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The ecological role of feeding disturbances of the Atlantic horseshoe crab, Limulus polyphemus

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THE ECOLOGICAL ROLE OF FEEDING DISTURBANCES OF
THE ATLANTIC HORSESHOE CRAB, *LIMULUS POLYPHEMUS*

BY

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DISSERTATION

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ABSTRACT

THE ECOLOGICAL ROLE OF FEEDING DISTURBANCES OF
THE ATLANTIC HORSESHOE CRAB, *LIMULUS POLYPHEMUS*

by

Wan-Jean Lee

University of New Hampshire, December, 2012

This study examined the influence of localized disturbances on the heterogeneity of ecological communities at multiple temporal and spatial scales. Foraging disturbances by the epibenthic predator, Atlantic Horseshoe Crab *Limulus polyphemus*, on the intertidal mudflats of Great Bay estuary, New Hampshire, USA were used as the study system.

This study overcame methodological hurdles in the study of small localized disturbance over extensive areas of soft-sediments. Using a novel, low-cost technique to monitor *Limulus* foraging disturbances, Great Bay’s tidal flats were found to be critical feeding habitats from late spring till fall. Foraging *Limulus* disturbed the benthos of Great Bay at high frequencies and intensities – disturbing 67-70% of the survey area more than once every four weeks over the intertidal foraging season. It was also found that *Limulus* disturbance within a single site exhibited a clustered spatial pattern over a spatial scale of 3 weeks and also over the entire intertidal foraging season.

Infauna densities in individual *Limulus* feeding pits were significantly lower than in undisturbed sediment, and recovered to resemble the structure of undisturbed communities within 28 days. The role of *Limulus* disturbance on infaunal community structure was confirmed by long-term exclusion experiments in 2009 and 2010. Removal
of *Limulus* disturbance resulted in significant increase in predatory polychaetes in both years, although there were no significant trends observed in abundance of total infauna or deposit feeding polychaetes and oligochaetes. On the other hand, bivalve, *Macoma balthica*, abundance and biomass were significantly higher within exclusions. On the scale of the estuary, *Limulus* disturbance was found to contribute between 24% to 91% of the variability of total infaunal abundance, and had similarly negative effects on the abundance of predatory and deposit feeding polychaetes across the estuary. However, *Limulus* disturbance patterns did not explain the variability of *Macoma* abundance and biomass across the estuary. Observational and experimental results revealed that *Limulus* is a critical factor structuring infaunal communities in Great Bay. However, the infaunal taxa that is most affected by *Limulus* foraging disturbance varies from the localized scale of individual disturbances to the landscape scale of the estuary.
CHAPTER 1

GENERAL INTRODUCTION

_Localised disturbance and ecological heterogeneity_

Disturbance is defined as damage, displacement or mortality caused by physical agents of incidentally by biotic agents (Sousa 2001). All communities and ecosystems exhibit some degree of spatial and temporal heterogeneity (Goldberg and Gross 1988, Collins and Smith 2006). Heterogeneity, refers to the point-to-point dissimilarity in environmental conditions, species composition, or process rates in space and/or time, which in turn have been shown to influence species diversity, coexistence and ecological thresholds in communities (Collins and Smith 2006). Therefore as ecosystems are increasingly homogenized through human activities, it is critical to understand the dynamics of disturbances and the causes and consequences of heterogeneity (Thrush et al. 2001, Collins and Smith 2006).

Ecology of disturbances in marine habitats have received increasing attention since the classic studies by Dayton (1971) and Sousa (1980). Large scale disturbances such as hurricanes, hypoxia, oil spills, dredging are the most conspicuous and dramatic examples of disturbances in marine environments and have been the subject of considerable research, particularly in sedimentary habitats (Dethier 1984, Norkko et al. 2006, Van Colen et al. 2008). However, small scale localized disturbance play a similarly
significant role in structuring ecological communities, in marine and terrestrial communities (Probert 1984, Goldberg and Gross 1988, Hall et al. 1994). Disturbance caused by biogenic agents is also referred to as bioturbation. In the broadest sense, bioturbation encompasses reworking of soils and sediments by all kinds of organisms, including microbes, rooting plants and burrowing animals, and has been described as ‘Darwin’s last idea’ (Meysman et al. 2006). Compared to large-scale disturbances, sediment reworking by animals are often cryptic to the human eye. Sousa defines a disturbed ‘patch’ as a contiguous area or volume in which resident organisms have been disturbed (Sousa 2001). While individual animal-generated patches may be small – in the order of cm$^2$ or m$^2$ – the collective actions of a large number of animals and/or a highly mobile population can result in disturbances over an extensive area (Hall et al. 1994).

Sound understanding of the role of disturbance in structuring ecological communities is underlied by information on the spatial and temporal patterns of disturbance and the trajectory of the impacted communities (Hall et al. 1993, Sousa 2001). While there is a considerable body of work studying the recovery of impacted communities (see Sousa 2001 for review), comprehensive understanding of the consequences of disturbances on communities has been limited by the lack of spatially explicit studies of disturbance regimes (Hall et al. 1994, Klaas et al. 2000). This information gap is particularly critical with regards to animal-generated disturbances. This is because animal activities are often driven by extrinsic factors such as predators or food availability, which are patchily distributed in space, and are therefore also expected to be unevenly distributed in space and time.
Most spatially explicit studies of disturbances have been conducted on forests (Goldberg and Gross 1988), probably as a result of the relative tractability of terrestrial plants and animals compared to marine organisms. On the other hand, marine habitats are more fluid, and usually exhibit greater ‘openness’ compared to terrestrial environments (Carr et al. 2003). Therefore, marine communities provide a system to study the patterns and impacts of localized disturbances under levels of key environmental variables different from those of terrestrial studies. This would be critical to understanding the conditions under which localized disturbances are significant in structuring communities. Furthermore, studies that examine localized disturbances at multiple scales (e.g. patch and landscape scales) are rare (Hall et al. 1994), but are necessary to gain a comprehensive understanding of the role disturbances play in structuring ecological communities. In a review of predation in soft-sediment communities, Wilson highlights that a major stumbling block to generalizing the role of predators in soft-sediment communities is the difficulty in understanding the dynamics of highly mobile predators (Wilson 1990b). A major limitation in previous quantification of epibenthic predator disturbance regimes and impacts were difficulty in monitoring the benthos over an adequate spatial and temporal extent and with sufficient resolution under prohibitive field conditions (bad visibility and/or soft-benthos which are disturbed by observer presence). This is a reflection of a general problem in benthic ecology, where there is an on-going need for the development of technology to quantify benthic characteristics and dynamics on local scales (Zajac 2008).
Animal-generated disturbances

Bioturbation results from similar animal activities in terrestrial and aquatic environments, which includes burrowing and excavation (Meysman et al. 2006, Berke 2010). It was suggested recently that effects of burrowing and excavating bioturbators be partitioned (Berke 2010). This partitioning is extremely useful when considering the diversity and range in scale of disturbance caused by burrowers and excavators.

Terrestrial invertebrates such as earthworms, ants and termites and vertebrates such as prairie dogs, gophers and moles are burrowers. While porcupines, skunks, canids and bears dig nesting or foraging holes (references in Berke 2010). Soil disturbance caused by foraging animals such as gophers in old fields and grasslands and porcupines in deserts (Goldberg and Gross 1988, Wilby et al. 2001, Hobbs et al. 2007), have been presented as model systems for the study of biogenic disturbances. Similarly, marine bioturbators include burrowers and excavators. Numerous invertebrate taxa burrow through sediments as a result of movements, or feeding on organic matter associated with sediments (Hall 1994). For example the lugworm, *Arenicola marina* inhabits in J-shaped burrows extending 20-40cm below the sediment surface, through which the surface sediment slides down the burrow to be ingested by the polychaete (Volkenborn et al. 2007). Burrowing amphipods were found to displace a substantial amount of sediments on a South Carolina mudflat (Grant 1983). On the other hand, epibenthic predators ranging from crabs, flounders to whales and walruses excavate sediments in search of buried prey (Probert 1984, Hall 1994). This final class of animal disturbances often leave conspicuous feeding traces that cover extensive areas of the benthos, but are difficult to repeatedly monitor in a sedimentary environment.
Disturbance Impacts & epibenthic predators

Disturbed patches often support a community that is different from undisturbed patches, consequently (Hall et al. 1993). On a broader spatial scale, presence of patches resulting from disturbances dating back from varying points in time result in a mosaic of patches at varying stages of recovery (references in Lohrer et al. 2004). The major factors that determine the impact of a disturbance beyond individual patches are frequency, areal extent and rate of recovery of disturbed patches (Hall et al. 1993, Sousa 2001).

Disturbances in marine sediments encompass a wide range of scales and intensities. At the largest scale, events such hypoxia and fish trawling impact contiguous areas on the scales of square kilometers (Probert 1984, Sousa 2001, Norkko et al. 2006). On the other end of the spectrum are biogenic disturbances, where individual disturbances are on the scale of cm² and m². Hall (1994) provides a detailed review of the spatial scales of biogenic disturbances on marine benthos. Size of individual biogenic disturbances range from 0.03m² pits dug by *Cancer pagurus* crabs to 18m² furrows created by walruses in the Bering Sea. Considering the combined impact of a population of bioturbators, and the greater frequency of animal activity relative to natural events such as hypoxia and storms, biological disturbances are just as likely to be significant in structuring benthic communities.

While all of the abovementioned classes of marine bioturbators are recognized to influence the sedimentary environment in different ways, and play significant roles in structuring benthic communities (Hall 1994), small invertebrate burrowers, suspension and deposit feeders have been the main model organisms in the study of marine
bioturbation (e.g. Michaud et al. 2005, Thrush et al. 2006b, Berkenbusch and Rowden 2007, Volkenborn et al. 2007).

**Epibenthic predator impact on soft-sediment communities & spatial patterns**

Disturbance by epibenthic predators have been repeatedly recognized to be important factors in structuring soft-sediment communities by numerous studies and reviews (Thrush 1999). Flounder and gray whales feed by taking bites out of the sediment surface; crabs dig pits on the benthos to access buried prey; others like walruses and rays uncover infaunal prey by disturbing the sediments with jets of water (Hall 1994, Thrush et al. 1994). Consequently, epibenthic predators influence benthic community structure through a combination of physical disturbance and predation. It is generally recognized that pit-digging by predators immediately result in a significant reduction and/or change in infaunal abundance and community structure (Hall et al. 1991, Thrush 1991, Commito et al. 1995). Zajac’s (2004) review of infaunal response to epibenthic predator activities showed that communities recover to be similar to that of undisturbed patches between 1 to more than 80 days. As mentioned before, the broader impact of disturbances are dependent on the frequency and intensity of disturbance and the rate of recovery of the disturbed communities. Few studies have combined a rigorous quantification of disturbance patterns with the response of disturbed communities (but see review by Zajac 2004). Thrush et al. (Thrush et al. 1991) examined the frequency of disturbance by feeding eagle rays *Myliobatis tenuicaudatus* and the rate of infauna colonization in pits and concluded that low disturbance frequency and intensity per unit area and high colonization rates limited the role of ray foraging in maintaining heterogeneity in the great infaunal community. Hall et al.’s (1991, 1993) study of *Cancer*
*pagurus* led to similar conclusions. On the other hand, other studies have reported relatively large areas of sediments impacted by epibenthic predators. Woodin’s (1978) monthly surveys of a 50m x 1m transect on a sandflat found that disturbance by *Limulus* and *Callinectes sapidus* peaked at 45% in the summer. Van Blaricom (1982) reported that rays disturbed up to 5% of a subtidal sandbed in California everyday in the summer and fall. While Levin’s (1984) weekly surveys of three 15m transects on a mudflat found between 40% to 90% of the benthos disturbed.

However, Hall (1994) also pointed out that measurements of areal extent disturbed by predators are difficult as disturbances occur patchily, so researchers may be biased towards areas of high disturbance, or spatial scales random sampling schemes fail to capture a representative area. Moreover, visibility of the water column above soft-sediments often limit, if not prohibit, adequate sampling of the benthos (Ambrose 1984a, Hall 1994, Hines et al. 1997). While intertidal sites are more accessible than subtidal systems, limited mobility on soft mud and disturbance caused by observers also limit the extent and frequency of surveys.

The broader scale consequences of small localized disturbance by epibenthic predators have also been examined with experiments where predators are excluded from plots, usually covering an area ranging from 0.25m$^2$ to 4m$^2$ (Wilson 1990b, Hall et al. 1993, Olafsson et al. 1994). The majority of the experiments showed that infaunal abundance increases in the absence of predators, although several reviews have pointed out that a substantial number of exclusion studies did not show significant effects (Raffaelli and Milne 1987, Thrush 1999, Rosa et al. 2008). Thrush’s (1999) reviews of previous studies of epibenthic predators showed that the spatial scale of investigation is
critical to the interpretation of results and that complex predator-prey interactions are also likely to vary with spatial scale. Impacts of epibenthic predators are usually reviewed on the basis of their impacts on all infauna in general (WILSON 1990a, Hall 1994, Olafsson et al. 1994). However, as it has also been shown that infaunal communities possess trophic complexities, such as infaunal predator-prey dynamics, that need to taken into consideration when assessing the role of epibenthic predators (Ambrose 1984a, b).

Epibenthic predators have been found to prey preferentially on predatory infauna because (1) many ubiquitous predatory infauna active feed on or near the sediment surface and (2) adult deep-living predatory infauna tend to be larger than non-predatory infauna (Ambrose 1986).

Patterns and Scales

The dominant ecological paradigm is that disturbance is unevenly distributed in space and time (Hall et al. 1993). In a 1994 review of disturbance dynamics, Hall calls for increased attention to analyses of spatial patterns by benthic ecologist (Hall et al. 1994). This is an important point to note when considering the findings of studies on the role of epibenthic predator disturbances. While frequency and intensity of disturbance are critical determinants of impacts of a particular kind of disturbance on a community, more temporally and spatially explicit information is required to understand the ecological role of disturbance (Moloney and Levin 1996). Moloney and Levin (1996) lists the three components essential to characterizing a disturbance regime and its broad scale impact: (1) the basic, non-spatial components: rate and intensity; (2) spatial components of individual disturbances: size and shape; and (3) spatial and temporal components of groups of disturbances: temporal and spatial autocorrelations among individual
disturbances. The non-spatial factors - rate and intensity - represent a mean-field approach historically used by studies of disturbance ecology, where patterns of disturbance in space and time are characterized as an average (Klaas et al. 2000, Menge et al. 2005). However, in representing disturbance rates as averages, local scale variability is critically ignored, and a disturbance regime that plays a significant role may be erroneously concluded as unimportant. The non-spatial measurements of mean rate and intensity determines the proportion of a landscape that is disturbed by a pulse of disturbance. However, the recovery of the landscape from disturbance and the resulting community structure of the landscape depends on the correlation structure of individual disturbances in space and time (Moloney and Levin 1996).

Mean-field approaches do not provide information on the spatial distribution of individuals disturbances. One of the most common ways to present spatial pattern in ecology is the description of a distribution as aggregated, regular or random (Perry et al. 2002). Nearest neighbor distance analyses and variance to mean ratios are two commonly used methods to determine whether spatial distribution of particular events or organisms are random, clumped or regularly spaced (Perry et al. 2002). On the other hand, indices such as Moran’s I estimates the autocorrelation, that is the degree of similarity in the variable of interests, between pairs of samples separated by a fixed distance in space or time (Fortin and Dale 2005). The spatial or temporal extent of a cluster (if present) can be examined by plotting Moran’s I values against a range of distance classes to produce a correlogram (Fortin and Dale 2005). In characterizing the community impacts of a disturbance regime, it is also critical to determine frequency of re-occurrence of disturbance. The figures represent a hypothetical system where disturbed patches recover
completely after 20 days. Disturbances that occur within a single day are clumped. shows a scenario where the clumped disturbances on Days 0 and 14 occur extremely close together, while shows a pattern where Day 14 disturbances occur in between clumps of disturbances from Day 0. On day 15, in the case of the resulting community is the presence of two distinct clusters consisting a mixture of communities recovering from disturbances that occurred one and fourteen days prior. While in b, there are four clusters of disturbed communities, with distinct clusters of communities that have been disturbed one and fourteen days prior. Another approach is the use of applying artificial disturbances that mimick natural disturbances at varying spatial arrangements and scales to examine the influence scale on recovery dynamics (e.g. Dernie et al. 2003, Norkko et al. 2006, Van Colen et al. 2008), but our understanding of disturbance ecology is incomplete without incorporating the natural spatial and temporal variability of disturbance.

