedented disease outbreak of sea stars, affecting at least 10 species, is currently underway along the west coast of North America from Alaska to California (http://www.eeb.ucsc.edu). The ecological effects of this mass mortality, which includes keystone predators such as *Pisaster ochraceus* and *Pycnopodia helianthoides*, are likely to be dramatic.

Notably, epizootics that cause mass mortalities of sea urchins have triggered phase shifts from so-called urchin barrens to kelp beds (or forests) on temperate rocky reefs (Scheibling et al. 2013, Filbee-Dexter & Scheibling 2014), and from coral reefs to macroalgal-dominated reefs in tropical regions (Lessios 1988a). These phase shifts occur due to the release of macroalgae from grazing pressure by sea urchins. Based on these observations, a purported increase in sea urchin disease (Ward & Lafferty 2004) is expected to have important consequences for marine ecosystems, if disease causes mass mortality in species that have important functional roles.

In this review, we examine the extent to which disease acts as an agent of population control in sea urchins that are dominant grazers in coastal marine ecosystems. We survey the scientific literature to determine whether epizootics are increasing among these species, and examine trends in the spatial extent of epizootics and subsequent phase shifts. We also identify gaps in our knowledge and suggest ways to plug these gaps and increase our understanding of the etiology of disease and the factors that influence its spread and severity. Finally, we discuss the importance of our findings as a model for exploring the broader ecological implications of a global increase in marine disease, and the feasibility of mitigation and management strategies.

### 8.3. Disease as a Control of Sea Urchin Populations

#### 8.3.1. Strongylocentrotus droebachiensis

Green sea urchins *Strongylocentrotus droebachiensis* inhabit a large geographic range, extending throughout the arctic and boreal oceans (Scheibling & Hatcher 2013). On both sides of the North Atlantic and in the Northeast Pacific, population outbreaks of
S. droebachiensis have led to destructive grazing of kelp and phase shifts to sea urchin barrens (Filbee-Dexter & Scheibling 2014), with important consequences for ecosystem productivity (Mann 1982). There is evidence that amoebic disease acts as a major agent of population control in S. droebachiensis in the Northwest Atlantic (Table 8.1). Macroparasitic disease has been recorded in S. droebachiensis in the Northeast Atlantic, but with limited evidence for an impact on populations (Table 8.1). There have been no reports of mass mortalities of S. droebachiensis due to disease in the Northeast Pacific.

In Nova Scotia, Canada, an amoebic infection by a facultative parasite, Paramoeba invadens, causes mass mortality of Strongylocentrotus droebachiensis in the shallow subtidal zone (Scheibling 1988, Scheibling et al. 1999, Fig. 8.1a). This parasite was first isolated and described as a new species in the early 1980s (Jones 1985), when mass mortalities of S. droebachiensis in barrens spanned 500 km of coast and accounted for 100s Kt of sea urchin biomass in areas of complete die-off (Miller & Colodey 1983, Miller 1985, Scheibling 1986). The identity of P. invadens was later confirmed by the analysis of small-subunit (SSU) ribosomal DNA (rDNA), following a disease outbreak in 2011 (Feehan et al. 2013). Recurrent outbreaks of this disease (paramoebiasis) have caused phase shifts from barrens to kelp beds along this coast over the past 3 decades, stabilizing the kelp bed state of the rocky subtidal ecosystem (Scheibling et al. 2013, Table 8.1). Epizootics in S. droebachiensis are associated with increasing tropical storm (hurricane) activity and post-storm sea temperatures, which appear to mediate the introduction and spread of the pathogenic agent (Scheibling & Lauzon-Guay 2010, Scheibling et al. 2013). Recurrent outbreaks of paramoebiasis likely have replaced predation as the major agent controlling populations of S. droebachiensis in this region (Feehan & Scheibling 2014a).

Mass mortality of sea urchins due to infection by Paramoeba invadens has not been recorded elsewhere in eastern Canada or in the northeastern USA, where Strongylocentrotus droebachiensis has been studied extensively. Urchin barrens in the Gulf of St. Lawrence and Newfoundland have been relatively stable over the past 3 decades (Keats et al. 1991, Gagnon et al. 2004). Dead or dying sea urchins and crabs were observed in 1999 in Long Island Sound during an outbreak of paramoebiasis in lobster (P. pemaquidensis comb. nov. (Feehan et al. 2013) was isolated from lobster
tissue) that was associated with abnormally high sea temperatures (Mullen et al. 2004). Although there was no microbial screening of sea urchins at that time, *P. pemaquidensis* subsequently was isolated from the tissues of moribund *S. droebachiensis* in the Gulf of Maine (Caraguel et al. 2007), where localized mass mortalities of *S. droebachiensis* periodically occur (T.A.C. De Graaf, Maine Department of Marine Resources, pers. comm.). A phase shift from barrens to kelp beds is purported to have occurred in the Gulf of Maine as a result of a poorly managed sea urchin fishery (Steneck 1997). These kelp beds currently are stabilized by predation of sea urchins by crabs, which are considered an apex predator following the extirpation of predatory fish by historical overfishing (Steneck et al. 2004). The absence of widespread epizootics in regions of the Northwest Atlantic outside of Nova Scotia is likely due to differences in coastal oceanography (water temperature, ocean currents) that may mediate the introduction and establishment of infective populations of *Paramoeba* (Feehan et al. 2012a).

In the early 1970s, *Strongylocentrotus droebachiensis* destructively grazed kelp beds (*Laminaria hyperborea*) along the central and northern coasts of Norway, creating barrens that have since dominated the northern region of the coast (Norderhaug & Christie 2009). Factors initiating this large-scale phase shift remain largely unknown (Norderhaug & Christie 2009). Kelp bed recovery began in central Norway in the late 1980s (Norderhaug & Christie 2009) and was initially linked to infection of sea urchins by an endoparasitic nematode, *Echinomermella matsi* (Jones & Hagen 1987, Table 8.1). Hagen (1987) documented a localized mass mortality of sea urchins in central Norway in 1983 and observed a trend of decreasing sea urchin density with increasing prevalence of *E. matsi*. He suggested that parasitism by *E. matsi* is a critical factor terminating sea urchin population outbreaks and causing shifts of barrens to kelp beds. However, subsequent studies indicate that although *E. matsi* reduces reproductive fitness (Hagen 1992, 1996, Sivertsen 1996, but see Stien et al. 1998) and survival (Stien 1999) of *S. droebachiensis*, nematode infection does not cause mass mortality or control sea urchin abundance (Stien et al. 1995). Localized mass mortalities of *S. droebachiensis* in central Norway in the early 1990s were attributed to an unknown waterborne agent(s) (Skadsheim et al. 1995, Table 8.1), although a pathogen was not detected in tissues of moribund urchins (Christie et al. 1995). Fagerli et al. (2013) provide indirect evidence
that an increase in larval mortality due to ocean warming may account for a decrease in
sea urchin density in barrens and the shift to kelp beds in central Norway since the 1980s.
Reduced fitness of sea urchins due to nematode infection (Norderhaug & Christie 2009)
and increased predation by crabs (Fagerli et al. 2014) may contribute to this decline and
confer resilience to the kelp bed state.

