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The impact of seasonal movements by ovigerous American lobsters (Homarus americanus) on egg development and larval release

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THE IMPACT OF SEASONAL MOVEMENTS BY OVIGEROUS AMERICAN LOBSTERS (HOMARUS AMERICANUS) ON EGG DEVELOPMENT AND LARVAL RELEASE

BY

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DISSERTATION

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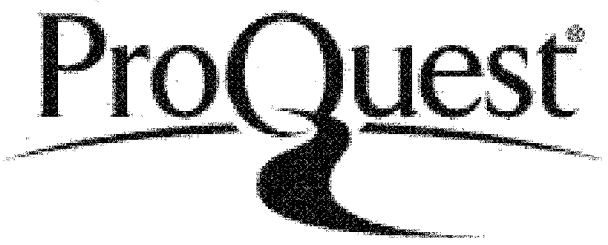

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DEDICATION

This work is dedicated to my parents, especially my father whose spirit and character will always be with me and the things I strive for in my life.

"How inappropriate to call this planet Earth when it is clearly Ocean."

- Sir Arthur C. Clarke

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ABSTRACT

THE IMPACT OF SEASONAL MOVEMENTS BY OVIGEROUS AMERICAN LOBSTERS (HOMARUS AMERICANUS) ON EGG DEVELOPMENT AND LARVAL RELEASE

by

Jason Seth Goldstein

University of New Hampshire, May, 2012

The American lobster (*Homarus americanus*) supports one of the most economically successful fisheries in the Gulf of Maine. The continued success of this fishery is attributed in part to vigilant broodstock conservation through the preservation of ovigerous (egg-bearing) females. Previous studies of ovigerous lobster movements indicate that some, if not most, display seasonal inshore-to-offshore movement patterns. While it has been assumed that these movements serve to expose eggs to thermal regimes that are optimal for development, this theory has never been rigorously tested. In Chapter 1, I present results from ultrasonic tracking studies designed to determine if lobsters in coastal New Hampshire waters exhibit this inshore-offshore pattern and also to identify where ovigerous females overwinter. In Chapter 2, I assess how the movements of ovigerous lobsters would influence the temperature regimes they experienced and thus the development of their eggs. I evaluate this question using a combination of laboratory and field experiments that expose animals to seasonally fluctuating water temperatures they would experience if they remained inshore or moved offshore; data from these

experiments were then used to determine the influence of these thermal regimes on egg development, time to hatch, and larval survival. Finally, in Chapter 3, I present results from a study using experimental ocean drifters deployed in areas where ovigerous females were located when their eggs hatched, to determine where these larvae might drift.

Ultrasonic tracking revealed that most lobsters move offshore in the fall and ovigerous lobsters tend to remain there until after their eggs hatch the following summer (Chapter 1). Eggs exposed to disparate thermal regimes (inshore and offshore) demonstrated that eggs carried by lobsters that moved offshore actually hatched later than those exposed to inshore temperatures (Chapter 2). Finally, most drifters released in offshore hatching locations were carried south or to offshore locations at the time when larvae would settle (Chapter 3). Taken together, these results suggest that seasonal movements of ovigerous lobsters have a strong influence on when and where eggs hatch and, subsequently, where larvae may settle. These findings have significant implications for population connectivity and management of the lobster fishery.

INTRODUCTION

Rationale and Objectives

The effective management of any commercial fishery is enhanced by studying its population dynamics and understanding the factors that influence its distribution and abundance. For large mobile crustaceans, like lobsters, their distribution and abundance is a function of: 1) their daily (e.g., homing and foraging) and seasonal (e.g., inshore to offshore) movements; 2) egg production and fecundity; 3) the release and transport of larvae to an appropriate (and hopefully favorable) settlement location (i.e., recruitment) and; 4) environmental factors that influence all of the above, such as temperature. This work seeks to test the overall hypothesis that ovigerous (berried) lobsters undertake seasonal migrations in order to expose their eggs to a thermal regime that optimizes egg development, the timing and location of larval hatch, and the dispersal and transport of larvae to areas that are best for settlement.

The data and results generated from this work will greatly improve bio-physically coupled models of lobster larval transport that are currently being produced by a number of marine biologists and oceanographers for marine fishes and decapods alike (Katz et al. 1994, Wolanski et al. 1997, Carr et al. 2004, Cowen et al. 2006, Incze et al. 2006, 2010, Leis 2007, Butler et al. 2011). As a result, scientists and fisheries managers will have an improved understanding of the potential sources of new recruits throughout coastal and

offshore waters of New England. Moreover, these data will be invaluable for aiding in the determination of potentially distinct regional lobster stocks, a critical component of an effective management strategy for the American lobster fishery. This work was aimed at carrying out a series of field and laboratory studies to test the overall hypothesis that seasonal lobster movements, in combination with seasonal water temperature fluctuations, strongly influence the rate of egg development, the time and location of larval hatching, and the initial dispersal of larvae.

My first objective was to track the seasonal movements of berried lobsters and non-berried lobsters, using ultrasonic telemetry, to test the hypothesis that there are differences between these groups of animals with respect to 1) their tendency to move offshore in the fall; 2) the timing of their offshore movements; and 3) the magnitude of these movements. Complementary to this, I quantified the thermal histories for some of these lobsters to determine if there were significant differences in the temperatures experienced by lobsters that remain inshore versus those that move offshore. My second objective was to examine how these seasonal movements might influence egg development, and time of hatch. I completed this objective by incubating berried lobsters both in the laboratory and in the field, and exposing them to the type of thermal fluctuations they would experience regardless of seasonal migrations. Finally, I used my knowledge of berried lobster movements and egg development rates, to determine where lobsters will be residing when larvae hatched in the late spring and early summer. I released surface ocean drifters in these locations to determine larval dispersal and the most probable settlement location for these larvae after they fall out of the water column.

Thus, I am testing the overall hypothesis that the seasonal movements of ovigerous lobsters have a significant impact on the time and location of larval hatch, as well as the probability that those larvae will settle in an area that is optimal for their survival.

These studies attempt to provide better resolution to several long-standing questions about the life history strategies of ovigerous lobsters and the adaptive significance (e.g., costs and benefits) in animals that adopt an inshore-offshore movement strategy. It is the first to determine if the movements of females have an impact on their reproductive output, specifically the location of ovigerous females when they are hatching. On a larger scale, my data could potentially aid in identifying habitats where ovigerous females release their larvae, making it possible to model the fate of larvae, identify the source of recruits, and determine which populations or stocks may overlap, contributing to the idea of marine connectivity. These data are essential in sustaining this valuable marine resource through the establishment of sound fishing practices and the development of proper management strategies. Finally, changes in lobster movement dynamics or responses by lobsters to small and changing seasonal temperatures may provide a unique insight into some of the alterations that could be associated with ocean-related climate change.

An Overview of the *H. americanus* Life-cycle

The life history of *H. americanus* includes a complex suite of embryonic, pelagic (larval), and benthic (juvenile and adult) developmental stages that are punctuated with a

dominant and often long-lived benthic period (see Lawton and Lavalli 1995 for overview; Fig. 1). Subsequent to mating, eggs are normally extruded anywhere from 1-6 months later and, as ova are released, they are fertilized externally by the spermatozoa stored in the seminal receptacle (Templeman 1936, Aiken et al. 2004). Freshly extruded lobster eggs are dark green and irregularly shaped and, as they develop, they increase in size and become elongated and lighter in color (Herrick 1909, Helluy and Beltz, 1991). The number of eggs in a given clutch ranges considerably (3,000 to 115,000) and is related to lobster size (Herrick 1909, Perkins 1971, Estrella and Cadrin 1995). Fertilized eggs become firmly attached to the pleopods where they develop for 9-11 months.

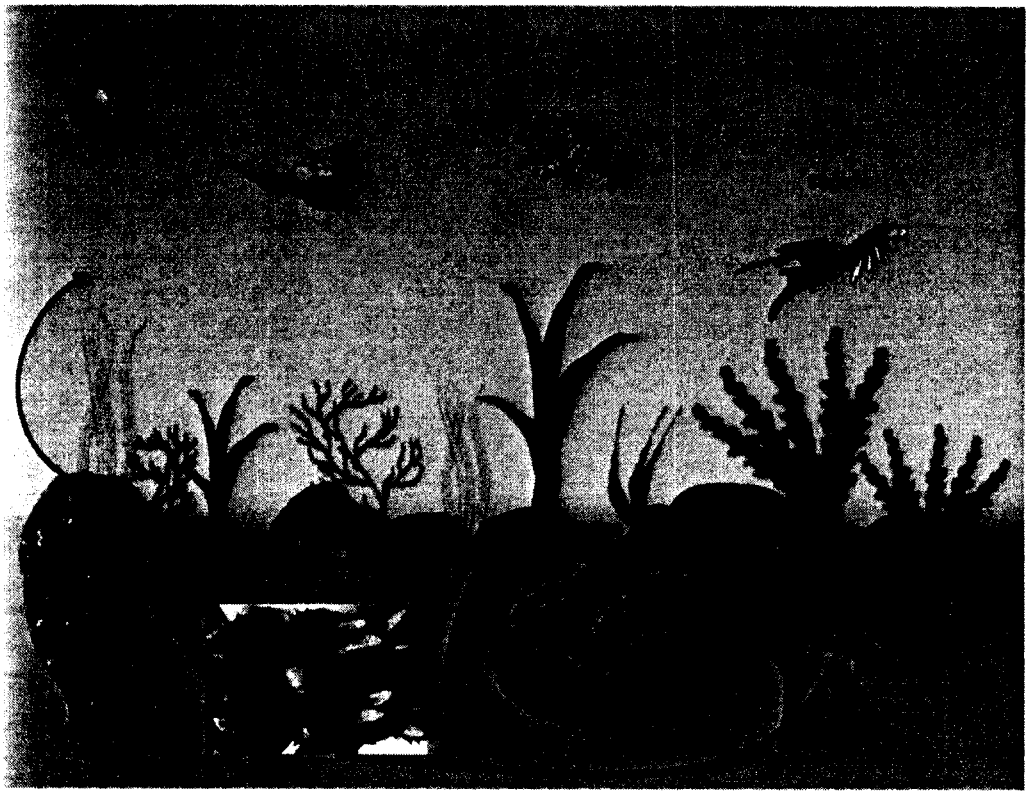

Fig. 1. An illustrative depiction of the American lobster life-cycle that includes a protracted egg development period (9-12 months), four pelagic larval stages (20-30 days), and several years of growth until harvestable size (figure adapted from Lavalli and Lawton 1995).

Temperature is a key factor that determines the length of time the eggs are carried (Templeman 1940, Aiken and Waddy 1980). For example, eggs develop to the 16 cell stage in two days at 18.5 °C, compared with 4.8 days at 10.5 °C (Templeman 1940). Peak hatching typically occurs in June and early July when surface water temperatures are generally > 12 °C (MacKenzie 1988).

Lobsters pass through one prelarval and four free-swimming larval (zoeal) stages (distinguished by morphological, behavioral, and physiological attributes) before settling to the bottom and molting into juveniles (Hadley 1908, Lawton and Lavalli 1995). All larval stages are normally completed in 25-35 days (Herrick 1895, Templeman 1940), but their pelagic duration is highly temperature dependent, and it has recently been argued that it is markedly shorter than previously thought (Annis et al. 2007). The distribution and abundance of larvae are affected by the locations of spawning females in tandem with a host of abiotic factors (e.g., temperature, salinity, light intensity, surface current and velocity, etc.; Phillips and Sastry 1980) that ultimately help to influence their final destination along with intrinsic larval behaviors (e.g., vertical migration and swimming, Harding et al. 1987, Ennis 1995). Late in Stage IV, postlarvae settle to the bottom and burrow into the substrate where they will eventually molt into benthic-dwelling juveniles (Cobb and Wahle 1994), although the presence of biologically relevant odor plumes and the presence of thermoclines have been reported to impact postlarval settlement (Boudreau et al. 1992, 1993). As in larvae, juveniles are distinguished by their ecological ontogeny until functional maturity and adulthood (see Lawton and Lavalli 1995).

Embryology

Early works by Herrick (1891, 1895) and Bumpus (1891) provided the most comprehensive studies and detailed descriptions of *H. americanus* embryology including developmental rates of eggs at various temperatures in the laboratory and predictions for approximating egg extrusion dates. Bumpus (1891) provided additional descriptive embryology leading to the first staging table for early lobster egg development; he observed that in early development, following fertilization, lobster eggs go through superficial cleavage and rapid cellular division before reaching the 16-cell morula stage. The nuclei of dividing cells, each surrounded by an amoeboid mass of protoplasm, divide within the yolk and approach the periphery. As development continues, constant cellular division results in the formation of a blastula leading to gastrulation. Gastrulation is then followed by the forming of the naupliar stage in the egg (oriented near the surface) that is situated dorsally, opposed to the yolk. The naupliar stage is the trademark developmental phase among crustacean decapods and is typified by a median eye along with three distinct appendage types: antennulae, antennae, and mandibles. 'Twitching' motions by the nauplius are typically observed by ~ 10 % development and indicates the existence of embryonic molting (Herrick 1895, Helluy and Beltz 1991).

Herrick (1891) further observed that lobster eggs undergo up to three embryonic molts prior to the occurrence of the lateral eye pigment. Therefore, evidence for a protracted prelarval embryonic molting scheme (based on setal staging in the telson) seems to be very similar to that of the molting cycle for both larval and juvenile lobsters and is thus

affected by environmental variables such as temperature (Aiken 1980). Templeman (1940) built upon this early progress by reporting the time from 16-cell development to eyespot formation at a variety of temperatures. However it was Perkins's (1972) work that produced a series of lobster egg development curves of *H. americanus* thereby giving us a predictive ability for lobster hatch at various temperatures resulting in the Perkins Eye Index (PEI) function.

The PEI has been modified and used for a variety of other lobster species to predict hatch (e.g., Richards and Wickins 1979, Charmantier and Mounet-Guillaume 1992). Most recently, a comprehensive reassessment of the *H. americanus* staging scheme, based on earlier studies, were used to incorporate detailed anatomical, morphological, and physiological descriptions as well as the characterization of 10 distinct embryonic stages (Helluy and Beltz 1991). In addition, this same study also included the conversion of PEI values into a percent-staging system along with descriptions of developmental landmarks through to hatch. As confirmed from the earlier works, Helluy and Beltz (1991) also substantiated the observation of two molts (within the egg) prior to hatch and the first larval stage, described as the beginning and end of the metanaupliar stage in the embryonic period.

The American Lobster Fishery

Early History

The American lobster, *Homarus americanus,* represents one of the most highly coveted marine species in the Northwest Atlantic and is one of the most productive and lucrative (> 40,000 mt in 2006; FAOStat 2008) commercial fisheries in the world. Although the geographic range of *H. americanus* occurs from Labrador, Canada to Cape Hatteras, North Carolina, USA, most commercially active fishing is concentrated from the Canadian Maritimes and into the New England States and encompasses one of the steepest latitudinal sea surface temperature gradients in the North Atlantic (Anthony and Caddy 1980, Fogarty 1995). The history of the American lobster fishery contains references to its earliest beginnings in colonial New England (see Nicosia and Lavalli 1999 for review, Corson 2004). Early reports describe the ease by which lobsters were captured, and their almost ubiquitous existence in both coastal and offshore waters to the degree that they were commonly used as bait and as agricultural fertilizer (Cobb and Castro 2006). Colonial fishing practices involved a variety of hand-aided gear types including dip nets, gaffs, spears and hoop nets used from small boats. Despite the seemingly over-abundance of lobsters in nearshore waters, some were keen to note that unregulated lobstermen and their fishing efforts could have profound impacts on the fishery, particularly for larger lobsters (Herrick 1895).

A shift to a trap or pot-based fishery was in full swing by the mid-1840's (Dow 1949) and enabled large numbers of lobsters to be caught, covering a wider range of fishing areas. As a result, by the mid-19th century, fishery declines were apparent thereby motivating individual states to enact protective legislation to ensure a continuous level of lobster catch (Rathbun 1884). Measures included resident-only permits, closed seasons, and the prohibition of catching, buying and selling of ovigerous (berried) lobsters (Dow 1949). The additional enactment of marking ovigerous lobsters by hole-punching their tails (i.e., uropods) was later replaced with V-notching them with the goal of preserving broodstock in the fishery (Miller 1995). Because New England lobster landings continued to decline precipitously in the late 1880's (e.g., more than 23 % between 1889 and 1892; Smith 1898), states enacted minimum sizes at catch to help mitigate the declining populations. These and other compensatory measures spurned a re-examination of the preservation of large, mature lobsters and their associated role in egg production. Herrick's (1895) extensive studies of lobster fecundity and maturity served as biological benchmarks considering the rigorous sample sizes and comprehensive data that he collected. Since then, other studies have offered fecundity models that essentially validate Herrick's work (Krouse 1973, Estrella and McKieran 1989, Estrella and Cadrin 1995).

As a result of fluctuations in catch coupled with a more panoptic view of lobster reproductive dynamics, additional studies were aimed at examining the contributing role that environmental factors (e.g., temperature, salinity, water quality, photoperiod, disease, etc.) had on catch fluctuations and broodstock condition (Herrick 1911, Aiken and

Waddy 1986). However, it was generally accepted that, of the existing variables, temperature elicited the most pronounced effect on all aspects of lobster biology including larval development, egg production, and size at maturity (Hughes and Mattheissen 1962, Aiken and Waddy 1980). With this insight in mind, along with a significant loss of berried lobsters from illegal scrubbing (Herrick 1895, Smith 1898), researchers and managers decided that artificial propagation of lobster larvae was a viable way to mitigate egg loss and rebuild lobster stocks (Ryder 1886, Rathbun 1892, Herrick 1895, Mead and Williams 1903, Scattergood 1949). These culturing efforts combined both field and laboratory-designed operations that included lobster parks, and the establishment of hatcheries with the goal of holding berried females, hatching their larvae, and seeding post-larval animals back into the sea (Nicosia and Lavalli 1999). Although the biological knowledge (e.g., growth, maturity, diet) gained from these efforts was exceptionally valuable, almost all hatchery and re-stocking efforts were cancelled due to their lack of *biological and economic suitability* (Nicosia and Lavalli 1999).

Current Fishery Status

Today, lobster fishery management and legislation has been streamlined by state and federal agencies into the Atlantic States Marine Fisheries Commission (ASMFC) (http://www.asmfc.org/). ASMFC manages lobster under Amendment 3 (enacted in 1997) of the Interstate Fishery Management Plan (FMP) and includes area-specific management through industry participation organized by Lobster Conservation Management Teams. ASMFC also issues a stock-assessment report for lobster on a

periodic basis that details many fishery and population-level parameters that are integral to the management of this species (Cobb and Castro 2006, ASMFC 2099). The continued success of this fishery is largely attributed to a variety of management measures that particularly single out the conservation of broodstock including license controls, size limits, and the preservation of egg-bearing females through the prohibition of their landings (Kelly 1992, Miller 1995). Many aspects of the U.S. lobster fishery have changed dramatically over the past few decades including consecutive increases in traps fished and the average vessel size. Additional changes such as the switch from wooden lathe traps to coated wire mesh traps, combined with major advances in technology (e.g., GPS, radar, sonar) have had major impacts on fishery-mediated changes including an increased catch efficiency and effort. The impacts of these changes in heavily exploited crustacean fisheries (such as lobster) could potentially influence the reproductive dynamics of ovigerous lobsters. It has been demonstrated that density-dependent reproduction can influence egg production (e.g., maturity at a smaller size, changes in fecundity and egg quality) towards compensatory mechanisms, as has been the case in spiny lobster (MacDiarmid 1989, Polovina 1989, DeMartini et al. 2003).

In the U.S., commercial lobster fishing is designated among three distinct stock management units that include the Gulf of Maine (GoM), Georges Bank (GB), and Southern New England (SNE). By far, the GoM supports the largest and most productive stock with a total of ~ 76 % of all U.S. landings between 1981 and 2007, and has subsequently accounted for over 87 % of the landings since 2007 (ASMFC 2009). GoM biomass has increased significantly along with fishing effort, especially into areas that

have not been fished regularly in the past (Fig. 2). Some surmise these rapid increases in abundance and catch are not only due to increases in fishing effort but also with enhancements in fishing technology, water temperatures that favor growth, and decreases in keystone predators (e.g., cod; Fogarty et al. 2008). Although the GB stock remains relatively stable, SNE has experienced drastic declines since 2002 accounting for only 9 % of all U.S. landings. A mean total of 11,900 permits were issued during the 1981-2007 period, with a fair amount of variability surrounding each of the New England States. In the GoM, the historical and recent success in lobster fishing (400 % increase since 1985) has created a virtual ecosystem monoculture that some suggest may negatively impact economic and social aspects of this fishery in years to come (Steneck et al. 2011).

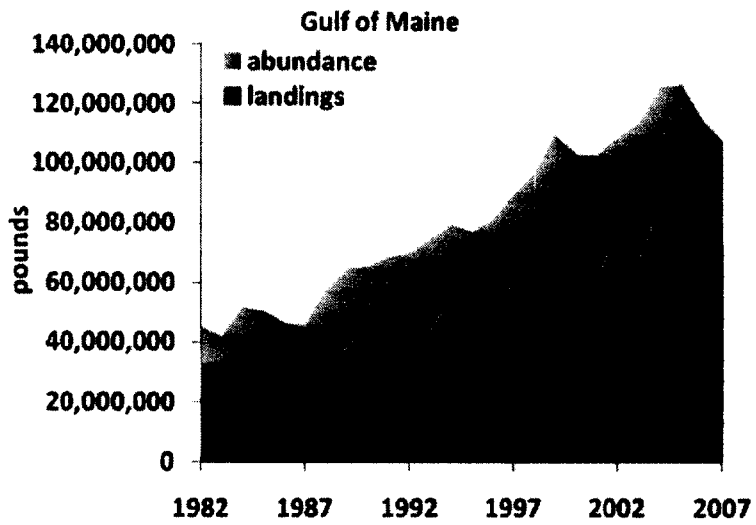

Fig. 2. Biomass and commercial lobster landings in the Gulf of Maine. Various reasons including favorable temperatures, increased exploitation effort, and decreased predator assemblages have been postulated for the significant increases of lobster. (source: NOAA 2009).

Influence of Temperature on Attributes of Lobster Life-history

Influence of Temperature on Ectotherms

Temperature affects all of an ectotherm's physiological processes mainly through the alteration in biochemical pathways (e.g., enzyme activation rates, macromolecule formation, acid-base regulation etc. (see Hochachka and Somero 2002 for review) and thereby dramatically influences animal distributions, thermal preferences, and survivorship. For crustaceans like lobsters, temperature is arguably one of the most pervasive factors influencing metabolism, activity levels, spawning, development, growth, and possibly life span (see Hawkins 1996 for review). Changes in temperature also have striking effects resulting in at least a twofold increase in overall biological processes with each 10 °C rise in temperature (i.e., Q_{10} temperature coefficient; Schmidt-Nielsen 1991). Temperature also has directed effects on processes such as gas exchange, acid-base regulation, and protein synthesis among others (Whiteley et al. 1997).

Remarkably, temperature can also operate as a selective force in many animals. Petersen and Steffensen (2003) found that juvenile cod (*Gadus morhua*) in Denmark waters could be distinguished by their hemoglobin genotype resulting in distributional differences based on their thermal preferences. The discovery by Liu et al. (1998) that small changes in temperature can entrain the biological clocks in reptiles and fungi or that subtle changes in seawater temperatures can trigger extensive coral bleaching events (Gates and Edmunds 1999) are some of the more dramatic examples. In these cases, thermal

dependence then becomes a 'universal currency' for most aquatic ectotherms, and many studies have sought to summarize and model these complex relationships examining growth and maturity in both temperate and tropical waters (Gillooly et al. 2002, Angilletta et al. 2004, Sponaugle et al. 2006).