**Description of spatial patterns of disturbances**

Goldberg and Gross noted in 1988 (1988) that the majority of spatially and temporally explicit patterns of disturbances were carried out in forests. Since then, ecologists have also used spatially explicit analyses to examine disturbance dynamics in grasslands, and modeling and empirical works show that the spatial and temporal structure of disturbances within a landscape plays a similarly significant role as the overall (mean) landscape-scale rate of disturbance (Hobbs and Mooney 1991, 1995, Moloney and Levin 1996, Hobbs et al. 2007, Questad and Foster 2007). While the importance of spatial scales and patterns in marine benthic ecology have been reiterated in numerous papers and reviews (Thrush 1991, Hall et al. 1994, Menge et al. 2005),

Though it appears that there is increasing effort to apply spatially explicit methods commonly used in terrestrial and landscape ecology in marine systems, studies such as the above mentioned are limited to the description of population and species distribution. To the best of my knowledge, the spatial structures of small localized disturbances have not been elucidated in marine benthic habitats. Previous studies on the role of disturbances on benthic communities focused on the responses of communities after disturbance. Some studies examined the ecology of natural disturbed patches (Hall et al. 1991, Thrush et al. 1991, Commioto et al. 1995). On the other hand, due to the patchiness and relative unpredictability of small localized disturbances, numerous studies of disturbance dynamics artificially induced disturbances, such as digging and raking
(Cowie et al. 2000, Dernie et al. 2003), inducing anoxia and defaunation with by placing plastic sheeting over the sediment (Norkko et al. 2006, Van Colen et al. 2008), and replacing natural sediment with defaunated sediment (Zajac and Whitlatch 2003). Lohrer et al. (2004) criticized such disturbance-recovery dynamics studies that examine completely defaunated sediment and do not take into account effects of repeated disturbances. Disturbances by epibenthic predators are good examples of natural disturbances that do not completely defaunate the infaunal community and where patches may be repeatedly disturbed. Therefore, studies that examine disturbance ecology of defaunated sediments from a single pulse of disturbance are not representative of a large class of natural disturbance. Though numerous studies documented rates of disturbances over space and time, disturbances are usually represented as an average percentage of the benthos (Woodin 1978, Grant 1983, Levin 1984, Cross and Curran 2000). Clustering of disturbances is usually described qualitatively (e.g. Grant 1983, Pearson et al. 2007), and Cross and Curran (2004) noted that new feeding pits were dug by daysatid rays over pre-existing pits. These observations strongly suggest significant within site spatial variability that is not represented by the measured average rates of disturbances, and require spatially explicit approaches to examine the underlying disturbance regime.

**Large-scale implications of small-scale disturbances**

Studies that have highlighted the importance of localized disturbance are typically conducted at local scales (tens to hundreds of meters). However the relative importance of physical and biotic factors in structuring community patterns vary with spatial scale (Legendre et al. 1997, Seitz and Lipcius 2001). Therefore to reach a general understanding of the influence of disturbance on community structure, the relationships
have to be examined at multiple scales. Effects of spatial scale can be interpreted in many ways. Norrko et al. (2006) examined the effects of the area of the disturbed patch on recovery dynamics. Another important consideration of scale in disturbance ecology is the spatial extent of the system in question.

It is generally recognized that large-scale spatial patterns are driven by environmental or abiotic factors such as nutrient levels, while small-scale patterns are driven by biotic processes such as predation or herbivory (Thrush 1991, Menge et al. 1997). For example, Menge et al. (1997) found that the food supply in the water column (represented by chlorophyll concentrations) do not vary within a single rocky shore, but there is significant variability in predation pressure within the site, but variations in chlorophyll among sites tens of kilometers apart accounted for differences in benthic community structure.

The spatial scale of the system has particularly important implications on the studies examining biogenic disturbances. This is because the spatial significance of the disturbance is likely to be related to the mobility of the bioturbator. For example, a crustacean predator is able to move within a tidal flat spanning tens of meters in relation to prey availability, but is unable to make foraging decisions among tidal flats separated by kilometers within the estuary (Seitz and Lipcius 2001). Furthermore, several abiotic factors such as sediment grain size and organic content have been found to be important determinants of infaunal community structure, and these variables can vary significantly within an estuary (references in Lenihan and Micheli 2001, Thrush et al. 2003).

While manipulative field experiments, such as predator exclusions, are effective in examining local processes, they are usually carried out at single sites (Hall et al. 1994,
Seitz et al. 2001). As mentioned before, exclusion experiments are the conventional approach to examine predator influence, but multi-site experiments can be logistically prohibitive (but see Quijon and Snelgrove 2005, Langlois et al. 2006). Moreover, it is difficult to examine effects of environmental factors over large scales with experiments (Thrush et al. 2003). All of the aforementioned work on disturbances by epibenthic predators were conducted at single sites. A few studies examine the large scale effects of epibenthic predators over a broad spatial scale. Kvitek et al. (1992) investigated the effects of otter foraging along a gradient of otter density in the Gulf of Alaska with sites separated between kilometers to tens of kilometers. Seitz et al. (2001) were the first to examine the importance of spatial scale in determining the relative significance of predation versus food availability in soft-sediment populations (*Macoma balthica* clams).

On the other hand, Hewitt et al. (2007) suggests using natural history information for initial guidelines for the scale of study and nesting manipulative studies within a correlative framework. Studies of small terrestrial mammal predators have shown that the spatial structure of predator activity over the range of an entire population (as opposed to within an arbitrary study site) has significant impacts over the variability of prey populations (Schauber et al. 2009). While, spatially explicit studies of epibenthic predators over a large spatial extent are rare, tracking of the Atlantic Horseshoe Crab *Limulus polyphemus* in enclosed estuaries in Maine, Massachussets and Great Bay, New Hampshire showed that a population of predators can move among various sub-embayments and coves of an estuary over the scale of kilometers, and over time scales of less than a month to seasons (James-Pirri et al. 2005, Moore and Perrin 2007, Schaller et
al. 2010). Furthermore, in these studies, there appeared to be spatial variability in the rate of visitations by tracked animals within an estuary.

**Research overview and objectives**

The Atlantic Horseshoe Crab *Limulus polyphemus* is frequently cited as an important bioturbator, and epibenthic predator in particular (Wilson 1990b, Hall et al. 1994, Lenihan and Micheli 2001, Meysman et al. 2006, Botton 2009). This study examines the role of foraging disturbances by *Limulus* in the Great Bay Estuary in New Hampshire. Just as importantly, this investigation addresses the lack of spatially and temporally explicit measurements of localized biogenic disturbances and discussion on the influence of scale on their effects.

The general objective was achieved by examining the (1) feeding ecology of *Limulus*, (2) regime of *Limulus* foraging disturbance and (3) its impacts on infauna on spatial scales of (a) individual pits, (b) within the habitat within reach by an individual predator and (c) over the scale of an estuary which is the range of a *Limulus* population. This study overcomes methodological limitations met by previous studies of disturbances in soft-sediment habitats with the development of a low-cost, novel low-level aerial survey method had to be developed to achieve objectives (1) and (2) which facilitated the understanding of the greater implications of findings from (3). Findings of this work will identify critical scaling issues that researchers need to be aware off when studying small disturbances which are ubiquitous in all environments.

Though *Limulus* is a well-known epibenthic predator, the species’ foraging ecology in enclosed embayments and estuaries such as Great Bay is not well understood. Chapter 2 describes *Limulus* foraging behavior in Great Bay, reports previously
unquantified usage of intertidal mudflats by the species as important foraging habitats, thereby establishing intertidal mudflats as critical *Limulus* habitats. Chapter 3 describes the methodology used in measuring the disturbance regime of *Limulus* and its impacts on infaunal communities. Complementing conventional approaches to investigating community impacts of disturbances are spatially explicit quantification of disturbance patterns, which better informs the overall role of *Limulus* foraging disturbance in the community. Chapter 4 is a presentation of the results. Chapter 5 is a discussion of the findings within context of current approaches and understanding of the role of localized disturbances and the influence of scale. Chapter 6 is a concluding discussion on the significance of this work, importance of scale illustrated by this study, contribution of this work to the field of ecology and the way forward in future resea
CHAPTER 2

DESCRIPTION OF STUDY SYSTEM

Introduction

Concerns over sustainability of current levels harvest of American horseshoe crab *Limulus polyphemus* (*Limulus* hereafter) by the eel and whelk fisheries for bait and biomedical industries have resulted in increased efforts to understand the ecology of exploited populations (Hooker et al., 2010). The majority of on-going monitoring and management strategies focus on the reproductive ecology and health of spawning habitats of the species (e.g. Smith et al. 2009, Hooker et al. 2010). However, there remains a lack of understanding of *Limulus* foraging ecology and habitat needed to support the trophic requirements of a *Limulus* population (reviewed by Botton 2003, but see Carmichael et al. 2004, Moore and Perrin 2007 for recent studies), which is necessary for the development of a comprehensive management strategy.

Foraging Ecology of *Limulus*

*Limulus* is an epibenthic predator that feeds on buried infaunal prey in soft-bottom habitats with a preference for thin-shelled bivalves (Botton 2009). *Limulus* feed by disrupting the sediment with their prosoma and digging with their legs (reviewed in detail by Botton 2003). *Limulus* possess chemoreceptors on the chelae and spines of
gnathobases, which have been found to be responsive to fish and bivalve extracts. The sedimentary nature of *Limulus* habitat and the dorsal-lateral location of the compound eyes and the ventral placement of the mouth, means that *Limulus* likely seek suitable prey via chemical and tactile rather than visual cues (Botton 2003). Smith (1953) observed *Limulus* digging selectively on plots of planted *Mya arenaria* clams, suggesting that an ability to precisely locate areas of dense prey. On the other hand, less preferred prey items, such as the small hard-shell clam *Gemma gemma*, have been found in *Limulus* gut in numbers that positively correlated with the volume sediment, suggesting that *Limulus* carries out nonselective feeding (Botton 1984a).

In the process of foraging, *Limulus* create pits frequently observed on tidal flats along the east coast of the United States (Smith and Chin 1951, Baptist et al. 1957, Woodin 1978, Shuster 1982, Sickley 1989, Botton 2003, 2009). However, the majority of the current understanding of foraging ecology of *Limulus* is based on work on mid-Atlantic and southern New England populations (Botton 2009). Due to the relative isolation of populations across its range, it is important to investigate *Limulus* ecology across its distributional range. Intertidal foraging by *Limulus* along the mid-Atlantic coast is usually associated with the narrow seasonal window of the species’ spawning period spanning late spring till summer, after which they are thought to return to deeper waters (reviewed by Botton et al. 2003, Botton 2009). However, the appearance of *Limulus* feeding pits after the spring breeding season has been reported elsewhere (Smith and Chin 1951, Baptist et al. 1957, Woodin 1978, Webster 1991). In addition, recent studies on the movements of *Limulus* in enclosed bays and estuaries revealed that *Limulus* remain active in intertidal areas beyond the limited spawning season, with activity
ceasing in the autumn (Moore and Perrin 2007, Watson et al. 2009, Watson and Chabot 2010). This evidence suggests that certain populations of \textit{Limulus} utilize intertidal habitats beyond the mating season, but there remains a paucity of studies on \textit{Limulus} behavior in intertidal habitats during the remainder of the year (Moore and Perrin 2007).

Watson and colleagues hypothesized that \textit{Limulus} make repeated excursions to tidal flats after the spawning season to forage (Watson et al. 2009, Watson and Chabot 2010). Studies of decapod predators have shown that tidal flats in estuaries are important foraging grounds, which are accessed at high tide (reviewed by Holsman et al. 2006). For these predators, the energetic cost of tidal migration is potentially outweighed by the abundance of infaunal prey in intertidal areas relative to subtidal habitats (reviewed by Holsman et al. 2006). \textit{Limulus} are commonly found in highly productive estuaries with extensive soft-bottom intertidal zones (e.g. Anderson and Shuster 2003, Carmichael et al. 2004, Moore and Perrin 2007, Watson et al. 2009). In the spring, \textit{Limulus} migrate to the upper intertidal zones to mate and spawn, when the animals are expected to exploit the food resources in adjacent intertidal habitats, as observed by previous studies (Anderson and Shuster 2003, Botton et al. 2003, Botton 2009). After the spawning season, abundant intertidal prey may still be energetically profitable for estuarine populations of \textit{Limulus} to continue making tidal migrations, thus explaining their continued presence in intertidal habitats (Smith and Chin 1951, Baptist et al. 1957, Woodin 1978, Webster 1991, Watson et al. 2009, Watson and Chabot 2010).

While presence of \textit{Limulus} feeding pits have been repeatedly reported by the aforementioned authors, there have been relatively few attempts to quantify the patterns of foraging behavior. Methodological difficulties are partly responsible for this
information gap, as visibility is usually low while the predators are feeding at high tide. Prior work quantified intertidal foraging by *Limulus* by counting the number of animals found on the intertidal at low tide (Smith and Chin 1951, Botton 1984b). However, as *Limulus* forage mostly at high tide, and retreat to the subtidal zone with the outgoing tide, the number of *Limulus* found exposed on tidal flats may be an underestimate of the level of foraging activity occurring at a particular tidal flat. Examination of predator tracks is a complementary method to assessing foraging activity (Hines et al. 1997, Schauber et al. 2009), but disturbance to the benthos caused by researchers’ footprints on fine-grain habitats prohibit repeated monitoring of mudflats for *Limulus* feeding pits. To the best of my knowledge, only Woodin (1978) has quantified *Limulus* pit digging pattern by examining the percentage area covered by pits on a sandflat in Virginia. However, Woodin did not report on the density of pits which would measure the number of foraging attempts an area of tidal flat can support.

Using a novel non-invasive technique to observe the presence of *Limulus* feeding pits, this study examines temporal and spatial pattern of *Limulus* intertidal foraging of a northern estuarine *Limulus* population. In particular, I ask the following questions: (1) how intensively and frequently is a mudflat used as a foraging ground; (2) does *Limulus* forage intertidally beyond the spawning season; (3) what is the foraging behavior of individual *Limulus*?

**Materials and Methods**

**Study site**

This study was conducted at Adams Point in the Great Bay estuary, New Hampshire, U.S.A., which has a large population of *Limulus* (Watson et al. 2009) and
extensive unvegetated fine-grained mudflats (>20% of 44 km²; Short 1992, Jones 2000). Short (1992) described the hydrology and hydrochemistry of Great Bay. The average tidal range of Great Bay ranges from 2.0 m to 2.7 m. Freshwater inputs to the estuary is relatively low, therefore overall water movement in the estuary is driven primarily by tidal currents. Typical of high latitudinal temperate estuaries, Great Bay’s surface water temperature ranges from -2.0°C to 27°C. Low winter temperatures result in significant ice formation from late December to March in parts of Great Bay, although continuous ice cover (thickness ~0.3 m) has been absent during warm winters. Apart from periods of major spring runoff events, salinities in the estuary is usually greater than 20 ppt.

Great Bay is close to the northern distributional limit of *Limulus* (Watson et al. 2009). It is characteristic of unvegetated fine-grained mudflats commonly found in Great Bay, consisting of poorly sorted fine to medium silt (Webster 1991). *Limulus* is the only epibenthic predator that that creates large feeding pits (approximately 20 cm in diameter, Figure 2.2) on the mudflats of Great Bay (Sickley 1989 pers. obs., Webster 1991). Foraging *Limulus* have been observed to dig elliptical pits surrounded by a raised rim of sediment usually with a rim broken on one side (Commito et al. 1995, Shuster 2001). Snorkeling surveys in Great Bay also found *Limulus* to excavate sediments while pivoting over one point, creating circular pits in the process (pers. obs). Bivalve shell fragments and large volumes of sediment have been found in the guts of *Limulus* from Great Bay (Lee unpubl. data), which corroborates with previous studies on mid-Atlantic populations showing that they are generalist predators with a preference for bivalves (reviewed by Botton 2009).
Spatial and temporal pattern in foraging activity

Intensity and frequency of intertidal foraging is defined as the level of activity of foraging *Limulus* over space and time respectively. Foraging intensity is measured by the number of feeding pits per unit area found at any one time, while the frequency is quantified by the appearance of new feeding pits in a certain area over time. While feeding traces are useful indications of the use of a tidal flat by a predator population, its utility as a surrogate for predator abundance is not known (but see Schauber et al. 2009).