8.3.2. Strongylocentrotus franciscanus and S. purpuratus

*Strongylocentrotus franciscanus* and *S. purpuratus* are ubiquitous in subtidal and
intertidal habitats of the Northeast Pacific, from Alaska, USA, to Baja California, Mexico
(Rogers-Bennett 2013). Population outbreaks of these species have led to destructive
grazing of kelp forests in Alaska and California, USA, and British Columbia, Canada
(North & Pearse 1970, Duggins 1980, Watson & Estes 2011). Epizootics have been
documented in *Strongylocentrotus franciscanus* and *S. purpuratus* from central California
to Mexico, and are attributed to at least 3 different pathologies (Table 8.1). ‘Bald sea
urchin disease’ is a cosmopolitan disease of sea urchins (Maes & Jangoux 1984) that was
identified in *S. franciscanus* in southern California in the early 1970s (Johnson 1971).
This disease is associated with bacterial infection (*Vibrio* sp. or *Aeromonas* sp.) of the
body wall of sea urchins that causes lesions and loss of spines (Gilles & Pearse 1986,
Table 8.1). However, Koch’s postulates have not been fulfilled, and it remains unclear
whether this disease is infectious (Gilles & Pearse 1986). In the mid-1970s, localized
mass mortality of *S. franciscanus* due to bald sea urchin disease led to kelp forest
recovery in an urchin barrens near Santa Cruz, California (Pearse et al. 1977, Pearse &
Hines 1979). In 1992, a second pathology termed ‘sea urchin wasting disease’ was
documented in *S. purpuratus* and *S. franciscanus* at Channel Islands National Park
(CINP), California. A monitoring program has been in place in CINP since 1982, and the
emergence of disease in 1992 was associated with warm water caused by an El Niño
event (Richards & Kushner 1994, Lafferty 2004). The causative agent of wasting disease
is believed to be bacterial; however, a pathogen has not been identified, and the mode of
transmission and origin of wasting disease remain unknown. Sea urchin wasting disease
causes a shortening or loss of spines and formation of lesions on the outer body wall
(Richards & Kushner 1994). Although sea urchins often recover from wasting disease, localized mass mortalities of *S. purpuratus* and *S. franciscanus* have occurred, resulting in small-scale phase shifts from barrens to kelp forests at CINP (Table 8.1). In 2005, a third pathology, termed ‘black spot disease’, was documented at CINP, but has not been associated with mass mortality of sea urchins.

Lafferty et al. (2004) found that the prevalence of wasting disease in *Strongylocentrotus franciscanus* and *S. purpuratus* (measured as the proportion of sites with infected sea urchins) increased from 1982 to 2001. This change in disease prevalence was not related to sea temperature (Lafferty et al. 2004). Wasting disease was positively related to sea urchin density, which was negatively related to the abundance of sea urchin predators, indicating that a trophic cascade may act to facilitate epizootics (Lafferty 2004). The overall relative importance of disease in controlling population densities of *S. franciscanus* and *S. purpuratus* is likely less than that of predation (Lafferty 2004, Kushner et al. 2004), which has caused large-scale shifts from urchin barrens to kelp forests throughout the Northwest Pacific (Tegner & Dayton 2000, Estes et al. 2010, Watson & Estes 2011). However, our analysis of 30 years of published data from the CINP Kelp Forest Monitoring Program, National Park Service (Kushner et al. 2013c), provides evidence that wasting disease may regulate populations of *S. purpuratus* in some parts of California (Fig. 8.1b).

**8.3.3. Diadema**

Sea urchins of the genus *Diadema* occur in warm temperate and tropical oceans worldwide (Muthiga & McClanahan 2013), and are important grazers of macroalgae on shallow reefs (Sammarco 1982, Benítez-Villalobos et al. 2008, Alves et al. 2003). Disease has been documented in 3 of 7 species of *Diadema*. Disease has had a profound effect on *D. antillarum* in the tropical western Atlantic, with important ecological consequences for coral reefs. In 1983, an epizootic resulted in mass mortality of *D. antillarum* throughout its Caribbean range (Lessios et al. 1984), resulting in a 93 % decline in abundance – it is considered the single greatest mass mortality of a marine animal (Lessios 1988a, Fig. 8.1c). The eradication of this key grazer resulted in a phase
shift from live coral to macroalgal dominance on many reefs (Liddell & Ohlhorst 1986, Hughes et al. 1987, Levitan 1988, Hughes 1989, 1994, Carpenter 1990, Ostrander et al. 2000, Aronson & Precht 2001a, Edmunds & Carpenter 2001). The pathogenic agent was not identified; however, the spread of the mass mortality suggests a water-borne pathogen (Lessios 1988a). The epizootic and the ensuing phase shift to macroalgal reefs were preceded by the collapse due to overfishing of herbivorous fish stocks (mainly parrot fish and surgeonfish) that historically provided functional redundancy as grazers on coral reefs (Hughes 1994). Subsequent localized mass mortalities of *D. antillarum* were documented in St. Croix, US Virgin Islands in 1985 (Carpenter 1990) and the Florida Keys in 1991 (Forcucci 1994). In some regions of the Caribbean, populations of *D. antillarum* have recovered, leading to localized reverse phase shifts to coral dominance (Edmunds & Carpenter 2001, Carpenter & Edmunds 2006). In general, however, recovery has been slow possibly due to an allee effect following the 1983 mass mortality, which may be limiting larval production (Lessios 2005, Chiappone et al. 2013).

The 1983 disease outbreak in *Diadema antillarum* in the tropical western Atlantic did not affect the populations of 2 congeneric species: *D. mexicanum* on the Pacific coast of Panama (Lessios et al. 1984) and *D. africanum* (previously *D. aff. antillarum*) in the eastern Atlantic (Tuya et al. 2005). In 2009, a localized mass mortality of *D. mexicanum*, with symptoms of disease similar to those observed in *D. antillarum* in 1983, occurred along the Pacific coast of Mexico (Benítez-Villalobos et al. 2009). In 2009 to 2010, an outbreak of bald sea urchin disease caused widespread mass mortality of *D. africanum* across > 400 km from Madeira, Portugal to the Canary Islands, Spain (Hernández et al. 2013). *Vibrio algynoliticus* and *Paramoeba branchiphila* comb. nov. (Feehan et al. 2013) were identified as pathogenic agents (Dyková et al. 2011, Hernández et al. 2013). *Paramoeba branchiphila* is closely related to *P. invadens*, the pathogen that infects *Strongylocentrotus droebachiensis* along the Atlantic coast of Nova Scotia (Feehan et al. 2013), although it is thought to be an opportunistic pathogen of *D. africanum*, infecting only diseased individuals (Dyková et al. 2011). *D. africanum* is a dominant grazer in the Canary Islands (Alves et al. 2001, 2003, Tuya et al. 2004), and phase shifts from urchin barrens to macroalgal beds have been observed in marine protected areas following the cessation of historical overfishing of sea urchin predators (Sangil et al. 2012). This
suggests that predation is an important agent of population control of *D. africanum* (Sangil et al. 2012, see also Clemente et al. 2010). The recent emergence of sea urchin disease in this region potentially could augment predatory controls, with important implications for the persistence of the macroalgal state.

### 8.3.4. Paracentrotus lividus

*Paracentrotus lividus* is found in intertidal and subtidal habitats throughout the Mediterranean Sea and in parts of the Northeast Atlantic. It is an opportunistic generalist, but can destructively graze macroalgae, altering benthic habitats (Boudouresque & Verlaque 2013). In the northwestern Mediterranean, predation and dislodgement by strong storms are major agents of mortality of *P. lividus* (Hereu et al. 2012), although pollution, harvesting, and disease also affect population abundance (Sala et al. 1998, Boudouresque & Verlaque 2013).

Bald sea urchin disease has been recorded in *P. lividus* in the Mediterranean, on the Atlantic coast of France, and in the Canary Islands, Spain (Table 8.1). Mass mortality of *P. lividus* due to this disease was observed in the late 1970s in the northwestern Mediterranean (Boudouresque & Verlaque 2013) and in 2003 at intertidal sites in the Canary Islands, where disease prevalence was positively associated with sea surface temperature and negatively associated with wave height (Girard et al. 2012). A mass mortality of *P. lividus* in the northwestern Mediterranean in the late 1970s led to a localized increase in filamentous epiphytes on seagrass in a *Posidonia oceanica* meadow (Boudouresque et al. 1980). In Corsica, France, *P. lividus* is infected by the metacercariae of a parasitic trematode (*Macvicaria crassigula*), which may increase the susceptibility of the sea urchin to predation by fish that are a secondary host to the adult trematode (Boudouresque & Verlaque 2013). *P. lividus* is also parasitized by a marine snail, *Vitreolina philippi*, in the Canary Islands (Rodríguez et al. 2001).
8.3.5. Other Herbivorous Sea Urchins

Disease and parasitism have been identified in a number of species of herbivorous sea urchin, with no associated mass mortality. In California, *Centrostephanus coronatus* is infected by parasitic nematodes with no apparent lethal effects (Byrne & Andrew 2013). *Lytechinus anamesus* experiences wasting disease in California (Richards & Kushner 1994), but there have been no recorded mass mortalities. In Southeast Australia, *Heliocidaris erythrogramma* is infected by trematodes and a parasitic tubellarian (Keesing 2013). *Eucidaris galapagensis* in the Galápagos (Sonnenholzner et al. 2011), *Arbacia lixula* and *Sphaerechinus granularis* in the Canary Islands (Rodríguez et al. 2001), and *Strongylocentrotus nudus* in Japan (Agatsuma 2013) are parasitized by snails. Other sea urchins in Japan (*Strongylocentrotus intermedius*, *Pseudocentrotus depressus*, and *Hemicentrotus pulcherrimus*) succumb to bald sea urchin disease in aquaculture, but mass mortality has not been documented in nature (Wang et al. 2013).