Reproduction and Maturity

There is a resounding influence of water temperature on most aspects of lobster reproduction including maturation, spawning, molt cycle, embryogenesis and hatching (see Waddy and Aiken 1995 for review). While elevated temperatures accelerate the onset of reproductive maturity, low temperatures tend to delay ovarian maturation (Templeman 1936, Waddy and Aiken 1995). If increases in springtime water temperature are delayed, the final stages of ovarian maturation are inhibited and spawning is, for the most part, lost for that year (Waddy and Aiken 1992). Likewise, the exposure of ovigerous lobsters to sufficiently cold winter temperatures helps to synchronize proper timing of spawning and molt. A few weeks at temperatures below 5 °C at the right time of year (typically Dec-Jan), insure spawning, and thermally sets the mode for other processes such as ovarian maturation and egg extrusion (Waddy and Aiken 1992). Differences in temperature also manifest themselves on much more localized scales (10s of km). For example, one area's lobster population may show markedly different reproductive dynamics (i.e. onset of molting or the start of spawning) compared with another area (Little and Watson 2005). Small variations among thermal regimes have been documented to influence lobster size at maturity in areas as close as

11 km away (Estrella and McKiernan 1989, Little and Watson 2005). Other studies, conducted along the coast and bays of Newfoundland, indicate that lobster populations differ thermally resulting in differential spawning (Squires et al. 1971, Ennis 1971).

Temperature also has a direct influence on the rate and development of eggs; however, egg attachment and even egg loss can be impacted as well. Under a normal temperature regime, Waddy (1988) was able to show that egg development can be reset anywhere from 3-17 months under controlled laboratory temperatures. Talbot et al. (1984) discovered that elevated winter temperatures prior to spawning have an adverse effect on egg retention resulting in only 2 of 23 lobsters retaining or hatching a significant number of eggs. Other long-term laboratory studies implicate elevated temperatures in the significant loss of extruded eggs as well as their attachment to the abdomen, ultimately influencing hatching success (Talbot and Harper 1984, Waddy 1988).

Physiology and Behavior

Lobsters are exceptionally adroit at responding to small changes in temperature as demonstrated in previous work (Crossin et al. 1998, Jury and Watson 2000), and they respond both behaviorally (e.g., movement) and physiologically (e.g., changes in cardiac cycle) to this highly influential environmental parameter (McLeese and Wilder 1958). Additionally, lobsters in a thermal gradient tank (Crossin et al. 1998) were shown to exhibit a final thermal preference of 15.9 °C, which is very similar to the value of 16.5 °C found by Reynolds and Casterlin (1979), using a similar method; lobsters also tend to

avoid water temperatures below 5 °C and above 18 °C (Crossin et al. 1998). *H. americanus* are reported to live in areas that range in water temperature from 5-20 °C (Aiken and Waddy 1986), although this is a synergistic relationship involving varying levels of salinity and oxygen as well. Early laboratory experiments by McLeese (1956) gave us insight into the survivorship of lobsters subjected to combinations of varying temperatures, dissolved oxygen, and salinity. Lobsters tend not be directly stressed by water temperatures below 20 °C as long as oxygen levels are maintained at > 2 mg O_2/L. However, Dove et al. (2005) more recently determined that the respiration rate of lobsters is significantly decreased at temperatures above 20.5 °C and this temperature has been used as an upper threshold for some lobster populations.

Field evidence has further elucidated the responses of lobsters to temperature and salinity and how they influence their distribution and abundance. For example, following a hurricane, estuarine lobsters tend to move into colder, deeper, higher salinity water (Jury et al. 2005). This change to their normal movement patterns (moving into the estuary in the spring and out in the fall) may be explained, in part, by their responses to these variables (Watson et al. 1999). What is not clear is whether lobsters change their responses to temperature during their life history (e.g., immature, sexually mature, ovigerous) and if so, does this cause changes in their population dynamics?

Temperature has certainly been implicated in influencing the behavior, activity, and movement patterns of lobsters throughout their range (reviews in Herrnkind 1980, Lawton and Lavalli 1995, Childress and Jury 2006). Temperature has also been shown to

significantly influence commercial lobster catch (Dow 1977) and as a possible cause for increased lobster abundances throughout the 1980s and early 1990s (Drinkwater et al. 1996). Furthermore, increased temperatures in the GoM within the ranges predicted under proposed alternative climate change scenarios hold the potential for increased lobster productivity (Fogarty et al. 2008; Fig. 3).

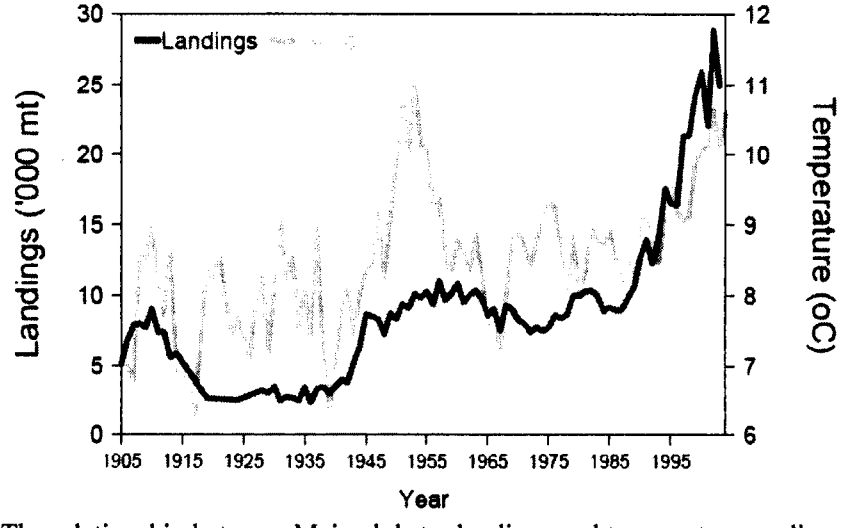

Fig. 3. The relationship between Maine lobster landings and temperature readings (taken at Boothbay Harbor over a 100-year period. Lobster landings have more than tripled in Maine over the last decade (source: Fogarty et al. 2008).

Temperature then can be thought of as an ecological resource that has boundaries within which animals can operate, and contains a directed (and perhaps selective) impact on the physical distribution of animals and their associated physiological processes and behaviors.

Lobster Movements

The concept of animal movements has been encapsulated in the general framework by Nathan et al. (2008) who describe the movement paradigm as having four essential

components: the internal state of the organism (i.e., intrinsic motivation to move), the motion and navigational mechanisms that define the animal's ability to move and influence where and when to move, and the broad range of external factors that affect movement. Addressing these kinds of questions allows for the exploration of the causes, mechanisms, and patterns of movement and the ecological and evolutionary implications (e.g., individual fitness) that follow. Movements that occur over a range of spatiotemporal scales can be used to answer a variety of questions.

Long-distance movements (for both plants and animals) can significantly impact community and local population dynamics (Kokko and Lopez-Sepulcre 2006, Nathan 2006) and plays a key role in species invasions, habitat fragmentation (Robertson and Butler 2009) responses to climate change (Polovina 2005, Brander 2010), and the spatial design of marine protected areas (MPAs) (e.g., lobsters; Kelly et al. 2002, Goñi et al. 2010). Technological advances (e.g., GIS, ultrasonic telemetry) now allow us to collect movement data at a high spatiotemporal resolution and infer these patterns on many different levels (Pittman and McAlpine 2003). The patterns, and mechanisms of lobster movements are varied and have been studied extensively (see reviews in Herrnkind 1980, Lawton and Lavalli 1995). The dynamics of lobster movements have a great impact on their distribution and abundance, and knowledge of these movement patterns is integral to the fisheries management of coastal habitats and our understanding of their continued ecological function and economic success.

Movements by Ovigerous Lobsters

Some brooding animals seem to utilize movements as a selective force that reflects their ancestral requirements linked to reproductive processes. This is seen consistently in many kinds of amphibians and terrestrial crustaceans that routinely return to waters to release their eggs or larvae (Dingle 1996, Adamczewska and Morris 2001). Certainly, some ovigerous marine crustaceans (spiny lobsters and crabs) that maintain external lecthiotrophic egg masses and hatch pelagic larvae have been reported to undergo brooding-related movements that serve to selectively position their progeny for transport away from deleterious environments and into areas that favor larval advection. The movements of gravid blue crabs (*Callinectes sapidus*) for example, to the mouths of estuaries and bays, allow crab zoeae to utilize offshore currents and avoid osmotic stress and predators (Forward et al. 2003). Booth (1997) compiled the long-distance movements by several spiny lobsters in the Pacific (*Jasus* spp.) and postulated that many of these inshore to offshore movement events were associated with molting or reproduction. Some of these movements are described as contranatant, acting to redress the dispersal of larvae back to maternal areas; this has also been the case in slipper lobster migrations as well (e.g., Stewart and Kennelly 1998). Finally, studies looking at the reproductive movements of late-stage ovigerous Caribbean spiny lobster (*Panulirus argus*) using ultrasonic telemetry determined that these animals made homing excursions from their dens on the reef to the reef edge to release their larvae (Bertelsen and Hornbeck 2009). Clearly, there is evidence that some ovigerous crabs and lobsters incorporate specific movements into their repertoire for purposes of reproduction.

Movement Effects on Egg Development and Larval Hatch

Ovigerous lobsters incubate their eggs on average for 9-11 months (Bumpus 1891) and are known to exhibit a high degree of maternal care in keeping their eggs healthy and viable (Aiken and Waddy 1980). However, temperature elicits a first-order influence on overall development, and this period can be reduced to 6 months, or lengthened to 13 months, depending on the thermal environment during incubation (Templeman 1940, Perkins 1972, Aiken and Waddy 1980, Chapter 2). Given that most ovigerous lobsters are exposed to temperatures below 11-12 °C while incubating their eggs through the winter, one can certainly imagine a number of different scenarios whereby lobster movements could influence hatching and larval survival. For example, by delaying inshore migration into warm water, a female could delay hatching, while a lobster inshore would be exposed to a rapid increase in water temperature in the spring so that hatching might occur before optimal conditions for larval survival. It is possible then, that ovigerous females have evolved a life history strategy that is based not on exposing their larvae to the warmest temperatures to achieve the fastest rates of development, but instead is based upon the need to time hatching so that it occurs at the right time and at the right place for optimal survival of their larvae.

Ultrasonic Telemetry

One way to ascertain both fine and large-scale movement patterns of animals in response to these cues is the use of ultrasonic telemetry. Ultrasonic telemetry is an excellent tool

for investigating the movements of aquatic species and lobsters in particular (Golet et al. 2006, Bowlby et al. 2007, MacArthur et al. 2008, Scopel et al. 2009, Schaller et al. 2010). Despite the widespread and more economical use of tag-recapture studies and seasonal SCUBA surveys, these methods are often inherently confounded by a number of factors including inconsistent effort and catch (with traps) and limited geographic scale (Freire and Gonzalez-Gurriaran 1998, Dunnington et al. 2005). Although telemetry is often financially limited to focus on a much smaller pool of individuals, it can be much more foretelling of the spatial and temporal resolution for individuals and provides reliable and consistent data over discrete time periods.

The use of fixed array telemetry systems, allows precise measurements of lobster movements on a minute-to-minute time scale with a resolution of ~ 1 meter. Combined with advances in multiscan ocean mapping techniques and GPS technologies, researchers and fisheries managers can now correlate fine scale movements with specific features of benthic habitats (e.g., Geraldi et al. 2009). As a result, we now have a window into the lives of lobsters that was not previously available, and we can take advantage of this opportunity to address several long-standing questions about the behavior of lobsters in their natural habitat.

CHAPTER 1

SEASONAL OVIGEROUS LOBSTER (HOMARUS AMERICANUS) MOVEMENTS ALONG THE NEW HAMPSHIRE SEACOST

Abstract

Like many large mobile crustaceans, American lobsters (*Homarus americanus*) exhibit daily and seasonal movement patterns at both local and regional scales. In the case of ovigerous (egg bearing, berried) lobsters, while there is conflicting evidence concerning the timing, patterns, and purpose of their movements, it is generally accepted that some or most, move offshore during colder months and inshore into shallower water in the summer. To determine if this pattern applies to lobsters in New Hampshire (southern Gulf of Maine) coastal waters, 45 individuals (20 egg-bearing females, 15 non-egg bearing females, and 10 males) were equipped with ultrasonic transmitters and tracked for an average duration of 250 days in 2006-2009. We sought to determine 1) if ovigerous lobsters express different seasonal movement patterns than males or non-ovigerous females; 2) what potential environmental triggers induce coastal lobsters to initiate offshore movements in the fall; and 3) the location of ovigerous lobsters when their eggs hatched. There were no significant differences ($p > 0.05$) in the seasonal movements of ovigerous lobsters compared with non-ovigerous females, but female and male movements were different, in the fall, spring and summer ($p < 0.01$). During the tracking period, a total of 82 % ($n = 37$) of lobsters showed movements (> 0.5 km) while the remainder (18 %) were resident. Of these animals, some moved small distances (< 5

km, n = 19, 51 %), some modest distances (5-10 km, n = 7, 19 %) while others (both large and small ovigerous and non-ovigerous females) moved greater distances offshore (> 10 km, n = 11, 30 %). As a group, large females (> 86 mm CL) moved the furthest (average = 7.3 km). Most lobsters that moved offshore in the fall initiated their movements between October and November, apparently cued by a combination of rapidly falling water temperatures ($r^2 = 0.85$, $p < 0.001$) and increased wave activity resulting from fall storms ($r^2 = 0.64$, $p = 0.040$). By the time eggs hatched in late spring/summer, ovigerous females were located at an average distance of 7.44 ± 1.38 km from their original inshore tagging location. Our working hypothesis is that the majority of lobsters move offshore in the fall/winter to avoid harsh coastal environments. These movements, in turn, influence the thermal history and hatching time of eggs carried by ovigerous females, as well as the location where these eggs will hatch. Therefore, the movements of ovigerous females ultimately influence the survival of their larvae, their dispersal and where new recruits potentially settle.

Introduction

The movements of marine decapod crustaceans are highly diverse, often dramatic, and serve a variety of purposes (Dingle 1996, Pittman and McAlpine 2003, Nathan 2008). In general, these animals move to: 1) acquire resources and shelter; 2) avoid suboptimal habitats and environmental perturbations (e.g., extreme temperatures, turbulence); 3) enhance growth and development by moving to areas with optimal temperatures; or 4) improve the dispersal of progeny (e.g., eggs and larvae) (Herrnkind 1980, Herrnkind and Thistle 1987, Levin 1992, Bowler and Benton 2005, Childress and Jury 2006). Likewise, crustacean movements can also serve a homeostatic purpose that may enhance survival of both adults and offspring (Leggett 1985).

American lobsters (*Homarus americanus*) are well known to exhibit daily and seasonal movement patterns at both local and regional scales (Cooper and Uzmann 1980, Haakonsen and Anoruo 1994, Lawton and Lavalli 1995, Scopel et al. 2009). While it is generally accepted that most lobster movements are local in nature, long-distance movements have also been documented (some of these could be considered migrations; see Hernnkind 1980 for definitions). In general, offshore lobsters appear to move the furthest, and some express homing tendencies and return migrations (Cooper and Uzmann 1971, 1980, Pezzack and Duggan 1986), suggestive of a panmictic stock in the Gulf of Maine (Fogarty 1995, Tam and Kornfield 1996). In contrast, tag-recapture studies of lobsters in coastal and estuarine waters indicate that they seldom move long-distances (> 10 km) (reviewed by Krouse 1980, Haakonsen and Anoruo 1994, Lawton

and Lavalli 1995, Watson et al. 1999, Commeau and Savoie 2001, Cowan et al. 2006, Bowlby et al. 2008). Nevertheless, the seasonal inshore-to-offshore movements by inshore lobsters are fairly predictable and may have a significant impact on their physiology and ecology. These movements are influenced by changes in water temperatures and are believed to constitute a form of behavioral thermoregulation (McLeese and Wilder 1958, Reynolds and Casterlin 1979, Crossin et al. 1998, Jury and Watson 2000).

Offshore water temperatures (below the mixing zone) typically remain warmer and more stable than inshore waters in winter; in contrast inshore waters tend to be warmer during the spring and summer (Flowers and Saila 1972, Oviatt 2004, Goldstein and Watson submitted). Therefore, lobsters that undertake seasonal migrations inshore in the summer and offshore in winter gain more degree-days which is likely to enhance their growth rate and may modulate other temperature-dependent processes (e.g., ovary maturation, molt cycle) (Campbell 1986, Waddy and Aiken 1995). For ovigerous (i.e., berried) lobsters, increased water temperatures will also enhance egg development rates (Perkins 1972, Campbell 1986, Talbot and Helluy 1995). Finally, it has been demonstrated that larval survival decreases significantly below 12 °C (MacKenzie 1988, Annis et al. 2007), so the movement of berried lobsters to warmer waters may enhance both embryonic development and larval survival.

Some, if not most, ovigerous lobsters move offshore during colder months and then move inshore or into shallower water in summer to incubate their eggs and release their larvae (Campbell 1986, Harding and Trites 1988, Robichaud and Campbell 1995, Lawton and Lavalli 1995, Cowan et al. 2006). The existing paradigm is that these movements are probably driven by the need to incubate eggs at the warmest possible temperatures (Cooper and Uzmann 1980, Campbell 1986, Lawton and Lavalli 1995, Comeau and Savoie 2001), to increase overall rate of egg development. However, this pattern may not be universal (Childress and Jury 2006, Goldstein and Watson submitted). Several studies have demonstrated that females with late-stage eggs move very little (MacKay 1929, Jarvis 1989, Watson et al. 1999). For example, late-stage ovigerous lobsters tagged off outer Cape Cod (Massachusetts) made northward movements (averaging 28 km) along the outer coastal periphery (Morrissey 1971), compared to smaller excursions (10 km or less) by ovigerous lobsters in shallow lagoons, semi-enclosed bays, and estuaries (Munro and Therriault 1983, Watson et al. 1999, Commeau and Savoie 2001). Ovigerous lobsters in offshore waters have been reported to undertake seasonal migrations, and in the southern end of their range these movements can be quite large (Fogarty et al. 1980, Childress and Jury 2006). In contrast, lobsters in more northern regions do not have to move as far from wintering areas in deep offshore canyons to reach shallower, warmer banks (e.g., Browns, Georges) where they reside in the summer and fall (Saila and Flowers 1968, Cooper and Uzmann 1980, Pezzack et al. 1992).

More recently, Cowan et al. (2006) addressed the question that ovigerous lobsters undergoing seasonal movements are exposed to disparate thermal histories by tracking

individual animals along the mid-coast of Maine and simultaneously monitoring the water temperatures these lobsters experienced. In general, smaller females moved shorter distances than larger lobsters. Furthermore, data from *in situ* temperature loggers demonstrated that inshore lobsters tended to experience larger and more rapid seasonal fluctuations in water temperature than those that moved offshore, even though both thermal histories resulted in a similar number of degree-days. Moreover, limited data from this study suggest that the duration of egg development was not always shorter for eggs exposed to the most degree-days. These data suggest that: 1) approximately one third of ovigerous females in coastal Maine waters move far enough to influence the temperature regime they experience, and these females tend to be > 93 mm carapace length; 2) lobsters that move offshore experience a more gradual decrease in water temperature in the fall, warmer water in the winter and a more gradual increase in water temperature in the spring; and 3) the duration of egg development is not simply a function of the number of degree-days. The primary goal of our study was to extend these findings to lobsters in coastal waters and determine if larger ovigerous females do, in fact, move into offshore, deeper waters than smaller ones. We also sought to determine if movements expressed by ovigerous females were indicative of a general pattern of movements expressed by all adult lobsters.

Other marine crustaceans are also known to exhibit shallow to deeper water movements in response to seasonal changes in water temperature and other physical perturbations (e.g., spider crabs; Gonzalez-Gurriaran et al. 2002). One of the most dramatic of these seasonal migrations is the sudden orchestrated movement of Caribbean spiny lobsters

(*Panulirus argus*). It has been proposed that this migration is triggered by large drops in temperature and increased turbidity, as the result of autumn storms on the shallow banks of the Bahamas (Kanciruk and Herrnkind 1978). American lobsters movements are also most pronounced in the fall, as water temperatures are dropping and the probability of storms increases. For example, following a hurricane, lobsters tend to move into higher salinity, colder, deeper water (Jury et al. 1995). In the laboratory, it has been demonstrated that lobsters can detect very small changes in temperature (Jury and Watson 2000) and salinity (Dufort et al. 2001), and they avoid hyposaline water as well as adversely high or low temperatures (Jury et al. 1994, Crossin et al. 1998). The normal movement patterns of some lobsters into estuaries (e.g., Great Bay, NH) in summer and out in the fall may be explained, in part, by their attraction to warmer water and avoidance of low salinity (Watson et al. 1999).

The most dramatic lobster movements generally occur in fall and spring, yet it is not clear what environmental factors might trigger these events (reviewed in Herrnkind 1980). Ennis (1984) observed that in the fall lobsters in a Newfoundland bay tended to move to deeper waters in response to increased storm turbulence and the breakdown of the thermocline. Environmental factors are very likely to have a strong influence on lobster movements and often correlate with specific times when they are more active (Gregory and Labisky 1986, Cockcroft 2001, Jury et al. 2005). A secondary goal of this study was to determine if the offshore movements expressed by lobsters along the New Hampshire coastline in the late fall were associated with distinct seasonal changes in the marine environment.

We used a variety of ultrasonic telemetry techniques to track seasonal movements of lobsters along the coast of New Hampshire in the Southern Gulf of Maine. Our major goals were to: 1) track the seasonal movements of ovigerous lobsters, male lobsters and non-ovigerous females, of different sizes, to determine if the movements of ovigerous females were unique; 2) evaluate potential environmental triggers that induce lobsters to initiate their seasonal movements; and 3) locate berried females when their eggs would be hatching to determine the influence of movements on the location where larvae are released.

We found that most of the lobsters studied, of all sexes and sizes, moved modest distances offshore in the fall. These fall movements appeared to be triggered by a combination of rapidly cooling waters and increased turbulence (waves) created by fall storms. Most of the animals investigated remained offshore throughout the winter and well into the spring. Thus, ovigerous females were in these offshore areas when their eggs hatched in the early summer. These data suggest that, while all lobsters appear to be responding to the same environmental cues and express similar patterns of movements, these events have a significant impact on the timing and location of hatch and larval release. This, in turn, might influence the ultimate pattern of recruitment and the extent to which different lobster populations overlap.

Materials and Methods

Study Site

All tracking took place along the seacoast of New Hampshire (NH), USA between the fall of 2006 and summer of 2009 (three seasons total) (Fig. 1). Lobsters were captured in standard lobster traps, fitted with ultrasonic transmitters as described below, and released in a small cove just off New Castle Island, NH, at the mouth of the Piscataqua River (43°04.912 N; 70°42.456 W). Local benthic habitats included a prominent shallow rocky reef complex (2-8 m depth), surrounded by a heterogeneous mixture of sand and fine sediment flats interspersed with patchy eelgrass (*Zostera marina*) beds. Adjacent to the reef was a deeper channel (18-20 m). Bottom water temperatures, monitored with HOBO temperature data loggers (model UA-002-64; Onset Computer Corp., Pocassett, MA) ranged from 2-18 °C during the course of the study. Current speeds and directions measured at 1 m from the bottom in the area where lobsters were released ranged from 0.03 to 28.9 cm^{s-1} throughout all tidal cycles (Golet et al. 2006). For purposes of this study, we considered inshore as areas < 5 km from shore corresponding to a depth range of 5-20 m (avg = 8 m) and offshore areas as > 5 km from shore at depths > 20 m (Fig. 1). The exception to this was areas around the Isles of Shoals (> 5 km from the tagging location) that had steep drop-offs and were considered offshore.