To examine the spatial and temporal pattern of *Limulus* intertidal foraging, the presence of feeding pits was quantified along a fixed 50 m × 2 m transect on a mudflat at Adams Point (43°5'29" N, 70°51'53" W) using a novel non-disruptive benthic survey method. Such cohesive sediments are inhibitive to the development of *Limulus* eggs, and therefore the study site is unlikely to be a *Limulus* spawning habitat. In addition, previous surveys over the site found few *Limulus* buried in the sediment (pers. obs.). Therefore all pits found within the monitored transect are unlikely to be spawning or resting pits and were regarded as feeding pits. Repeated monitoring of the transect was carried out without disturbing the benthos by recording a video of the transect with a digital camera moving along a cable suspended 3 m above the mudflat (Figure 2.3). The transect was located in the upper intertidal zone 5 m from the mudflat edge to reduce potential edge effects. The transect was monitored once a month during a daytime low tide from June to October 2009 after which the monitoring setup was taken down to prevent equipment damage by freezing winter temperatures. Monitoring resumed in May to June 2010. Each video was processed using Adobe Photoshop® CS5 Extended to extract still frames with approximately 90% overlap, which were aligned and overlaid to produce a mosaic of the
transect. The images are scaled by measuring the distance between two fixed objects at two ends of the cable. The survey area was divided into 25 contiguous 2 m×2 m quadrats, and the number of Limulus pits present within each quadrat was quantified visually. 

*Limulus* feeding pits in Great Bay persist between one to two weeks (pers. obs.). In addition, comparisons of consecutive months’ mosaics showed that spatial arrangements and shapes of individual pits were unique to that particular month. Therefore, it was inferred that physical traces of feeding pits disappeared within a month, and pits found in each month’s transect were dug not more than one month earlier. It should be pointed out that pits dug between survey dates could have been ‘overwritten’ by more recent pits, therefore the number of pits observed per month reported here may be an underestimate.

**Foraging behavior of individual *Limulus***

Snorkelling surveys were conducted at high tide over a mudflat near Adams Point (43°5'50" N, 70°52'3" W) to determine the rate of pit-digging by individual *Limulus* and the spatial extent of individual foraging activity. Surveys were carried out on 21 July and 6 August 2009. During each survey, the mudflat was searched visually in a haphazard pattern until individual or pairs of *Limulus* were found and subsequently followed. The locations where the predators excavated the benthos were marked with thin bamboo stakes. Individuals/pairs were tracked until the visibility and/or water depth made it impossible to continue observations. The distances between consecutive pits made by individual or pairs of *Limulus* were then measured at the subsequent low tide.
Results

Spatial and temporal pattern in foraging activity

*Limulus* feeding pits were found in all seven months surveyed between June 2009 to May 2010. *Limulus* foraging exhibited a seasonal pattern on Great Bay’s mudflats in 2009 (Figure 2.4). The total number of pits differed significantly among months [ANOVA of SQRT(X+1) transformed data, F$_{6,168} = 25.35$, $p < 0.001$]. Feeding pits were already present when the survey began in June 2009, and peaked in August 2009. Out of the five months surveyed in 2009, feeding pits were most abundant in August and September, with activity peaking in August (Student-Newman Keuls’ test, $P<0.01$, Figure 2.4). In the following year (2010), the activity level in May 2010 was similar to that in low-activity months of June, July and October 2009, but increased significantly one month later in June 2010, where the density of *Limulus* pits were as high as the density observed in August 2009 (SNK, $P<0.05$, Figure 2.4).

Visual inspection of the spatial pattern of pits in the months of low activity (June, July and October 2009 and May 2010) suggested a clustered distribution. This clustering was less apparent during the months of high foraging activity (August, September 2009 and June 2010) where many 2m×2m quadrats were completely occupied by pits. In August 2009 and June 2010, pits appeared to be distributed evenly throughout the transect at high densities (Figure 2.2). The maximum number of pits found within the 2 m×2 m quadrats was 21. This density was observed in six of the twenty quadrats surveyed in August 2009 and one of the quadrats in June 2010. Examination of the spatial arrangement of pits in these quadrats showed that 21 pits might be the upper limit of the
pit density, as the areas between adjacent pits are smaller than that of individual pits (Figure 2.2).

**Foraging behavior of individual *Limulus***

A total of ten *Limulus* individuals/amplexed pairs were observed on 6 August and 22 July 2009. Unattached males and females and amplexed mating pairs were not differentiated during the snorkelling surveys. However, all three types of predators were found actively foraging and each individual/pair were observed to dig more than one pit. The distances between consecutive pits dug by the same foraging individuals/pairs ranged from 1.3 m to 17.6 m. The mean distance between pits was 6.2 (±1.56 SE) m and the median distance was 5.0m. Disturbance to the benthos is minimal in between pits, except for small shallow tracks made by trailing *Limulus* tails and walking legs. Moving *Limulus* also left small perforations in the sediment, which appeared to be made by legs probing into the sediment.

**Discussion**

This study presents the first quantification of *Limulus polyphemus* foraging activity by measuring the spatial and temporal patterns of foraging excavations. The results revealed intense and frequent use of an intertidal habitat by foraging *Limulus*. While *Limulus* foraging occurs throughout a tidal flat at high tide, *Limulus* typically mate and spawn along the shoreline at high tide (Brockman 1990). A census of *Limulus* along the shore of five sites in Great Bay (including Adams Point – this study’s location) at high tide from May to July 2009 found *Limulus* only in May and June (NMFS 2010). It is likely that spawning ceases by July (Watson pers. comm.). While spawning activity in
Great Bay usually peaks in June, intertidal foraging observed in this study peaked in August. This study shows that *Limulus* intertidal foraging activity not only persisted but increased, after the May-June breeding season. While this study examined only one site in Great Bay, presence of feeding pits on other mudflats in Great Bay (pers. obs.), together with results of this study show that *Limulus* are actively foraging on Great Bay's tidal flats from late spring till at least early autumn. These findings contrast with studies of mid-Atlantic *Limulus* that suggest that they forage intertidally and are significant agents of physical disturbance only during the spawning season (Kraeuter and Fegley 1994, Botton 2009). Intertidal foraging by *Limulus* at Adams Point exhibited a distinct seasonality. Seasonal movements such as foraging excursions into the intertidal zones are common among mobile estuarine species (Watson et al. 2009). My findings support Watson et al.'s (2009) and Watson and Chabot's (2010) reports of high *Limulus* locomotory activity between subtidal and intertidal zones in Great Bay from May till August, and their hypothesis that *Limulus* are making foraging excursions on tidal flats. Tidal flats in Great Bay where *Limulus* foraging is evident are separated between >1km to >10km apart (pers. obs.). It is not known whether *Limulus* foraging activity peaks at the same time at all locations or move to forage in other parts of the estuary. The latter is possible as *Limulus* were found to move downstream in Great Bay in the autumn (Watson et al. 2009, Schaller et al. 2010). On the other hand, the area surveyed in this study was repeatedly used as a foraging ground during the one-year study period. Sixteen of the 20 quadrates monitored contained feeding pits on all the sampling dates, while three and one quadrates contained no pits in one and two months respectively. Therefore, it is apparent that the study site is an important feeding ground that is repeatedly utilized by *Limulus* on
the scale of months, and possibly years. Temporal persistence of foraging 'hot spots' has also been reported in other systems (Schauber et al. 2009). However, it is not known whether the same individuals are returning to the same site to feed within the duration of this study – although Watson et al.’s (2009) study at the same location reported Limulus returning to site within days during the spawning season.

At low tide, Limulus individuals are extremely rare on Great Bay’s tidal flats relative to the abundance of pits present (per. obs.). Therefore, most of the animals that dug the pits described here migrate between subtidal and intertidal zones within a high tide. Watson et al. (2009) suggested that an endogenous tidal clock, which can be triggered by an increase in temperature in spring, drives such movements. The positive relationship between tidal migratory behavior and temperature may explain the spike in foraging activity observed in June 2010. Higher than average ambient temperatures in the spring of 2010 in New Hampshire (USDA 2010) might have caused the early onset of the Limulus intertidal foraging. 21 pits per 4 m² appear to the upper limit of density for Great Bay’s Limulus population. The size of feeding pits is likely to correlate with prosomal width. As prosomal widths of northern Limulus populations tend to be smaller than those found on the mid-Atlantic, density of feeding pits found on mid-Atlantic habitats are predicted to be lower.

Predator activity has also been found to correlate positively with prey density (e.g. Seitz et al. 2001). Consequently, the drop in Limulus foraging rate at Adams Point might have been due to prey depletion. In addition, foraging activity can vary within a single mudflat as shown by the presence of clustering of pits in months of low activity, and such aggregative response might be caused by the spatial variability in prey density.
(Sutherland 1996). The apparently uniform distribution of high pit density in August 2009 and June 2010 follows the prediction of the Ideal Free Distribution of predators, where consumers will move into patches with lower prey density as predator density or activity increase (Fretwell and Lucas 1970). Though the spatial pattern of prey density at the study site remains to be determined, Smith (1953) reported a *Limulus* detecting and excavating into plots of enriched *Mya arenaria* located 1 m apart, suggesting that *Limulus* are able to detect patches of high prey densities on a small spatial scale. While it is not known whether *Limulus* can detect cues from infaunal prey in the water column, probing of sediment observed in this study and reported by earlier work (Caster 1938, Shuster and Botton 1985) showed that *Limulus* probably detect prey on a small spatial scale with chemical and tactile cues.

Observations of individual *Limulus* behavior at Adams Point showed that individual and attached pairs dig multiple pits within a single intertidal excursion. In the course of this study, some animals were found to dig multiple pits in quick succession (<10 min per pit) before digging a pit for an extended period of time (up to 15 min). At spring low tides, Great Bay’s mudflats typically span 100 s of m along the shore and between the high and low water line. The distances between pits made by foraging individuals/pairs located during snorkelling surveys indicated that *Limulus* foraged on a spatial scale smaller than that of individual mudflats. As a result, individual/pairs of *Limulus* might have dug multiple pits observed within the 50 m×2 m transect monitored in this study. However, because the minimum distance between pits was 1.3 m, the tightly clustered pattern of pits shown in Fig. 1 is the result of multiple predators foraging adjacent to each other or previous excavations. *Limulus* located during snorkelling
surveys were at least 100 m away from the spring low water level. Regular tidal migration on this scale is possible given the movement rates of Great Bay Limulus reported by Watson and Chabot (2010), while Dungeness crabs Cancer magister have been found to carry out 1.2 km roundtrips to the littoral zones (Holsman et al. 2006). Display of this energetically expensive behavior outside of the spawning season, provided strong support for the hypothesis that benefits of exploiting intertidal food sources outweighs the cost of tidal migrations (Holsman et al., 2006).

In addition to evidence of frequent and intensive Limulus intertidal foraging activity presented here, the absence of Limulus eggs at the study sites (Lee unpubl. data) suggest a segregation of spawning and feeding habitats. Current Limulus management practices emphasize the quality of spawning habitats (Hooker et al. 2010), but an effective management plan needs to consider habitats used by the species at other stages of its life history. The potential use of different parts of the intertidal zone at different stages of Limulus’s life history in Great Bay underscores the importance of healthy diverse littoral habitats to support the reproductive and trophic requirements of resident estuarine populations. Consequently, findings of this study have important implications on the management of this economically and ecological valuable species in estuarine systems. It is especially critical to include protection of intertidal habitats in management efforts because of the increasing threat of anthropogenic influences on estuaries (PREP 2010). Holsman et al. (2003) speculated that loss of intertidal habitats in San Francisco Bay might explain the decline in Dungeness crab production in the region, which rely on intertidal areas as foraging grounds. Similarly, recent declines of Limulus populations should also be examined within the context of foraging habitat quality.
Figure 2.1. *Limulus* feeding by disrupting the sediment with its prosoma.
Figure 2.2. Photograph taken on 14 August 2009, showing high density of *Limulus* foraging pits.
Figure 2.3. Schematic diagram showing setup of a cable suspended 3m above a mudflat anchored from two trees approximately 70m apart. A digital camera (Cannon Powershot A540 6.0 megapixel) is hung from the cable pointing downwards onto the mudflat, and is moved along the cable by a movable line, which can be pulled from either side of the cable.
Figure 2.4. Density (mean±SE) of *Limulus* pits on 7 dates. Letters a-c indicate results of Student-Newman-Keuls test.
CHAPTER 3

METHODS

Disturbance regime of *Limulus* foraging

The spatial and temporal pattern of *Limulus* intertidal foraging was further examined by using the cable-video survey method described in Chapter 2. To investigate the importance of *Limulus* foraging disturbance, the percentage area covered by *Limulus* pits, the sizes and number of pits were determined from video mosaics using the method described in Chapter 2. The temporal resolution of this study is finer than the preliminary analyses presented in Chapter 2. In 2009, the rate of disturbance was examined on the scale of days and weeks. The study periods are presented in Table 3.1. For example, to determine the level of disturbance over two days between August 4 and 6, the video mosaics of August 4 and 6 were compared and the pits present on August 6 but not August 4 were traced digitally and its dimensions measured. Only 23 out of the 25 2m×2m quadrats along the transects had consistently clear images for analyses, therefore n=23 quadrats was used in this study. The site of the video surveys will be referred to as JEL (for Jackson Estuarine Laboratoy which is situated next to the mudflat) from here onwards (Figure 3.1).

Analyses of even finer spatial resolution was carried out to examine the frequency disturbance of an area on the mudflat comparable to that of a *Limulus* feeding pit.
(diameter ~20cm) in 2009 and 2010. The presence or absence of pits in 0.2m×0.2m quadrats spaced 0.5m apart (measured from center of adjacent quadrats) were noted using video mosaics from the dates listed in Table 3.1. The temporal scale of this analysis is one week (7-9 days), and spans the beginning, peak and waning of Limulus foraging activity in Great Bay in two years (July 6 to September 16, 2009, June 22 to September 29, 2010). In 2009, two roughly parallel transects with 50 quadrats along each transect were monitored while in 2010, 75 quadrats along a single transect were monitored.

**Impacts of Limulus foraging on infauna community**

**Infauna sampling**

The impacts of Limulus foraging disturbance on infaunal community structure were assessed on multiple spatial scales. All infauna sample cores mentioned in this section measured 10cm in diameter and 10cm in depth. Infauna cores were sieved with a 500μm mesh, fixed in 5% formalin and stained with Rose Bengal. The retained infauna was dominated by polychaetes which were identified down to family level, small crustaceans were identified to order or family level, bivalves were identified to species level. This approach shortened the time needed to process samples, facilitating the collection of a greater number of replicate samples. It is unlikely to have affected the usefulness of the data in demonstrating spatial patterns in benthic assemblages; similar patterns of benthic assemblages are often found when coarse or fine levels of taxonomic resolution are used (Bishop et al. 2006). Due the large size of the most common bivalve *Macoma balthica* compared to the dominant polychaetes, separate larger cores were sampled when abundance of *Macoma* was quantified. All *Macoma* cores were
20cm×20cm and 15cm in depth and sieved with a 5mm mesh. The empirical relationship between *Macoma* size (maximum shell length) and tissue dry mass was determined from 34 individuals collected from ten sites across Great Bay ($r^2 = 0.89, p < 0.0001$, Figure 3.2), and the relationship was used to calculate the biomass of *Macoma* collected from the exclusion experiments and estuary-scale survey.

**Impacts of individual feeding pits**

To examine the effect of individual *Limulus* foraging disturbance, the infaunal community in pits were compared with that of the surrounding undisturbed sediment. Freshly excavated pits less than one day old were easily distinguish at low tide because of the presence of grayish sediment surrounding the pits – which indicated that sub-surface anoxic sediment had been exposed recently (Commito et al. 1995).