Mass mortality of *Evechinus chloroticus* in New Zealand (Shears & Ross 2009) and *Echinometra lucunter* in Brazil (Granéli et al. 2002) has been associated with harmful algal blooms. Whether these mortalities were due to the toxic nature of the microalgae or indirect effects of the bloom is unknown (Shears & Ross 2009).
Table 8.1. Reports of disease outbreaks in herbivorous sea urchins, indicating environmental and biological correlates (positive, +; negative, −) with disease, urchin density prior to disease outbreak (mean ± SD, where available), whether disease resulted in a mass mortality (defined as ≥ 50 % mortality) and estimated % mortality (mean ± SD, where available), the scale of mass mortality, the dominant ecosystem state prior to mass mortality, and whether mass mortality was associated with a phase shift to an alternative ecosystem state.

<table>
<thead>
<tr>
<th>Species/Location</th>
<th>Disease (agent)</th>
<th>Environmental/biological correlates</th>
<th>Year(s)</th>
<th>Urchin density (m$^{-2}$) (no. of sites/reefs sampled)</th>
<th>Mass mortality? (% mortality)</th>
<th>Scale (km)</th>
<th>State</th>
<th>Phase shift?</th>
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<td></td>
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<td></td>
<td>1982</td>
<td>128 ± 62 (1)</td>
<td>Yes (~70)</td>
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<td>Miller (1985)</td>
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<td>Brady &amp; Scheibling (2005)</td>
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<td>ND</td>
<td>Scheibling &amp; Lauzon-Guay (2010)</td>
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<td>Scale (km)</td>
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<td>Phase shift?</td>
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<td>1992</td>
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<td>Skadsheim et al. (1995)</td>
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<td>Barrens</td>
<td>ND</td>
<td>Christie et al. (1995)</td>
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<td><em>Strongylocentrotus purpuratus</em> (<em>S.p.</em>) and <em>S. franciscanus</em> (<em>S.f</em>)</td>
<td>Bald sea urchin disease (bacterial)</td>
<td>ND</td>
<td>1971</td>
<td>ND</td>
<td>ND</td>
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<td>Barrens</td>
<td>ND</td>
<td>Johnson (1971)</td>
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<td>1976</td>
<td>2–6.5 (2)</td>
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<td>Yes</td>
<td>Pearse et al. (1977), Pearse and Hynes (1979)</td>
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*Notes:*
- ND: Not determined
- 1977?: Median year
- Source(s): Additional references not provided in the table.
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<th>Year(s)</th>
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<th>Scale (km)</th>
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<th>Phase shift?</th>
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<td>Urchin density (+), Strong El Niño? (+)</td>
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<td>1996</td>
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<td>1997</td>
<td>S.p.: 45 ± 19; S.f.: 8.4 ± 6.5 (2)</td>
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<td>Yes</td>
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<td>S.p.: 4.3; S.f.: 7.5 (1)</td>
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<td>1999</td>
<td>7.8 (1)</td>
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<td>2002</td>
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<td>Yes</td>
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<td>Year(s)</td>
<td>Urchin density (m(^2)) (no. of sites/reefs sampled)</td>
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<td>Scale (km)</td>
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<td>Phase shift?</td>
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<td>Lester et al. (2007)</td>
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<td>2005</td>
<td>16 (1)</td>
<td>No (19)</td>
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<td>Kushner et al. (2012, 2013a)</td>
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<td>2006</td>
<td>54.4 ± 28.8 (2); S.f: 13 (1)</td>
<td>No (S.p.: 0, S.f.: 23)</td>
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<td>Kushner et al. (2013a), Moore et al. (2013)</td>
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<td>2007</td>
<td>83 (1); S.f.: 12 ± 2.8 (2)</td>
<td>No (S.p.: 0, S.f.: 0)</td>
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<td>Moore et al. (2013), Sprague et al. (2013a)</td>
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<td>2008</td>
<td>90; S.f.: 11 (1)</td>
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<td>Sprague et al. (2013b)</td>
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<td>2009</td>
<td>137.5 ± 2.9; S.f.: 7.2 ± 1.0 (2)</td>
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<td>1983</td>
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<td>Coral</td>
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<td>1985</td>
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<td>Transition to turf</td>
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\(^a\) The 2004 event at California/Pacific Mexico is an outlier.
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<thead>
<tr>
<th>Species/Location</th>
<th>Disease (agent)</th>
<th>Environmental/biological correlates</th>
<th>Year(s)</th>
<th>Urchin density ((m^2)) (no. of sites/reefs sampled)</th>
<th>Mass mortality? (% mortality)</th>
<th>Scale (km)</th>
<th>State</th>
<th>Phase shift?</th>
<th>Source(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diadema mexicanum</strong></td>
<td>Pacific (Mexico) Unknown</td>
<td>ND</td>
<td>2009</td>
<td>ND (1)</td>
<td>Yes (&gt;99)</td>
<td>&lt;1</td>
<td>Coral</td>
<td>ND</td>
<td>Benítez-Villalobos et al. (2009)</td>
</tr>
<tr>
<td><strong>Diadema africannum</strong></td>
<td>Canary Islands Bald sea urchin disease (bacterial)</td>
<td>ND</td>
<td>2010</td>
<td>ND (2)</td>
<td>No (21?)</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>Dyková et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>Canary Islands &amp; Madeira Island Bald sea urchin disease (bacterial)</td>
<td>Sea temperature (+)</td>
<td>2009-2010</td>
<td>ND</td>
<td>Yes (50)</td>
<td>100s</td>
<td>ND</td>
<td>ND</td>
<td>Hernández et al. (2013)</td>
</tr>
<tr>
<td><strong>Paracentrotus lividus</strong></td>
<td>Mediterranean (France) Bald sea urchin disease (bacterial)</td>
<td>Urchin density (+)</td>
<td>1979</td>
<td>~1–40 (1)</td>
<td>Yes (55 ± 28)</td>
<td>&lt;1</td>
<td>Seagrass</td>
<td>No</td>
<td>Boudouresque et al. (1980), Azzolina (1987)</td>
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<td></td>
<td></td>
<td></td>
<td>2006</td>
<td>ND (1)</td>
<td>No (0.1)</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>Becker et al. (2008)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>ND</td>
<td>ND</td>
<td>No (ND)</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>Hobaus et al. (1981)</td>
</tr>
<tr>
<td></td>
<td>Atlantic (France) Bald sea urchin disease (bacterial)</td>
<td>ND</td>
<td>2006</td>
<td>ND (1)</td>
<td>No (1.0)</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>Becker et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Canary Islands Bald sea urchin disease (bacterial)</td>
<td>Wave action (-), sea temperature (+)</td>
<td>2003</td>
<td>ND (3)</td>
<td>Yes (~10–95)</td>
<td>10s</td>
<td>ND (intertidal)</td>
<td>ND</td>
<td>Girard et al. (2012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2004</td>
<td>ND (1)</td>
<td>N (&lt;10)</td>
<td>–</td>
<td>ND (intertidal)</td>
<td>–</td>
<td>Girard et al. (2012)</td>
</tr>
</tbody>
</table>
ND No Data