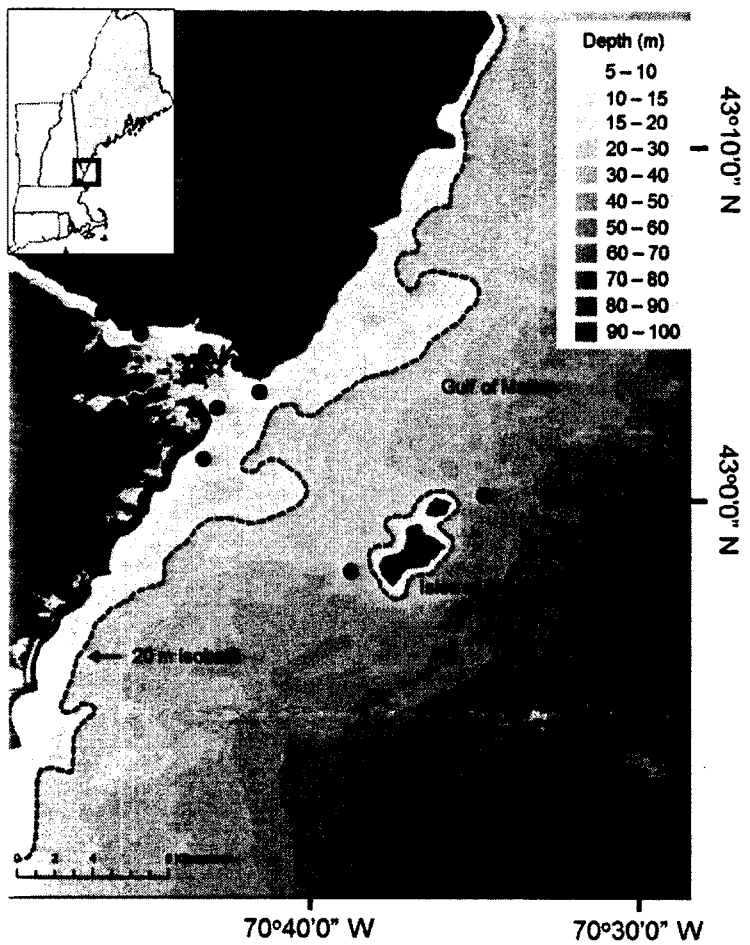

Fig. 1. Study area and release location (star) for lobsters tagged over three successive seasons, 2006- 2009. Also pictured are locations where telemetry receiver/loggers (VR2s) were positioned (black circles). Dashed line indicates the approximate location of the 20 m isobath, used to delineate inshore from offshore areas.

Tagging Protocol

Lobsters were captured using conventional baited commercial lobster traps and then their sex, size (carapace length, CL, to 1 mm increments using calipers) and molt stage was determined. Only lobsters that were postmolt (stage 'C'; Waddy and Aiken 1992) were used for this study because, unlike sphyrion tags, ultrasonic transmitters are lost when a lobster molts. For most ovigerous lobsters, a small sample (10-15) of eggs was removed

from the abdomen and placed in a 1.5 mL tube with 4 % formalin-seawater for later determination of developmental stage (Helluy and Beltz 1991).

Lobsters were fitted with VEMCO V13-1L coded tags (69 kHz, 13 mm diameter, 36 mm long, 6 g in water, estimated battery life > 600 days, VEMCO-AMIRIX Systems Inc., Halifax, Canada). Animals were also tagged with small (19 mm diameter) vinyl laminated disc tags (Floy Tag Co., Seattle, WA) containing contact information, and a message requesting lobstermen to either keep or release lobsters depending on their time at large. A select number of lobsters (ovigerous; n = 10) were also fitted with HOBO Tidbit temperature loggers (Onset Computer Corp.) that recorded temperature every 30 minutes and could be downloaded using a PC-based software package (HOBOware Pro v. 3.0) upon recapture.

Ultrasonic tags were secured by gluing them inside a piece of Tygon® tubing then attaching the tubing to each lobster using a cable-tie fastened between the second and third pair of walking legs (Fig. 2; Golet et al. 2006, Scopel et al. 2009). The disc tag and temperature logger (where applicable) were then cable-tied onto the main transmitter harness using a combination of smaller cable ties. Finally, a small amount of cyanoacrylate glue and 1.5 cm duct-tape squares were fastened from the cable tie to the carapace to prevent the backpack from slipping. The entire tagging process took ~ 3-5 minutes.

After tagging, lobsters were placed into old standard single-parlor lobster traps that had their doors and vent removed to facilitate escape. After lobsters were lowered to the

bottom, they gradually left the traps and took up residence in the same vicinity (verified by tracking with a high resolution VRAP telemetry system, VEMCO-AMIRIX Systems Inc., Golet et al. 2006, Scopel et al. 2009). This approach seemed to reduce the tendency of lobsters to move large distances after being handled. While we did not directly examine the influence of transmitter backpacks on lobster behavior and locomotion, distances traveled by tagged and untagged lobsters in both field and laboratory settings were not significantly different (Jepsen et al. 2002, Golet et al. 2006, Scopel et al. 2009).

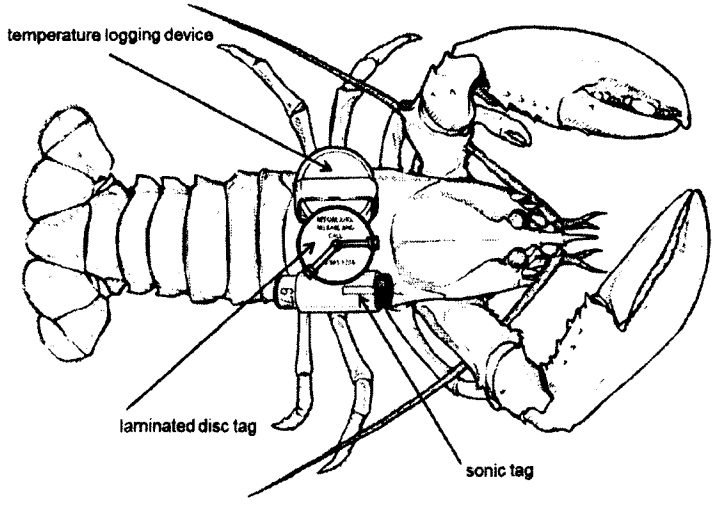

Fig. 2. Attachment of an instrument backpack to a lobster. All animals were fitted with an ultrasonic transmitter and laminated disc tag; some were also equipped with a temperature logger.

We tagged a total of 53 lobsters. However, only 45 lobsters (18 females and 0 males in 2006; 12 females and 6 males in 2007; 5 females and 4 males in 2008; n = 10 ovigerous animals with temperature loggers; Fig. 3) yielded sufficient data to be included in our final analyses. Most lobsters were fitted with transmitters in the late summer and fall and tracked throughout the winter and the following spring and summer. Although the primary focus was to determine if there were differences between the movements expressed by ovigerous and non-ovigerous lobsters, more ovigerous lobsters were tagged

for two reasons. First, movement data obtained from egg-bearing lobsters was also part of a companion egg development study (Goldstein and Watson submitted). Second, ovigerous lobsters caught by fishermen must legally be released, so they remain at large longer than those without eggs.

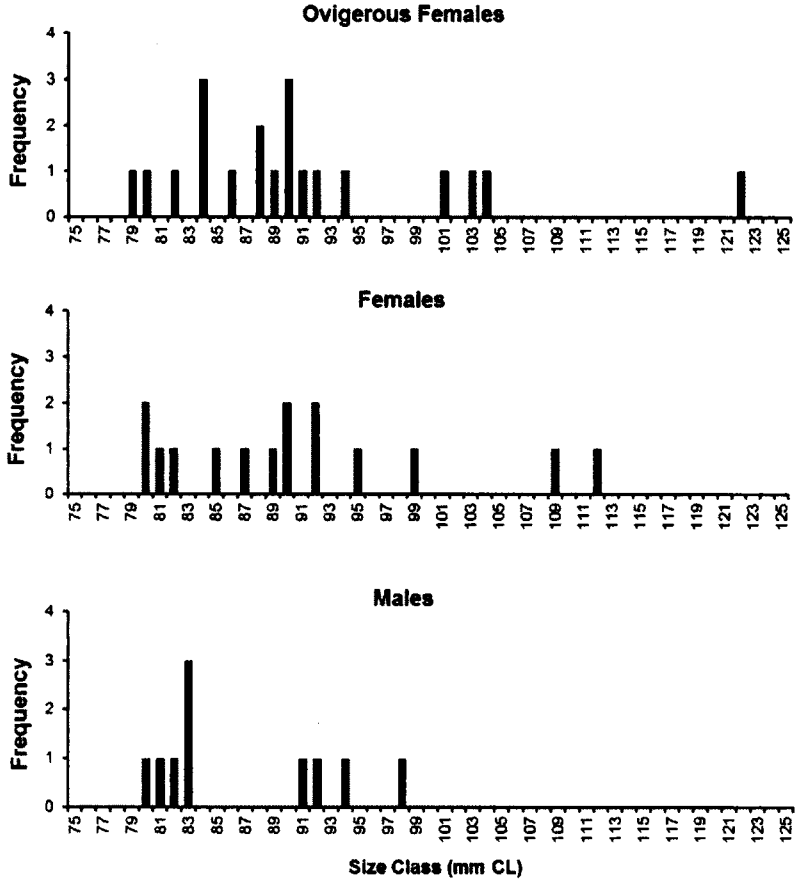

Fig. 3. Size frequency distribution for all lobsters fitted with transmitters in 2006-2009. Lobsters are sorted by class and include: ovigerous females (n = 20, CL_{avg} = 87.7 ± 2.3), females (n = 15, CL_{avg} = 90.9 ± 2.5), and males (n = 10, CL_{avg} = 86.7 ± 2.0).

Ultrasonic Telemetry

Lobsters were tracked throughout the study using three types of commercially available hydrophone-receiver instrumentation. First, a series of fixed underwater acoustic

receivers (VR2s) that function as low resolution, high-coverage (300-400 m radius) 'gateways' along constrained hydrodynamic features (e.g., river channels, land points, islands). Seven single receiver listening stations (VR2s) were moored along the NH coast and out to the Isles of Shoals (Fig. 1). These self-contained units detected the presence of transmitters within ~ 400 m and logged the time and transmitter ID. Second, a mobile acoustic receiver (VR100) was connected to an omni-directional hydrophone and towed behind a research boat on a custom-made harness at a depth of 3-4 m. This receiver provided medium-scale resolution (within 20-100 m of a tag) and enabled us to locate animals in virtually any location. Finally, a fixed array listening system (VRAP) utilized a triangulation algorithm to locate animals within the range of the array with a resolution of a few meters. All telemetry equipment and associated software was obtained from VEMCO-AMIRIX Systems Inc. (Halifax, Nova Scotia, Canada, http://www.vemco.com).

The high resolution fixed array radio-acoustic positioning system (VRAP) was deployed in the fall of 2006 to track lobster movements during the fall to winter transition. The goal was to determine exactly when lobsters initiated their offshore movements during this time of year. The VRAP system consisted of a three-buoy array and a base station. The buoys were moored ~ 150 m apart in an equilateral triangle (Fig. 4). Details of this tracking system are found in Golet et al. (2006) and Watson and Chabot (2010). Briefly, each buoy contains a hydrophone that detects the ultrasonic transmissions, and a radio that communicates with an onshore base station (receiver/computer). The computer then triangulates the position of each transmitter based upon signal arrival times at each buoy;

the system is accurate to within 1-3 m under optimal conditions. Data are obtained for each lobster in the vicinity of the array approximately every 5 minutes.

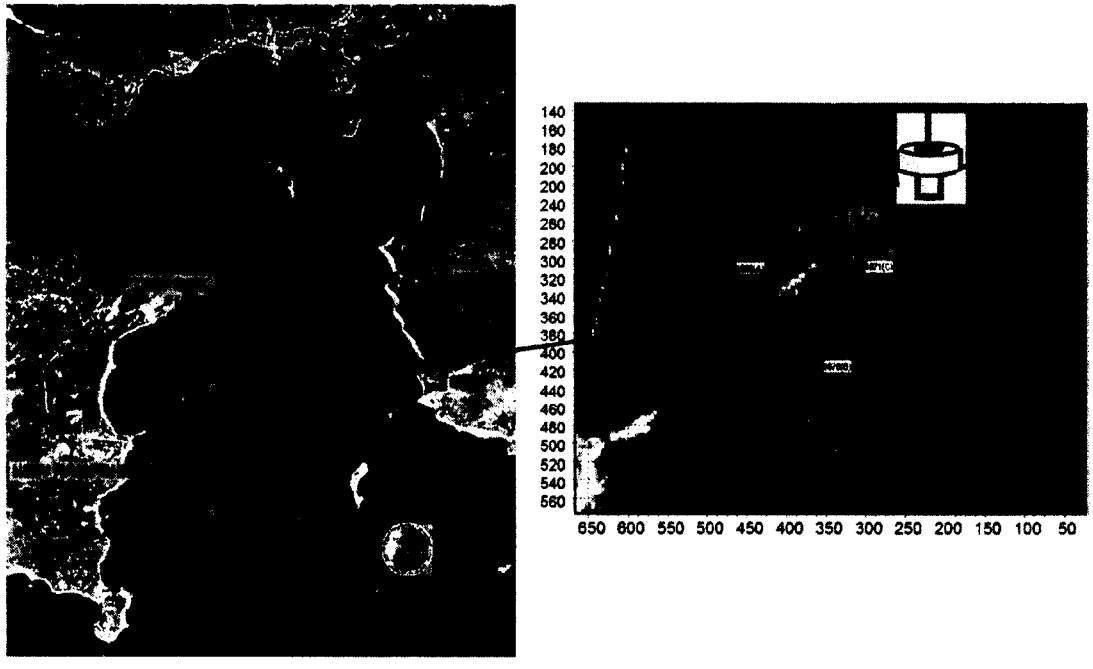

Fig. 4. Release location (arrow) and position of VRAP fixed acoustical system (2006 only, triangle covering the tagging site) consisting of a three-buoy array positioned as an equilateral triangle (scale in meters), and base station (onshore at the UNH Coastal Marine Lab (CML). This system plotted real-time positions of tagged lobsters based upon signal arrival times received by each buoy. See Golet et al. 2006 for details.

Manual Tracking

It took approximately one month to cover the entire grid using manual tracking methods (Fig. 5). Manual (VR100) tracking was conducted at weekly, or sometimes biweekly (wintertime) intervals, throughout the year; tracking areas were covered in a grid-like fashion (lines separated by ~ 300 m). However, positions of animals inshore, around the release location, and from downloads of the VR2s, were obtained on a weekly basis. Therefore, while weekly data were available for some animals, seasonal movements were generally analyzed using monthly data.

The accuracy and reliability of the manual (VR100) telemetry system were verified in two ways. First, a simple range test was conducted to determine the distance at which a particular tag could be detected (Webber 2009). A tag was mounted on a small brick with underwater marine epoxy and placed at-depth in the vicinity of the release location. The hydrophone was towed away from the tag location behind a small boat in each of the four cardinal directions until the signal could no longer be detected (i.e., the receiver could not identify the tag number). Tags in the study area could be heard and identified within 300-500 m of their location.

A second test determined the most probable location of a lobster, given that the manual tracking system often logged multiple fixes for a given animal as the hydrophone was being towed past the animal. Data obtained using the VRAP system were used to ground-truth the actual position of a given lobster/transmitter, and this position was then compared to GPS locations obtained using the towed system. Averaging all the GPS coordinates obtained for a given lobster as the vessel passed by it yielded single GPS locations within 10-15 m of the animal's actual position. However, since the manual system was not always towed right over the transmitter, data obtained with the manual tracking system were most conservatively accurate to within 30-50 m of the pinger's location.

In addition to data from telemetry, we frequently received positional information from lobstermen who caught tagged lobsters and phoned or emailed this information (per instructions on the disc tags). Informational flyers were distributed among lobster

pounds, fishermens' co-ops, and lobster wholesalers in the area to alert fishermen to the details of this study.

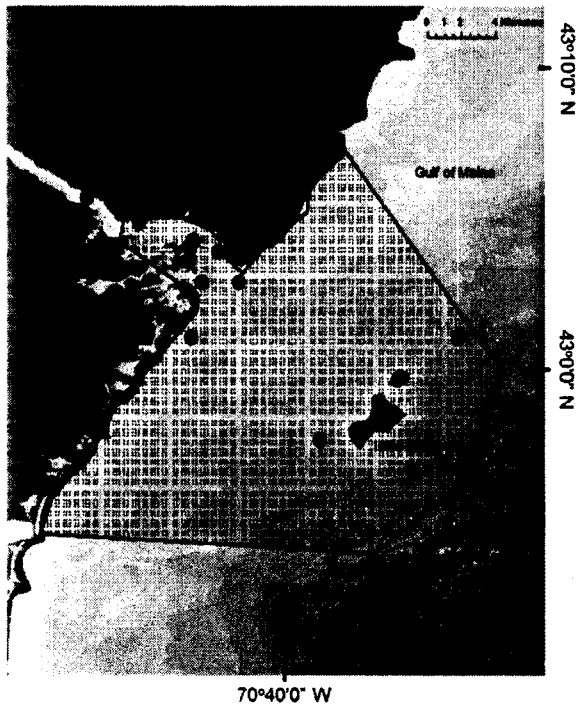

Fig. 5. Area monitored during study duration. The total area (~ 375 km^2) was covered monthly, primarily using manual tracking methods and the downloading of VR2 units. Telemetry receiver/loggers (VR2s) are indicated by black circles (see Fig. 1).

Environmental Data

Daily water temperature data were acquired using a combination of HOBO pendant temperature loggers attached to VR2s in fixed locations and HOBO Tidbit temperature loggers recovered from some recaptured lobsters. Wave height measurements, indicative of storm events, were obtained from ocean observation buoys for the fall of 2006. Wave height data was queried as daily averages and downloaded for selected time frames from the Gulf of Maine Ocean Observing system (GOMOOS; http://www.gomoos.org) and two GOMOOS buoy locations: 1) NOAA CMAN IOSN3 (Isles of Shoals, 42°58'12" N,

70° 37'12" W); and 2) Buoy B01 (Western Maine Shelf, 43°10'51" N, 70° 25'40" W). Relationships between lobster emigration, temperature and wave heights were analyzed using pairwise correlations in JMP v. 9.0.3 (SAS Institute, Cary, NC).

Data Processing and Analysis

Lobster positional information was plotted using the ArcGIS v.9.3 software package (ESRI Inc., Redlands, CA). If more than one position was obtained on a given day during manual tracking or from the VRAP system, GPS fixes were averaged to yield a single location per day. Positional fixes based on VR2s were considered to be the location of the VR2 unit.

To calculate the distance a given animal moved from one season to the next we used the two points (one from each season) that yielded their maximum distance. The three seasonal time periods (4 months each) chosen for our calculations included fall (Sept 1-Jan 1), winter (Jan 2-April 1) and spring (April 2-July 1). The partitioning of seasons in this manner also corresponded to periods when: 1) temperatures were decreasing the most (fall); 2) temperatures remained < 6 °C (winter); and 3) lobsters generally re-initiated their movements (temperatures > 6 °C; spring). Calculations beyond this time frame were confounded by transmitters that were expiring; however, we did track some animals into August and September when possible. Additionally, lobsters that were recaptured by fishermen provided added data as well. If animals moved away from the coast, distances were recorded as positive values, while movements towards the coast were recorded as

negative values. To calculate 'net' distances positive and negative values were summed, or averaged. For comparison of distances traveled by individual lobsters of different categories animals were grouped as large females (86-120 mm CL; n = 10), small females (70-85 mm CL; n = 5), large ovigerous females (n = 14), small ovigerous females (n = 6), large males (n = 4) and small males (n = 6).

To establish the time at which lobsters left the VRAP array (in 2006) we utilized the 'Playback' command module in the VEMCO software package (VRAP v. 5.1.4), which allows playback and visualization of the tracking history for each individual. For each individual, the Julian day of movement outside of the VRAP array and towards offshore locations was noted. All movement analyses were conducted using the statistical software package JMP v. 9.0.3. Directional data were analyzed from a Rayleigh's Z-test using Oriana v. 3.0 software (Kovach Computing Services, UK). Data that did not meet parametric assumptions were analyzed using non-parametric Mann-Whitney U-tests. All means are given ± se.

Results

A total of 45 lobsters were tagged and tracked in each of three successive seasons: 2006 (n = 18), 2007 (n = 14), and 2008 (n = 7) (Table 1). A total of 82 % (n = 37) of lobsters showed movements (> 0.5 km) while the remainder (13 %) was resident over a 150 day period. Of these animals that moved, some moved small distances (< 5 km; n = 19, 51 %), some modest distances (5-10 km; n = 7, 19 %) while others (both large and small

ovigerous and non-ovigerous females) moved greater distances offshore (> 10 km; n = 11, 30 %). As a group, large females (> 86 mm CL) moved the furthest (avg. = 7.3 km).

Days-at-large (DAL)

Overall, all 39 lobsters were at large for an average of 216.3 days. However, ovigerous females exhibited slightly longer DAL (mean = 248.2 ± 17.1) than non-ovigerous females (mean = 220.9 ± 19.7) or males (mean = 180.0 ± 24.1; Fig. 6). This was presumably because lobstermen were legally required to release any ovigerous lobsters captured. Nevertheless, DAL was not significantly different among the three lobster classes (ANOVA; $F = 2.69$, $df = 2,44$, $p = 0.081$). There were also no differences when comparing the same DAL for large versus small lobsters within and between classes (2-factor ANOVA; $F = 1.20$, $df = 5,44$, $p = 0.326$; Fig. 7). Interestingly, small males (mean = 161.5 ± 32.0) were not at large significantly more than large males (mean = 207.3 ± 39.1), or either ovigerous or non-ovigerous females (range = 217.2 – 251.1) (Tukey HSD; $q = 3.01$, $p > 0.05$, $\alpha = 0.05$). A total of 37 lobsters (82.2 %) were caught at least once by commercial fishermen; eight lobsters were caught more than once.

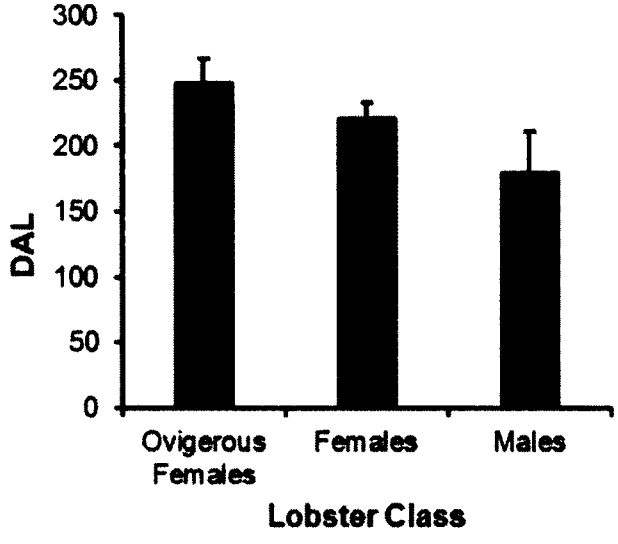

Fig. 6. Days-at-large (DAL) for three lobster classes: ovigerous females (n = 20), females (n = 15) and males (n = 10) tagged over three consecutive fall seasons and tracked through successive summers, 2006-2009. There were no significant differences among the three lobster classes (ANOVA, F = 2.69, df = 2,44, p = 0.081). Means are expressed ± se.