The immediate impact of *Limulus* disturbance was investigated in 2007. Between June 2 and 18, sixteen freshly dug pits were located within a 50m×50m area on a mudflat at Adams Point (API, Figure 3.1). One infaunal core was collected from each pit and a corresponding core was collected from adjacent undisturbed sediment 0.5m from the pit. To understand the recovery trajectory of infaunal communities after horseshoe crab disturbance, pits had to be monitored. To examine the short-term recovery of infauna after disturbance, on July 14, 2007, twenty freshly dug pits were located within another 50m×50m area at Adams Point, and marked with two thin bamboo stakes placed 0.5m from each side of a pit. One infaunal core was collected from four fresh pits and corresponding core was collected from adjacent undisturbed sediment 0.5m from the pit. Two low tides (~1 day) later, four pits were relocated, infaunal cores were collected from four pits and undisturbed sediment 0.5m away from each pit. Finally, three pits were
relocated 3 days later and infaunal cores were collected from four pits and undisturbed sediment 0.5m away from each pit.

The recovery trajectory of infaunal communities in *Limulus* foraging pit was examined up to 28 days in 2008. On August 1, 2008, twenty-six freshly dug pits were located within a 50m×50m area on the same mudflat. Two thin bamboo stakes were placed 0.5m from each side of each pit as markers. Out of the twenty-six, eight pits were sampled three days after marking, another eight were sampled 16 days after marking, and finally another ten pits were sampled 28 days later.

**Effects of *Limulus* disturbance within a single habitat**

To gain a mechanistic understanding of the effects of *Limulus* disturbance on infaunal communities and examine the collective and longer term impacts of *Limulus* disturbance within a single habitat (i.e. mudflat), a long-term exclusion experiment was setup at a nearby mudflat at Adams Point in the summer of 2009 and 2010 (AP1, Figure 3.1). Exclosure plots (2m×2m×0.6m cages) were constructed with PVC pipes and safety barrier with 10cm mesh (Figure 3.3). A 0.2m lip protruded from the top of the cages to prevent encroachment by *Limulus* that might climb up the cage panels. The lower 10cm of the cage panels were pushed below the mud surface to prevent entry by digging *Limulus*. Six cages were set up on May 12, 2009. Due to the large sizes of the cages, and the logistical difficulty of transporting caging material onto mudflats, cage controls were not constructed. In addition, the mesh size of 10cm is significantly larger than cages used in studies, which have found significant caging artifacts (Hall et al. 1990, Olafsson et al. 1994), and therefore, caging artifacts in this studies might have been significantly reduced. The cages were located in a haphazard pattern within a 50m×50m area. The
exclosures were checked weekly and detritus such as salt marsh plant material, seagrass and macroalgae were removed from the panels. No Limulus pits were observed within the cages throughout the experiment.

Almost 4 months later, between September 4 and 5, 2009, two infauna cores were taken from a 2m×1m area within each exclosure, such that half the exclosure remained undisturbed by sampling. Two larger Macoma cores were collected and clams larger than 10mm (maximum length from anterior to posterior edge of shell) were measured (maximum length from anterior to posterior edge of shell) and counted. Similar sets of cores were collected from six haphazardly selected 2m×1m plots outside the cages. The cages were taken down on October 15, 2009, at the end of the Limulus intertidal foraging season and before the onset of winter where there is significant ice formation and movement in the estuary. The corners of the cages’ locations were marked with PVC pipes driven 15cm into the mud, with a 10cm segment protruding from the surface. The cages were set up again on May 13, 2010, at the locations where the cages were in 2009. Four months later, two infauna and two Macoma samples were collected from the unsampled 2m×1m portion of the exclosures and six 2m×1m plots outside the cages.

**Importance of Limulus disturbance on an estuary-scale**

To determine the relative importance of Limulus disturbance as a driver of infaunal community patterns across Great Bay, the influence of Limulus disturbance and key abiotic sedimentary properties were examined at ten sites in the estuary. The ten sites selected were in the order of kilometers apart (Figure 3.1). All ten sites possess characteristics associated with Limulus foraging habitats, including large area of exposed unvegetated mudflat at low tide, sheltered location away from strong currents and waves
and presence of *Limulus* pits from preliminary surveys. All ten sites are separated by water even at the lowest spring tides, and are therefore considered separate and independent habitats in terms of *Limulus* foraging, because individual *Limulus* foraging in a single high tide are limited to a single mudflat (see Chapter 2 for explanation).

Sampling at the ten sites took place at low tide between August 9 and 24, 2010. Levels of foraging activity were measured in ten 2m×2m quadrats along a 100m transect. The distance between the center of adjacent quadrats was 10m. Due to the limited extent of the sampled area relative to the entire mudflat, sampling was concentrated where *Limulus* activity was obvious on the day of sampling. Therefore, the first quadrat was placed at a spot where a significant cluster of *Limulus* pits was observed. Digital photographs were taken by a camera mounted on a 3m pole in a setup similar to the one shown in Figure 3.4. The photographs were then rectified with Adobe Photoshop® CS5 and the percentage area covered by *Limulus* pits were determined. Sediment samples to examine biotic and abiotic variables were collected from the first five quadrats. One infauna and *Macoma* core were taken from each of the 5 quadrats. After infauna samples were identified taxonomically, the samples were dried in a 75°C for 8 hours to determine the biomass. *Macoma* were counted and measured for maximum shell length. One 5cm diamater, 5cm deep core was collected from each quadrat for mud content analyses, another similarly sized core was taken for organic content analyses. One 2cm diameter, 1cm deep core was collected, wrapped in aluminium foil immediately and placed in an insulated chilled container immediately in the field and frozen at -40°C upon return to the laboratory before being analysed for chlorophyll *a* content. Mud content (percentage of total mass) is analysed following the methods described by Folk (1974). Organic content
is measured by percentage loss in mass upon ignition. Samples were dried overnight in at 75°C and placed in a muffler furnaces at 450°C for four hours. The percentage mass loss at 450°C is the organic content. Chlorophyll \( \alpha \) is measured using the spectrophotometric method described by Lorenzen (1967).

**Analyses**

**Analyses of spatial patterns of *Limulus* foraging disturbance**

There are numerous methods for quantifying spatial structures, and while many approaches are computationally similar and answer similar questions, variations and uses of specific techniques are the result of their origin in different disciplines (Perry et al. 2002). A widely used measure of spatial structure, commonly referred to as patchiness, is spatial autocorrelation, which measures the similarity of a variable among pairs of locations spaced a given distance apart (Kraan et al. 2009). The Moran’s \( I \) coefficient is a frequently used index, and patch sizes can be estimated by plotting the degree of spatial autocorrelation against various lag distances to produce a correlogram (Hall et al. 1994, Kraan et al. 2009).

The cumulative frequency of *Limulus* disturbance observed from June 22 to September 29, 2010 at each of the 75 0.2mx0.2m quadrats spaced 0.5m apart at Adams Point (Table 3.1) will be used to analyze the spatial structure of *Limulus* foraging disturbance. Analyses will be carried out with Moran’s \( I \), to determine whether *Limulus* disturbance within a mudflat exhibited a clustered (aggregated) pattern (Fortin and Dale 2005). The Moran’s \( I \) indices was computed for distance lags up to 15m, which were than plotted into a correlogram using the Spatial Analysis in Macroecology software (SAM,
Rangel et al. 2010). The index ranges between +1 and -1, where +1 indicates strong positive spatial autocorrelation, while 0 indicates randomness, and -1 indicates negative spatial autocorrelation. The distance over which $I$ is positive is an estimate of the dominant patch size (Perry et al. 2002).

**Infaunal community analyses**

The effects of *Limulus* disturbance on infaunal community within a habitat (mudflat) were also examined on the scale of individual pits, and larger spatial scale of 2m×2m exclusion cages with univariate and multivariate analyses. To determine whether foraging disturbances caused by *Limulus* impacts on infauna is similar across different trophic levels, polychaetes found in infauna samples were divided into predatory and non-predatory families. The review by Fauchald and Jumars (1979) was used to separate carnivorous and omnivorous polychaetes from deposit feeders. Oligochaetes was a dominant taxa in Great Bay’s infaunal community, and are regarded as dominant deposit feeders in my analyses. Differences in infauna abundance and number of infauna taxa between pits (<1d, 1d, 3d, 15d, 28d old) and adjacent undisturbed sediments were analysed using negative bionomial regression with pit as factors (PROC GENMOD: SAS Institute Inc.). Similarly, differences in infauna abundance, number of infauna taxa, *Macoma* abundance in the exclusion experiments in 2009 and 2010 were analysed using negative binomial regression with cage and year as factors (PROC GENMOD: SAS Institute Inc.). Shannon-Wiener and Pielou’s evenness indices were also calculated from the infauna data collected in the pit surveys and exclusion experiment and analysed using ANOVA.
The biomass of *Macoma* collected from the exclusion experiments were calculated and analysed using ANOVA. Multivariate analyses of infauna between (1) pits and undisturbed sediment and (2) exclusion plots and naturally disturbed sediment were carried out with non-metric multi-dimensional scaling (nMDS). A Bray-Curtis similarity index was calculated using non-transformed data, and the resultant similarities used in the nMDS analyses. Analyses of similarities (ANOSIM) were used to test for significance of differences of pits versus undisturbed sediments, and in sediments within cages versus uncaged plots of sediment. The contribution of taxa to dissimilarities between treatments were than determined with the SIMPER routine. Multivariate analyses were performed using the PRIMER software (Clarke and Warwick 2001).

**Importance of Limulus disturbance on an estuary-scale**

To examine the importance of *Limulus* disturbance, relative to key abiotic sedimentary variables, as a driver of infaunal community structure across the estuary of Great Bay. One site (AP2) had a disproportionately large number of *Gemma gemma* (487.2±166 per 78cm², compared to an average of 3.3±2.1per 78cm² across all the other sites). Also, *Limulus* have been found to actively avoid *Gemma gemma* as a prey (Botton 1984a), therefore, it is expected that *Limulus* foraging would not have a significant impact on *Gemma* abundance. Consequently, to reduce the influence of *Gemma gemma* on the overall infaunal community patterns, *Gemma* was removed from the total abundance of infauna in the analyses. I used stepwise multiple regressions to determine which variables best explains the variation in (1) total infauna abundance (excluding *Gemma gemma*), (2) total predatory infauna abundance, (3) total deposit feeding
polychaetes and oligochaetes abundance, (4) infauna biomass, (5) infauna taxonomic richness, (6) Macoma clam abundance and (7) Macoma biomass. The means of all variables across replicates taken from each site were used. Percentage area disturbed, sediment chlorophyll $a$ content, sediment organic content, percentage mud content and salinity were used in the regressions analyses (Table 3.2). All variables were examined to determine whether they met assumptions of normality and natural-log(sqrt) transformations were performed when necessary (Table 3.2). Mixed stepwise regression was performed in JMP v9.0 (SAS 2010) where forward and backward steps were alternated. The process includes the most significant independent variable that satisfied the probability to enter ($p=0.25$) and removes the least significant term satisfying the probability to leave ($p=0.10$). Variables are removed until the remaining terms are significant and then changes to the forward steps. Akaike’s Information Criterion (AIC) was used to select the best model. All possible combinations of the five independent variables were constructed in JMP and the corrected AIC ($AIC_c$) calculated for each model. As the count data for the total number of taxa found at ten sites across the estuary was not normally distributed. Generalized linear models (GLM) in the form of Poisson regression were used to examine the effects of Limulus disturbance and abiotic variables on the number of taxa.

Limulus disturbance had an extremely strong effect on total infauna (excluding Gemma) where 91% of its variability in total infauna abundance (excluding Gemma) was explained by percentage area occupied by Limulus pits (Table 4.9). There are many other factors that can drive spatial variability of total infaunal abundance across the estuary that was not accounted for by the suite of independent variables, but may be strongly
correlated with *Limulus* disturbance. For example, infaunal recruitment, annual variability in salinity and food availability. This study was carried out in an estuarine environment where environmental gradients are expected to be strongly correlated with the above factors. Therefore to get a better estimation of the role of *Limulus* disturbance in determining total infauna abundance, further analyses was conducted to remove possible influences of variables unaccounted for by the sampling design. Distance from the mouth of the estuary is used as a proxy for the factors unaccounted for in the sampling scheme. Distance from the mouth of estuary is measured as the distance of study sites to the General Sullivan Bridge (Figure 3.1). Residuals from the regression analyses between distances of each of the ten sites and total infauna abundance (excluding *Gemma*) was then used in the stepwise regression analyses as a new response variable.
### Tables and figures

Table 3.1. Dates, temporal and spatial scales of video surveys of *Limulus* disturbance.

<table>
<thead>
<tr>
<th>Year</th>
<th>Start date</th>
<th>End date</th>
<th>Duration (Temporal scale)</th>
<th>Spatial scale</th>
</tr>
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<td>August 4</td>
<td>August 6</td>
<td>2 days</td>
<td>23 contiguous 2m×2m quadrats</td>
</tr>
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<td>August 7</td>
<td>1 day</td>
<td></td>
</tr>
<tr>
<td></td>
<td>August 7</td>
<td>August 9</td>
<td>2 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>August 9</td>
<td>August 12</td>
<td>3 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>July 12</td>
<td>July 20</td>
<td>One week (8 days)</td>
<td></td>
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<tr>
<td></td>
<td>August 4</td>
<td>August 12</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>September 2</td>
<td>September 9</td>
<td>One week (7 days)</td>
<td></td>
</tr>
<tr>
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<td>July 6</td>
<td>July 13</td>
<td>One week (7 days)</td>
<td>2 parallel transects</td>
</tr>
<tr>
<td></td>
<td>July 13</td>
<td>July 20</td>
<td>One week (7 days)</td>
<td>50 0.2m×0.2m quadrats</td>
</tr>
<tr>
<td></td>
<td>July 20</td>
<td>July 27</td>
<td>One week (7 days)</td>
<td>0.5m apart along each transect</td>
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<tr>
<td></td>
<td>July 27</td>
<td>August 5</td>
<td>One week (9 days)</td>
<td></td>
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<td>August 5</td>
<td>August 12</td>
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<td>September 16</td>
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<tr>
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<td>July 6</td>
<td>One week (7 days)</td>
<td></td>
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<td>September 22</td>
<td>September 29</td>
<td>One week (7 days)</td>
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Figure 3.1. Locations of large scale sampling survey conducted in August 2010. JEL is the location of the video surveys of *Limulus* disturbances. AP1 was the location of study of impacts of individual *Limulus* disturbances (pits), and cage exclusion experiment. RC, NH, AP1 and AP2 were locations of aerial surveys using radio-controlled airplane in October 2010.
Figure 3.2. Regression graph of maximum shell length and dry mass of *Macoma balthica*. $r^2=0.90$, $p<0.0001$, $y=-0.042 + 0.0054496x$.

Figure 3.3. 2m×2m×0.6m exclusion cage.
Figure 3.4. Overhead camera setup. Camera was mounted on a 3m pole, and pointed down towards a 2mx2m quadrat on the mudflat.

Table 3.2. Variables and transformations used in multiple regression analyses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>Transformation</th>
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<tr>
<td>Total infauna abundance</td>
<td>Count per 78cm²</td>
<td>natural log x</td>
</tr>
<tr>
<td>Total infauna abundance excluding Gemma gemma</td>
<td>Count per 78cm²</td>
<td>natural log x</td>
</tr>
<tr>
<td>Infaunal biomass excluding bivalves</td>
<td>g per 78cm²</td>
<td>natural log x</td>
</tr>
<tr>
<td>Macoma abundance</td>
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<td>natural log (x+1)</td>
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<tr>
<td>Macoma biomass</td>
<td>g per 0.04m²</td>
<td>natural log x</td>
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</tr>
<tr>
<td>% organic content,</td>
<td>Percentage</td>
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<td>Percentage</td>
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</tr>
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<td>Distance from mouth of estuary</td>
<td>km</td>
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</tr>
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</table>
CHAPTER 4

RESULTS

Disturbance regime

404 Limulus pits were measured in August and September 2009. The pits measured on average 26(±0.4) cm in length and 22(±0.3) cm in width. There was significant monthly variation in the percentage coverage and abundance of pits from July to September 2009 (Figure 4.1). Both percentage cover by pits and number of pits peaked in August (28(±2.3)% and 27(±1.9) pits per 4m² respectively), while the intensity of disturbance were similar between July and September (Figure 4.1). Table 4.1 summarizes the frequency of Limulus disturbance observed in 2009 and 2010. The average frequency of disturbance was once every 3.8(±0.22) weeks in 2009 and 3.18(±0.25) weeks in 2010. The modal frequency of disturbance was lower, where 33% of the survey was disturbed every 3.3 weeks in 2009 while 21% of the area was disturbed every 2.7 weeks in 2010. On the whole, 70% of the surveyed area was disturbed more than once every four weeks in 2009 while 67% of the surveyed area was disturbed more than once every four weeks in 2010.