aDisease decimated sea urchins in an advancing grazing front along the margin of a kelp bed
bDisease was observed in sea urchins experimentally transplanted into a kelp bed
cLocalized barrens shifted to kelp beds (C.J. Feehan pers. obs.)
dMass mortality likely due to unidentified waterborne agent, not nematode infection (see Stien et al. 1995). Nematode infection is likely chronic in *Strongylocentrotus droebachiensis* in Norway, but with varying prevalence
eSite remained *Strongylocentrotus purpuratus* barrens
fOnly 1 site shifted to developing kelp forest following sea urchin decline. The other site remained barrens, but switched from *Strongylocentrotus purpuratus* to *S. franciscanus* dominance
g*Strongylocentrotus franciscanus* barrens maintained, although *S. purpuratus* decreased dramatically
h*Strongylocentrotus* spp. continued to dominate the site
iThe ecosystem was already transitioning to a macroalgal state as a result of the 1983 *Diadema antillarum* mass mortality, in combination with overfishing and hurricanes (Hughes 1994)
jPreviously *Diadema* aff. *antillarum* (see Rodríguez et al. 2013)
kCo-infection with amoeba *Paramoeba branchiphila* found
lThere was overgrowth of epiphytes on *Posidonia oceanica* leaves following sea urchin mass mortality
Fig. 8.1. Annual fluctuations in mean sea urchin density (m$^{-2}$) and occurrence of disease (arrows). (a) *Strongylocentrotus droebachiensis* (amoebic disease) at 3 sites and 6 habitats along the Atlantic coast of Nova Scotia, Canada (squares eagle head, gray fill cobble bed, no fill boulder field; circles Little Duck Island, Mahone Bay, no fill urchin front, gray fill newly formed urchin barrens, black fill transitional kelp beds; black triangles St. Margarets Bay, kelp beds). ND no data. (b) *S. purpuratus* (wasting disease) at 2 sites at Santa Barbara Island, Channel Islands National Park, California (arch point: closed symbols, solid line; Cat Canyon: open symbols, dotted line). Three different types of arrows indicate disease at one of these sites (Cat Canyon; dotted arrow, arch point; gray arrow) or at both sites (black arrow). (c) *Diadema antillarum* (unknown disease) in Panama (mean + SE, n = 3 – 11 sites). Dashed lines in (a) and (c) indicate gaps in the data record. Data sources: (a) Scheibling (1988 & unpubl data), Scheibling et al. (1999), Feehan & Scheibling (2014a); (b) Kushner et al. (2013c), (c) Lessios (2005).
8.4. CHANGES IN THE FREQUENCY OF SEA URCHIN EPIZOOTICS

Ward & Lafferty (2004) present indirect evidence for an increase in disease in sea urchins as a group (and in other marine taxa) between 1970 and 2001, based on an increase in the proportion of scientific publications (in the primary literature) on a given taxon that report disease. They used the proportion rather than the actual number of reports to standardize for an increase in publication rate over time (Ward & Lafferty 2004). To determine whether a trend of increasing disease outbreaks holds for sea urchin species that have important functional roles as grazers (Strongylocentrotus droebachiensis, S. purpuratus and S. franciscanus, Diadema antillarum, D. mexicanum, and D. africanaum and Paracentrotus lividus; see Disease as a Control of Sea Urchin Populations), we conducted an exhaustive review of the scientific literature (primary and gray) from 1970 to 2012 (Table 8.1).

To identify specific disease events for the 7 species mentioned above, we searched titles, abstracts, and entire articles using each species’ name or “urchin*” as the search terms, in combination with the disease string used by Ward & Lafferty (2004): “disease* or parasit* or pathogen* or infect* or bleaching* or prevalence or virus* or bacteri* or viral or fung* or nematod* or cestod* or trematod* or acanthoceph* or ectoparasit* or endoparasit* or worm* or protozoa* or protist* or (mass and mortalit*).” We searched the following databases: ISI Web of Science; Aquatic Sciences and Fisheries Abstracts; WAVES database, Department of Fisheries and Oceans Canada (DFO); Science Direct; Zoological Record; and the Directory of Open Access Journals (DOAJ). We also manually searched all publications from the CINP Kelp Forest Monitoring Program, National Park Service database. For each article encountered for each species, we determined the location of a disease event, pathology and disease agent (if known), year of infection, sea urchin density prior to disease, and percent mortality due to disease (difference in density before and after disease outbreak). Sea urchin density prior to disease is used to examine whether epizootics were density-dependent. For disease outbreaks that caused mass mortality (defined as ≥ 50 % mortality, Scheibling & Lauzon-Guay 2010), we also recorded the spatial scale of mass mortality, ecosystem state at the time of the epizootic, and whether mass mortality was associated
with a phase shift to an alternative state (Table 8.1). This enabled us to investigate temporal trends in the spatial extent of disease and consequent phase shifts (see Ecosystem-level Effects of Sea Urchin Epizootics).

Studies uncovered by our exhaustive review were used to generate annual records of disease outbreaks for each species of sea urchin (Table 8.1). Records of disease in *Strongylocentrotus purpuratus* and *S. franciscanus* are pooled, as these species are sympatric and both are often impacted by a single disease event. Mass mortalities due to epizootics were identified for the 7 species in 9 regions: *S. droebachiensis* in Nova Scotia, *S. purpuratus* and *S. franciscanus* in California, *Diadema antillarum* in the Caribbean and the Florida Keys, *D. mexicanum* in Pacific Mexico, *D. africanum* in the Canary Islands and Madeira, and *Paracentrotus lividus* in the Mediterranean and the Canary Islands (Table 8.1). To examine temporal trends in epizootics, we constructed cumulative curves for each species based on annual records (Table 8.1, Fig. 8.2a–d). Only records of mass mortality were used in our analysis, as these events are conspicuous and likely to be observed and recorded during regular monitoring of species. Disease outbreaks resulting in mass mortality are of particular interest because they often are associated with phase shifts.

Each of the above species has been extensively studied since the early 1970s (*Paracentrotus lividus*, Boudouresque & Verlaque 2013; *Diadema antillarum* and *D. mexicanum*, Muthiga & McClanahan 2013; *Strongylocentrotus purpuratus* and *S. franciscanus*, Rogers-Bennett 2013; *S. droebachiensis*, Scheibling & Hatcher 2013), except for *D. africanum* and *P. lividus* in the Canary Islands, which have been monitored since the early 1980s (Boudouresque & Verlaque 2013, Muthiga & McClanahan 2013). Therefore, we constructed a cumulative curve of epizootics from 1970 to 2012 for each species (from 1980 to 2012 for *D. africanum* and *P. lividus* in the Canary Islands). This interval encompasses the first scientific report of a disease outbreak in a sea urchin (bald sea urchin disease in *S. franciscanus* in California in 1971, Table 8.1) and the period over which Ward & Lafferty (2004) recorded an increase in disease for sea urchins as a group (1970 – 2001). To control for the addition of new monitoring sites at CINP between 1982 and 2012, we considered data from only 2 sites (Arch Point and Cat Canyon, Santa Barbara Island) monitored every year from 1982 or 1986 to 2012, respectively. Mass
mortalities of *S. droebachiensis* in Norway and the eastern USA (Table 8.1) were not included in this analysis, as there is insufficient evidence to conclude that these mortalities were due to disease (see Disease as a Control of Sea Urchin Populations).

Cumulative curves of mass mortality due to disease differ among species of sea urchin (Fig. 8.2a–d). For *Strongylocentrotus droebachiensis* along the Atlantic coast of Nova Scotia, recurrent outbreaks of disease, resulting in mass mortality, have occurred periodically in 3 waves: early 1980s, late 1990s to early 2000s, and from 2009 onwards, with no reports of disease in the intervening years. There is a trend of decreasing length of the intervening periods without disease (plateaus in curves), suggesting that periods of recurrent disease may be increasing in frequency (Fig. 8.2a). This is consistent with the results of a statistical model by Scheibling & Lauzon-Guay (2010), which indicates that epizootics in *S. droebachiensis* are associated with increasing tropical storm (hurricane) activity and warm sea temperatures (see also Scheibling et al. 2013). The periodicity of disease outbreaks appears to conform to the dynamics of phase shifts between kelp beds and urchin barrens along this coast. Widespread epizootics have occurred in years when sea urchins were abundant and the system was predominantly in the barrens state (e.g. 1980 – 1983 and 1993 – 1999), punctuated by subsequent years when sea urchins were rare and kelp beds were dominant (e.g. 1984 – 1993 and 2003 onwards). Localized disease outbreaks from 2009 to 2012 occurred in patchy barrens or among sea urchins that were experimentally transplanted into a kelp bed (Table 8.1 and references therein). Anecdotal evidence from fishers indicates that mass mortalities of *S. droebachiensis*, and reciprocal fluctuations in kelp and sea urchin abundance, have occurred sporadically along the coast of Nova Scotia in the 1920s, 1930s, and 1950s, prior to any scientific reporting of disease (Scheibling & Stephenson 1984, Miller 1985, Scheibling 1986).