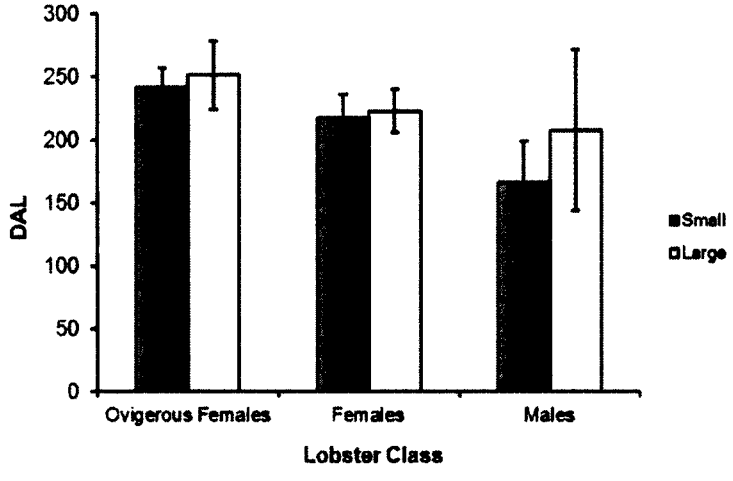

Fig. 7. Days-at-large (DAL) for three lobster classes by size: ovigerous females (n_{small} = 6, n_{large} = 14), females (n_{small} = 5, n_{large} = 10) and males (n_{small} = 6, n_{large} = 4) tagged over three consecutive fall seasons and tracked through successive summers, 2006-2009. Means are expressed ± se.

Year	Sex	Size (CL)	Date Tagged	Days-at-Large (DAL)	Max. Absolute Dist. Traveled	Egg Dev. %
2006						
	F(o)	79	12/06/06	211	7.73	62
	F(o)	82	10/03/06	216	0.28	38
	F(o)	84	09/22/06	315	11.35	58
	F(o)	84	11/02/06	206	19.59	52
	F(o)	86	08/30/06	250	20.73	0
	F(o)	88	09/26/06	99	0.54	0
	F(o)	89	09/19/06	370	1.65	0
	F(o)	90	09/22/06	296	7.40	34
	F(o)	103	10/26/06	336	5.90	43
	F(o)	104	10/04/06	309	14.44	0
	F(o)	122	09/19/06	345	11.72	0
	F	82	11/09/06	264	12.49	
	F	87	11/02/06	180	0.97	
	F	92	09/26/06	217	2.64	
	F	95	09/29/06	124	21.82	
	F	99	09/29/06	220	1.98	
	F	109	09/26/06	223	9.20	
	F	112	10/13/06	206	5.80	
2007						
	F(o)	90	10/26/07	250	15.07	30
	F(o)	90	10/16/07	252	3.57	15
	F(o)	91	10/22/07	260	10.05	12
	F(o)	101	10/16/07	204	10.79	
	F	80	07/20/07	152	5.20	
	F	80	11/14/07	241	4.03	
	F	85	10/26/07	229	22.54	
	F	89	10/16/07	340	1.15	
	F	92	10/22/07	247	3.17	
	M	80	10/26/07	221	0.31	
	M	81	11/09/07	207	3.41	
	M	82	10/22/07	39	0.62	
	M	91	10/22/07	58	0.50	
	M	94	10/23/07	196	2.81	
2008						
	F(o)	80	09/30/08	225	0.58	8
	F(o)	84	09/30/08	275	7.73	66
	F	81	09/30/08	200	3.23	
	F	90	10/14/08	232	0.77	
	M	83	09/30/08	250	1.20	
	M	83	10/14/08	208	0.98	
	M	92	10/14/08	308	3.10	

Table 1. Inventory of all lobsters tagged and tracked (n_{total} = 39) in each season: 2006 (n = 18), 2007 (n = 14), and 2008 (n = 7). Categories include ovigerous females, F(o), females (F), and males (M). Egg assessment is based on the Perkins Eye Index (Perkins 1972) and calculated for a subset of eggs. Eggs with 0 % development lacked discernible eyespots, and were not measured.

Movement and Distance

The maximum absolute distances traveled by the three classes of lobsters were significantly different (Fig. 8; ANOVA; $F = 3.43$, $df = 2,44$, $MS = 130.5$, $p = 0.041$). Distances traveled by ovigerous lobsters were the greatest (7.44 ± 1.38 km), compared with non-ovigerous females (6.33 ± 1.59 km) and males (1.29 ± 1.95 km). Furthermore, the mean distance traveled by ovigerous females was significantly different from the mean distance moved by males, but not significantly different from non-ovigerous female movements (Tukey HSD; $q = 2.42$, $p < 0.05$; Fig. 8).

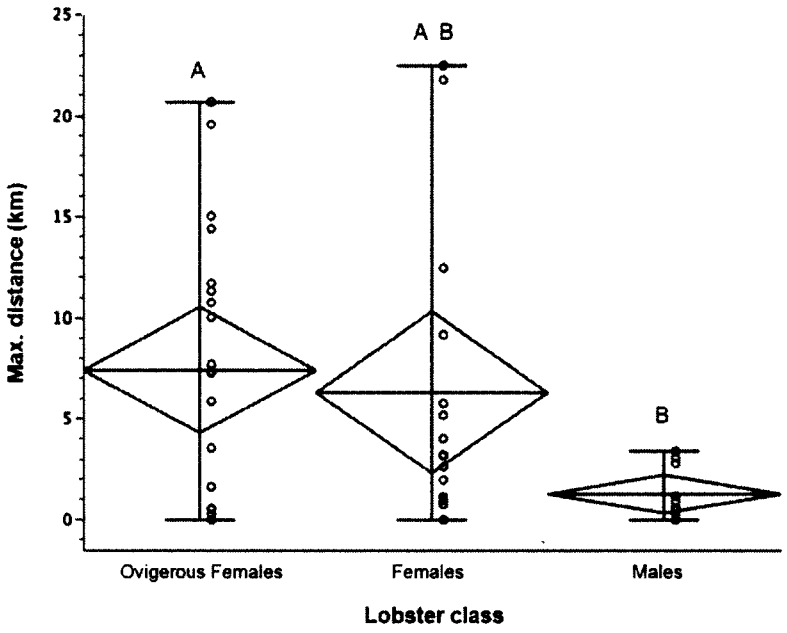

Fig. 8. Maximum distance traveled by three classes of lobsters (all sizes combined). Diamonds indicate 95 % confidence intervals, with horizontal lines representing means for each class. Different letters above bars indicate significant differences ($p < 0.05$, $\alpha = 0.05$).

Within each season there were moderate differences in movement by lobster type (ANOVA; $F = 3.43$, $df = 2,44$, $p = 0.051$), but no differences by lobster size (ANOVA; F

= 0.46, df = 1,41, p = 0.97). However, there were strong differences in lobster movement across different seasons (ANOVA; F = 4.79, df = 2, 84, p = 0.011; Fig. 9). From fall-winter both types of females moved significantly farther than males. Between December and March (winter-spring) minimal movement by all lobsters was observed, including comparatively diminished movements by males (Fig. 9). By late spring and early summer, there were dramatic movement events observed for ovigerous females (avg. = 2.55 ± 0.66 km) compared with non-ovigerous females (avg. = 1.56 ± 0.64 km) and males (0.41 ± 0.31 km). These seasonal differences by each lobster class are shown in Figure 10. An example of seasonal movements of two individual lobsters (ovigerous and non-ovigerous females) is given in Figure 11.

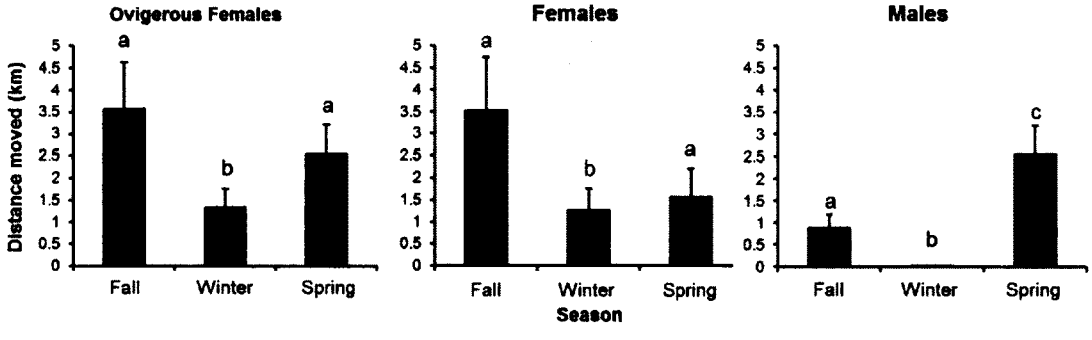

Fig. 9. (Top): Net movements by lobster class for animals moving > 0.5 km, and at large for at least 150 days, for each of three seasons (all sizes combined): fall, winter and spring. Means expressed ± se. Shared letters above bars indicate no significant differences ($p > 0.05$, $\alpha = 0.05$).

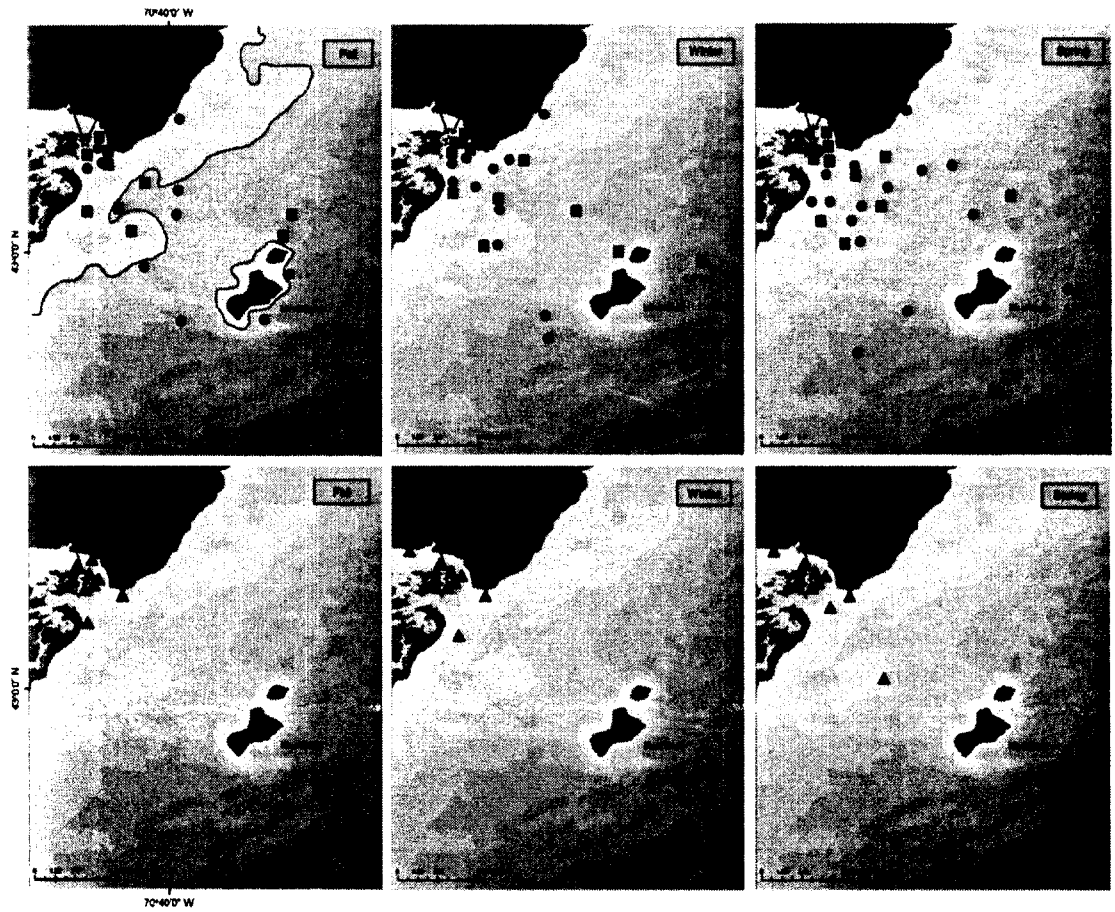

Fig. 10. Composite seasonal movements by lobster type, ovigerous, non-ovigerous, and male, sizes combined, over three seasons, 2006-2009. Panel A depicts both ovigerous females (n = 20, circles) and females (n = 15, squares) across each of the three seasons while Panel B includes all male lobsters (n = 10, triangle) over the same three seasons. The original tagging location is designated by a star, and the dashed black line indicates the 20 m isobath, used to delineate inshore from offshore movements (also see Fig. 1).

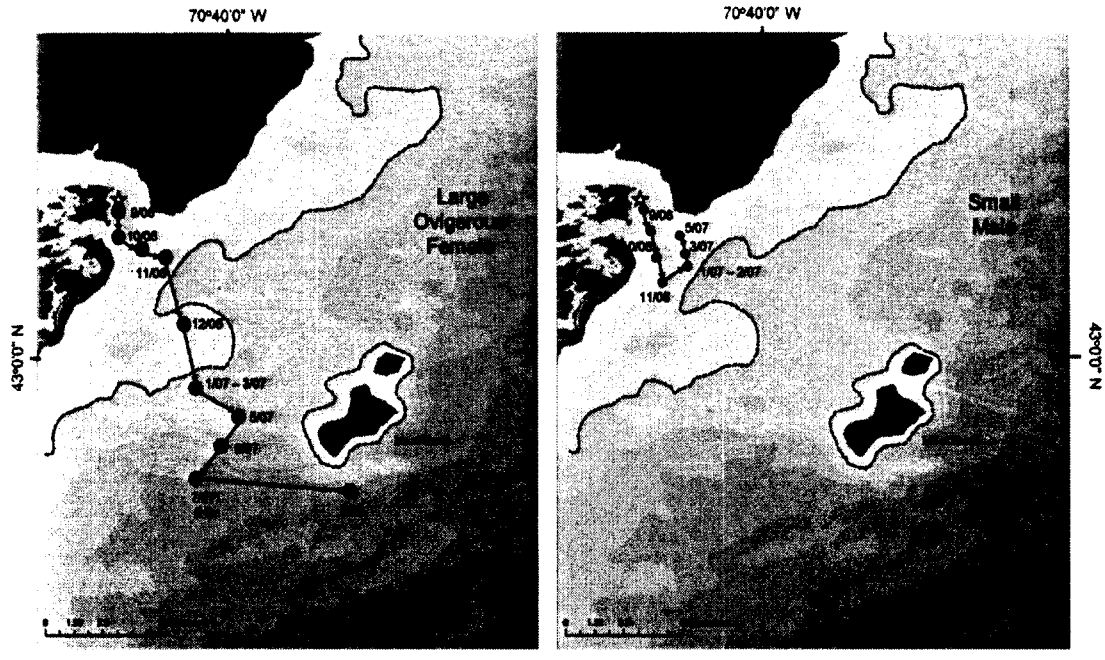

Fig. 11. Contrasting seasonal movements between a large ovigerous female (CL = 90) and a small male lobster (CL = 82) in 2006-2007. The track of the large ovigerous female is fairly typical for this group, characterized by large rapid offshore movements in the fall, a stationary period in the winter months, and movement after the predicted hatching date (H). While some smaller non-ovigerous females expressed the same seasonal movements as some large ovigerous animals, most males (large and small) remained exclusively within inshore waters.

Initiation of Fall Offshore Movements

A total of 18 lobsters were tracked with the high resolution VRAP system in the fall of 2006 (11 ovigerous, CL_{mean} = 92.0 ± 8.3 mm and 7 non-ovigerous, CL_{mean} = 95.6 ± 11.7 mm). Most lobsters (n = 16) moved away from the VRAP array (Fig. 4) over a period of 21 Julian days (avg. = Nov-1; 95 % CI = Oct-22 to Nov-11; Fig. 12). Lobsters tagged before Oct-5 (n = 13 or 72.2 %) expressed strong site fidelity, remaining near the tagging for 39.5 ± 6.6 days before moving offshore. In contrast, those tagged after Oct-5 remained in the area for significantly less time (1.5 ± 1.5 days; t = 3.781, df = 17, p = 0.0015). There were no differences between ovigerous and non-ovigerous lobsters in

terms of their propensity to leave the tagging site (Mann-Whitney test; $U = 35.0$, $p = 0.68$). Initial lobster movements offshore were in a SSE direction, with a mean vector of 159° (Rayleigh test; $Z = 25.35$, $p < 0.001$). Many of the tagged lobsters showed daily and sometimes longer movement patterns within the periphery of the array; however, they were not considered as having vacated the array until they were undetected by the VRAP array for > 24 hr.

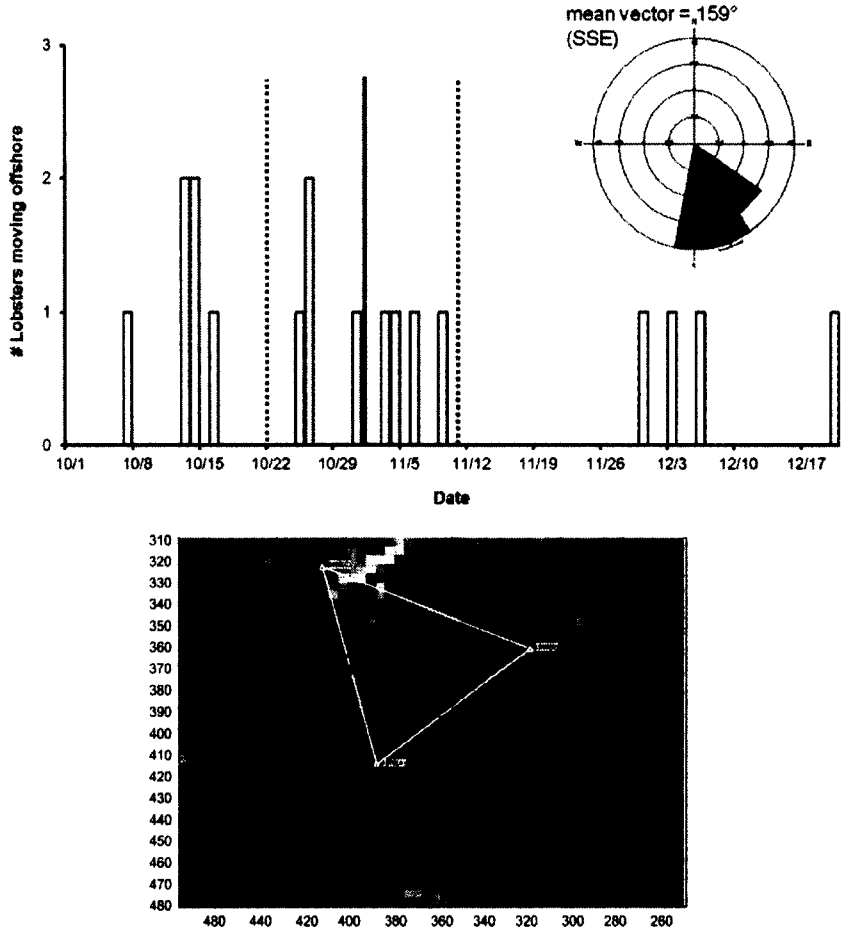

Fig. 12. Top: Number of lobsters moving out of the VRAP array towards offshore waters by date. The solid black line indicates the mean date of departure (Nov-1) for all tagged lobsters, and dotted lines the 95 % CI (range = 295-315 Julian days). On average, lobsters moved offshore in a SSE direction, with a mean vector of 159°. Bottom: Example of the fine-scale movements (> 1,500 positional readings) of an ovigerous lobster (89 mm CL, tagged on 8/29/06) on the day it left the VRAP array (triangle, Oct-31) and continued to move offshore (scale in meters). This lobster remained in this area for 40 days before leaving.

Environmental Cues

Since drops in water temperature and storm events may trigger the offshore movements of lobsters, we monitored water temperatures and wave heights before, during, and after the period when lobsters left the tagging location (Sept-Dec of 2006) to test for a correlation between these environmental cues and lobster movements (Fig. 13). Water temperatures were generally stable in the period leading up to the initiation of offshore movements (avg. = 14.1 ± 0.3 °C; range = 12.9-15.7 °C). However, starting in mid-October, when offshore movements began, there was a significant decrease in temperature (-28.4 %; avg. = 10.3 ± 0.5 °C; range = 9.0-12.8 °C, over the period when lobsters emigrated from the VRAP array (Mann-Whitney U-test; $\chi^2 = 12.9$, df = 1, p = 0.0003; Fig. 13).

Likewise, wave heights during the two time periods were significantly different (Mann-Whitney U-test; $\chi^2 = 5.8$, df = 1, p = 0.0160), averaging 0.8 ± 0.08 m (range = 0.43-1.17 m) before the migration period and 1.2 ± 0.13 m (~ 36 % increase; range = 0.58-1.77 m) after offshore movements commenced (Fig. 13). A strong relationship was evident between temperature and week ($r^2_{adj} = 0.851$, $p < 0.001$) over the tagging time period (21 weeks).

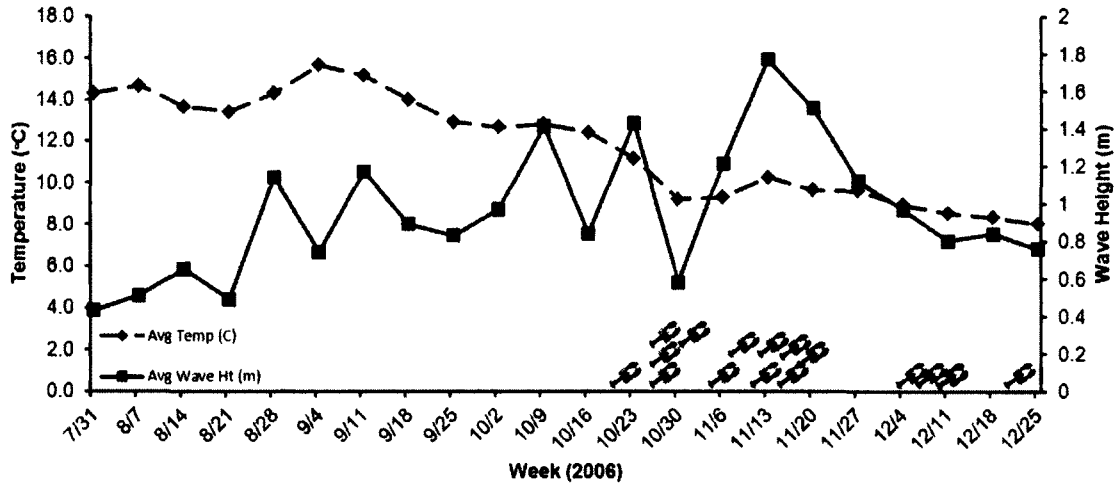

Fig. 13. Weekly water temperatures and wave heights in the fall of 2006 during the time period before, and during lobster offshore movements. Temperature data were obtained from local temperature logging devices at the inshore tagging location, while wave height data were obtained from a local oceanographic buoy (GoMOOS databse, see methods). Lobster symbols indicate movement events away from inshore waters by individual animals. A total of 75 % of the lobsters (n = 16) in the area left between Oct 22 and Nov 21.