Figure 4.2a and b show the spatial structures of Limulus foraging disturbance within a single site in 2009 and 2010 in the form of correlograms. Limulus disturbance in 2009 had significant positive spatial autocorrelation at distances up to 2m, and showed
significant negative long-range autocorrelation at distances of 11-13 m (Figure 4.2a). In 2010, *Limulus* disturbance had significant positive autocorrelation up to 10 m, and showed significant positive autocorrelation between 10 m and 13 m, beyond which (13-15 m) autocorrelation was significantly negative (b). The spatial structures of *Limulus* disturbance in 2009 and 2010 as revealed by the correlograms were similar in that disturbances were autocorrelated at the small spatial scale of up to 2 m, and significantly negatively autocorrelated at a larger spatial scale of 11-13 m.

Figure 4.3a and b show the spatial structures of *Limulus* disturbance on a finer spatial scale of three weeks, as revealed by correlograms. Cumulative disturbances observed over both 3-week periods showed significant positive autocorrelation at short distances of ~1-2 m (0-3 m for June 22-July 15, 0-2 m for July 15-August 6), and also over a longer range of ~6-7 m (5-8 m for June 22-July 15, 6-7 m for July 15-August 6).

**Impacts of *Limulus* foraging on infauna community**

**Impacts of individual feeding pits**

Figure 4.4 and Figure 4.5 show the impacts of *Limulus* disturbance on total number of infauna individuals, dominant taxa, total number of infauna taxa, equitability (Pielou’s evenness $J'$) and diversity (Shannon-Wiener diversity index $H'$) less than 24 hours after disturbance (Day 0). On Day 0, the total density of all infauna were significantly lower in pits than in control (undisturbed) sediments (Figure 4.4a, $\chi^2 = 24.22$, $p<0.001$). The number of taxa found in pits were also lower than the number found in undisturbed sediments (Figure 4.5a, $\chi^2 = 17.10$, $p<0.001$). Consequently, the Shannon-Wiener diversity index of pit infauna communities were lower than those of control.
communities (Figure 4.5c, Table 4.5.). However, evenness among taxa were similar in both communities as indicated by the Pielou’s evenness (Figure 4.5b, Table 4.5.). Multivariate analysis with non-metric multi-dimensional scaling (MDS) showed that the community structure of control and pit communities were distinct, with more variability among pits than among samples taken from control (undisturbed) sediment (Figure 4.6). Analysis of Similarities (ANOSIM) revealed a significant difference between control and pit communities ($R=0.351$, $p = 0.001$). SIMPER analyses that identified the contribution of each taxa to the overall dissimilarity between pit and control communities are summarized in Table 4.7. Capitellidae contributed most to the average dissimilarity between Day 0 pit and control (23%). This is the result of a drastic reduction of Capitellids by *Limulus* disturbance (Figure 4.4b). The density of other dominant deposit feeders, Cirratulidae and Spionidae, were also significantly reduced in pits less than 24h old compared to control undisturbed sediments. The number of predatory polychaetes were very low in both pit and control communities and were therefore summed across taxons (Nereidae, Phyllodocidae, Lumbrineridae, Orbiniidae). Similarly negative impacts caused by *Limulus* foraging were observed in predatory polychaetes and deposit feeding polychaetes and oligochaetes (Figure 4.4c).

Figure 4.7 shows impacts of *Limulus* disturbance on total infauna, dominant deposit feeders and total predatory polychaetes <24h, 1 day and 3 days after disturbance in July 2007. Results showed that the significant reduction of total infauna abundance less then 24h after *Limulus* disturbance was maintained up to 3 days (Figure 4.7a). The average number of total predatory polychaetes (Nereidae, Phyllodocidae, Lumbrineridae, Orbiniidae) per 78cm² ranged from 0.25 to 1.5. As a result of the small numbers, no
substantive inferences can be made on the influence of *Limulus* disturbance on predatory polychaetes. SIMPER analyses that identified the contribution of each taxa to the overall dissimilarity between pit and control communities are summarized in Figure 4.3. Spionids contributed most to the average dissimilarity between Day 0 pit and control (42%) and was significantly reduced by *Limulus* disturbance (Figure 4.7b). Capitellidae contributed most to the average dissimilarity between Day 1 pits and control (39%) and was also significantly reduced in pits 1 day after disturbance (Figure 4.7c). Significantly lower number of Spionids, and Oligochaetes were also observed in pits compared to control sediments a day after *Limulus* disturbance (Figure 4.7c). Three days after first disturbance, total infauna abundance remained significantly lower in pits compared to undisturbed sediment, and which was the result of a significant reduction in Capitellids in pits (Figure 4.7a and d).

Figure 4.8 and Figure 4.9 shows the total number of infauna individuals, total number of infauna taxa, equitability (Pielou's evenness $J'$) and diversity (Shannon-Wiener diversity index $H'$) of pits that were disturbed 3, 15 and 28 days prior to infaunal sampling and control (undisturbed sediment). The total abundance of infauna in pits remained significantly reduced compared to undisturbed sediment (control) 3 day ($\chi^2=6.05$, $p=0.01$) and 15 days ($\chi^2=7.12$, $p=0.01$) after disturbance. While infauna abundance were similar after 28 days ($\chi^2=0.99$, $p=0.38$) (Figure 4.8a). On the other hand, there were no statistical differences of the other three variables between the pit and control communities (Table 4.5.). MDS plots corroborates with the trend shown in the total number of infauna, whereby community structures of Day 3 and 15 pits were significantly different from that of undisturbed communities, while Day 28 pits were
similar to those of undisturbed communities (Figure 4.10, Day 3: \( R=0.18, p<0.5 \); Day 15: \( R=0.40, p<0.01 \); Day 28: \( R=-0.027, p>0.05 \)). Table 4.4. summarizes the taxonomic composition of the infaunal communities found in pits 3, 15, and 28 days after disturbance and that of the corresponding undisturbed communities. The most dominant taxa in pit and corresponding control communities three and 28 days after disturbance were Cirratulidae (Figure 4.8b and d). Spionidae were exceptionally abundant in control samples on Day 15 (Figure 4.8c). SIMPER analyses that identified the contribution of each taxa to the overall dissimilarity between pit and control communities are summarized in Table 4.4. The dominant taxa in 2008, Cirratulidae, was more than 50% less in Day 3 pit communities compared to control communities and contributed to 58% of dissimilarity between Day 3 and control communities (Figure 4.8b, Table 4.4.). The abundance of Cirratulidae between Day 15 and 28 pits and control communities were similar (Figure 4.8c and d). Spionidae, contributed the most to the dissimilarities found between Day 15 and 28 pits and their corresponding control communities (49% and 35% respectively, Table 4.4).

**Within habitat effects of Limulus disturbance**

Impacts of Limulus disturbance over a temporal scale beyond 28 days and spatial scale beyond individual pits were examined with cage exclusion experiments in 2009 and 2010. Results from the exclusion experiments in 2009 and 2010 provides a mechanistic understanding of Limulus role in structuring infaunal communities (Figure 4.11 to Figure 4.13). nMDS plots in Figure 4.11a show the general community patterns of infauna communities in cages versus control plots with natural levels of Limulus disturbance in 2009 and 2010. ANOSIM revealed that cage and control communities were similar in
2009 ($R=0.089, p=0.17$). Total abundance of infauna did not show significant treatmentxyear interaction ($\chi^2 = 0.02, p<0.9018$). The total abundance of infauna though appeared to be reduced compared to control sediment in both years (Figure 4.12a and Figure 4.13a). The difference was not statistically significant ($\chi^2 = 3.55, p<0.06$).

Examination of abundance of predatory polychaetes and deposit feeding polychaetes and oligochaetes showed that long-term *Limulus* disturbance impacted the two trophic groups differently. Long-term *Limulus* disturbance significantly reduced the abundance of total predatory polychaetes and two out of four predatory families in 2009 (Figure 4.12d, Total: $\chi^2 = 6.99, p<0.01$, Orbiniidae: $\chi^2 = 0.51, p=0.5$, Nereidae: $\chi^2 = 3.89, p<0.05$, Phyllodocidae: $\chi^2 = 1.88, p=0.1705$, Lumbrinidae: $\chi^2 = 4.37, p<0.05$). On the other hand, long term disturbance by *Limulus* results in a non-significant reduction in total abundance of deposit feeding polychaetes and oligochaetes, which was reflected in the trends shown by three out of four deposit feeding taxa (Figure 4.12e, $\chi^2 = 1.29, p=0.26$, Spionidae: $\chi^2 = 0.35, p=0.55$, Oligochaete: $\chi^2 = 0.37, p=0.54$, Cirratulidae: $\chi^2 = 2.67, p=0.10$, Capitellidae: $\chi^2 = 5.43, p=0.0198$).

General community patterns of infauna communities in cages versus control plots with natural levels of *Limulus* disturbance in 2010 were significantly different (Figure 4.11b, $R=0.25, p=0.02$). A greater reduction in total predatory polychaetes by *Limulus* disturbance was observed in 2010 than 2009, which was the result of a drastic reduction of Lumbrinidae by *Limulus* disturbance in 2010 (Figure 4.13d). There were significant differences in the general structure of infaunal communities between years ($R=0.657, p=0.001$).
Figure 4.14 shows the total number of infauna individuals, total number of infauna taxa, equitability (Pielou's evenness $J'$) and diversity (Shannon-Wiener diversity index $H'$) of infauna found within cages and in sediment with natural levels of Limulus disturbance (control) in 2009 and 2010. The total abundance of infauna did not show significant treatment×year interaction ($\chi^2 = 0.02, p = 0.9018$). The number of taxa also did not show significant treatment×year interaction ($\chi^2 = 1.74, p = 0.2$, Figure 4.14), and there was a significant difference in the number of taxa between cage and control communities ($\chi^2 = 4.02, p = 0.04$) but not between years ($\chi^2 = 0.00, p = 0.9$). There were no differences in the Pielou’s evenness $J'$ and Shannon-Wiener diversity index $H'$ between cage/control and years (Figure 4.14). Figure 4.15 shows the consequences of the Limulus exclusion on the abundance and biomass of Macoma. There were no significant treatment×year interaction for both variables ($\chi^2 = 0.92, p = 0.3$ and ANOVA respectively). Exclusion of Limulus resulted in significant increase in Macoma abundance ($\chi^2 = 13.7, p < 0.001$) and biomass (ANOVA, $p = 0.005$). There was no significant difference in the size of clams found in cages and control areas in 2009, however, the sizes of clams found in cages were significant smaller than those found in control in 2010 ($F = 7.2, p < 0.05$).

**Importance of Limulus disturbance on an estuary-scale**

Table 4.9 summarizes the results of the stepwise regression analyses of the influence of Limulus disturbance and four other abiotic variables on infaunal community response variables. Most strikingly, stepwise regression of total infauna abundance without Gemma gemma showed that Limulus disturbance intensity – as measured by percentage area covered by pits – explained 91% of variation of total infauna (minus Gemma gemma) among the ten sites ($p < 0.001, F = 83.2$, Figure 4.16). Limulus disturbance
appeared to have similarly negative influence on deposit feeding oligochaetes and polychaetes ($R^2=0.39$, $F=5.2$, $p=0.05$) and predatory polychaetes ($R^2=0.56$, $F=10.06$, $p=0.01$).

To obtain a better estimation of the role of *Limulus* disturbance in determining total infauna abundance, residuals from the regression analyses between distances of each of the ten sites from the estuary mouth and total infauna abundance (excluding *Gemma*) was then included in the stepwise regression analyses as a response variable. The influence of *Limulus* disturbance on total infaunal abundance is reduced (from $R^2=0.91$) when distance of study sites from the mouth of the estuary was removed from the overall model ($R^2=0.24$, $F=2.15$, $p=0.15$), but remained more important than the other variables (Table 4.10). There are other drivers such as recruitment variability, that would correlate with distance between site and estuary mouth. Therefore, it should also be noted that the significant relationship between *Limulus* disturbance and total infauna abundance does not demonstrate a direct causation.

On the other hand, $R^2=0.24$ is likely to be an underestimate of *Limulus*' significance, because *Limulus* disturbance correlates significantly with distance from the mouth of the estuary ($R^2=0.70$, $p<0.01$, Figure 4.17), therefore removing the distance of sites from the mouth of the estuary would perhaps unfairly reduce the influence of *Limulus* disturbance on total infaunal abundance. Also, exclusion experiments conducted at a single site demonstrated the causal influence of *Limulus* disturbance on infaunal abundance. As a result, *Limulus* is likely to explain between 24% and 91% of the variability observed in total infaunal abundance (excluding *Gemma*) among the ten sites.
Occasional large bivalves *Macoma balthica* and *Mya arenaria* found in 10cm diameter infauna cores disproportionately skewed the total infaunal biomass of samples, and therefore bivalves were removed in the calculation of infaunal biomass, and bivalve biomass were calculated with the dry mass of *Macoma* sampled from 20cm×20cm cores. *Limulus* disturbance explained 67% of non-bivalve infaunal biomass. A model with only percentage mud content that explains 36% of the total variability of *Macoma* abundance has the lowest AIC\(_C\) value, while models that included *Limulus* disturbance did not possess substantially increased the explanatory power and also resulted in increased AIC\(_C\) values. Similarly, a model with only salinity explained 29% of the total variability of *Macoma* biomass and had the lowest AIC\(_C\) value. While models that included *Limulus* disturbance did not increase the predictive power of the models and reduce the goodness of fit (as indicated by AIC\(_C\) values). Poisson regression modeling showed that none of the measured variables sufficiently explain estuary-wide variability of taxonomic richness (Table 4.11).
Figures and tables

Figure 4.1 (a) Percentage area covered by pits (b) number of pits per unit area. Data are mean ± SE.
Table 4.1. Disturbance regime of *Limulus* foraging disturbance. Disturbance frequency indicates the frequency a given patch of 20cmx20cm sediment was disturbed.

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<thead>
<tr>
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<th>2009</th>
<th>2010</th>
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</thead>
<tbody>
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<td>Average frequency of disturbance</td>
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<td>3.18(±0.25) weeks</td>
</tr>
<tr>
<td>Modal frequency of disturbance</td>
<td>3.3 weeks</td>
<td>2.7 weeks</td>
</tr>
<tr>
<td>% of survey area disturbance once</td>
<td>33%</td>
<td>21%</td>
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<tr>
<td>every &lt;4 weeks</td>
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Figure 4.2 Correlogram showing spatial structure of cumulative disturbance frequencies recorded from July 6 to September 16, 2009 and June 22-September 29, 2010. Shaded bars indicate p<0.05.
Figure 4.3. Correlogram showing spatial structure of cumulative disturbance frequencies recorded over two 3-week periods in 2010 (June 22-July 15 and July 15-August 7). Shaded bars indicate p<0.05.
Figure 4.4. Abundance of (a) total infauna, (b) dominant deposit feeding polychaete families and (c) total predatory polychaetes found in control (undisturbed) sediments and in pits dug less than 24h before sampling. Data are mean ± SE.
Figure 4.5. (a) Total number of taxa, (b) Pielou's index of evenness and (c) Shannon-Wiener diversity index found in control (undisturbed) sediments and in pits dug less than 24h before sampling. Data are mean ± SE.
Figure 4.6. Results of non-metric multi-dimensional scaling (nMDS) analyses showing community structure of infaunal communities in control (undisturbed) sediments relative to communities in pits dug less than 24h before sampling.
Figure 4.7. (a) Abundance of total infauna, found in control (undisturbed) sediments and in pits dug <1, 3 and 3 days before sampling. (b-d) Abundance of dominant deposit feeding taxa in pits dug <1, 3 and 3 days before sampling. Data are mean ± SE.
Table 4.2. Taxonomic compositions of infaunal communities in control (undisturbed) sediment vs in pits dug less than 24h before sampling in June 2007.

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Table 4.3. Taxonomic compositions of infaunal communities in control (undisturbed) sediment vs in pits dug less than 24h, 1, 3 days before sampling in July 2007.