There is a trend of decreasing spatial extent of disease outbreaks in *Strongylocentrotus droebachiensis* in Nova Scotia (Table 8.1, Fig. 8.2a). Epizootics in the 1980s and 1990s resulted in coastal-scale mass mortality, while outbreaks since 2003 have been localized (Table 8.1, Fig. 8.2a). This decrease in the extent of die-offs follows a progressive reduction in urchin barrens along this coast, as a result of recurrent epizootics since the early 1980s (Scheibling et al. 2013). Percent mortality of *S. droebachiensis* due to disease is mediated by sea temperature at the time of infection.
(Scheibling & Stephenson 1984) and does not appear to be related to local sea urchin density (Table 8.1).

The spatial extent of epizootics in *Strongylocentrotus purpuratus* and *S. franciscanus* has remained relatively small (Table 8.1, Fig. 8.2b). A sharp increase in sea urchin mass mortalities occurred in 1992 due to the emergence of wasting disease at CINP (Table 8.1, Fig. 8.2b). Most mass mortality events involved the more abundant species, *S. purpuratus*, and the severity of these events increased with sea urchin density, suggesting density-dependent mortality (Table 8.1, Fig. 8.3). This is consistent with Lafferty’s (2004) observation of a positive relationship between the probability of an epizootic and sea urchin density (pooling densities of *S. purpuratus*, *S. franciscanus*, and *Lytechinus anamesus*) at CINP from 1992 to 2001. Percent mortality of *S. purpuratus* due to wasting disease also was higher overall in years when disease was preceded by a strong El Niño event, and 5 out of 6 mass mortality events involving *S. purpuratus* occurred in these years (Table 8.1, Fig. 8.3). Our analysis supports Lafferty’s (2004) proposal that the emergence of wasting disease in 1992 was linked to a large El Niño event and suggests that warm water associated with El Niño facilitates the propagation of wasting disease (Fig. 8.3).

Disease outbreaks in *Diadema antillarum* in 1985 and 1991 occurred on a spatial scale that was 2 orders of magnitude less than that of the 1983 event, which encompassed the geographic range of the species in the tropical western Atlantic (Table 8.1, Fig. 8.2c). There have been no recurrent epizootics in *D. antillarum* since the 1990s, indicating a decrease in the frequency of disease outbreaks (Table 8.1, Fig. 8.2c). This is likely due to the minimal recovery of populations following widespread mass mortality in 1983. However, the observed epizootics of *D. antillarum* do not suggest density-dependent mortality (Lessios 1988a). There have been no records of recurrent mass mortality of *Paracentrotus lividus* in the Mediterranean since an initial outbreak in 1979 (Fig. 8.2d), although these population have been extensively monitored (Hereu et al. 2012, Table 8.1). Epizootics in *D. mexicanum* in Pacific Mexico and *D. africanaum* and *P. lividus* in the Canary Islands have been documented only in the past decade (Fig. 8.1c,d), and may reflect emerging diseases of sea urchins.
The cumulative curve of epizootics for all of the above species combined indicates that severe disease events among ecologically important sea urchins have occurred fairly regularly since the mid-1970s, with ~ 5-year gaps in the mid- to late 1980s/early 1990s and the mid-2000s (Fig. 8.2e). This trend is driven largely by 3 multiyear periods of recurrent outbreaks of paramoebiasis in *Strongylocentrotus droebachiensis* in Nova Scotia since 1980 (Fig. 8.2a). It is strengthened by the emergence of wasting disease in *S. franciscanus* and *S. purpuratus* in California in the early 1990s (Fig. 8.2b), and the emergence of bald sea urchin disease in *Diadema mexicanum* along the Pacific coast of Mexico (Fig. 8.2d) and in *D. africanum* and *P. lividus* in the Canary Islands over the past decade (Fig. 8.2c,d), and countered by an absence of recurrent disease in *P. lividus* in the Mediterranean since the 1980s or in *D. antillarum* in the tropical western Atlantic since the mid-1990s. It should be noted that mass mortalities of *S. droebachiensis* in Norway and the eastern United States, which were excluded from this analysis due to a lack of evidence for disease, occurred in 1983 and from 1991 to 1999 (Table 8.1) and therefore would strengthen the trend indicated by our cumulative curve (Fig. 8.2e). An apparent decrease in the spatial scale of epizootics since the early 1980s (Fig. 8.2e) mainly reflects situations where the range of host populations declined due to recurrent epizootics (e.g. *S. droebachiensis* in Nova Scotia and *D. antillarum* in the tropical western Atlantic).

Frequent disease outbreaks in *Strongylocentrotus droebachiensis* in Nova Scotia are contingent on the ability of this species to recolonize benthic habitats following mass mortality. Refuge populations, unaffected by epizootics, act as a source for repopulation of disease-affected areas through adult migration or larval recruitment. A sharp thermal threshold for outbreaks of amoebic disease in *S. droebachiensis* results in the survival of sea urchins in deeper water (> 18 m) where temperatures are below 12 °C (Scheibling & Stephenson 1984). The lack of refuge populations of *Diadema antillarum*, following mass mortalities in the Caribbean in the 1980s, may explain the low recurrence of disease in this region (Fig. 8.1c).

Change in publication rate can potentially influence temporal trends in disease outbreaks based on a literature review (Ward & Lafferty 2004). To examine this possibility, we compiled a time series of publication records for 5 species that
experienced recurrent epizootics over the course of our analysis (1970 – 2012), using an indexed database. This method is commonly used to examine temporal trends in scientific effort on a particular research topic (Ward & Lafferty 2004, Smale et al. 2013). We searched ISI Web of Science for articles with titles containing each species’ name (S. droebachiensis, S. purpuratus or S. franciscanus, Diadema antillarum, and Paracentrotus lividus). Only titles were searched, as abstracts have only been available for most articles in ISI Web of Science since the 1990s. We present these data as 3-year running averages for each species to account for a lag (~ 3 years) between the completion of a study and the publication of its findings (Ward & Lafferty 2004).

We found no evidence of an effect of publication rate on trends in disease outbreaks for each of the above species. Publication rates generally have increased for all species (Fig. 8.4). For Strongylocentrotus droebachiensis, there is no evidence for waves of publication that could account for those observed in disease reporting, and publication rate decreased from 2009 to 2012, when disease outbreaks were recorded annually (Fig. 8.2a). For S. purpuratus and S. franciscanus, publication rate increased between 1970 and 1990, and then declined over the following decade. This pattern is opposite to the trend in disease outbreaks, which increased between 1990 and 2000 (Fig. 8.2b). Importantly, this trend in disease outbreaks is based largely on the data from the CINP Monitoring Program (gray literature), which is not considered in the time series from ISI Web of Science (primary literature only). However, we controlled for an increase in research effort in the CINP Monitoring Program by considering data from only 2 regularly monitored sites (1982 and 1986 – 2012). For Diadema antillarum, publication rates exhibited a strong cyclicity with peaks in the late 1980s and late 2000s that did not conform to the trend of decreasing disease outbreaks after the major event in 1983 (Fig. 8.2c). Finally, for Paracentrotus lividus, a progressive increase in publication rate over the period of our analysis was not associated with an increase in reports of disease (Fig. 8.2d).
Fig. 8.2. (a–e) Cumulative number of annual mass mortality events (≥ 50% mortality) of sea urchin species due to the outbreaks of disease in published studies from 1970 to 2012. (f–i) Cumulative number of epizootics in (a–e) that resulted in an ecosystem phase shift (urchin barrens to macroalgae for Strongylocentrotus spp.; coral reefs to macroalgae for D. antillarum). Vertical bars show the spatial extent of an epizootic or epizootic-induced phase shift on a log scale (km). The starting date of the analysis is 1970 for all species except for Diadema africanum and Paracentrotus lividus in the Canary Islands, which begin in 1980. Data for D. africanum in (c) are shifted by +0.5 for visual clarity. The spatial extent of disease outbreaks for D. africanum and D. mexicanum in 2009 is shown in dark and light gray, respectively. See Table 8.1 for data sources.
Fig. 8.3. Relationship between percent mortality due to wasting disease and sea urchin density (m$^{-2}$) (mean ± SD, where available) for *Strongylocentrotus purpuratus* (squares) and *S. franciscanus* (diamonds) at two sites (Arch Point and Cat Canyon) at Channel Islands National Park, California, from 1992 to 2012. Lines indicate a logarithmic relationship for *S. purpuratus* for years when disease was preceded by a strong El Niño event (closed symbols, solid line) or not (open symbols, dashed line). Analysis of covariance (ANCOVA) indicates a significant effect of sea urchin density ($F_{1,10} = 5.486$, $p = 0.0412$) and whether or not disease was preceded by an El Niño ($F_{1,10} = 22.329$, $p < 0.001$) on percent mortality of sea urchins (log-transformed data). Error bars are SE of mean sea urchin density (n = 2 sites) or percent mortality (n = 2 sites). See Table 8.1 for data sources.
Fig. 8.4. Running averages (3 year) of the number of studies published for 5 species of sea urchin from 1970 to 2012 based on a search of titles in ISI Web of Science.
8.5. ECOSYSTEM-LEVEL EFFECTS OF SEA URCHIN EPIZOOTICS