Movements and Egg Development

During 2006-2009, we tracked a total of 17 ovigerous females carrying eggs that were 25.9 ± 6.0 % (range = 0-66 %) developed when first tagged. There was no relationship between distance traveled by these lobsters and the initial stage of the eggs they were carrying ($r = 0.24$, Spearman $\rho = 0.26$). The thermal histories of six ovigerous lobsters (attached temperature loggers, see methods) that were tracked in the fall of 2006 and re-captured were also analyzed. Of these six animals, two remained inshore, while four moved offshore in the fall. The predicted hatch for each animal was determined from 1) tracking; 2) the recapture and report of tagged lobsters by commercial fishermen; and 3) calculations of egg development from starting egg values, water temperatures, and use of a modified Perkin's (1972) eye index (PEI) (Goldstein and Watson submitted). Inshore

lobsters were predicted to hatch between Jul-15 and Jul-27 compared to August 1-14 for offshore lobsters (Fig. 14).

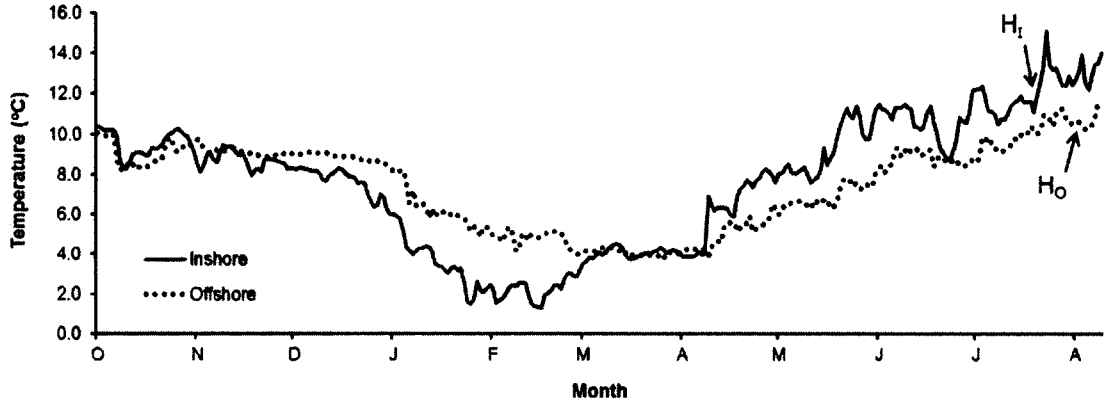

Fig 14. Example thermal histories for the seasonal movements by two ovigerous lobsters described in Table 1. Arrows indicate the average predicted hatch time (H_I, inshore; H_O, offshore) for each group of lobsters, based on lab data from a previous study (Goldstein and Watson submitted) and observations by fishermen when these lobsters were recaptured in traps. Lobsters that remained inshore, hatched inshore. Likewise, lobsters that moved offshore hatched offshore.

Discussion

Moderate to long distance movement is typically driven by a number of different biotic and abiotic factors. This study augments and complements previous work on the seasonal movements of ovigerous lobsters and offers a new perspective. We demonstrated that most ovigerous lobsters (60 %) move offshore in the winter. However, we also demonstrated that 46 % of all adult lobsters move offshore in the winter, regardless of their sex or reproductive status. These movements appeared to be triggered by a combination of rapidly cooling waters and increased turbulence and waves caused by fall storms. Taken together, these data suggest that offshore movements are likely

adaptive for all types of lobsters, and they might be seeking a more stable environment to inhabit during cold months when they tend to be less active and require less food.

We found many lobsters remained offshore throughout winter and well into spring. Thus, ovigerous females were in these offshore areas when their eggs hatched in the early summer. These data suggest that, while most lobsters appear to be responding to the same environmental cues and express similar patterns of movements as other lobsters, these events have a significant influence on the timing and location of hatch and larval release. This, in turn, might influence recruitment patterns and the extent of overlap between different populations.

Reliably documenting seasonal patterns of lobster movements depends on the area studied as well as the type and duration of the study (Pittman and McAlpine 2003). By combining three different ultrasonic telemetry techniques, we documented both the small-scale movements of lobsters as they initiated their offshore movements in the fall, and larger scale seasonal movements over multiple seasons. We found that while VR2s are very useful in locations such as the coast and at the mouth of bays and rivers, once animals move into the open ocean too many are required to cover the entire area. Therefore, while time-consuming, manual tracking is required. Lobster movements are easier to study than those of other aquatic species because telemetry data are complemented by data from fishermen who routinely capture lobsters in traps. Fishermen were also able to provide additional status of tagged animals that were recaptured, especially ovigerous lobsters and their eggs.

Previous reports on the seasonal movements of lobsters have included a number of patterns including mature lobsters moving further than immature ones, return movements by some lobsters back into either inshore waters or shallow banks, and local movements of other lobsters (e.g., homing) (Campbell 1986, Pezzack and Duggan 1986, Bowlby et al. 2007, Scopel et al. 2009). Most lobsters moved short (< 5 km) to moderate (5-10 km) distances including limited movements by all lobsters in the winter (Dec-Mar; Fig. 9), mirroring the general trend seen in many other lobster movement studies. In addition, we found no statistically significant differences in the movements of lobsters by size and this has also been documented in at least one other study (Watson et al. 1999). Finally, environmental factors that appear to initiate the autumnal movements of American lobsters have not been documented very often, especially compared to other lobster species.

The Northwestern Atlantic Ocean has only recently been recolonized by *H. americanus* (~ 10,000 years ago); thus, lobster populations have had a relatively short time (~ 1,000 generations) to evolve strategies to optimize their survival and recruitment of their eggs and larvae (Kenchington et al. 2009). Thus, the motivations and associated mechanisms of seasonal movement patterns in all lobsters are still largely undetermined. A variety of factors acting alone or synergistically may influence patterns of ovigerous lobster movements and include: 1) optimizing thermal regimes for biological processes; 2) sex-biased movements; 3) habitat structure and marine landscapes; 4) foraging activity; and 5) movements associated with larval hatch and dispersal.

Movements to Optimize Thermal Regimes

Lobsters can detect small changes in temperature and avoid water that is either to hot or too cold (behavioral thermoregulation; Crossin et al. 1998, Jury and Watson 2000), and most studies of lobster movements have proposed that temperature is the primary driver (Drinkwater et al. 1996; Watson et al., 1999). The seasonal movements of *H. americanus* are widely thought to be temperature- and depth-dependent, rather than distance-dependent (Lawton and Lavalli 1995).

The association of seasonal movements with maximum optimal temperatures (i.e., degree days) needed for biological functions has been well documented, especially in ovigerous lobsters (Waddy and Aiken 1992, Waddy et al. 1995). Evidence suggests that ovigerous lobsters move as a strategy to subject their eggs to a sufficient and optimal number of degree-days in offshore waters to allow their eggs to develop and hatch inshore during the following summer (Campbell 1986, Cowan et al. 2006). However, a companion study has recently shown that in coastal New Hampshire waters, degree days between inshore and offshore locations do not differ enough to elicit such a pattern. Instead, the rate of temperature increase in the spring had the greatest effect on the timing of hatch (Goldstein and Watson submitted), with inshore lobsters actually hatching sooner than offshore lobsters. Offshore movements actually delay hatching and ovigerous lobsters remained offshore until after their eggs hatched in the summer, even though inshore movements would have accelerated hatching. Together, these findings suggest lobsters that undertake inshore-offshore movements in the NH region of the Gulf of Maine do not necessarily do so in order to gain degree-days and accelerate development. However, it

remains unknown if such a selective pressure originally evolved to facilitate the acquisition of an optimal number of degree days.

Sex-biased Movements

Sex-biased movements have been documented for a variety of animals including birds (Greenwood 1980), butterflies (Baguette et al. 1998) and fishes (Hutchings and Gerber 2002), and are linked to mating and reproductive strategies. In all these cases sexual selection strongly shapes mating strategies by modifying behavior, leading to divergent movement patterns between sexes. Other studies have shown differential sensitivity to environmental cues, reflecting differences in the evolutionary pressures to disperse (Benton and Bowler 2005). Breeding migrations in crabs have also been documented and show a variety of patterns as well (Stone et al. 1992, Stone and O'Clair 2002, Carr et al. 2004). The movements of female gravid blue crabs (*Callinectes sapidus*) for example, to the mouths of estuaries and bays, allow crab zoeae to utilize offshore currents and avoid osmotic stress and predators (Forward et al. 2003). These studies suggest differential movements can result in skewed sex ratios.

One goal of our study was to ascertain if seasonal movement patterns expressed by ovigerous lobsters (to deeper, colder, more stable habitats) were different from those of their male or non-ovigerous female counterparts. Even though this was not entirely the case, we still observed that the movements by ovigerous lobsters were disproportionately longer (60 % moved > 5 km) compared with males (no male moved > 4 km). This result

is somewhat contrary to other work, which reported male-biased (Jury et al. 1994) and female-biased (Campbell and Stasko 1986) movements of *H. americanus* in some coastal waters.

Sex-biased movements are most frequently attributed to selective pressures acting on reproductive strategies or physiological requirements that differ between sexes (Haakonsen and Auoruo 1994, Jury et al. 1994). For example, male lobsters exhibit a wider range of physiological tolerances in estuarine waters (e.g., Great Bay estuary, NH) that reflects the timing and magnitude of their movements into such locations (Howell et al. 1999, Watson et al. 1999). Such patterns in coastal waters exist but are not as pronounced. Campbell and Stasko (1985) and Templeman (1940) found that mature females moved greater distances than mature males in some areas, but not others. These patterns are consistent with previous reports of greater mobility of male spiny lobster (MacDiarmid and Butler 1999, Goñi et al. 2010). However, Fogarty et al. (1980) and Krouse (1981) found no differences in movement as a function of sex or size, possibly because those lobsters were immature. On Cape Cod, Morrissey (1971) reported that ovigerous females moved further and faster than other lobsters.

More recently, den Heyer et al. (2009) found a small, but significant difference in the movement (displacement) rates between male and female lobsters, in Northumberland Strait Canada. Although we did not compare the movements of ovigerous females to that of males in the first year of this study, we did observe that all female lobsters were capable of moving out of the tagging array (VRAP array 2006), and most did so,

regardless of their reproductive state (Figs. 12, 13). Female-biased lobster movements may be associated with mating and reproduction. By comparison, male lobsters are territorial and defend dens, especially during the mating season (Karnofsky et al. 1989). Therefore, males may be less mobile than females, especially during certain times of year (e.g., molting, den acquisition) but may increase their activity in the spring when temperatures warm and they are foraging (Golet et al. 2006). Because we followed all types of lobsters throughout the year including during their breeding season, it is possible that some differences between male movements (and their counterparts) are driven by mating-related behaviors including the defense of dens.

Movements and Habitat

The presence of appropriate habitats is likely to have a significant influence on the tendency of lobsters to move. For example, Comeau and Savoie (2001) suggest that lobster movements in Northumberland Strait are facilitated by an overall flat homogeneous bottom. The topography and shape of the Strait also provide a large area conducive to movement. Cooper and Uzmann (1980) proposed that the shallowly-sloped continental shelf would also favor movement. Both Watson et al. (1999) and Estrella and Morrissey (1997) found that lobsters were more likely to move (and did so rapidly) when presented with suboptimal habitats in Great Bay Estuary and outer Cape Cod, respectively.

More recently, Geraldi et al. (2009) determined that lobster movements were dependent on the quality of habitats through which they were moving. Even in some estuarine environments, complex hard-bottom areas between soft-sediment patches (e.g., eelgrass beds) can serve as corridors and passageways (see Micheli and Peterson 1999) for lobsters and crabs engaged in short- or long-term movements (Selgrath et al. 2007, Goldstein unpub. data). Movements by other crustaceans (e.g., spider and king crabs) have also been tied to habitat selection on a seasonal basis (Stone et al. 1992, Gonzalez-Gurriaran et al. 2002).

We observed no differences in movement patterns between lobsters with varying degrees of egg development stage (0-66 %), however Jarvis (1989) documented strong resident behavior in late-stage ovigerous lobsters in areas of suitable habitat, compared with more transient behavior where habitats were described as featureless (i.e., sand flats). Likewise, Watson et al. (1999) observed a similar pattern in an estuary. This phenomenon has been described in Dungeness crab (*Cancer magister*), and has been attributed to habitat correlates (substrate type) that enhance brooding for developing embryos (Stone and O'Clair 2002).

Our choice in tagging sites was characterized predominantly by boulder-gravel complexes (male shelters were also observed) in the general area that likely afforded lobsters ample structure for residency and foraging (Poppe et al. 2003, Scopel et al. 2009, CCOM-JHC, 2012). Alternatively, had this study been conducted at a nearby site dominated by flat, sandy habitats, we surmise that lobsters would exhibit much more

transient behavior (Geraldi et al. 2009). Lobsters may switch from one type of habitat to another on a seasonal basis, move when habitats are limiting, or come back to areas when conditions improve. These cyclical patterns are particularly evident in estuaries and bays where physical conditions can change very dramatically (Watson et al. 1999). Finally, the tendency of lobsters in this study to leave the tagging site with similar directionality suggests that their movements are not arbitrary. For those animals that undertake seasonal movements, it is highly likely that habitat features (e.g., depth contours, substrate composition) act as signposts and influence the trajectories and movement rates in these animals (Herrnkind and Thistle 1987). Clearly, more work is needed to more accurately determine the correlation between habitat quality, marine landscape, and movements of lobsters (both transient and resident). These data will be especially relevant to the design and establishment of marine protected areas (Goñi et al. 2010).

Movements and Foraging

There are a variety of explanations for offshore movement in the winter, but the motivation to move inshore in the spring and summer is less clear. Movements in marine organisms are often linked to the procurement of food resources over a range of habitat scales (Polis et al. 1997). As a result, many lobsters are considered key predatory species, exerting a strong influence on the ecosystem dynamics through their foraging activity on benthic communities (Langlois et al. 2005). American lobsters frequently influence the dynamics in local benthic communities as evidenced by the broad spectrum of food items that they consume (Scarratt 1980, Conklin 1995, Palma et al. 1998).

Feeding activity in lobsters has also been linked with temperature and movement patterns (Lawton and Lavalli 1995). On a daily basis, foraging activity by lobsters accounts for small movements on the order of 1-2 km (typically 100-300 m or less, Cooper and Uzmann 1971, Ennis 1984, Watson et al. 2009). Although lobsters do not move very far during their daily foraging excursions, it is possible that their larger seasonal migrations are motivated by a need to move into areas with a high abundance of prey. While there is no direct evidence that lobsters exhibit seasonal movements inshore related to food availability, they often shift their prey based on a spatiotemporal scale and this may drive some patterns of movement (Scarratt 1980, Elner and Campbell 1987).

Environmental Perturbations

The cues that initiate and control seasonal, directed movements in American lobsters are largely unknown. Movements are typically correlated with seasonal changes in temperature and, in locations such as estuaries, fluctuations in salinity (Wahle 1993, Jury et al. 1995, Watson et al. 1999). However, Cooper et al. (1975) observed that lobsters typically moved from shoal waters (5-20 m) to deeper waters (30-60 m) over strong wind events and increasing wave heights. Others report that a combination of ice scour and wave action to shallow bottom sediments (coupled with low water temperatures) triggered lobsters to shelter beneath hard substrate features or in deep water (Ennis 1984, Karnofsky et al. 1989). Some of the best evidence for environmental triggers for seasonal lobster movements comes from studies on spiny lobsters (Herrnkind 1980). The occurrence of frequent autumnal storms (waves and turbidity), coupled with decreasing

water temperatures, was enough to trigger the mass migration of Caribbean spiny lobster, *Panulirus argus* (Kanciruk and Herrnkind 1978, Nevitt et al. 1995).

Two environmental parameters (water temperature and wave height) were quantified during the first year of this study, when we were able to obtain high resolution tracks of lobsters to accurately assess when they left the coast. Changes in temperature and wave height provide a good metric of environmental disturbance and have been used to assess the changes in benthic communities in other studies (e.g., Schiebling and Gagnon 2010). Both factors were strongly linked to the propensity of lobsters to leave the tagging site. In our study, we observed a clear window of opportunity when lobsters tended to move offshore and this was associated with an increase in storm events and rapidly falling temperatures (Fig. 13). Additionally, because most lobsters were tagged well before this event (~ 40 days prior), we conclude that fall movements offshore were not an artifact of our tagging protocol.

A follow-up analysis for all three tagging seasons (combined fall, 2006-2009) indicated a strong correlation between these two parameters (temperature and wave height) and the emigration of lobsters ($r = 0.72$, $p = 0.028$). Interestingly, in a nearby estuary (Great Bay, NH) over the same years (2006-2009) drops in fall water temperatures are even more extreme, but wave action is minimal and lobsters moved very little (Goldstein et al. unpub.). This suggests that perhaps seasonal movements are not initiated by changes in temperature *per se* but are more influenced by a thermal threshold that elicits such movements. Triggers that are implicated in the fall movements of lobsters are not well

understood but may include associated cues such as seasonal changes in photoperiod (Aiken 1969), alterations in barometric pressure and internal changes in endocrine processes, which work to modulate lobsters' physiological state (Kanciruk and Herrnkind 1978, Herrnkind 1980). Therefore, our working hypothesis is that lobsters tend to move from coastal to offshore waters in the fall because this is when they are most likely to encounter a combination of falling temperatures and increased wave action. However, multi-year studies designed to look at a variety of cues both in the field and laboratory would be beneficial in future studies.

Movements Related to Larval Hatch and Dispersal

Many ovigerous marine decapods (spiny lobsters and crabs) that maintain external lecithotrophic egg masses and hatch pelagic larvae undergo brooding-related movements that are thought to selectively position larvae for transport away from deleterious environments. For example, the movements of gravid blue crabs (*Callinectes sapidus*) to the mouths of estuaries and bays allow crab zoeae to be transported in offshore currents to avoid osmotic stress and predators (Carr et al. 2004). Booth (1997) compiled information about the long-distance movements by several Pacific spiny lobsters and postulated that inshore-to-offshore movement events were associated with reproduction and molting. Other such lobster movements are described as contranatant, acting to redress the dispersal of larvae back to maternal areas. Movements of late-stage ovigerous Caribbean spiny lobsters (*Panulirus argus*) using ultrasonic telemetry determined that some individuals make homing excursions from their dens to the reef edge to release their

larvae (Bertelsen and Hornbeck 2009). There is clear evidence that some ovigerous crabs and lobsters incorporate brooding-specific movements into their repertoire for purposes of larval release.

Most ovigerous *H. americanus* are exposed to temperatures well below 11 °C while incubating their eggs throughout the winter; a number of different scenarios involving movements to warmer waters could influence the timing of hatch and larval survival. For example, by delaying inshore migration into warm water, a female could delay hatching, while a lobster inshore would be exposed to a rapid increase in water temperature in the spring and hatching might occur before optimal conditions for larval survival. Ovigerous lobsters may have evolved a life-history strategy that is based not on exposing their larvae to the warmest temperatures to achieve the fastest rates of egg development, but rather upon the need to time hatching so that it occurs at a time and location conducive to optimal larval dispersal and survival.

Superimposed on these biological patterns are the attributes of both local (10s of km) and regional (100s of km) oceanography that further influence larval dispersal and ultimate settlement locations. This is particularly true in the Gulf of Maine (GoM) where features such as river run-off and the seasonal strength of coastal currents can affect larval dispersal between inshore and offshore locations on both temporal and spatial scales (e.g., Mountain and Manning 1994, Pringle 2006). Because larval hatch and dispersal may be constrained by locally influential physical events (e.g., tidal fronts, eddies, convergence zones), the locations of ovigerous lobsters near their time of hatching is of significant value. Thus, even modest movements (10s of km) can influence these

processes. This has been shown in other marine species with planktonic larvae, particularly larval marine fishes, which exhibit self-recruitment back to natal reefs (Almany et al. 2007, Paris et al. 2007). Therefore, an understanding of adult lobster movements and their locations of hatching could help to further elucidate the degree to which larval dispersal occurs and the scale of marine connectivity for *H. americanus*.

Evolutionary Perspective

Many studies suggest that long-distance movements of mature lobsters provide evidence for a panmictic population throughout the GoM. This has been confirmed by a variety of genetic studies (isozymes, mitochondrial DNA, microsatellite tags) showing low levels of genetic variability between American lobster populations, including inshore and offshore groups (Tracey et al. 1975, Tam and Kornfield 1996, Harding et al. 1997). Recently evidence for the existence of sub-populations of *H. americanus* that are attributable to morphological differences among some geographical regions has been documented (Harding et al. 1993, Atema et al. unpub. data). Therefore, at present, evidence suggests that the movement patterns expressed by lobsters and their effect on larval dispersal may be sufficient to maintain a single, homogenous biological lobster stock in the GoM.

Long-distance movements (for both plants and animals) can significantly impact community and local population dynamics (Kokko and Lopez-Sepulcre 2006) and plays a key role in species invasions, habitat fragmentation, responses to climate change (Brander 2010), and the spatial design of marine protected areas (Goñi et al. 2010). Technological advances in telemetry now allow us to collect movement data at a high

spatiotemporal resolution and infer these patterns on many different levels. The dynamics of lobster movements have a great impact on their distribution and abundance and knowledge of these movement patterns is integral to the fisheries management of coastal habitats and our understanding of their continued ecological function and economic success.

CHAPTER 2

INFLUENCE OF NATURAL INSHORE AND OFFSHORE THERMAL REGIMES ON EGG DEVELOPMENT AND TIME OF HATCH IN THE AMERICAN LOBSTER, HOMARUS AMERICANUS

Abstract

Some egg-bearing (ovigerous) lobsters (*Homarus americanus*) make seasonal inshore-to-offshore movements that expose their eggs to a different thermal regime than eggs carried by lobsters that do not migrate. The overall aim for this study was to determine if these different thermal regimes influence the rate of egg development, and the time to hatch. We subjected ovigerous lobsters to natural inshore or offshore water temperatures from September-August, either in the laboratory (n = 16/each, inshore and offshore), or in the field (n = 16/each, inshore and offshore), combined for two consecutive years. Temperatures averaged 7.1 ± 0.19 °C (range = 2.1-14.2) for inshore laboratory simulations, compared with 6.4 ± 0.17 °C (range = 2.8-12.4) for the offshore thermal regime. There were no significant differences between natural or simulated inshore or offshore thermal regimes or mean temperatures ($p > 0.05$). Likewise, cumulative growing degree-days (GDD) over the full course of egg development did not differ significantly between the two treatments ($mean_{inshore}$ = 938.0 ± 10.3 GDD, $mean_{offshore}$ = 904.7 ± 13.0 GDD, $p = 0.061$). Although the rate of egg development between inshore

and offshore conditions did not differ significantly in the fall ($p \sim 0.570$), inshore eggs developed faster in the spring ($p < 0.001$). As a result, eggs exposed to inshore thermal regimes hatched ~ 30 days earlier (mean = June 26) than offshore eggs (mean = July 27), and their time of development from extrusion to hatch was significantly shorter (inshore = 287 ± 11 days vs. offshore: 311.5 ± 7.5 days, $p = 0.034$). These results suggest that seasonal movements of ovigerous lobsters strongly influence both the time and location of hatching. This finding has important implications for the transport and recruitment of larvae to coastal and offshore locations.

Introduction

Water temperature modulates the physiology and behavior of most marine ectotherms and influences the movements of many mobile species. Both temporal and spatial fluctuations in water temperature can have a substantial impact on species distributions, including both population-level responses (e.g, dispersal, allocation of resources, survivorship) and the adaptations of individuals to changing environments (Whiteley et al. 1997, Pittman and McAlpine 2003, Nathan 2008). For crustaceans like lobsters, temperature is arguably one of the most important factors influencing their metabolism, growth, life cycle, and possibly even life span (reviews in Talbot and Helluy 1995, Waddy et al. 1995, Hawkins 1996).