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Figure 4.8 (a) Abundance of total infauna, found in control (undisturbed) sediments and in pits dug dug 3, 15 and 28 days before sampling. (b-d) Abundance of dominant deposit feeding taxa in pits dug dug 3, 15 and 28 days before sampling. Data are mean ± SE.
Figure 4.9 Total number of taxa, Pielou’s index of evenness and Shannon-Wiener diversity index found in control (undisturbed) sediments and in pits dug 3, 15 and 28 days before sampling. Data are mean ± SE.
Figure 4.10 Results of non-metric multi-dimensional scaling (nMDS) analyses showing community structure of infaunal communities in control (undisturbed) sediments relative to communities in pits dug (a) 3, (b) 15 and (c) 28 days before sampling.
Table 4.4. Taxonomic compositions of infaunal communities in control (undisturbed) sediment vs in pits dug 3, 15 and 28 days before sampling.

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Table 4.5. Results of ANOVA of Pielou’s evenness and Shannon-Wiener diversity index of infaunal communities in control (undisturbed) sediment vs in pits dug less than 24h, 3, 15 and 28 days before sampling.

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Figure 4.11 Results of non-metric multi-dimensional scaling (nMDS) analyses showing community structure of infaunal communities in Cages and Control plots in (a) 2009 and (b) 2010.
Table 4.6. Taxonomic compositions of infaunal communities in cages and control plots in 2009 and 2010.

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<tr>
<td></td>
<td></td>
<td>Mya</td>
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<td>12</td>
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<td></td>
<td>Lumbrinidae</td>
<td>3</td>
<td>3</td>
<td>87</td>
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Figure 4.12 Abundance of (a) total infauna, (b) predatory polychaetes and (c) total oligochaetes and deposit feeding polychaetes, (d) individual predatory polychaetes families, (e) oligochaetes and individual deposit feeding polychaete families in cages and control (naturally disturbed) sediment in 2009. Data are mean ± SE.
Figure 4.13 Abundance of (a) total infauna, (b) predatory polychaetes and (c) total oligochaetes and deposit feeding polychaetes, (d) individual predatory polychaetes families, (e) oligochaetes and individual deposit feeding polychaete families in cages and control (naturally disturbed) sediment in 2010. Data are mean ± SE.
Figure 4.14. Total number of taxa, Pielou's index of evenness and Shannon-Wiener diversity index found in cages and control in 2009 and 2010. Data are mean ± SE.
Table 4.8. Cage vs Control Pielou’s and Shannon-Wiener diversity index

<table>
<thead>
<tr>
<th>Variable</th>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
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<td>0.04</td>
<td>13.42</td>
<td>0.00</td>
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<tr>
<td></td>
<td>Cage/Control</td>
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<td>0.00</td>
<td>0.21</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Year*Cage/Control</td>
<td>1</td>
<td>0.01</td>
<td>1.95</td>
<td>0.18</td>
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<td></td>
<td>Residual</td>
<td>20</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shannon-Wiener diversity index ( H' )</td>
<td>Year</td>
<td>1</td>
<td>0.33</td>
<td>8.76</td>
<td>0.01</td>
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<td>Cage/Control</td>
<td>1</td>
<td>0.01</td>
<td>0.39</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>Year*Cage/Control</td>
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<td>0.10</td>
<td>2.79</td>
<td>0.11</td>
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<tr>
<td></td>
<td>Residual</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
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</table>

Figure 4.15. *Macoma* clam abundance and dry mass in cages and control plots in 2009 and 2010.

Data are mean ± SE.
Table 4.9 Akaike information criteria (AIC) model results of stepwise regressions comparing alternative models and the variation they explain. Independent variables used are: percentage area disturbed, chlorophyll a content, sediment organic content, sediment mud content and salinity. ΔAIC is the difference between the AIC value of the "best" model and that of each consecutive model and is an indication of relative model performance.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Model</th>
<th>$R^2$</th>
<th>AIC</th>
<th>ΔAIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total infauna abundance</td>
<td>% area disturbed</td>
<td>0.91</td>
<td>90.8</td>
<td></td>
</tr>
<tr>
<td>excluding <em>Gemma gemma</em></td>
<td>% area disturbed, Chl a (ug/m2)</td>
<td>0.94</td>
<td>92.2</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>% area disturbed, % mud</td>
<td>0.93</td>
<td>94.6</td>
<td>2.40</td>
</tr>
<tr>
<td>Deposit feeding oligochaetes and polychaetes</td>
<td>% area disturbed</td>
<td>0.39</td>
<td>30.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% area disturbed, Chl a (ug/m2)</td>
<td>0.52</td>
<td>34.1</td>
<td>3.80</td>
</tr>
<tr>
<td></td>
<td>% area disturbed, % mud</td>
<td>0.48</td>
<td>34.8</td>
<td>0.70</td>
</tr>
<tr>
<td>Predatory polychaetes</td>
<td>% area disturbed</td>
<td>0.56</td>
<td>29.8</td>
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<tr>
<td></td>
<td>% area disturbed, salinity</td>
<td>0.65</td>
<td>33.5</td>
<td>3.66</td>
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<tr>
<td></td>
<td>% area disturbed, Chl a (ug/m2), salinity</td>
<td>0.87</td>
<td>32.7</td>
<td>-0.80</td>
</tr>
<tr>
<td>Infaunal biomass</td>
<td>% area disturbed</td>
<td>0.67</td>
<td>18.23</td>
<td></td>
</tr>
<tr>
<td>excluding bivalves</td>
<td>% area disturbed, % organic content</td>
<td>0.74</td>
<td>22.08</td>
<td>3.85</td>
</tr>
<tr>
<td></td>
<td>% area disturbed, % organic content, % mud content</td>
<td>0.74</td>
<td>30.73</td>
<td>8.65</td>
</tr>
<tr>
<td><em>Macoma</em> abundance</td>
<td>% mud content</td>
<td>0.36</td>
<td>29.03</td>
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<tr>
<td></td>
<td>% mud content, salinity</td>
<td>0.44</td>
<td>33.53</td>
<td>4.50</td>
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<td>Chl a (ug/m2), asin mud, salinity</td>
<td>0.50</td>
<td>41.54</td>
<td>8.01</td>
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<tr>
<td></td>
<td>% area disturbed, % mud content, salinity</td>
<td>0.45</td>
<td>42.43</td>
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<tr>
<td><em>Macoma</em> biomass</td>
<td>salinity</td>
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<td>-0.4</td>
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<tr>
<td></td>
<td>% mud content, salinity</td>
<td>0.39</td>
<td>0.2</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>% area disturbed</td>
<td>0.03</td>
<td>2.7</td>
<td>2.50</td>
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</table>
Table 4.10. Akaike information criteria (AIC) model results of stepwise regressions comparing alternative models and the variation they explain.

Independent variables used are: percentage area disturbed, chlorophyll a content, sediment organic content, sediment mud content and salinity. ΔAIC is the difference between the AIC value of the "best" model and that of each consecutive model and is an indication of relative model performance.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Model</th>
<th>$R^2$</th>
<th>AIC</th>
<th>ΔAIC</th>
</tr>
</thead>
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<tr>
<td>Residuals of regressing (Total infauna abundance excluding <em>Gemma gemma</em>) on (distance from estuary mouth)</td>
<td>% area disturbed</td>
<td>0.24</td>
<td>103.4</td>
<td></td>
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<tr>
<td></td>
<td>sediment % organic content</td>
<td>0.08</td>
<td>105.3</td>
<td>1.90</td>
</tr>
<tr>
<td></td>
<td>% mud content</td>
<td>0.02</td>
<td>105.9</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>% area disturbed, chl a (ug/m2)</td>
<td>0.27</td>
<td>108.9</td>
<td>3.01</td>
</tr>
<tr>
<td></td>
<td>% area disturbed, salinity</td>
<td>0.26</td>
<td>109.1</td>
<td>0.20</td>
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</table>
Figure 4.16. Linear regression between total infauna abundance (excluding *Gemma gemma*) and distance from estuary mouth ($R^2=0.60$, $p<0.01$).

Figure 4.17. Percentage area disturbed and distance from estuary mouth ($R^2=0.70$, $p<0.01$).
Table 4.11. Likelihood ratio statistics of Type 1 Poison regression analysis.

<table>
<thead>
<tr>
<th>Source</th>
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<th>$\chi^2$</th>
<th>$p$</th>
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<td>Intercept</td>
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<td></td>
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<tr>
<td>arcsine-sqrt (% disturbance)</td>
<td>2.19</td>
<td>1</td>
<td>0.53</td>
<td>0.47</td>
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<tr>
<td>chl a</td>
<td>2.16</td>
<td>1</td>
<td>0.03</td>
<td>0.86</td>
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<tr>
<td>arcsine-sqrt (% organic content)</td>
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<td>1</td>
<td>0.82</td>
<td>0.37</td>
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<tr>
<td>arcsine-sqrt (% mud content)</td>
<td>0.95</td>
<td>1</td>
<td>0.4</td>
<td>0.53</td>
</tr>
<tr>
<td>Salinity</td>
<td>0.94</td>
<td>1</td>
<td>0</td>
<td>0.96</td>
</tr>
</tbody>
</table>
CHAPTER 5

DISCUSSION

There is no lack of prior studies examining the role of localized disturbances on structuring ecological communities using marine epibenthic predators as study models (Woodin 1978, Van Blaricom 1982, Thrush et al. 1991, Hall et al. 1993, Commito et al. 1995). These studies have contributed to the discourse on the importance of localized disturbances in creating heterogeneity, which is integral to all ecological communities (Probert 1984, Goldberg and Gross 1988, Hall et al. 1994). However, while previous work on epibenthic predator disturbance have all qualitatively recognized the importance of epibenthic predators in changing the physical topography of soft-sediment benthos, workers that quantified the intensity, frequency, impact of predator disturbances and recovery trajectory of impacted communities, frequently find that at least one of those four critical determinants to fall short of resulting in equivocal conclusions about the significance of the role of predator disturbances on soft-bottom communities (e.g. Hall et al. 1991, Thrush et al. 1991, Hall et al. 1993). A critical limitation is the low temporal and spatial resolution of studies, lack of temporal and spatial explicit measurements of disturbance patterns, and also logistical difficulties in examining predator impacts over multiple scales – especially at multiple sites impacted by a predator population (Quijon and Snelgrove 2005). This study examines the activity of a model organism (Atlantic
horseshoe crab *Limulus polyphemus*) within a spatially and temporally explicit framework. The discussion of this study’s results within the context of current approaches and understanding will contribute towards developing a more comprehensive framework to examine the overall significance of small-scale disturbances, which are often overshadowed by conspicuous large-scale anthropogenic disturbances (Dethier 1984).

**Disturbance regime**

The frequency of disturbance and intensity of a disturbance determines the collective impact of disturbances (Hall et al. 1994). For example, in a landscape where the time interval between most disturbances (frequency) are longer than the time required for the disturbed community to recover, the presence of patches is fleeting. On the other hand, if the time interval between disturbances are shorter than the recovery time, disturbed patches would persist in the landscape. Furthermore, if disturbances re-occur in patches still recovering from previous disturbance, the community structure present would be the result of recovering communities subjected to multiple ‘setbacks’ (disturbances) and would likely be different from that of a disturbed patch with no history of disturbance or a disturbed patch that does not experience new disturbances. The intensity of disturbance, frequently expressed as the percentage area disturbed, determines the importance of the disturbances in creating heterogeneity across the habitat or landscape. To the best of my knowledge, this is the first time natural small-scale disturbances on soft-sediments have been observed over such a fine temporal (weekly) and spatial (0.5m intervals) scale at the same time. Furthermore, this study was conducted
over 2 months in 2009 and 3 months in 2010, which allowed repeated monitoring within a season and over two years.

The results show that pit-digging by foraging *Limulus* physically disturbs a significant portion of a mudflat in the Great Bay estuary of New Hampshire, U.S.A., thereby confirming that *Limulus* foraging plays a key role in structuring infaunal communities. Mean rates of disturbance intensity and frequency observed during this study are at the higher end of the range of intensities and frequencies reported by previous studies (Table 1 in Zajac 2004). I found that *Limulus* disturbed 15%, 28%, and 13% over three one-week survey periods in July, August and September respectively. These levels of disturbance intensities translate to daily rates of 2% in July, 4% in August, 2% in September; and monthly rates of 45% in July, more than 112% in August, and 42% in September. Hall et al. (1991) estimated that 0.0015% of the subtidal sandbed on the west coast of Scotland was disturbed by *Cancer pagurus*. While Thrush et al. (1991) reported that eagle rays *Myliobatis tenuicaudatus* disturbed 1.4% of sandflats in New Zealand per day. On the other end of the spectrum, rays disturbed up to 5% of the subtidal benthos per day in California in the summer and fall (Van blaricom 1982), and 40-90% of a mudflat in Mission Bay, California was found disturbed every week (Levin 1984).

Frequency of disturbance refers to the number of times a particularly patch is expected to be disturbed over time. In studies of benthic systems, frequency of disturbance is often measured as turnover rates – where frequency of disturbance as the inverse of the proportion of benthos disturbed over a certain time period. For example, Based on a daily disturbance rate of 1.4%, Thrush et al (1991) calculated the sediment
turnover rate by rays in New Zealand to be ~70 days. Based on Woodin's (1978) monthly observations of epibenthic disturbance at Tom’s Cove, VA, that peaked at 45% in July, Commito et al. (1995) inferred that there is a high turnover rate of sediments caused by epibenthic predators. However, these calculations of disturbance frequencies from mean intensities could be misleading. These calculations assumed that new disturbances would first occur on previously undisturbed and neglect the possibility that animal activities such as foraging can be temporally autocorrelated, where animals visit ‘hot spots’ repeatedly (Schauber et al. 2009). Positive temporal autocorrelation of patchy disturbance patterns at a particularly area can result in a restricted sub-area being subjected to intense disturbance, conversely, negative temporal autocorrelation results in the dispersion of the total disturbance over a larger area. This study’s method of estimation of *Limulus* disturbance frequency, by monitoring quadrats with areas comparable to those of *Limulus* pits (20cm×20cm versus 26cm×22cm respectively) at weekly intervals provides a more accurate quantification of disturbance frequency than previous works. The results show a high frequency of *Limulus* disturbance, where the majority (70%) of the sampled area was disturbed more than once every 4 weeks (Table 4.1).

Furthermore, mean-field approaches of examining disturbance regimes, historically adopted by studies of benthic disturbances, do not account for the influences of disturbance spatial structures on the recovery dynamics of disturbed communities. As Hall et al. (1991) pointed out that predator disturbances were usually studied in isolation on the scale of individual disturbances, whereby recovery of the impacted community was examined, particularly to determine the time required for the community to resume the structure similar to that prior to disturbance. This duration needed for recovery was
then juxtaposed with the disturbance frequency (sediment turnover rates in many instances), and if recovery time was shorter than disturbance frequency, disturbed patches were deemed ephemeral features on the landscape. However, if disturbance frequency was greater than the recovery time, a substantial proportion of the landscape would possess communities at some stage of recovery (Hall et al. 1991). Rather than calculating the mean rate of disturbance by *Limulus*, this study showed that a significant (67-70%) of the survey area is re-disturbed by monitoring an array of closely located quadrats.

Examination of the general spatial structure of a disturbance regime is critical in revealing the extent of clustering among individual disturbances. Numerous workers have reported qualitatively the clustering of large number of epibenthic predator excavations, but did not quantify the degree of clustering. Information on the disturbance aggregation is necessary when considering the possible recovery trajectory of disturbed infaunal communities. This is because it has been found in many cases, disturbed infaunal communities had significantly lower abundances compared to undisturbed sediment immediately after disturbance, and recover via passive transport of adult and post-larval individuals from adjacent sediment into the disturbed patch (Hall et al. 1991, Thrush et al. 1991, Commito et al. 1995). Therefore, a disturbed patch surrounded by similarly disturbed patches (forming a cluster) would take longer to recover via passive transport of individuals than a isolated disturbed patch which is surrounded by undisturbed sediments with larger densities of infauna. This hypothetical discrepancy in disturbance spatial structure and recovery rates, even if disturbance frequencies are similar, can produce two landscapes with different levels of infaunal community heterogeneity. The
period and temporal resolution over which disturbances by epibenthic predator is examined is finer in this study than the above-mentioned surveys.