Mass mortalities of sea urchins have had profound effects on the structure of marine ecosystems (Lessios 1988a, Scheibling et al. 2013). These events have led to phase shifts from rocky barrens and coral reefs to macrophyte-dominated communities throughout the world (Table 8.1). The cumulative curve of phase shifts from barrens to kelp beds following sea urchin epizootics, based on our exhaustive review of the literature (Table 8.1), indicate that these shifts occurred repeatedly following die-offs of *Strongylocentrotus droebachiensis* along the Atlantic coast of Nova Scotia from 1980 to 2000 (Table 8.1, Fig. 8.2a,f). This did not occur following localized mortalities of *S. droebachiensis* in 2003, when disease decimated sea urchins at an advancing grazing front along the margin of a kelp bed, or in 2009 and 2010 when disease occurred among groups of sea urchins that were experimentally transplanted to a kelp bed (Table 8.1, Fig. 8.2f). On each occasion, these localized mortalities occurred when the system was in a kelp-dominated state (Table 8.1). The spatial extent of phase shifts also decreased from 1980 to 2012, reflecting the decreasing extent of sea urchin barrens along this coast (Table 8.1, Fig. 8.2f). These observations provide evidence that recurrent epizootics are stabilizing the kelp bed state in Nova Scotia (Scheibling et al. 2013, Feehan & Scheibling 2014a).

In California, phase shifts from barrens to kelp forests have occurred following mass mortalities of both *Strongylocentrotus franciscanus* and *S. purpuratus* due to wasting disease or bald sea urchin disease (Table 8.1, Fig. 8.2g). However, they did not occur when large numbers of one or the other of the species survived (Table 8.1). Functional redundancy of the dominant herbivores in the Californian system may render it more resilient to perturbation by disease than the Nova Scotian system with a single species of herbivorous sea urchin. Disease outbreaks in California also have occurred over smaller spatial scales than in Nova Scotia (Fig. 8.2a,b, Table 8.1 and references therein). Patchy barrens along the coast of California result in discontinuous populations of *S. purpuratus* and *S. franciscanus* at relatively small spatial scales, which may limit the transmission of wasting disease over broad areas.
In many parts of the Caribbean, a phase shift from coral- to macroalgal-dominated reefs resulted from the mass mortality of *Diadema antillarum* in 1983 (Lessios 1988a). Populations of *D. antillarum* have failed to recover throughout the species’ range decades after this event (Lessios 2005). Recurrent disease outbreaks, like those observed in 1985 and 1991, may be partially responsible for this lack of recovery (Lessios 2005, Table 8.1). There was no further change in ecosystem state following these subsequent outbreaks, as many reefs were already transitioning toward macroalgal dominance after the 1983 event (Table 8.1, Fig. 8.2c,h). These results indicate that recurrent disease may also act in part to stabilize a macroalgal state in the Caribbean. The impact of multiple disturbances and stressors on coral reefs, such as physical damage from hurricane activity, coral diseases, and bleaching associated with ocean warming, combined with the lack of functional redundancy due to overfishing, have contributed to the long-term dominance of a macroalgal state (Hughes 1994, Harvell et al. 2007, Mumby et al. 2007, Fung et al. 2011).

The cumulative curve of disease-induced phase shifts for all of the above species combined provides evidence that the frequency of phase shifts, relative to the number of epizootics, has decreased over the past few decades (Fig. 8.2e,i). This mainly reflects situations where the macroalgal state was already in place during recurrent disease outbreaks (e.g. localized mass mortalities of *Strongylocentrotus droebachiensis* in Nova Scotia since 2000 and of *Diadema antillarum* in the tropical western Atlantic since 1983) and suggests that periodic recurrence of sea urchin epizootics may stabilize the macroalgal state. The spatial extent of these phase shifts also appears to have decreased (Fig. 8.2i). This mainly reflects a reduction in the spatial extent of sea urchin populations due to recurrent disease outbreaks.

Various positive feedback mechanisms stabilize the macroalgal state. For example, increased algal cover on reefs can cause recruitment failure of corals (Hughes et al. 2010). High algal cover also can decrease available habitat for sea urchin settlement (Lessios 1988a), and predatory fish and crabs associated with kelp beds can increase post-settlement mortality of sea urchins (Steneck et al. 2002). Recurrent disease outbreaks, combined with these feedback mechanisms within kelp beds, limit the recovery of the sea urchin populations and the likelihood of a reverse shift to barrens.
(Filbee-Dexter & Scheibling 2014). Consequently, a decrease in the severity and extent of epizootics of *Strongylocentrotus droebachiensis* in Nova Scotia over the past decade has not been associated with a phase shift back to barrens (Table 8.1).

On Caribbean reefs, coral disease contributes to the resilience of the macroalgal state (Hughes et al. 2010). Some shifts to a macroalgal state in the Caribbean in the 1980s were attributed to diseases of corals in areas where fishing pressure was light and densities of *Diadema antillarum* were low before and after the 1983 mass mortality (Aronson & Precht 2001b, 2006, but see also Hughes et al. 2010). Small-scale shifts to macroalgae also have been associated with coral disease since the 1983 mass mortality of *D. antillarum* (Nugues & Bak 2008).

### 8.6. Conclusions and Directions for Future Research

Frequent outbreaks of disease among strongylocentrotid urchins in temperate ecosystems are stabilizing a kelp bed state (Fig. 8.2), which generally is considered desirable from a management perspective. In Nova Scotia, for example, kelp beds provide habitat for lobster (*Homarus americanus*), which constitute a valuable fishery (Wharton & Mann 1981). Coastal ecosystems throughout the North Atlantic have been heavily impacted by historical overfishing of top predators (Jackson et al. 2001). Disease likely has replaced predation in controlling sea urchin populations in Nova Scotia (Feehan & Scheibling 2014a), offsetting some of the impacts of overfishing. A similar situation has been observed for sea urchins in California (Lafferty 2004). In contrast, a widespread disease outbreak in *Diadema antillarum* in the Caribbean had devastating effects on the structure and functioning of coral ecosystems (Lessios 1988a, Hughes et al. 2010). Recovery of *D. antillarum* in some regions of the Caribbean has resulted in reverse shifts from macroalgal to coral dominance. However, this is rare, likely due to continuing stresses and disturbances to coral reefs and positive feedback mechanisms that stabilize the macroalgal state (Hughes et al. 2010). Given that *D. antillarum* is subject to an allee effect, a large increase in the sea urchin population likely is required for recovery of this species and this may take decades (Lessios 2005). Management of coastal fisheries
is expected to positively affect Caribbean reefs through the recovery of herbivorous fish (Edwards et al. 2011).