North American lobsters (*Homarus americanus*), are endemic to coastal and offshore waters from Labrador, Canada to North Carolina, USA, and occupy a variety of thermal niches over a steep gradient (Fogarty 1995, ASMFC 2009). Lobsters can sense small changes in water temperature (Jury and Watson 2000), and they behaviorally thermoregulate by moving into water that is at their preferred temperature (Reynolds and Casterlin 1979, Crossin et al. 1998). Thermal preferences are thought to influence lobster movements and thus overall distribution within gradients ranging from inshore to offshore, and shallow to deep waters (reviewed in Cooper and Uzmann 1980, Lawton and Lavalli 1995). For egg-bearing (ovigerous) lobsters in particular, it has been proposed that seasonal movements from coastal to offshore waters in the winter serve to expose their eggs to elevated temperatures during the colder months (Cooper and Uzmann 1971,

Campbell 1986, Pezzack and Duggan 1986, Cowan et al. 2006). This, in turn, is *associated with maximizing degree-days needed for molting, growth, gonad development, egg extrusion, and egg development* (Campbell 1986).

Water temperature has a pervasive influence on *H. americanus* egg development (Templeman 1940, Pandian 1970, Perkins 1972, Aiken and Waddy 1980). Both Perkins (1972) and Helluy and Beltz (1991) quantified the effect of temperature on embryonic development and time to hatch in the laboratory, by exposing eggs to a constant temperature and measuring embryonic eye (pigmentation) size as an index of development. Additional studies at constant temperatures served as a basis for temperature-specific growth models for egg development in other lobster species, such as the European lobster, *Homarus gammarus*, (Charmantier and Mounet-Guillaume 1992) and New Zealand rock lobster *Jasus edwardsii* (Tong et al. 2000). Although these studies are relevant to the management of captive broodstock and the operation of year-round hatcheries, they do not address how naturally fluctuating temperatures influence the rate of egg development and therefore the time from egg extrusion to hatch, which may have critical ecological implications.

Herrick (1895, 1909) and Bumpus (1891) provide the most comprehensive descriptions of *H. americanus* embryology, including developmental rates of eggs at various temperatures. Bumpus (1891) was the first to create a staging table for early lobster egg development. Herrick further noted that lobster eggs undergo up to three embryonic molts prior to the appearance of the lateral eye pigment (i.e., eyespot). This protracted

prelarval embryonic molting scheme seems to be very similar to the molting cycles of both larval and juvenile lobsters, and they appear to be influenced by environmental variables such as temperature (Aiken and Waddy 1980). Templeman (1940) reported the time between the 16-cell stage of development and eyespot formation at a variety of temperatures. Finally, Perkins's (1972) described a series of development curves for lobster eggs exposed to different temperatures. This, in turn, gave rise to the Perkins Eye Index (PEI) function. Hence, Perkin's work is fundamental to all subsequent efforts to predict lobster hatch dates for eggs exposed to a range of temperatures.

Lobster egg development 'pauses' when development is both 50 % and 80 % complete, based on PEI (Helluy and Beltz 1991). Delayed hatching is also common in frogs, salamanders, and salt marsh fishes (reviewed in Martin 1999). This temporal developmental plasticity may help developing embryos compensate for, or adjust to, sub-optimal environmental conditions (e.g., temperature, air exposure). For example, if lobster eggs are in an elevated thermal environment (> 11 °C), they will proceed continuously through development (Perkins, 1972). If an embryo reaches 80 % development and the temperature is suboptimal (< 4 °C), it ceases to develop and remains in stasis at the 80 % development plateau until ambient temperatures increase (Helluy and Beltz 1991, Waddy and Aiken 1992). However, the embryo remains metabolically active while development is curtailed and continues to use valuable yolk reserves (Sasaki et al. 1986, Sibert et al. 2004). Thus, the longer the eggs remain in stasis, the fewer energy reserves will be available to the larvae upon hatching (Attard and Hudon 1987).

Given that most egg-bearing American lobsters are exposed to temperatures below 11-12 °C while incubating their eggs throughout the winter (Waddy and Aiken 1995), the well-documented mobility of lobsters, along with spatial and temporal variability in water temperatures inshore and offshore, suggest that lobster movements could influence their time of hatching through several different scenarios.

Lobster movements both at seasonal (e.g., inshore-to-offshore migrations) and local, (e.g., homing and initial dispersal) scales may influence the thermal profiles they experience (Cooper and Uzmann 1980, Lawton and Lavalli 1995, Watson et al. 1999, Bowlby et al. 2007, Scopel et al. 2009). Even relatively short migrations or movements (< 15 km) can significantly affect growth and molting (Waddy and Aiken 1995), egg development (Ennis 1984, Campbell 1990, Cowan et al. 2006) and size at maturity (Landers et al. 2001, Little and Watson 2005). While numerous studies have documented the long distance migrations and local movements of American lobsters (reviewed by Krouse 1980, Cooper and Uzmann 1980, Haakonsen and Anoruo 1994, Lawton and Lavalli 1995), few have focused on ovigerous lobsters and how movements may influence egg development and time to hatch. The existing paradigm is that ovigerous lobsters seek deeper (offshore) waters in the fall and winter because these areas tend to be warmer or more stable than inshore habitats during the colder months (Chapter 1). Lobsters move back inshore in spring and summer to gain the advantage of seasonally warmer inshore waters. It has been proposed that these seasonal movement patterns allow eggs to gain sufficient degree-days to maximize egg development, leading to

earlier hatch (Campbell 1986, Pezzack and Duggan 1986; although see Gendron and Ouellet 2009).

Cowan et al. (2006) further examined the relationship between ovigerous lobster movements and water temperature using ultrasonic telemetry. They found that small (< 93 mm carapace length) ovigerous females tended to remain closer to shore than larger ones and, as a result, their eggs were exposed to more extreme thermal fluctuations. However, no previous study has tested the hypothesis that seasonal movements of ovigerous lobsters to offshore waters lead to enhanced egg development and earlier hatching of their eggs. To test this hypothesis, we investigated the effects of naturally fluctuating thermal profiles on the development of American lobster eggs.

We sought to determine whether natural seasonal fluctuations in water temperature, characteristic of inshore and offshore habitats, influenced the duration of egg development and thus, the time of hatch. We found that lobster eggs exposed to typical inshore thermal regimes, which are characterized by more rapid cooling in the fall and warming in the spring, hatched significantly earlier than those exposed to more stable offshore thermal regimes. We also show that typical offshore movements of some ovigerous females in the fall led to a delay in the time of hatching, as well as a change in the location where larvae will be released.

Materials and Methods

Lobsters and Egg Assessment

Ovigerous lobsters were collected in late August and early September in 2006 and 2007 along the New Hampshire (NH) seacoast, near Rye, NH and Gunboat Shoals (43 °.0274 N; 70 °.6938 W; Fig. 1), by permitted commercial lobstermen using standard baited traps. Lobsters were transported to the University of New Hampshire (UNH) Coastal Marine Laboratory in Newcastle, NH and initially held in large 1,200 L fiberglass tanks containing PVC shelters. Tanks were exposed to ambient light and received sand-filtered ambient seawater (average temp = 15.3 ± 0.5 °C). A subset of the eggs in each clutch were viewed under a dissecting scope and staged according to the methods outlined by Helluy and Beltz (1991). Only lobster embryos whose initial eye index was less than 18 % were used for this study. Lobster carapace lengths (CL) were measured to the nearest 1 mm using digital calipers (Mitutoyo IP 65, Mitutoyo Corp., Japan). A single, circular, laminated disc tag (diameter = 2.0 cm; Floy Tag Inc., Seattle, WA) was fastened to the claw knuckle of each adult animal for individual identification during the study.

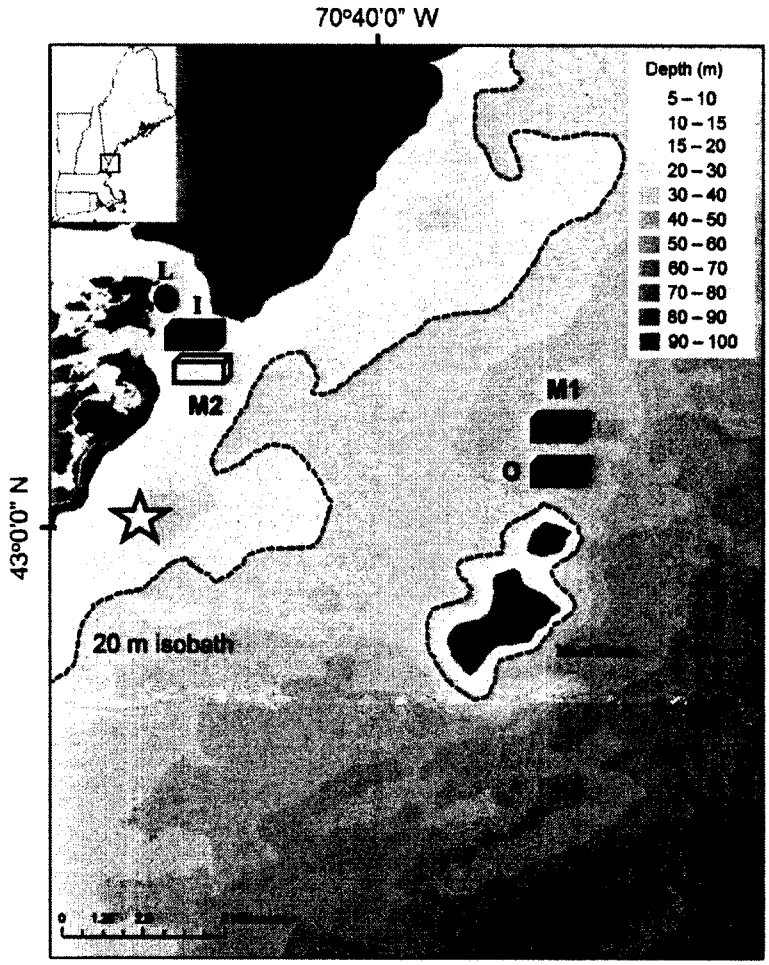

Fig. 1. Locations where lobsters were obtained and maintained along the NH Seacoast. The collection location for all lobsters, near Rye, NH (Gunboat Shoals), is indicated by a star. Additional symbols show locations of incubation cages (rectangles): inshore (I) cages were near New Castle Common, and offshore cages (O) were near Duck Island, ME (Isles of Shoals). Also indicated on the map is the location of the UNH Coastal Marine Laboratory, where the laboratory component of the study was conducted (black circle, L). The mixed thermal treatment involved moving lobster cages that resided offshore at Duck Is. (M1) from fall to winter to the inshore location (M2) in the spring. (see methods for detailed descriptions). Dashed line indicates delineation (20 m isobaths) between inshore and offshore locations.

Simulated Inshore and Offshore Conditions in the Laboratory

For purposes of this study, inshore locations (shallow and coastal) were delineated as 2-5 km from shore (8-10 m depth), while offshore locations were designated as 12-20 km from shore (20-30 m depth) (Fig. 1; see Chapter 1 methods). A series of four 0.91 m

diameter (600 L) tanks were used to hold lobsters under simulated inshore or offshore temperature regimes. Trials were repeated twice, using two groups of 16 lobsters, between the fall of 2007 and summer of 2009, with two tanks / treatment (n = 32 total). Lobsters averaged 91.2 ± 2.39 mm CL (range = 76-117, mode = 80). All tanks were insulated with Formular® 5 cm-thick insulation (r-value = 10; Owens Corning Co., Toledo, OH) and were divided into four sections with coated lobster trap mesh wire (Fig. 2).

Incoming seawater from an intake pipe at a depth of 8 m was run through sand-filtration, UV sterilization, (model E120S, Emperor Aquatics, Pottstown, PA), and 100 μm cotton-wound filter canisters before being distributed into either inshore or offshore (600 L) header-tanks (Fig. 2). Inshore tanks were run primarily as a flow-through system (no heating or cooling) so lobsters were exposed to ambient coastal seawater. Seawater for offshore tanks was pre-treated (heated or cooled) in each header tank before being fed into the individual tanks holding the lobsters, and then pumped (Maxijet 1200, 295 GPH, Aquatic Ecosystems, Apopka, FL) back into the header tanks. A steady trickle of fresh seawater was fed into the offshore system as well, creating a semi-closed system.

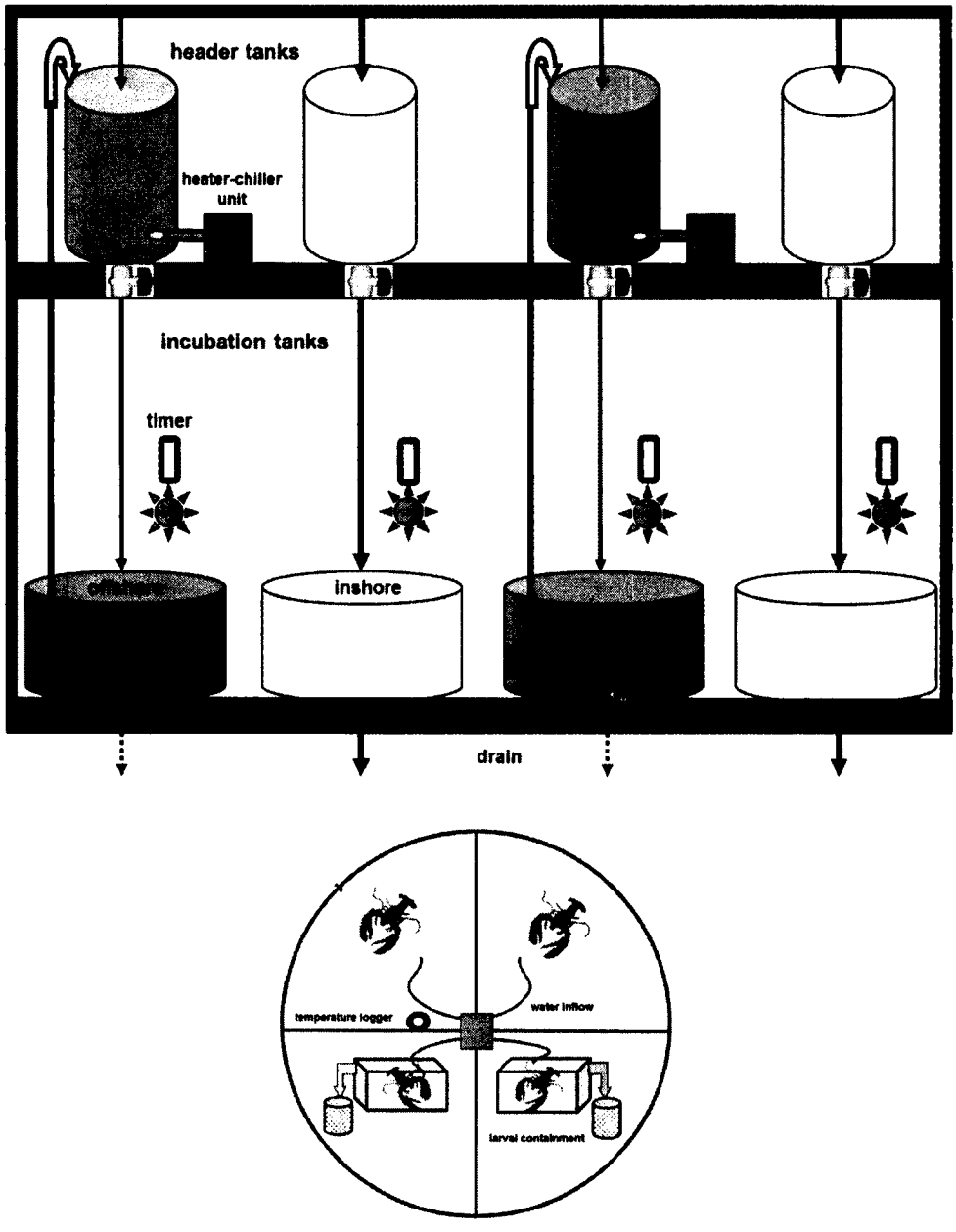

Fig. 2. Tank arrangement for holding lobsters. Top: Tank design for exposing lobsters to simulated inshore and offshore temperature regimes. Shaded containers had simulated offshore conditions, with incoming seawater pre-treated (heated or cooled) in the header tanks before being gravity-fed into treatment tanks (0.91 m diameter, 600 L) holding lobsters. Inshore tanks received ambient seawater. All tanks were maintained on a seasonal photoperiod using programmable timers. Bottom: Incubation chambers housing individual lobsters. Prior to the time when the eggs were due to hatch, lobsters were maintained in separate areas of the tank using mesh dividers. Close to hatch time, lobsters were placed into tanks with individual seawater inputs (bottom two lobsters in figure), and larvae drained through a small one-way valve into a collection basket

Seawater was heated using two 1,500 W titanium immersion heaters (model QDTYL5, Cleveland Process Corp., Homestead, FL) and cooled with portable bath cold finger chiller units (Cyclone model CY-2 1/5 hp, Aqualogic Inc., San Diego, CA). Heating and cooling regimes were controlled with digital thermostat controllers (model Nema type 4X, Aqualogic Inc., San Diego, CA) and adjusted as needed. Offshore thermal regimes were based on temperature data obtained from: 1) buoys (GoMOOS Buoy B, 20 m depth) situated at the western edge of the Gulf of Maine (http://www.gomoos.org); and 2) HOBO pendant temperature loggers (model UA-002-64, Onset Computer, Bourne, MA) mounted on commercial lobster traps (20-30 m depth) at the Isle of Shoals (43°.0050 N, -70°.5905 W), logging temperature at 30-minute intervals. Water temperatures in each treatment tank were monitored using submersible HOBO temperature loggers (accurate to ± 0.47 °C), as well as real-time temperature readouts from small digital thermometers (Coralife CD-18773, Aquatic Ecosystems, Apopka, FL). Temperature data were downloaded at weekly intervals using a PC-based software (HOBOware Pro. v. 3.0).

Photoperiod was controlled using an astronomic timer (model SS8, Intermatic, Inc., Spring Grove, IL) adjusted for seasonal changes in photoperiod. Lighting was provided using 20 cm diameter 40 W hooded lamp-lights filtered with Roscolux colored lighting gels to simulate natural daylight (# 61 for inshore, transmission = 62 %; # 388 for offshore, transmission = 76 %, Rosco Labs, Inc., Stamford, CT).

All lobsters were provided with shelters (clay flower pots) and fed fresh squid, shrimp, or mussels twice weekly. Tanks were cleaned once per month and uneaten food was removed to maintain water quality. All tanks were continuously aerated.

When embryos were close to hatching, ovigerous adults were placed into individual holding tanks (32 cm x 18 cm x 12 cm, L x W x D) with separate seawater supplies and drains for each tank. Tanks were designed such that hatching larvae would exit the drain line and collect in attached screened baskets (Fig. 2). This also served to eliminate any conspecific chemical cues associated with hatching (e.g., Ziegler and Forward 2007). The first signs of hatch (observed in collection baskets) provided a benchmark date that was then used to estimate the date when 50 % of the larvae were released, and the 50 % hatch date was used to calculate the mean hatch date for each lobster.

Lobster Incubation Cages (Field Component)

In parallel with the lab-based study, we assessed the development of eggs exposed to natural thermal regimes in the field. A total of 16 ovigerous lobsters (CL_{avg} = 95.3 ± 5.0 mm, range = 77-131, mode = 79) were held in cages in two locations: 1) off Newcastle common (depth ~ 8-10 m, inshore); and 2) near Duck Island, Maine, Isle of Shoals (depth ~ 30 m, offshore). Two cages were placed at each location with four lobsters/cage (n = 16 total; Fig. 1). In addition, there was also a 'mixed' treatment containing two cages with three lobsters each (n = 6 total) designed to simulate a scenario where lobsters migrate offshore in fall, and then move back inshore in spring and summer (Campbell

and Stasko1986, Campbell 1990). For this treatment cages were located ~ 2 km from Duck Island in fall and winter and were moved to Newcastle Common (inshore) the following spring (15-March) by transporting traps using a commercial fishing vessel (Fig. 1). Transport handling and stress were minimized by removing lobsters from their cages and immediately placing them onboard holding tanks with running ambient seawater for transport to inshore locations; this process took a total of 50 minutes.

For all field treatments, lobsters were held in standard vinyl-coated lobster traps (1.2 m x 0.6 m x 0.4 m, 3.8 cm square mesh) constructed without vents or entrances and divided into four sections by the insertion of additional coated mesh wire. A single lobster was placed into each compartment along with a bait bag holder. Offshore traps were weighted with concrete blocks to minimize their excessive movement from offshore winter storms. Temperature loggers were fastened with cable ties to each trap. Fortnightly, all cages were pulled and lobsters were rapidly checked and fed with fresh bait. Offshore cages were maintained by commercial lobstermen throughout most of the year. When lobster eggs reached late-stage development (typically one month prior to hatch), individual females were isolated in screened baskets within traps so that hatched larvae would be retained.

Egg Staging and Morphometrics

For all lobsters a set of 10-15 eggs were removed at monthly intervals, placed in plastic 2.0 mL storage tubes, preserved in a 4 % formalin and sterile seawater solution (see

Ouellet and Plante 2004), and stored at 4 °C. Bi-weekly samples were taken during the first and last months to account for the rapid changes that occur during early development and close to hatch (see Sibert et al. 2004). Eggs from each sample were staged according to Helluy and Beltz (1991). For each sample a digital picture was taken for each egg under a dissecting microsope (Nikon SMZ-2T, Nikon USA Inc., Melville, NY) at a magnification of 25x using a scope-mounted Nikon Coolpix 995 digital camera. All egg image files were imported into an image processing software (Image J v.1.35; see http://rsb.info.nih.gov/ij/) and digital measurements were taken of each egg's eyespot's maximum length and width to generate an eye index (0-570 μm, PEI; Fig. 3). All eyespot measurements were made to the nearest 0.01 mm (then converted to μm) and values for all 10-15 eggs in each sample were averaged (mean ± se).

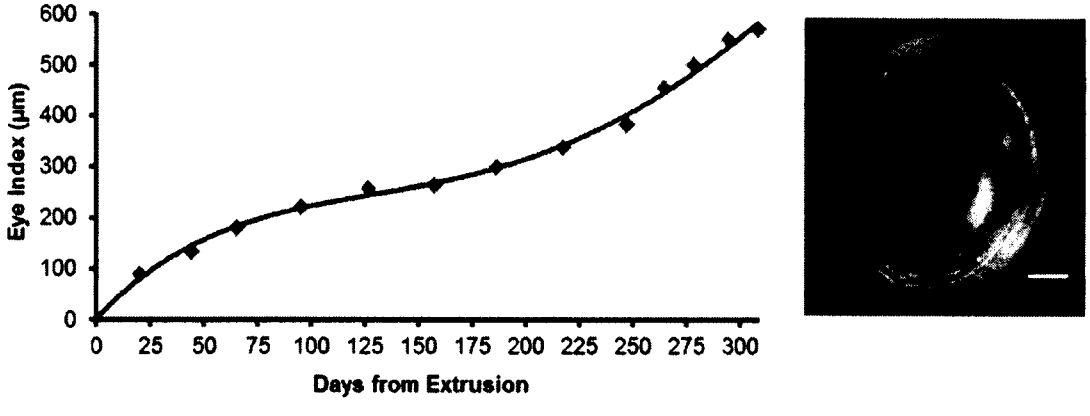

Fig. 3. Method used for determining day of extrusion for eggs. A curve was fit to the growth data for all the egg clutches in the inshore treatment (all females and associated eggs were obtained from inshore waters, see Fig. 1), and the curve was constrained to have a y-intercept of zero on day zero (equation: $y = -1e\text{-}07x^4 + 0.0001x^3 - 0.0325x^2 = 4.4703x$, $r^2 = 0.995$). This curve was then used to determine how many days it took for an egg of a given eye index to reach that size, and these data were then used to calculate when each clutch of eggs was extruded. For example, inshore lobster #503 had eyespots that were 57 μm on Oct-1. Solving the equation for 'x' (days from extrusion) gives a calculated value of 11 days old, or a predicted extrusion date of Sept-21. Right: Digital measurements used to stage lobster eggs. Arrows indicate the longest length and width measurements (eye size) obtained using Image J software. Measurements from all eggs in each sample were averaged together (longest length and width / 570, the eye index prior to hatch) to obtain a mean Perkins Eye Index (PEI). Scale bar = 300 μm.