The results show that 67-70% of the survey area was disturbed at least once every four weeks, which suggests that some of the disturbances occurring every four weeks would overlap each other. It was also found that it took up to four weeks for infaunal communities in pits to recover to resemble community patterns found in adjacent undisturbed sediment (see Chapter 4: “Impacts of individual feeding pits”). This reoccurrence of disturbances in a significant portion of a mudflat within four weeks, before the complete recovery of the disturbed infaunal community, is a strong indication that *Limulus* is a major agent of disturbance in the benthic community. Firstly, because the average (3.8 weeks) and modal (3.3 weeks) disturbance frequency by *Limulus* at the study site is shorter than the rate of recovery of disturbed infaunal communities (ca. 4 weeks), therefore patches of infaunal communities at some stage of recovery from *Limulus* would persist in 67-70% of the benthos. Furthermore, it was observed *Limulus* re-disturbed certain patches of sediment within four weeks, which would result in the presence of a mosaic of patches at different stages of recovery (Hall et al. 1993).

Results of this study contrasts with previous findings where disturbed patches created by epibenthic predators were ephemeral features in the benthos (Hall et al. 1991, Thrush 1991). This study’s method of estimation of *Limulus* disturbance frequency, by monitoring quadrats with areas comparable to those of *Limulus* pits (20cm×20cm versus 26cm×22cm respectively) at weekly intervals provides an accurate quantification of disturbance frequency. In this study, the spatial structure of *Limulus* disturbance was examined at one site over the *Limulus* foraging seasons in 2009 and 2010. *Limulus*
disturbance was examined over a season as a whole for both years, and over a shorter
temporal scale of three weeks, which was the estimated time required for disturbed
infauna to recover (see Results Chapter). The oscillating patterns of correlograms plotted
for Limulus disturbance accumulated over 2-3 months (Figure 4.2) and three weeks
(Figure 4.3) indicate that Limulus disturbances occurring over these time scales occurred
in multiple small clusters (Kraan et al. 2009). Presence of small clusters of Limulus
feeding pits suggest a the actual extent of a disturbed patch is total area of multiple
feeding pits, thus increasing the sizes of disturbed patches, which in turn would influence
patch-size dependent recovery processes.

Though, it is widely recognized that the greater ecological impacts of small-scale
disturbances is determined by the frequency, areal extent, and the rate of recovery of
disturbed patches (Hall et al. 1993, Sousa 2001). The repeated disturbances of fixed areas
of soft-sediments on a time scale shorter than the rate of recovery have rarely been
documented over an extended period of time. Such information on the spatial and
temporal structures of small-scale disturbances is critical to understanding the importance
of disturbances beyond individual disturbances. This study confirmed that epibenthic
predators can redisturbed significant portion of the benthos that is still recovering from
previous disturbances, thus resulting in a landscape that consisting of a mosaic of
disturbed patches at different stages of recovery (Connell 1978).

**Impacts of Limulus foraging on infaunal community**

Furthermore, the ecological impacts of Limulus disturbance are manifested on
multiple spatial scales as a result of the disturbance regime, and the recovery trajectory of
the infaunal community.
Impacts of individual feeding pits

Results of the examination of infaunal communities in pits created less than 24h prior to sampling and adjacent undisturbed sediment demonstrated that disturbance by *Limulus* immediately created localized patches with reduced density of infauna. Reductions in the abundance of infauna were likely the consequence of displacement, predation and emigration (Hall et al. 1991). Displacement occurs when surface sediments and the associated infauna are pushed out of the pit; predation occurs when infauna is consumed by *Limulus*; and emigration occurs when individuals move out of the disturbed patch. Apart from a reduction in abundance, there was a significant reduction in taxonomic richness as reflected in the total number of taxa and Shannon-Wiener indices of <1 day old pit and control communities (Figure 4.5). The significant immediate impact of *Limulus* disturbance is also evident from multivariate analyses of the two communities (Figure 4.6). The main taxa that contributed to the overall differences in infaunal community differences between <1 day old pit and control communities were Capitellidae and Cirratulidae (26% and 16% of overall dissimilarity respectively). These two taxa were ranked identically among all the taxa found in pit and control communities (Table 4.7), therefore the community difference between pits and controls are due to difference in abundance between the two community types. While it was not within the scope of the study to investigate whether the observed differences between pit and control communities was the result of consumption or physical disturbance, examination of differences in infaunal community structure in *Limulus* pits and undisturbed sediment, and the spatial and temporal patterns of *Limulus* disturbance provides a integrated understanding of the consequences of *Limulus* foraging disturbances.
Following the initial reduction in total infauna abundance, the relative importance of other processes will determine the recovery trajectory of pits. The significant reduction in total infauna abundance by *Limulus* disturbance compared to undisturbed sediments was maintained three and fifteen days after disturbance, but the significant difference was not observed 28 days later. The difference in infaunal diversity (total taxa and Shannon-Wiener diversity index) caused by *Limulus* disturbance was short-lived, as there were no significant difference in the total number of taxa and Shannon-Wiener diversity indices in 15d and 28d old pits compared to undisturbed sediments. The recovery trajectory of pits after disturbance could be the result of detrital accumulation, larval recruitment, adult migration and competitive and reproductive processes within the disturbed patch (Hall et al. 1991). Infaunal recolonization into pits have been shown to be the result of active migration in some studies (Comitto et al. 1995), and prior studies of pits created by epibenthic predators have highlighted the opportunistic response of certain taxa utilizing elevated levels of food resources, resulting in densities increasing beyond those of undisturbed sediments (Thrush 1991). However, there was no evidence in this study of disproportionate increases in the abundance of certain taxa as predicted by the competitive release hypotheses (Grassle and Sanders 1973) or food accumulation model where accumulation of organic matter in sediment depressions are exploited by opportunistic infaunal species, which increase in abundance (Thistle 1980, 1981, Van blaricom 1982). In contrast, the dominant taxa and their respective dominance (in terms percentage of total infauna) of pits of all ages examined were similar to those of corresponding control communities (Table 4.2, Table 4.3, Table 4.4).
These results are consistent with a large number of studies of infaunal community responses to small-scale disturbances where disturbed patches recover to possess communities similar to those in adjacent communities (reviewed by Thrush and Whitlatch 2001). In all these cases, it appears that feeding and competitive interactions within disturbed patches were not altered by the disturbances, and therefore relative abundance of taxa were maintained (Hall et al. 1991). Even though taxa, such as Capitellidae and Spionidae, that are known to exhibit opportunistic colonization behavior dominated undisturbed and disturbed patches, the pit communities did not exhibit the opportunistic response predicted in classic succession models of soft-sediment communities (Norkko et al. 2006). This is likely because conditions within disturbed patches were not conducive for supporting an opportunistic response (Zajac 2004). In cases where recolonizing communities of pits possess similar taxonomic composition to those of undisturbed sediment, recolonization of disturbed sediment most likely occurred through passive movement of animals from surrounding sediments (Commito et al. 1995, Thrush and Whitlatch 2001).

In systems, such as the abovementioned and the one examined in this study where recolonization of small disturbed patches occur through passive transport, site history has been highlighted as an important determinant of the recovery trajectory, and consequently the overall significance of localized disturbances (Whitlatch et al. 2001). Site history refers to the history of disturbance of the sediment surrounding a disturbed patch, which will determine the community structure of the closest source of recolonizers. Just as important but often neglected when considering small disturbances of fixed dimensions such as predator disturbances, are the variability in disturbed patch sizes as a result of clustering.
of small disturbances. This study revealed significant clustering of *Limulus* foraging pits which would result in significant clustering of disturbed patches at various stages of recovery and increase the effective area of a disturbed patch. Therefore given the ubiquity of soft-sediment communities that experience localized disturbances such as epibenthic predator excavations and recover through passive transport, it is especially critical to examine explicit spatial and temporal structure of the localized disturbances, so as to elucidate the collective impacts of these disturbances over a larger scale.

**Effects of *Limulus* disturbance within a single habitat**

Cage exclusion experiments conducted in this study provide a mechanistic understanding of *Limulus* disturbances’ impacts on the infaunal community over the temporal scale of a season and beyond the scale of individual disturbances. In this study, the reduction in the total abundance of infauna (though not statistically significant, Figure 4.13a) was observed after *Limulus* were excluded over two foraging seasons. This finding corroborates with previous exclusion experiments, where removal of predators, resulted in increases in infaunal abundances (reviewed by Wilson 1990b, Thrush 1999, Woodin 1999). Though, infaunal community structure was significantly different only in 2010 but not 2009, removal of *Limulus* disturbance for four months resulted in significant increase in predatory polychaetes but not deposit feeding polychaetes and oligochaetes in both years (Figure 4.12, Figure 4.13). The taxa that contributed the most to the dissimilarity between cage and control communities was Lumbrinidae - which was more than three times more abundant in cages than in control sediments in 2010 (Figure 4.13d). These finding suggests that *Limulus* foraging disturbance impacts predatory infauna more than deposit feeding infauna, supporting Commioto and Ambrose’s (1985) model that
predatory infauna should be regarded separately in soft-sediment community models.

According to Ambrose (1984a), differential impacts of epibenthic predators on predatory and deposit-feeding infauna could be the consequence of (1) preferential predation on predatory infauna by epibenthic predators; (2) preferential predation on predatory infauna and predation by predatory infauna on other infauna; (3) equal predation on predatory and non-predatory infauna with additional predation by predatory infauna on other infaunal species; or (4) competition between predatory and non-predatory infauna with predatory infauna out-competing non-predatory infauna. Competition is an unlikely explanation for the increased abundance of predatory polychaetes, but not deposit feeders observed in exclusion experiments conducted in unvegetated mudflats such as the ones found in Great Bay (reviewed by Lenihan and Micheli 1998). The increase in abundance of predatory polychaetes in the absence *Limulus* suggest that *Limulus* is preferentially feeding on predatory polychaetes. On the other hand, the absence of a significant increase in non-predatory polychaetes in cages is also likely the result of increased predation by predatory polychaetes that were released from *Limulus* predation.

The impact of *Limulus* was also apparent in the significant increase in abundance and biomass of *Macoma* as a result of *Limulus* exclusion (Figure 4.15). Though no cage controls were used in these experiments, the trends observed in polychaetes and *Macoma* abundance within and outside exclusion cages indicate that cage artifacts are negligible. Cage artifacts are probable causes of increase in infaunal abundance after removal of epibenthic predators because cages tend to reduce water flow, which results in accumulation of fine sediment and organic matter, which favor the deposit feeding infauna (Ambrose 1984b, Olafsson et al. 1994). But, the absence of a disproportionate
increase in deposit feeding oligochaetes and polychaetes suggest that the cages did not result in strong cage artefacts. Moreover, it is hypothetically possible for small epibenthic predators such as Carcinus maenas to move through the 10cm mesh, or for the cages to act as a reef that attracts exceptionally large number of small epibenthic predators, which would have resulted in a decrease in infaunal and clam abundance. The absence of a significant reduction in infaunal and clam abundance inside cages indicates that the cages did not result in a higher level of predation by small predators.

The difference in impacts of Limulus disturbance among the different infauna taxa was likely the consequence of the difference in life history traits between the major taxa. Numerically, the infaunal communities sampled were dominated by deposit feeding polychaetes and oligochaetes. These are opportunistic taxa with more extended reproductive seasons, than Macoma and predatory polychaetes (Ambrose 1984b, Grizzle et al. 1999). Therefore over the course of Limulus' intertidal foraging season, infaunal colonizers were likely to be adult deposit feeding polychaetes and oligochaetes bearing broods (Levin 1984) or larval recruits from the water column. Adult colonizers also in turn release larvae with limited planktonic duration and dispersal range, and were therefore likely to remain within the patches originally settled upon by the parents, contributing to localised infaunal abundances, reducing the difference in infaunal abundances between caged and control plots. On the other hand, the Macoma and predatory polychaete populations would be gradually decreased as predation by Limulus occurred throughout the season. Though Macoma recruitment may occur in the spring and fall, the recruits may not replace the portion of the adult population lost to predation within a season.
*Limulus* is generally regarded as a generalist predator with a preference for soft-shell bivalves (reviewed by Botton 2009). On the other hand, *Limulus* have been shown to be capable of separating less preferred small *Gemma gemma* clams from preferred larger *Mya arenaria* and *Mulinia laterelis* from a sandy benthos (Botton 1984a). Therefore it is possible that the different impacts of *Limulus* disturbance on *Macoma*, predatory and deposit feeding infauna was the result of avoidance of small deposit feeding polychaetes and oligochaetes.

In Great Bay, *Limulus* were commonly found moving tens of meters over a mudflat before settling into a patch of sediment where the animal spends more than ten minutes digging into the benthos (pers. obs.). Given the greater burial depth of the most common bivalve, *Macoma balthica* and predatory polychaetes found at the study site, Adams Point, compared to the other common small polychaetes (such as *Capitellidae* and *Spionidae*), and the increased abundance of *Macoma* and predatory polychaetes in the absence of *Limulus*, it is within reason to assume that *Limulus* were targeting *Macoma* and predatory polychaetes in Great Bay. This assumption about the *Limulus*’ preferred prey is also supported by the presence of bivalve shell fragments in gut contents of *Limulus* collected in Great Bay (Lee unpubl. data) and mid-Atlantic coast (reviewed by Botton 2009). Therefore patterns of *Limulus* disturbance and associated impacts on communities are most likely the result of foraging behavior driven by bivalves and predatory polychaete distribution. On the other hand, the co-occurrence of large volumes of sediment in *Limulus* guts also point to the possibility that Great Bay’s *Limulus* consume less-preferred small prey such as small deposit feeding polychaetes and *Gemma*.
*gemma* together with sediment in the absence of preferred prey – as was observed in *Limulus* from mid-Atlantic populations (Botton 2003).

The top two contributing taxa to the community difference between pits and controls were small surface-deposit feeders, that would not require *Limulus* to dig deep into the benthos, as observed on numerous occasions in Great Bay (pers. obs.), therefore, the reduction of Cirratulidae and Capitellidae observed in <1day old pits could be the result of consumption by *Limulus*, lethal injury or displacement by *Limulus* digging for bivalves and predatory polychaetes. Though this study does not parse out the mechanisms through which *Limulus* affects predatory and deposit feeding polychaetes differently, results from two years’ of experiments confirmed that *Limulus* foraging disturbance and significantly change the trophic structure of the infaunal communities by changing the proportion of predatory and deposit-feeding infauna in the communities.

**Importance of *Limulus* disturbance on an estuary-scale**

This study indicated that *Limulus* plays a significant role in determining the overall abundance of small infauna, accounting for between 0.24 to 0.91 of variability in total infauna abundance across the estuary. There are some notable differences between the influence of *Limulus* disturbance on infaunal community patterns on the scale of the estuary and a single mudflat in the estuary. Though, *Limulus* disturbance accounts for a significant proportion of variability of small infauna abundance across the estuary (Table 4.6), but within a mudflat, exclusion of *Limulus* resulted in significant increase in predatory infauna but not abundance of total infauna or deposit feeding polychaetes and oligochaetes (Figure 4.15a). On the other hand, across the estuary *Limulus* foraging also appeared to exert a greater negative impact on predatory polychaetes, than on deposit-
feeding polychaetes and oligochaetes – similar to findings from exclusion experiments carried out at a single site. In contrast, *Limulus* disturbance was not statistically significant in explaining bivalve *Macoma balthica* abundance and biomass across the estuary (Table 4.6), while *Macoma* abundance and biomass were significantly greater within exclusion cages than in control plots with natural levels of disturbance (Figure 4.15).

Variability in the spatial and temporal scales over which physical and biotic processes act is likely the key to understanding how *Limulus* disturbance affect bivalves and polychaetes differently. Firstly, the level of *Limulus* disturbance in the large-scale study was quantified by the areal coverage by *Limulus* pits found at each site, which are short-term snap-shots of the level of *Limulus* activity at each site. *Limulus* pits in Great Bay were generally visible for up to two weeks after excavation (pers. obs.), therefore the levels of *Limulus* disturbance measured in the large scale study reflected the spatial variability of *Limulus* disturbance over the two weeks prior to the surveys, were mostly likely to correlate with community variables that were influenced by short-term disturbances. On the other hand, the patterns of variability in infauna and *Macoma* observed between exclusion and control plots in the cage experiment reflect the cumulative impacts of *Limulus* disturbance over an entire season.