Evidence that disease-induced phase shifts can lead to a stabilized ecosystem state suggests that reversing the impact of disease, where this is desirable, can be difficult in marine systems (Filbee-Dexter & Scheibling 2014). Altering conditions that could lead to disease-induced phase shifts may be a more effective approach to management than attempting to reverse these shifts once they occur. Disease outbreaks resulting in phase shifts in marine systems often are preceded by ecosystem degradation (Jackson et al. 2001), and population outbreaks and associated epizootics in sea urchins seem to be linked with overfishing of sea urchin predators (Lafferty et al. 2004, Uthicke et al. 2009). These observations indicate that the establishment of marine reserves and management of coastal fisheries could increase the resilience of ecosystems to perturbations such as disease. The frequency or severity of epizootics in sea urchins has been linked to changes in storm activity, El Niño events, and increasing sea temperature associated with ocean warming (Lafferty 2004, Girard et al. 2012, Hernández et al. 2013, Scheibling et al. 2013). Climate change clearly plays a key role in the dynamics of marine disease (Burge et al. 2014). Unfortunately, in some cases (e.g. coral reefs), the predicted impacts of climate change may surpass our ability to manage coastal ecosystems to mitigate these impacts at local or regional scales (Edwards et al. 2011).

Although Ward & Lafferty (2004) conclude that disease is increasing for sea urchins as a group (as well as for other taxa), our analysis indicates that this trend does not hold for ecologically important species in some of the most intensively studied marine systems (Fig. 8.2). The question of whether disease is increasing in the ocean is difficult to answer, given that we lack baselines for a “natural” level of disease. Our results indicate that the answer also will depend on the scale over which the question is addressed. Indirect approaches, such as standardized literature analysis, are useful for analyzing trends in disease at a global scale, for which data are abundant (Ward & Lafferty 2004). However, at the local and regional scales of communities or ecosystems, over which ecological hypotheses generally are tested, data on marine diseases generally are limited and alternative approaches are required.
We have shown that an exhaustive review of the scientific literature can be a useful approach for investigating trends in disease outbreaks in sea urchins and associated ecosystem phase shifts. However, this approach relies on the existence of long-term datasets across large spatial scales that characterize marine communities and ecosystems (Table 8.1). This underscores the need for further longitudinal studies to effectively monitor disease and its ecological impacts on a global scale. Our findings may prove insightful for future research on disease trends in other marine organisms that also are impacted by climate change and other anthropogenic stressors. A better understanding of the dynamics of disease outbreaks in marine ecosystems, and the role of disease as a driver of ecosystem change, is required for effective management and governance of coastal resources.

There are many cases in marine systems where a causative agent of disease has not been identified (Table 8.1). This is problematic, as reliable identification of new and existing diseases is key to tracking changes in disease frequency and subsequent ecosystem-level effects (Table 8.1). Application of Koch’s postulates to confirm causative agents of disease should be an important component of ongoing research. Molecular genetic techniques increasingly are being used to identify pathogens in marine organisms (Feehan et al. 2013). Genetic probes, developed from previously isolated pathogens, allow rapid detection and identification of pathogens in environmental and biological samples. When combined with coastal circulation models, this can greatly advance our understanding of infective source populations and transmission pathways that govern the spread of marine disease.
8.7. ACKNOWLEDGEMENTS

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CHAPTER 9

DISCUSSION

9.1. Perspectives on the Dynamics of the Rocky Subtidal Ecosystem of Nova Scotia

Along the Atlantic coast of Nova Scotia green sea urchins Strongylocentrotus droebachiensis destructively graze kelp beds leading to the formation of less productive sea urchin barrens (Johnson & Mann 1988, Scheibling et al. 1999, Lauzon-Guay & Scheibling 2007a). This phase shift occurs through the shoreward migration of sea urchins from the deep subtidal zone (> 18 m), which exist within a thermal refuge from an infectious disease (paramoebiasis) (Brady & Scheibling 2005), or by the development of grazing aggregations of sea urchins within kelp beds (Chapter 2; Feehan et al. 2012b), which may arise by recruitment of sea urchins via larval settlement (Lauzon-Guay & Scheibling 2010). A reverse phase shift from sea urchin barrens to kelp beds occurs following outbreaks of paramoebiasis that cause mass mortality of sea urchins in shallow barrens (Miller 1985, Scheibling 1986s). Since the early 1980s, shifts between these alternative ecosystem states have occurred on an approximately decadal scale (Scheibling et al. 1999). However, observations over the course of my PhD research (2009 – 2015) suggest that this ecosystem is transitioning towards a new configuration, with the stabilization of a macroalgal state (Scheibling et al. 2013). This is related to an increase in the frequency of mass mortalities of S. droebachiensis due to paramoebiasis (Scheibling & Lauzon-Guay 2010, Chapter 7; Feehan & Scheibling 2014b) that is preventing the establishment of sea urchin grazing fronts and aggregations (Chapter 5; Feehan & Scheibling 2014a).

Evidence from my research (Chapter 4) and related studies (Feehan et al. 2013, R. Buchwald, C.J. Feehan, R.E. Scheibling, A.G.B Simpson, in review) indicates that the causative agent of disease, Paramoeba invadens, has remained functionally and physiologically stable over a 35-year period since the early 1980s. Outbreaks of paramoebiasis are linked to strong storms and warm sea temperatures (Scheibling &
Lauzon-Guay 2010, Scheibling et al. 2010, Chapter 3; Feehan et al. 2012a), which may play a role in the introduction of *P. invadens* to the coast of Nova Scotia from distant offshore source populations (Chapter 7). An increase in the frequency of outbreaks of paramoebiasis since the early 1980s is associated with an increase in storm activity and peak sea temperatures (Scheibling & Lauzon-Guay 2010). These results suggest that climate change is altering the environmental ‘landscape’ on which phase shifts between kelp beds and sea urchin barrens occur, leading to a new equilibrium or ‘domain of attraction’ that favours the macroalgal state (Scheibling et al. 2013, Filbee-Dexter & Scheibling 2014).

Predation on juvenile *Strongylocentrotus droebachiensis* by benthic invertebrates, such as cancrid crabs, may limit the recovery of sea urchin populations in Nova Scotian kelp beds following outbreaks of disease (Chapter 6; Feehan et al. 2014). However, my research suggests that, overall, disease has replaced predation as the major agent controlling sea urchin populations in this region (Chapter 5; Feehan & Scheibling 2014a). This likely is due in part to historical overfishing of sea urchin predators, such as ground fish (Scheibling 1996). Correlative evidence of an increase or emergence of disease following the removal of sea urchin predators exists in other overfished regions of the world, including the Caribbean and California, lending further support for this paradigm shift (Chapter 8; Feehan & Scheibling 2014b). A reduction in sea urchin grazing pressure as a result of disease has led to profound changes in the structure and functioning of marine ecosystems (Chapter 8; Feehan & Scheibling 2014b).

Over the course of my PhD research, profound changes have been observed in the kelp bed ecosystem along the coast of Nova Scotia. Notably, we have seen a widespread replacement of the dominant canopy-forming kelps (*Saccharina latissima, Laminaria digitata*) by opportunistic filamentous turf-forming macroalgal species (K. Filbee-Dexter & R.E. Scheibling unpublished manuscript). This phase shift to turf-algal dominance is linked to long-term reductions in kelp cover and biomass associated with the synergistic effects of increasing peak sea temperatures (E.J. Simonson, R.E Scheibling, A. Metaxas, unpublished manuscript), increased intensity of grazing by mesograzers (O’Brien et al. 2015), proliferation of an invasive bryozoan (*Membranipora membranacea*) that encrusts kelp fronds (Scheibling & Gagnon 2009), and increased intensity of strong storms that
cause kelp breakage (Krumhansl & Scheibling 2011). The turf-algal state may represent an alternative stable state of the kelp bed ecosystem, as it appears to be stabilized by positive feedback mechanisms (e.g. accumulation of sediment by turfs can limit kelp recruitment, K. Filbee Dexter & R.E. Scheibling unpublished manuscript). Thus, a release of kelps from sea urchin grazing pressure, due to recurrent disease outbreaks documented during my research, is unlikely to return the system to a kelp-dominated state. This is in contrast to 3 decades of observations of cyclical alternations between sea urchin barrens and kelp beds along this coast (Scheibling et al. 2013). It is possible that, historically, low-density populations of sea urchins within kelp beds mediated the competitive relationship between kelp and turf species fostering coexistence, and that the removal of sea urchins within these beds by recurrent disease outbreaks in recent years (Chapter 6) has facilitated the phase shift to turf. It remains to be determined whether reestablishment of sea urchin populations in turf-dominated beds could potentially facilitate the reinstatement of kelp beds; however, it is anticipated that Allee effects at low kelp density could prevent recovery of kelp populations (O’Brien et al. 2015, and J. O’Brien unpublished data).