Egg Development Calculations

The major hypothesis that was being tested in this study was that eggs exposed to different temperature regimes would hatch at different times. Egg extrusion (the time when eggs are fertilized and deposited along the underside of the female), was estimated when the eyespot size equaled zero. For lobsters in all treatments, % development was described using the Perkin's Eye Index (PEI; Perkins, 1972) (from Oct-1 to the time of hatch). Then, for each treatment, mean growth rate of the eggs was averaged and the slope of the curve was used as a representative of the rate of development of those eggs (Fig. 3). A best fit ($r^2 > 0.99$ in all cases) equation was determined for the line using a fourth-order polynomial (JMP 9.0.3; SAS Institute, Cary, NC). The intercept was set to (0,0), so that 0 eye size corresponded to 0 days of development. The equation for the curve was then used to estimate an extrusion date for a given clutch by calculating how many days it would have taken for an egg exposed to that range of water temperatures to reach the earliest stage we observed. Estimated extrusion date was then used as the 'birth date', enabling us to calculate the entire incubation duration, from extrusion to hatch for each clutch of eggs.

Egg staging used the PEI equation: $Z_{i\to h} = (W_h - W_i)/(-8.3151 + 2.6019T_{i^{\circ}C})$, where the number of weeks necessary for a lobster egg to hatch ($Z_{i\to h}$) is determined based on W_i, an average measurement of eye diameter, W_h, the size at complete development, and $T_{i^{\circ}C}$, temperature (Perkins 1972). The size of a lobster's eyespot at hatching (W_h) ranges from 560-580 μm (Helluy and Beltz 1991). We chose a median PEI value of 570 μm (based

on our observations of previous eggs) for a fully developed egg; an egg with PEI = 285 μm would be 50 % developed. Using the above equation, it can be shown that an egg at 50 % development requires 15.8 weeks to fully develop at 10 °C, but only 9.1 weeks at a temperature of 15 °C. The median hatch date (50 %) for each clutch of eggs was determined and converted to a Julian day (1-365), to average hatch dates for each clutch of eggs in each treatment. A Student's t-test was used to determine if mean hatch dates differed between treatments.

Cumulative heat-related growth or growing degree-days (GDD) was estimated for the duration of egg development (from extrusion to hatch) as daily temperature minus a thermal threshold value*the total days of development. We used a value of 4 °C as our thermal threshold value, derived from a compilation of reproductive-based studies summarized in Waddy and Aiken (1995) and Aiken and Waddy (1992).

Egg development was analyzed using ANOVA in the statistical software JMP v. 9.0.3. Where the parametric assumptions of normality and homogeneity of variance were not met, data were transformed and re-evaluated. All post-hoc tests were conducted using Tukey's HSD tests. A split-plot repeated measures ANOVA was used to investigate the effects of thermal treatment (inshore and offshore; factor 1) by month (12 levels; factor 2) with the potential effects of tank (whole-plot factor) and individual lobster (sub-plot factor). Associated temperature data (including GDD) were analyzed using a series of t-tests, and in all cases met parametric assumptions.

Larval Measurements and Survivorship

A secondary goal for this study was to assess if there were differences in larval quality between eggs incubated under inshore or offshore thermal regimes. Two assays were used to provide an index of larval quality. In the first, standard carapace lengths (CL_{STD}, from the posterior margin of the eye socket to the posterior edge of the median dorsal line of the carapace) were measured for Stage I larval lobsters (n = 15/female) from a randomized sample of 6 lobsters from both inshore (lab and field) and offshore (lab and field) treatments (n = 12 lobsters or 180 larvae). Larvae were removed from collection vessels by washing them with seawater into individual sample jars at ~ 50 % hatch. Larval CL_{STD}'s were averaged (± se) and compared between inshore and offshore (lab and field combined) treatments using a Mann-Whitney U test for non-parametric data.

The second assay measured the survivorship of first-stage larvae by determining how long they could survive without food (e.g., Mikami and Takashima 1993, Abruhosa and Kittaka 1997). For each larval cohort (n = 15 larvae x 12 females, total = 180), larvae were added in triplicate to clusters of six 15 mL individual wells (Costar 3516 culture clusters, Corning Inc., Corning, NY) (experimental unit = well; replicate = cluster). Clusters were labeled on one side and screened on the other to ample water exchange and circulation. All clusters were floated in a well-aerated temperature-controlled (18 °C) aquarium at 32-35 psu and exposed to a 10:14 L:D lighting regime. Water changes were conducted every few days while temperature was monitored via a digital data logger (HOBO, Onset Computer Corp.). Mortalities were checked daily, for two weeks by

observing larval activity and movement. Non-responsive larvae (mortalities) were removed from cells immediately. Larval survival was analyzed with a Kaplan-Meier survival algorithm (Sokal and Rohlf, 1995) using the PROC LifeTest algorithm with SAS v. 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Laboratory Temperatures

A total of 14 ovigerous females (two mortalities from 16 original) (six in 2007 and eight in 2008) were held in the lab from September-July of 2007-8 and 2008-9 under thermal regimes comparable to those they would experience if they resided in inshore waters. Inshore water temperatures (Fig. 4) averaged 6.7 ± 0.18 °C (min = 2.8 °C, max = 13.5 °C) in 2007-08, and 7.3 ± 0.22 °C (min = 1.5 °C, max 15.0 °C) in 2008-09. There was no significant difference in the mean water temperatures between years (paired t-test; $t_{1,11}$ = 2.015, p = 0.072), so data were combined for subsequent analyses.

In parallel to inshore temperature studies, a total of 16 ovigerous females (8 each year) were held at simulated offshore water temperatures (Fig. 4). Offshore water temperatures averaged 6.9 ± 0.10 °C (min = 2.6 °C, max = 13.7 °C) in 2007-08 and 7.2 ± 0.10 °C in 2008-09 (min = 2.6 °C, max 13.1 °C). There were no significant differences between the mean offshore temperature regimes for either year (paired t-test; $t_{1,11}$ = 1.902, p = 0.065), so these data were also pooled for subsequent analyses.

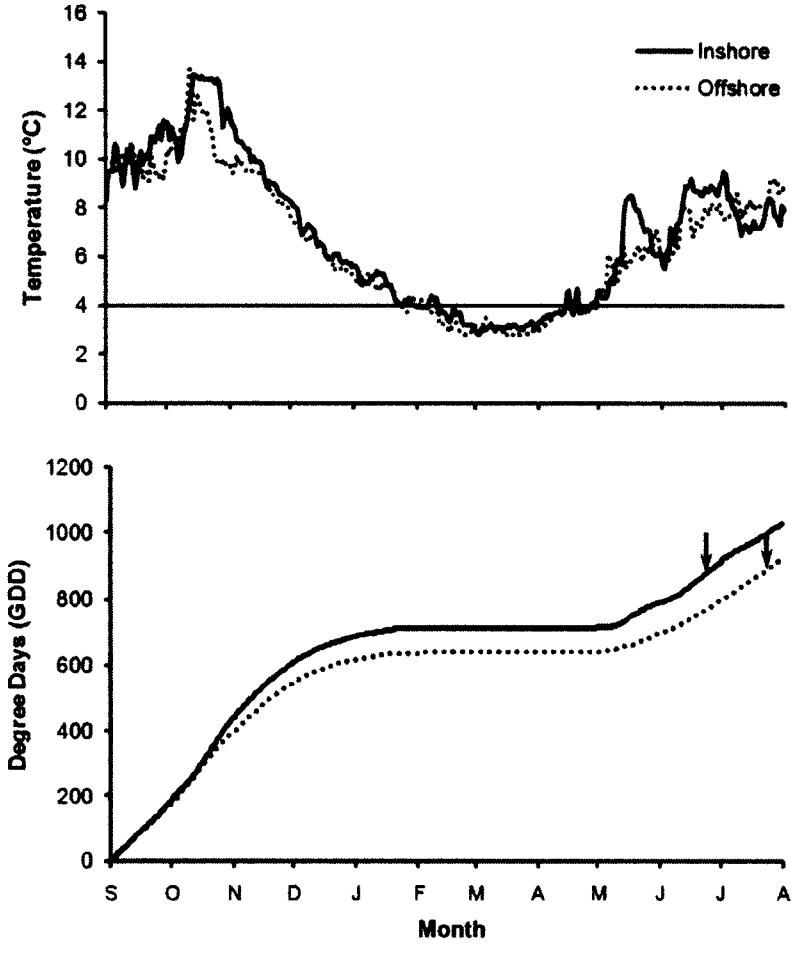

Fig. 4. Thermal regimes experienced by ovigerous females. Top: Average inshore and offshore temperatures (both lab and field, combined) from September to August, 2007-2009, with the 4 °C threshold temperature (solid line). Bottom: Associated growing degree-days (GDD) over the same time frame (calculated by subtracting the threshold temperature from the actual temperature each day and then adding all the days together). Data for each treatment terminated upon the average hatching date (vertical arrows), which was sooner for inshore lobsters (also see time to hatch section).

Field (Cage) Temperatures

A total of 16 ovigerous females were held in holding cages submerged in ambient seawater *in situ* inshore (n = 8; four in 2007-8 and four in 2008-9) and offshore (n = 8; 2007-8 = 3; 2008-9 = 5). Lobsters were held in these locations during the same time period, September to August, as for the laboratory studies. Inshore temperatures were the

same as in the laboratory trials (i.e., same source of water; see Fig. 4); however, offshore temperatures averaged 6.8 ± 0.40 °C (min = 2.8, max = 11.2 °C) in 2007-08 and 6.4 ± 0.32 °C (min = 3.1 °C, max = 10.6 °C) in 2008-09. These mean temperatures were slightly cooler than those simulated in the laboratory but differences were not statistically significant ($p > 0.05$).

In an attempt to simulate the natural movements of lobsters to offshore waters in the fall (move in Sep-Oct and remain offshore until March) and then back to inshore waters in the spring (move Apr-May and remain until hatching in May-June), the cages of the mixed treatment group of animals (n = 6) were moved back into inshore waters in mid-March in each year of the study, resulting in a large spike in water temperature and cumulative GDD of 840.1 (Fig. 5).

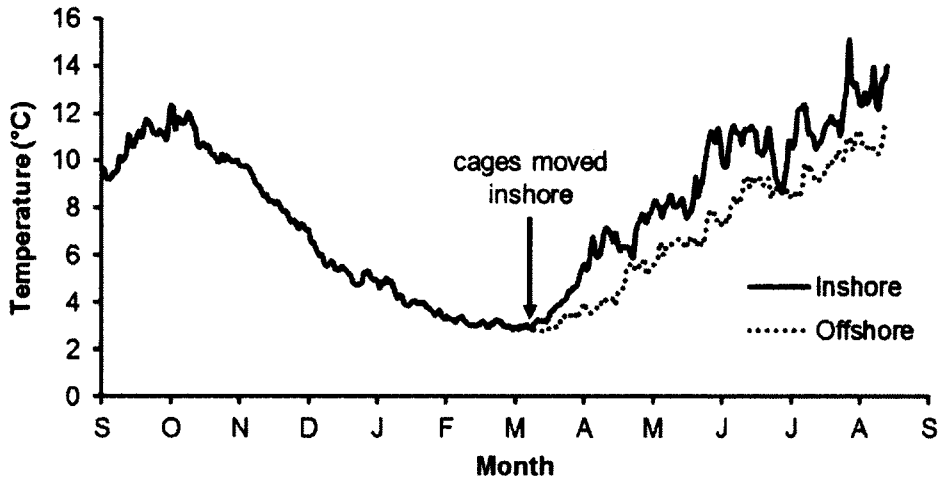

Fig. 5. Temperature profiles for those lobsters subjected to offshore conditions in the fall and winter and to inshore thermal conditions in the spring and summer ('mixed' treatment, field-study). Arrow indicates when cages were moved from offshore to inshore in the spring (15-March), leading to a sudden change in the thermal profile (dotted line). The offshore profile is continued for comparison. This scenario was designed to address the temperature changes that ensue for lobsters that undergo seasonal movements.

Inshore vs. Offshore Water Temperatures

During October-March inshore and offshore water temperatures were similar, while from April-September, there were significant differences (ANOVA; $F_{1, 24} = 2.20$, MS = 2.85, p = 0.045; Tukey HSD; $p < 0.05$, $\alpha = 0.05$; Fig. 6). Average monthly seawater temperature for the months of September, April, May, June, and July was significantly warmer inshore, while in January and February temperatures were slightly warmer offshore. As a result of being exposed to inshore water temperatures that were much warmer in April-July, inshore lobsters accumulated 336.3 GDD during this time period in comparison to only 163.8 GDD for offshore lobsters. In addition, inshore lobster eggs grew at a faster rate than offshore eggs during this time period.

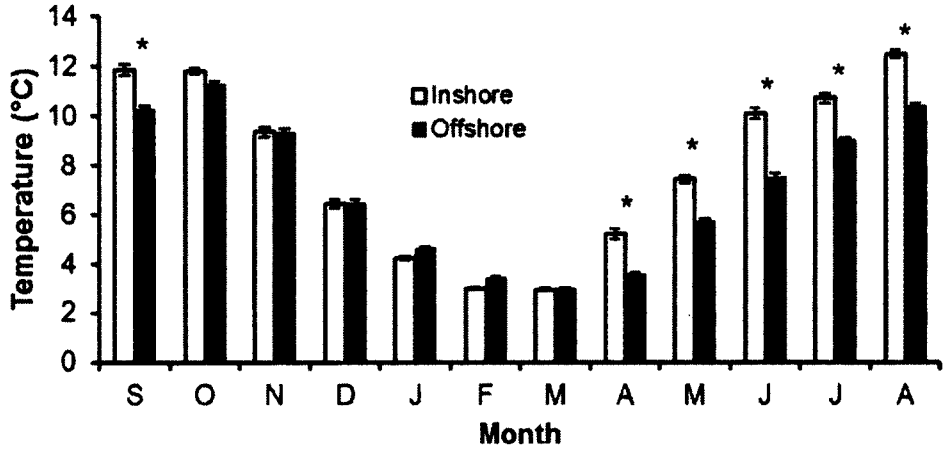

Fig. 6. Mean (± se) water temperatures for inshore and offshore locations during the months when eggs were developing (2007-09). While inshore and offshore water temperatures were similar from October through March, from April-July inshore temperatures were as much as two degrees warmer than offshore temperature. Asterisks (*) above treatment month comparisons denote significant differences ($p < 0.05$, $\alpha = 0.05$).

Egg Development

Developmental rates of eggs exposed to inshore and offshore thermal regimes were significantly different (ANOVA; $F_{1, 604} = 37.37$, MS = 23458, $p < 0.0001$, $\alpha = 0.05$). However, there were no significant differences between the rates in egg development by lobsters in the laboratory vs. those held in the field, under the same thermal regimes (ANOVA block effect in analysis; $F_{2, 604} = 1.37$, MS = 858.1, $p = 0.256$, $1-\beta = 0.58$). The development of all eggs (inshore and offshore) followed the same general trend each year, with rapid development in the fall, followed by a plateau at ~ 50 % development (PEI ~ 250-300 μm) during colder months, and then a rapid increase in developmental rate just prior to hatching in warmer months (Figs. 8, 9). Differences in the rate of egg development between inshore and offshore treatments paralleled those between mean monthly temperatures (Fig. 7). When water temperatures were similar between locations, as in the late fall and winter, developmental rates were also similar. However, when inshore waters warmed up more rapidly than offshore in spring and summer, growth rates diverged (Table 1; Fig. 7). While cumulative GDDs did not differ significantly between inshore and offshore thermal treatments ($mean_{inshore} = 938.0 \pm 10.3$ GDD, $mean_{offshore} = 904.7 \pm 13.0$ GDD; $t_{1,38} = 2.01$, $p = 0.061$; Table 2) over the total duration of development, they were acquired more rapidly by eggs exposed to inshore thermal regimes in the spring and early summer (Figs. 5, 9). For example, between April 1 and July 1 inshore sites yielded a total of 336.3 GDD compared to 163.8 for offshore sites.

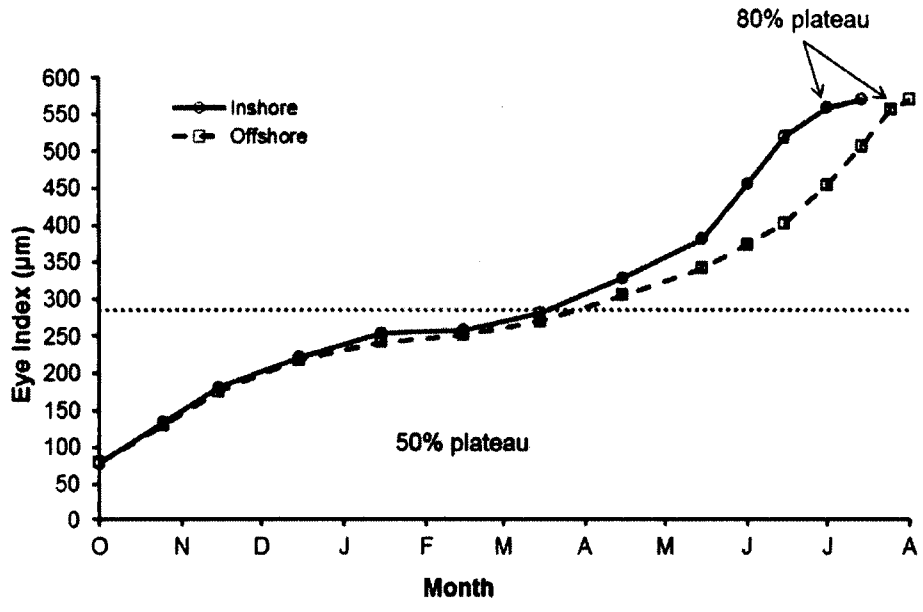

Fig. 7. A comparison of mean egg growth rates (eye size) for inshore and offshore treatments. Both data sets show a clear pattern, with very limited growth in the winter (45-50 % plateau, shaded area). Horizontal dotted line indicates PEI = 285 um, 50 %. A second, but more abbreviated plateau is seen at 80 % preceeded by increased growth in the spring and early summer.

Month	Temp (p-value)	Egg index (p-value)
Sept	0.047	0.127
Oct	0.603	0.937
Nov	0.665	0.490
Dec	0.696	0.637
Jan	0.574	0.100
Feb	0.517	0.332
Mar	0.696	0.098
Apr	**0.045**	**0.021**
May	**0.060**	**< 0.001**
Jun	**0.005**	**< 0.001**
Jul	**0.016**	**< 0.001**
Aug	0.008	0.592

Table 1
Results (p-values) from ANOVAs comparing the mean monthly water temperatures and egg development index (PEI), between inshore and offshore treatments. The shaded portion of the table indicates months (spring and early summer) when both temperature and egg development were significantly different between the two treatments. Underlined values indicate when there were only significant differences in temperature between the inshore and offshore thermal regimes.

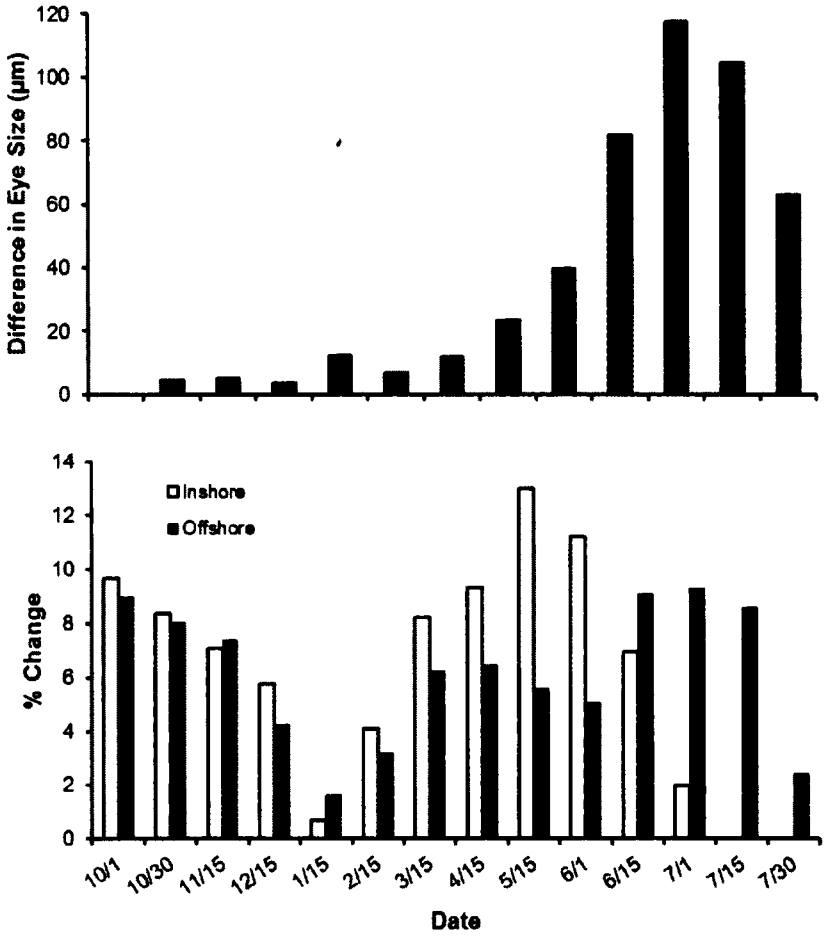

Fig. 8. A comparison of the growth rates of eggs exposed to inshore vs. offshore thermal regimes. Top: Differences (in µm) between inshore and offshore eye size (Perkins Eye Index, PEI) at monthly and biweekly intervals throughout egg development. Eye size was comparable for both inshore and offshore treatments from October through March and then eggs subjected to inshore water temperatures increased in size dramatically from April-July. Bottom: Percent-change in eye size over the course of development for both inshore and offshore lobsters (lab and field data, combined). While growth was similar in the fall and winter months, differences in egg development rates were most noticeable starting in March and were significantly different by May. Offshore growth extended through July as a result of their longer development and delayed hatch.

Time to Hatch

Eggs incubated inshore hatched earlier and over a shorter time period compared to eggs from offshore lobsters (Fig. 9). Egg incubation times in both the lab and field trials

showed clear differences in hatch as a function of temperature regime ($F_{2,67} = 64.73$, MS = 183.55, $p < 0.0001$, $\alpha = 0.05$). Subsequent pairwise comparisons showed differences between all three thermal treatments (Tukey HSD; $q = 8.4$, $p < 0.0001$) (inshore, offshore and mixed; Figs. 10,11). Eggs that were incubated under inshore temperature regimes hatched earlier (time at 50 % hatch) (mean = 177 ± 2.2 Julian days, median = 175, range = 161-196; or June 10-July 15, 35 days total) than those in the offshore treatment (mean = 208 ± 3.3 Julian days, median = 211, range = 184-230, or July 3-August 18, 46 days total; Fig. 10). Lobsters that underwent simulated seasonal movements (mixed treatment) hatched the earliest (mean = 165 ± 2.8 Julian days, median = 165, range = 141-200 Julian days, or May 21-July 19; Fig. 10) and over the longest period of time (59 days).