As it was observed that small infaunal communities in Great Bay required more than 15 days to recover from individual *Limulus* disturbances (see Results), therefore, if *Limulus* disturbance was a significant influence across the estuary, infaunal abundance and biomass would be closely related to the levels of *Limulus* disturbance that occurred within two weeks prior to infaunal sampling. Conversely, the snap-shot of *Limulus*...
disturbance recorded across the estuary may not be reflective of the spatial variability of cumulative disturbance levels over the entire Limulus foraging season, and thus did not produce statistically significant correlations with Macoma abundance and biomass. It is also of note that excluding Gemma gemma from the infaunal community in the analyses produces significant results with regards to the role of Limulus disturbance. Commito (1995) found that Gemma gemma abundances in Tom’s Cove, Virginia, were reduced within 24h of Limulus disturbance, but recovered to pre-disturbance levels 24 hours later. Commito’s findings suggests that in habitats with dense Gemma populations, Gemma may have a more rapid colonization rates than other small infauna, and thus was not significantly impacted by Limulus disturbance over the temporal scale greater than days. Lastly, recruitment levels of infauna are more likely to vary among sites in Great Bay and at a single site, such as where the exclusion experiments were conducted (Olafsson et al. 1994). Consequently, variability in infauna recruitment is a likely explanation for discrepancies in the relationship between Limulus disturbance and infaunal abundance at a single site versus across Great Bay.
Findings based on (1) individual *Limulus* disturbance (pits), (2) removal of *Limulus* disturbance over four months at a single site, and (3) *Limulus* impacts over weeks across an estuary showed that *Limulus* disturbance has significant impacts in structuring the infaunal community at multiple spatial scales. The nesting of multiple spatial scales and examination of disturbance impacts over different time scales has enabled the detection of impacts of *Limulus* disturbance on infauna taxa with different life history characteristics.

**Innovative Low-Cost Aerial Photography of Tidal Flats**

Wilson (1990b) noted in a review of predation dynamics in soft-sediments the difficulty of studying mobile epibenthic predators, which hinder the development of a predictive model of epibenthic predator foraging. Presented here is a system consisting of an epibenthic predator that produces highly visible foraging traces, similar to other predatory systems studied (e.g. Grant 1983, Oliver et al. 1984, Oliver et al. 1985, Dumbauld et al. 2008). However, intertidal mudflats such as Great Bay tend to be logistically difficult environments for rigorous investigations, and are especially problematic for tracking epibenthic foraging behavior due to the disturbance caused by researcher access to the study area. Feeding disturbances by epibenthic predators often
result in dramatic topographical features on tidal flats obvious even to the casual observer from the shore (Lee pers. obs.). However, as discussed above, the environmental characteristics of estuarine *Limulus* habitats hinder effective monitoring efforts with the appropriate spatial and temporal resolution. In developing a cost-efficient, aerial survey system that could repeatedly monitor intertidal soft-sediments at very fine spatial and temporal scales, this study overcame a major methodological hurdle in the study of soft-sediments. While the low-level aerial videography used in this study requires a specific environmental setting for the construction of a zipline, it shows the altitude at which *Limulus* foraging traces are visible from the air.

Aerial surveys have been proposed to be a potentially useful method to examine large areas of mudflats rapidly, without disturbing the benthos (Crawford et al. 2006, Sypitkowski et al. 2010). Compared to conventional aerial photography, low-altitude aerial photography (LAAP) from unmanned aircrafts radio-control aircrafts allow large-scale surveys at a significantly lower cost, higher spatial resolution, and with more control over the timing of the surveys. Therefore, LAAP has been increasingly adopted by environmental scientists and workers (Verhoeven 2009). By the use of an innovative aerial monitoring method, this study has overcome a major methodological roadblock to the further study of *Limulus* as a model predator.

Aerial photography has been utilized for a wide range of ecological and environmental studies (Watts et al. 2010). Over the past decade, sophisticated flight, GPS and photographic equipment has been rapidly miniaturized and decreasing in cost (Laliberte et al. 2010). Consequently, radio control (RC) airplanes, helicopters and drones, represent an inexpensive, versatile and controlled way to conduct low altitude
aerial photography (Thome and Thome 2000). To test the utility of radio-control airplanes as low-cost aerial platforms for the monitoring of *Limulus* feeding pits, qualitative survey flights were conducted on October, 10, 2010, at low tide at 4 of the ten large-scale sampling sites (Figure 3.1). The plane was flown over each mudflat at low tide at a constant altitude of ~10m, and it took less than five minutes to survey a 50m × 50m area. The still images extracted from the aerial videos revealed clearly distinct *Limulus* disturbances over large areas of a mudflat. Though there was no precise scale applied to the images, the variability in the density of pits among the photographs taken from four sites is apparent (Figure 6.1 to Figure 6.4).

Small disturbances are ubiquitous in soft-sediment habitats. Apart from *Limulus*, such low-level aerial surveys can be used to monitor other benthic fish species. An immediate application would in the management of threatened Green Sturgeon (*Acipenser medirostris*) in northern California and the Pacific Northwest, which was listed as threatened under the US Endangered Species Act (ESA) (Adams et al. 2007), and Cownose Rays (*Rhinoptera bonasus*) in the mid-Atlantic in the Chesapeake bay (Virginia Sea Grant 2006). Foraging by both these species create a highly visible depressions on tidal flats, and are therefore prime candidates for study using low-altitude aerial photography. Findings presented here show that low-aerial photography using consumer-grade equipment on low-cost aerial platforms is an extremely promising way to examine epibenthic predator dynamics.

**Importance of scale on the impacts of predation and associated disturbances**

The importance of spatial variability and scales is one of the most frequently cited point in ecological literature (Hall et al. 1994, Thrush et al. 1996, Schneider et al. 1997,
Schauber et al. 2009). And in soft-sediment benthic ecology, the influence of epibenthic predators as consumers and agents of physical disturbance is also another axiom (e.g. Wilson 1990b, Woodin 1999, Lenihan and Micheli 2001). However, our understanding of the overarching effects of both phenomena remain incomplete.

The strength of predictions of ecological phenomenon depends on studies and interpretation at the relevant temporal and spatial scales (Thrush 1991). In addition, processes operating at different scales can potentially interact, therefore small scale variability needs to be addressed for comprehensive development of larger scale models (Constable 1999). There is sufficient evidence – ranging from qualitative to the increasingly quantitative – that ecological communities, particularly soft-sediment communities, possess significant variation at different spatial scales (references in Zajac 2008). Thrush, Hewitt and co-workers (Thrush 1999, Hewitt et al. 2007) discussed case studies of predator community impacts with counterintuitive and variable results, which were consequence of difference in the spatial and temporal scales over which predators and prey organisms act.

Manipulative experiments, such as cage exclusions, have been the de rigueur, hypothesis-centered and reductionist approach in examining predator and disturbance ecology (Woodin 1999). However, despite undisputable physical evidence of intense and widespread topographical impact of large epibenthic predators on soft-sediment habitats, experiments and reviews provided equivocal conclusions of the phenomena’s significance (Hall et al. 1991, Thrush et al. 1991, Hall et al. 1993, Thrush 1999). Hewitt et al. (2007) suggested nesting small-scale manipulative studies within a larger correlative observational framework. Furthermore, the importance of observational
natural history information in identifying the appropriate scale and magnitude of heterogeneity in ecological studies has also highlighted repeatedly (Woodin 1999, Hewitt et al. 2007). Basic information on natural history is especially important in understanding predator impacts on communities. This is because the role of predator over a broader temporal and spatial scale is dependent on the life history and behavior of predators and associated communities. While, experiments provide critical information on the small-scale impacts of predators, rigorous observations of natural patterns are required to truly understand the extent of the processes exhibited in experiments.

Micheli (1997) made some pertinent points with regards to the differences in the focus of behavioral ecologists and community ecologists, which are relevant to this discussion on the importance of scale. She pointed out that the former are primarily interested in predators’ effects on community structure, while the latter examine factors and behavior that allow predators to maximize energy intake, and that individual predator behavior can have significant implications for community structure. More recently, Kuhlmann and Hines (2005) discussed the problems of applying findings of laboratory studies of individual predator behavior to natural predator populations, because of spatial and temporal variability in predator and prey. It should also be noted that foraging decisions for a population of predators would operate on a much different scale from individuals and thus affect communities on both the local and landscape scales. Another aspect of natural history that is critical in understand predator disturbance impacts is the life history characteristics of the impacted communities, as the response of each species is dependent on the scale of disturbance and its life history traits (Levin 1984), and this is especially important in soft-sediment communities because of the diversity of body-sizes.
and life history patterns of organisms that inhabit a closely knitted sedimentary matrix. Therefore, it is critical to examine the impacts of predators over a range of scales with the appropriate methods.

**Case study of a marine epibenthic predator**

This study is an effort to address the points discussed above with regards to the influence of scale in the role of localized predator disturbances in structuring community. It combined an examination of the natural feeding behavior of the Atlantic Horseshoe Crab, *Limulus polyphemus*, with the ecological consequences of *Limulus*’ disturbances at multiple scales to obtain a more integrated understanding of the importance of biogenic disturbances. Disturbances caused by feeding *Limulus* on soft-sediment benthos are representative of a large class of biogenic disturbances present in terrestrial and marine environments (Meysman et al. 2006). Estuarine *Limulus* populations, such as the one studied here in Great Bay, New Hampshire, presents a tractable study system, where it is possible to locate the exact locations of predator disturbance with sufficient replication over a practical spatial and temporal extent. This is in comparison to other analogous predators, previously studied which presented logistical challenges due to their large body sizes (Oliver et al. 1984, Oliver et al. 1985, Klaus et al. 1990) or environments with poor access or visibility (Ambrose 1984a, Hall 1994).

There have been numerous attempts to derive models for soft-sediment communities that can be generalized within larger ecological theories. However, frequently, the conclusion is that our understanding of the nature of soft-sediment communities remain incomplete and limits generalizations. Soft-sediment systems do not possess characteristics of a model study system, like intertidal rocky shores, that are
highly accessible and amenable to complex manipulations within the reductionist framework. However, as this study illustrates, marine sedimentary benthos encompass complexities present in many ecological systems, which requires workers to be cognizant of and explain the interplay between multiple factors and scales pertinent to ecological work in general. In this way, studies of soft-sediment communities such as this are important to the general advancement of ecological research.

The methods used in this study enabled the repeated monitoring of a sizable area of mudflat without disturbing the benthos. This study provides an account of disturbance dynamics by an epibenthic predator with fine temporal and spatial resolutions compatible with the predator foraging behavior and recovery dynamics of the impacted infauna. An accurate quantification of the extent and intensity of sedimentary disturbance is critical, because of its wide ranging impacts on communities and biogeochemistry. The results presented here confirmed the complex community impacts of disturbance by a large epibenthic predator. While the biogeochemical impacts caused by *Limulus* was not examined, the high rate of sedimentary turnover is likely to result in significant biogeochemical changes. While biogeochemical impacts of bioturbation by infauna has been the subject of numerous studies (e.g. Botto et al. 2005, Thrush et al. 2006a, Volkenborn et al. 2007), investigations on the effects of *epibenthic* disturbance are relatively rare (but see D'Andrea et al. 2002).

Biogeochemical impacts of direct disturbance to the benthos by epibenthic predators can be likened to disturbance caused by bottom trawling – though to a much less severe extent. Extensive and intensive epibenthic disturbance by *Limulus* to the benthos is expected to affect the sedimentary organic matter decay and nutrient budgets.
Firstly, anaerobic sediment would be exposed to the aerobic conditions, particularly when exposed to the air at low tide (Pilskaln et al. 1998). This impact is especially important in a fine-grain benthic environment like Great Bay, as the redox potential discontinuity layer (RPD) is a few millimeters thick, and *Limulus* can excavate between 5 to 11cm into the sediment. The findings from this study show that *Limulus* can uncover 67-70% of a mudflat every 4 week, which would have tremendous influence on the decomposition rate of buried organic matter in the estuary. In addition, changes in sediment geochemistry caused by *Limulus* disturbance may deter the recolonization of disturbed patches by infauna, thus delaying recovery till up to 28 days as observed in this study.

Furthermore, direct disturbance on the benthos results in an upward flux of nutrient by pore-water release that is greater than that caused by infauna bioturbation (Pilskaln et al. 1998). Underwater observations of *Limulus* foraging in Great Bay showed digging animals creating sustained sediment plumes (pers. obs.). It has been estimated that resuspension of only 1mm of sediment in a coastal storm can double or triple the nutrient flux and turbidity (Fanning et al. 1982). Therefore, the resuspension of 67-70% of a mudflat’s surface area by *Limulus* every four weeks would contribute significantly to the estuary’s nutrient inputs. Finally, the significant reduction of burrowing infauna by *Limulus* indirectly impacts the biogeochemical contributions by infauna.

This study combines conventional and innovative approaches at multiple spatial and temporal scales to further the understanding of the influence scale on the consequences of localized and animal-generated disturbances. Investigations of the influence of *Limulus* foraging disturbance in structuring the soft-sediment communities of the Great Bay estuary in New Hampshire necessitated field-based observational
studies of the predator. Findings revealed previously undocumented behavioral patterns, which then formed the bases for investigations into the predator’s community impacts. The results from small-scale studies of individual disturbances and exclusion plots were indispensable in the interpretation of large-scale correlations. Also paramount was the incorporation of information on the natural history of the infaunal community impacted by *Limulus* disturbance, because small and soft-bodied polychaetes responded to disturbances over a shorter time-scale than larger bivalves.

This study has advanced the methodology of studying epibenthic predators on soft-sediment, understanding of epibenthic predator feeding ecology and its impacts on soft-sediment communities on multiple scales. The findings facilitated an examination of the conditions and scales over which small disturbances can cause significant impacts over a broader scale. Hewitt et al. (2007) remarked that ecologists tended to ‘simply document that nature is variable’ – an inclination commonly found among studies of predator disturbances, where clustering disturbances were noted but not quantified (Grant 1983, Cross and Curran 2004, Pearson et al. 2007). It appears that the lack of quantification of the spatial and temporal structure of epibenthic predator disturbances limited scaling-up of previous studies. The next step is to incorporate predator interactions and their impact on prey communities into the study of biogenic disturbances, as predator behavior will have significant influences on the aggregation of predator disturbances, and the thus the overall influences on the landscape.

Finally, there has been increasing concerns over the homogenization of the ecological complex seafloor as a consequence of anthropogenic removal of benthic organisms (Thrush and Dayton 2002). *Limulus* is harvested by the eel and whelk fisheries.
for bait and biomedical industries for the production of *Limulus* amebocyte lysate (LAL).

Though research on *Limulus* ecology has improved the management of the species, there remained a critical lack of understanding of its feeding ecology. This study revealed that tidal flats are important *Limulus* feeding habitats from spring till fall in New Hampshire. This study developed a method to quantify the level of epibenthic predator foraging activity supported by a habitat that has historically been logistically difficult to study. This information will be critical in facilitating further studies on the bioenergetic and habitat requirements of *Limulus* and also the carrying capacity of its feeding habitats.

Just as importantly, observational and experimental investigations found *Limulus* to play a critical role in creating spatial heterogeneity in benthic communities. Consequently, unsustainable harvest of *Limulus* would likely result in the loss of an important benthic disturbance agent, and the homogenization of the benthic community. Furthermore, in systems where epibenthic predators exhibit aggregated foraging behavior like *Limulus*, understanding of the relationship between predator abundance the feeding behavior is critical to the prediction of how changes in predator abundance will affect the prey and associated communities. This study highlights the pervasiveness and importance of seemingly small and localized disturbances, which often escape the attention of scientists, managers and policy-makers, when compared to large scale catastrophic disturbances such as oil and sewage spills or sedimentation.
Figures

Figure 6.1. Aerial photograph of mudflat taken from a radio-control airplane from ~10m at AP1 on October 10, 2010. Individual depressions made by *Limulus* are highly visible.

Figure 6.2. Aerial photograph of mudflat taken from a radio-control airplane from ~10m at AP2 on October 10, 2010. Individual depressions made by *Limulus* are highly visible.
Figure 6.3 Aerial photograph of mudflat taken from a radio-control airplane from ~10m at RC on October 10, 2010. Individual depressions made by *Limulus* are highly visible.

Figure 6.4. Aerial photograph of mudflat taken from a radio-control airplane from ~10m at NH on October 10, 2010. Individual depressions made by *Limulus* are highly visible.
REFERENCES


Virginia Sea Grant. 2006. Regional Workshop on Cownose Ray Issues: Identifying Research and Extension Needs. Sea Grant Virginia, VA Institute of Marine Science, Gloucester Point, VA.