We observed high spatial and temporal variability in disease outbreaks in a field experiment conducted over 5 years (Chapters 3 and 7), and some evidence for spatial variability in the pathogenicity of Paramoeba invadens isolated from infected sea urchins in 2011 (Chapter 4). The spatial extent of a disease outbreak following introduction of P. invadens likely is mediated by the extent of the susceptible host population (for propagation of the disease) and by ocean currents that transport the pathogen along the coast. It is also possible that strong hurricanes that follow candidate storms spread the pathogen following initial introduction (Chapter 7). Future work should examine the distribution of P. invadens in the environment (sediments and seawater) inshore and offshore of Nova Scotia, before, during, and after strong hurricanes. If P. invadens is patchily distributed in the water column, this could account for a time lag in the onset of disease among sites, or the introduction of multiple infective strains of P. invadens of varying pathogenicity. New technologies are needed to examine such hypotheses, including a genetic probe to test for P. invadens in the environment and the tissues of marine organisms, quantitative real-time polymerase chain reaction procedures (qPCR) to
examine changes in the concentration of the pathogen, and measurements of *in situ* ocean currents using ocean gliders. These data, paired with high-resolution hydrodynamic models, will be essential to ultimately determine the mode(s) of introduction of *P. invadens* and mechanism(s) of spread along the coast.

9.2. Integrating Disease into Community Dynamics: Building a New Conceptual Framework

The concept of a ‘trophic cascade’ (Paine 1980) is well entrenched in theoretical ecology. To date this terminology has been used almost exclusively to describe predator-prey interactions. Recently, trophic cascades resulting from host-pathogen interactions involving keystone herbivores and predators (*sensu* Paine 1966) have been identified in both marine (Chapter 8; Feehan & Scheibling 2014b) and terrestrial (Hollings et al. 2014) ecosystems, although such interactions have yet to be incorporated into a common conceptual framework. Disease is one of the most complicated and pressing issues currently facing scientists and society (Burge et al. 2014). Gaining insight into biotic and abiotic processes that mediate disease outbreaks and their population- and community-level effects is crucial to conservation and management of marine ecosystems.

Recent research has focused mainly on changes in the frequency of disease outbreaks and on identifying drivers of emerging diseases (Harvell et al. 2002, Plowright 2008, Burge et al. 2014), with less effort invested in understanding the ecosystem-level effects of disease (Chapter 8; Feehan & Scheibling 2014b). I propose broadening our conceptual framework of community dynamics to include host-pathogen interactions that can lead to disease-induced trophic cascades. Outbreaks of amoebic disease and wasting disease in strongylocentrotid sea urchins in Nova Scotia (since the 1980s) and in California (since the 1990s), respectively, and an outbreak of an unknown disease in *Diadema antillarum* in the Caribbean in 1983, are prime examples. These disease outbreaks have caused or contributed to phase shifts from sea urchin barrens and coral reefs to macroalgal dominated reefs in temperate and tropical regions respectively (Chapter 8; Feehan & Scheibling 2014b). However the relative importance of disease and predation in controlling sea urchin populations and mediating phase shifts can vary within and between regions (Fig. 9.1). Other examples that would be considered under
this framework include: 1) diseases of seagrasses (e.g. *Zostera marina*) that have caused
dramatic changes to benthic community structure and function in soft sediments habitats
(Muehlstein 1989, Short et al. 1987, Robblee et al. 1991); 2) devil facial tumor disease
(DFTD) of the Tasmanian devil *Sarcophilus harrisii* (terrestrial carnivore), which has led
to an increase in the abundance of mesopredators and concomitant loss of smaller
predators (Hollings et al. 2014); and 3) rinderpest disease of wildebeest (terrestrial
herbivore) in the Serengeti, which has led to the loss of trees on African savannas due to
increased wildfires as a result of a build-up of unconsumed grasses (Dobson et al. 2011).

Within these examples there are typical drivers of disease-induced trophic
cascades. Often these are related to human activities, such as overfishing or overhunting,
that result in the loss of top predators or species that provide functional redundancy in
marine (Jackson et al. 2001, Chapter 8; Feehan & Scheibling 2014b) or terrestrial
(Hollings et al. 2014) ecosystems. For example, phase shifts from coral to turf-algal
dominance on reefs in the Caribbean following a disease outbreak in *Diadema antillarum*
in 1983 were preceded by the collapse due to overfishing of herbivorous fish species that
historically provided functional redundancy as grazers on reefs (Hughes 1994). Similarly,
changes in the abundance of mesopredators following catastrophic loss of Tasmanian
devils from DFTD were preceded by the extinction due to overhunting of the thylacine
(*Thylacinus cynocephalus*), a former apex predator in Tasmania. A conceptual framework
that includes disease-induced trophic cascades would integrate information on the
ecosystem-level consequences of major shifts in trophic interactions. Furthermore it
would aid in organizing the expanding body of information on infectious diseases and
increase our understanding of how human activity is altering ecosystems globally.
Fig. 9.1. Host-pathogen interactions may be overlooked as drivers of trophic cascades in marine and terrestrial ecosystems. Trophic cascades driven by predation (classical top-down trophic cascade model) and disease (model discussed here) in herbivorous sea urchins in 3 regions lead to changes in benthic community structure by altering the abundance of macroalgae on temperate and tropical reefs. Macroalgae compete with coralline algae and corals, resulting in shifts in the dominance of these groups. Solid lines indicate direct negative (-) interactions. Dashed lines indicate indirect positive (+) interactions. The size of the text indicates the relative importance of drivers in each region. The strength of the negative effect of disease on sea urchins may increase when predation pressure is reduced, due to density-dependence of disease.
**APPENDIX A**

**SUPPLEMENTAL MATERIALS**

Table A.1. Experimental sites (and abbreviations) within and immediately outside of St. Margarets Bay, Nova Scotia. A checkmark indicates that a site was used in a particular year.

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<th>Year</th>
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<th>Mill Cove (MC)</th>
<th>Croucher Island (CI)</th>
<th>Luke Island (LI)</th>
<th>Paddy’s Head (PH)</th>
<th>Cranberry Cove (CC)</th>
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Fig. A.1. (following pages): Time series of oceanographic and meteorological variables measured at Halifax Harbour Buoy (HHB) and Sable Island (SI) in late June to early November of 2009–2014 (a–f), showing wind speed cubed ((m s\(^{-1}\))^3), winds isolated into positive (red) and negative (blue) U (across-shore) and V (alongshore) components relative to 60 °T (m s\(^{-1}\)), time-integrated U (blue line) and V (red line) component winds cubed ((m s\(^{-1}\))^3) for Sable Island, atmospheric pressure (hPa), air temperature (°C), bottom orbital velocity (m s\(^{-1}\)) at 8 and 60 m depth, and significant wave height (m), and sea temperature (°C) measured at 4 depths at The Lodge in St. Margarets Bay (4, 8, 12, 18 m). The vertical dashed line indicates the date when a candidate storm was closest to the coast of Nova Scotia. In (a–c) and (f) a gray band indicates the period of mass mortality of sea urchins in cages within and immediately outside of St. Margarets Bay defined as the date when ≥ 50 % morbidity or mortality of sea urchins was first observed to the date when mortality reached ≥ 90 % or began to plateau. In (d) the finely hatched band indicates the period of mass mortality of a natural sea urchin population at Point Pleasant Park, the solid gray band indicates the period of mass mortality of sea urchins in cages at 2 sites immediately outside of the bay, and the loosely hatched band indicates the period of mass mortality of sea urchins in cages at a single site within the bay and at Splitnose Point (Fig. 7.1) (for detailed site-specific results see Fig. 7.2).
Wind Speed (m s\(^{-1}\))

Wind: V (m s\(^{-1}\))

Wind: U (m s\(^{-1}\))

Atm. Pressure (hPa)

Atm. Temperature (°C)

Sig. Wave Height (m)

Water Temperature (°C)

Orb. Velocity (m/s)

Date (2013)

APPENDIX B

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