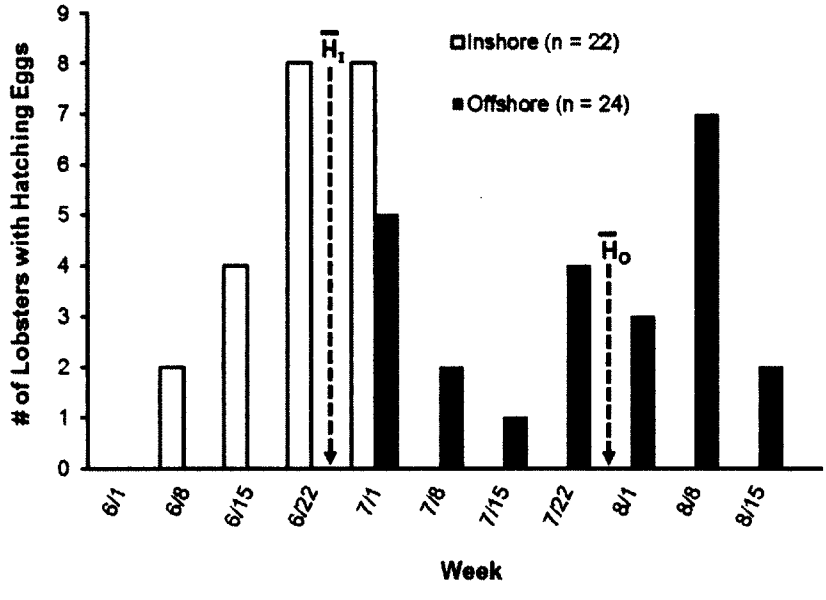

Fig. 9. The total number of lobsters with eggs hatching each week between June and August, for 2008 and 2009. Data are combined for animals exposed to laboratory and field conditions and exposed to either inshore or offshore temperatures. Eggs exposed to offshore temperatures not only hatched later, but over a longer period of time, compared with those exposed to inshore temperatures. Symbols (H_I and H_O) denote the mean hatching date for inshore and offshore treatments, respectively.

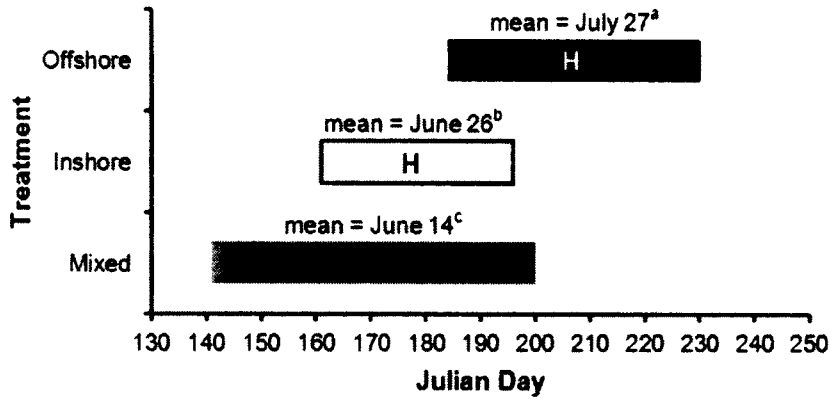

Fig. 10. The duration of time when eggs were hatching from lobsters exposed to one of three thermal treatments: inshore, offshore, or mixed. Mean hatching dates for each group of animals (H) are encompassed by their associated range of hatching times. Notice that inshore lobsters hatched earlier and over a shorter period of time. The mixed treatment group (simulating offshore to inshore migration) hatched first and exhibited the longest overall hatching time. Different letters above mean dates indicate significant differences ($p < 0.0001$, $\alpha = 0.05$).

Estimating Total Duration of Development

Although all the lobsters used in this study were captured between August-15 and September-20, not all eggs were at the same growth stage when trials were initiated in September. There were no significant differences between the mean initial stages of the eggs in the inshore group (PEI = 77.5 ± 5.8, or 14 % developed) compared to the offshore group (PEI = 78.5 ± 6.1, or 15 % developed) (unpaired t-test; $t_{1,46} = 0.068$, $p = 0.946$). To more precisely determine if exposure to different water temperatures influenced the total duration of egg development, we estimated the date of extrusion for the eggs carried by each lobster and then used that estimated extrusion date to calculate the total time for egg development (see methods). As expected, the mean duration of egg development for eggs experiencing inshore temperatures was significantly shorter (287 ± 11 days for

inshore vs. 311.5 ± 7.5 days for offshore) than the total duration of egg development for eggs incubated at offshore temperatures (p = 0.034; Fig. 11).

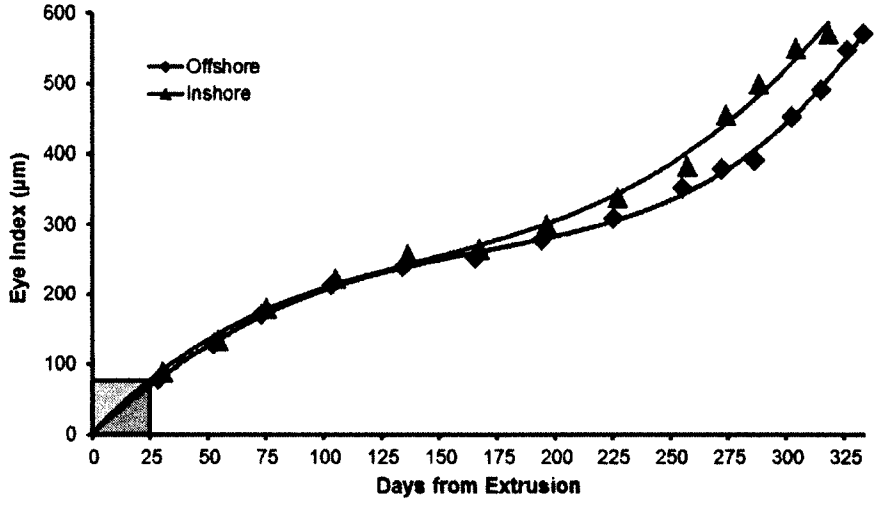

Fig. 11. Egg growth, from the day of extrusion, for eggs exposed to inshore and offshore temperatures. Data were combined from: 1) both years of the study; and 2) both eggs from ovigerous lobsters held in the field as well as those maintained in the lab. Data points represent empirical egg size information from our study. The growth curve and day of extrusion (eye size = zero) were calculated by fitting the empirical data to a fourth-order polynominal equation. The shaded area represents the portion of the curve that was extrapolated based on this equation.

	Mean Total to Hatch	Period I	Period II	Period III	Total GDD (300 day period)
Inshore	938	612	26	383	1021
Offshore	905	564	36	200	800
Mixed	840	612 (in)	36 (off)	383 (in)	1031

Table 2
Associated GDD values for each treatment, (in)shore, (off)shore, and mixed, over each of the 3 developmental time periods (100 days each). 'Total to hatch' values represent average GDD totals ($range_{inshore}$ = 818-974, mode = 974; $range_{offshore}$ = 780-1005, mode = 850) through to hatching for each treatment.

Larval Size and Survivorship

There were no significant differences in mean larval size (CL_{STD}) between inshore (mean $CL_{STD} = 1.90 \pm 0.018$) and offshore (mean $CL_{STD} = 1.98 \pm 0.015$) thermal treatments (Mann-Whitney; $U = 15002$, $p = 0.060$). Starvation trials revealed no apparent differences between inshore and offshore with respect to survivorship (SAS PROC LifeTest; $\chi 2 = 1.765$; $df = 1$; $p = 0.216$); a starting sample 90 larvae / treatment, resulted in a mean survival of 45.3 ± 4.1 % for inshore larvae and 47.5 ± 3.6 % for offshore larvae over the 14-day trial period.

Discussion

Many lobster species carry out seasonal migrations, and numerous explanations for these movements have been suggested. However, there remains a great deal of speculation and many exceptions to the rules. For example, it is generally accepted that the inshore-offshore migrations of ovigerous American lobsters enhance egg development because the deeper offshore waters are warmer and more stable in the late fall and winter. Thus, eggs should be subjected to more GDDs and hatch sooner. However, we found the opposite to be true: eggs exposed to offshore water temperatures took longer to develop and hatched later in the summer compared to eggs at inshore water temperatures. We focused on the existing paradigm that some ovigerous lobsters make directed seasonal movements, setting up potentially differing outcomes with respect to when and where larvae hatch, and the significance (if any) of seasonal movements that augment this

process. Importantly, it appears that this difference is due to the rapid warming of inshore waters in the spring, and not from overall differences in water temperature between inshore and offshore locations. This study utilized a comprehensive approach including field- and lab-based designs to examine the differences in egg development and timing of hatch over spatially disparate thermal environments while simulating local conditions under which lobsters would remain in one location year-round (inshore), undergo seasonal movements in the fall (offshore), or move and come back (mixed).

Patterns of Egg Development and Growth

Our findings corroborate previous work demonstrating the strong connection between water temperature and the development of lobster eggs (Templeman 1940, Perkins, 1972, Helluy and Beltz 1991, Gendron and Ouellet 2009). The pattern of egg development we observed generally followed those described in previous studies (Bumpus 1891, Herrick 1895, Templeman 1940, Helluy and Beltz 1991, Sibert et al. 2004, Gendron and Ouellet 2009): 1) rapid development in the fall, when water temperatures were decreasing; 2) a protracted period of developmental latency over the winter months at water temperatures < 4 °C; 3) a pronounced increase in growth rate in the spring as water temperatures increased; and 4) a brief pause in development about one month before hatch. Interestingly, despite the differences in thermal profiles, inshore and offshore egg development trajectories were very similar until the spring (Fig. 4). The rate of temperature increase from May through August was significantly different between inshore and offshore treatments (Table 1), and it was during this time period that egg

development diverged. In all treatment groups we observed a well-defined ~ 3.5-month plateau in growth at ~ 50 % (PEI = 285 μm) over the winter months and a shorter and less well defined one at 80 % (PEI = 455 μm), that occurred in late spring and early summer.

Developmental plateaus, during which neither the growth of the eye or the cephalothoracic segment is evident, have been documented in both crabs (Stevens et al. 2008) and lobsters (Helluy and Beltz 1991). The earlier, more prolonged, developmental plateau at 50 % could be the result of the culmination of the majority of morphological development and organogenesis that occurs before water temperatures decrease to sub-optimal growth levels in the late fall and winter. However, Sibert et al. (2004) determined that almost 65 % of the live biomass (total proteins) of hatching larvae accumulated during the last few weeks of development, indicating that a significant amount of growth and development is occurring at this time. Most likely, the lobsters in this study exhibited a plateau at 50 % because of exposure to colder winter temperatures (Gendron and Ouellett 2009).

The later plateau at 80 % seems to be related to the transition between the premolt metanaupliar stage, as the larva prepares for hatch, comparable to pre-molt pauses in growth in juvenile lobsters (Aiken 1973, Helluy and Beltz 1991). Additionally, Helluy and Beltz (1991) observed that developmental plateaus were observed at a variety of stages (PEI = 350-450, ~ 60-80 %). A developmental plateau is evident at 80 % even at constant temperatures (Helluy and Beltz 1991) (but not at 50 %), and the causes of this

80 % plateau remain largely unknown. During a prolonged 'resting period' (not considered a true diapause phase like in other marine crustaceans (e.g., copepods; Hansen et al., 2010), no measurable growth was recorded presumably because temperatures were low enough to subdue growth. These 'biological zero' points (Wear 1974) may, however, have imperceptible growth that is not captured by standard egg size measurements. Some species of Pacific crabs, for example, can continue growth at temperatures below 1 °C (Shirley et al. 1990, Stevens and Swiney 2007). Although other studies document suspended lobster egg growth below 5 °C (Pandian 1970, Perkins 1972), one study documented growth at temperatures as low as 1-1.5 °C in *H. americanus* (Gendron and Ouellett 2009). One possible explanation for this discrepancy may be the genetic variability associated with differing thermal habitats that is evident in lobsters from disparate geographic locations (Hedgecock et al. 1976, Hochachka and Somero 2002).

A variety of other factors also influence overall growth and egg development in lobsters and may manifest themselves in both the rate and the success of development. For example, thermally-induced hormonal changes (Talbot and Helluy 1995), the timing of reproductive cycles (successive- vs. alternate-year spawning; Waddy and Aiken 1986), overall reproductive history of ovigerous lobsters (primiparous or multiparous; Comeau and Savoie 2001), maternal effects (nutrition; Moland et al. 2010), and female size (Attard and Hudon 1987) all may influence how long eggs remain at a particular plateau.

Degree-days and Growth

The growing degree-days (GDD) metric is one way to quantify the influence of fluctuating water temperatures on the growth of ectotherms (Neuheimer and Taggart 2007). Historically, GDD has been used as an index of the thermal history experienced by ovigerous lobsters, and has included a cold threshold value below which, no growth is assumed to occur (4 °C threshold, this study). Degree-day calculations have been used to approximate total egg development and time to hatch in *H. americanus* in a variety of locations such as: Grand Manan, Canada (1,832 GDD; Campbell 1986), mid-coast Maine (952-983 GDD; Cowan et al. 2006), Massachusetts Bay (807-1,490 GDD; Tlusty et al. 2008), and the Magdalen Islands in Quebec (1,300-1,440 GDD; Gendron and Ouellet 2009). We obtained GDD from extrusion to hatch of 905 (coastal) and 938 (offshore) that are lower than any previously reported values (Table 2). However, making direct comparisons of GDD across studies is difficult since most do not estimate total development time from extrusion to hatch.

Additionally, lower threshold values for the calculation of GDD can vary based on local thermal profiles, helping to explain dramatic differences in lobster growth (along with environmental heterogeneity) that temperature elicits over both regional (e.g., northern vs. southern ranges) and local (10's of km) scales (Little and Watson 2005; Wahle and Fogarty 2006, Bergeron 2011). Clearly, the relationship between temperature and growth needs to be evaluated at several different scales that reflect a realism associated with changes in growth over time. We found no significant differences in GDD values for

lobsters incubated at inshore and offshore temperatures, even though mean monthly water temperatures were different at certain times of the year (Fig. 6). Contrary to other studies (e.g., Campbell 1986), we found that lobsters that remained inshore not only acquired enough GDD to complete development, but also hatched earlier than offshore lobsters (Fig. 10), suggesting that offshore movements do not necessarily maximize the rate of egg development. However, the combination of seasonal movements offshore in the winter and inshore in the spring does maximize egg growth and development. While the GDDs of our 'mixed' group were not significantly different from either inshore or offshore treatments, these animals hatched almost two weeks earlier (comparing means, Fig. 10).

We suggest that the key factor influencing the time of hatch in *H. americanus* eggs is the rate of increase in water temperature, and therefore GDD, during the spring and early summer, rather than the total GDD accumulated throughout the entirety of development. Both mean water temperature and GDD were very similar between our treatments during the first two-thirds of egg development, but then differed significantly during the last third, leading to earlier hatch for eggs exposed to the most rapid increase in water temperature.

Moreover, animals that moved inshore from offshore waters (mixed treatment) hatched even earlier because they were exposed to offshore water temperatures in the winter and then also experienced the rapid warming of inshore waters in the spring (Fig. 6). If spring hatching is advantageous, then a pattern of inshore to offshore migrations in the

fall, combined with offshore to inshore movements in the spring would be optimal. Such a pattern of seasonal migration has already been documented in some locations (Cooper and Uzmann 1980, reviewed in Lawton and Lavalli 1995). However, in coastal souther Gulf of Maine waters few lobsters that move offshore in the fall moved back inshore in the spring while they were carrying eggs (Goldstein and Watson in prep.). Rather, most remained offshore until their eggs hatch. This observation suggests a need to reexamine the adaptive significance of seasonal movement patterns in lobsters.

Time to Hatch

Our data do not support the hypothesis that offshore movements of ovigerous females result in a greater accumulation of GDD and therefore earlier hatching of larvae. Rather, eggs exposed to inshore coastal New Hampshire water temperatures hatched an average of four weeks earlier than eggs incubated at offshore thermal regimes. Hatching earlier in the spring/summer may be significant for a combination of reasons that are framed by two long-standing hypotheses: Hjort's (1914) critical period hypothesis contends that the presence and strength of larval year-classes are determined by the availability of food during a 'critical period' while Cushing's (1990) match-mismatch hypothesis states that variations in larval food supply are a function of the timing of the spring phytoplankton bloom when larval hatch occurs.

Although these hypotheses have been difficult to support, new innovations in remote sensing technologies (e.g., ocean color analysis) are helping to reveal the potential

relationships between the timing of larval hatch, the availability of food resources and subsequent larval survival. For example, Ouellet et al. (2007), found that the larvae of successful shrimp (*Pandalus borealis*) year classes tended to hatch during periods when colder sea-surface temperatures (SSTs) were followed by the rapid warming of the surface layers. While embryonic development rate in lobsters is most strongly influenced by temperature, the timing of the spring plankton bloom in coastal waters also depends on a combination of other factors including changes in photoperiod and seasonal circulation patterns. Alterations in such timed events could not only influence survivorship and hatchability of marine crustacean larvae (including lobster), it could also shift the time frame over which larval hatch is critical to survival in the plankton (Edwards and Richards 2004). Therefore, if movements of ovigerous females have evolved to ensure that hatching occurs at the right time of year, when both SSTs (> 12 °C; Mackenzie 1988, Annis 2005) as well as phytoplankton and associated zooplankton blooms are optimal for larval survival, then these events should correlate with hatch times of offshore, rather than inshore larval hatch. Just as critical is the exposure of larvae to thermal conditions that are conducive to larval survivorship and optimal growth in the plankton.

MacKenzie (1988) demonstrated in a series of laboratory rearing studies that larvae hatching at 10 °C can develop successfully through Stages I and II; however, warmer water is needed to complete development to Stage IV and the early benthic juvenile phase, Stage V (4 % larval survivorship at 10 °C vs. 56 % at 12 °C larval survivorship, MacKenzie, 1988). Similarly, Sastry and Vargo (1977) reported significantly lower

survivorship to Stage V below 10 °C, and Harding et al. (1983) found that larval hatching usually occurred when water temperatures rose above 12 °C. The possible and intentional movement towards such thermal regimes by some ovigerous lobsters may optimize larval development, growth, and survival, but this remains largely untested.

We found that the average water temperature when inshore larvae hatched was 11.5 °C, compared to 10.5 °C offshore at the same time (Fig. 6). So, to some extent, the differences in hatch date allowed lobsters in both areas to hatch when water temperatures were fairly favorable for growth and survival. Further investigation is needed and should shed light both on Cushing's match-mismatch hypothesis, and the overall adaptive significance of seasonal lobster movements.

While Perkins's (1972) equations for predicting hatch in eggs incubated at constant temperatures have been very useful for laboratory- and hatchery-based research, it is not as functional for animals in natural habitats characterized by large seasonal fluctuations in water temperature (Jarvis 1989). We sought to test the usefulness of the Perkins equations for eggs exposed to naturally fluctuating water temperatures by using the mean temperature for the duration of the incubation period as the constant temperature (Fig. 12). The discrepancy between predicted hatch dates and those empirically observed (in this study) suggest that is difficult to rely solely on Perkin's (1972) equations to predict the hatch dates of eggs *in situ*, and alternative models are necessary that take into account the effects of changing temperatures on overall egg development.

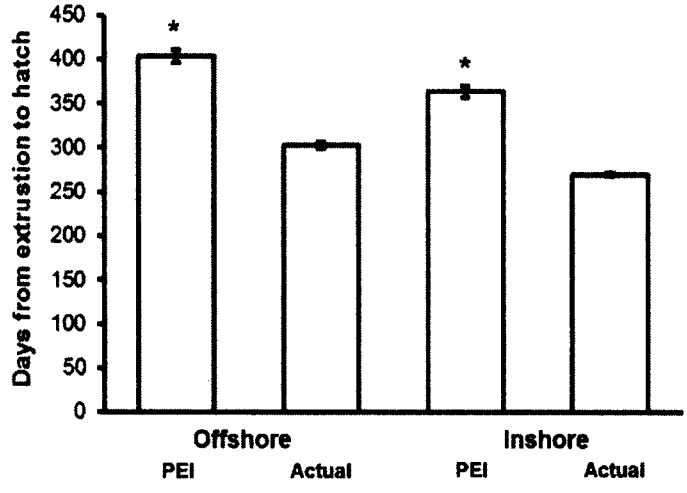

Fig. 12. Days from extrusion to hatch for a subset of ovigerous lobsters (n = 4, size range: 84-95 mm CL), whose egg hatch dates were calculated from either: 1) Perkin's (1972) PEI equation, using an average temperature (6.2 °C offshore, 6.7 °C inshore) over which egg development occurred (October-July); and 2) through empirically-derived egg development data from this study. The remaining weeks until hatch were calculated and added to the October-1 date to calculate predicted hatch. For animals subjected to offshore waters, PEI predicted eggs would hatch on November-7, 93 days later from the average observed hatch compared to inshore eggs hatching on September-28 or ~ 100 days from observed hatch. Differences were significant using a goodness-of-fit test for both offshore ($G = 14.27$, $p < 0.001$) and inshore ($G = 17.31$, $p < 0.001$) treatments and indicates the need for egg development models that take into account changing temperatures eggs encounter under natural conditions.

Larval Dispersal and Survivorship

We found no apparent differences between inshore and offshore larval size or survival. Various indicators of larval variability in other crustacean and fish larvae have included size at hatch and lipid profiles, among others (reviewed in Jaeckle 1995). For example, after raising spiny lobster larvae (phyllosomas) at various thermal profiles Smith et al. (2002) reported that Stage I phyllosomas cultured at warmer temperatures were smaller. Changes in incubation temperatures have also been shown to affect larval size in other lobsters (e.g., *Jasus edwardsii)* (Tong et al. 2000) and crabs (Shirley et al. 1987). One

advantage of *H. americanus* eggs developing more slowly at colder temperatures could be the conservation of metabolic reserves (e.g., lipids) during development, leaving more energetic reserves available during the first few days as larvae (Appendix A). However, this may not be a major issue, because even lobster eggs cultured at elevated temperatures contain residual yolk at the time of hatching (Sasaki et al. 1986, Appendix A). Therefore, differences in the thermal regimes between inshore and offshore New Hampshire waters do not appear to influence the viability of the larvae that hatch from eggs, and thus the offshore movements of ovigerous females might have evolved to serve another purpose.

Another possible explanation for the movement of ovigerous lobsters offshore in the late fall and winter is that these movements serve to position them in areas that will enhance larval survival and transport to optimal settlement areas; this has been conjectured for spiny lobsters (see Booth 1997 for review). According to tracking data, most ovigerous lobsters that migrate offshore remain there until after their eggs hatch the following spring/summer (Goldstein and Watson in prep.). Furthermore, preliminary data from oceanic drifters released in offshore areas at the time of hatching, indicate that larvae from NH waters are most likely transported to coastal locales in Massachusetts (to the south), where they will settle ~ 3-4 weeks later (Goldstein unpub. data). In contrast, drifters released in inshore areas were frequently and rapidly transported farther inshore, presumably too soon for developing larvae to reach a stage at which they are competent to settle (Goldstein unpub. data).

Our working hypothesis that the offshore movements of ovigerous females may optimize larval survivorship is important for two reasons. First, their larvae hatch when sea-surface temperatures are best for their survival. Second, larvae that hatch in offshore areas will typically spend at least 2-3 weeks drifting, feeding and growing in the plankton, and will be competent to settle, by the time they reach a variety of suitable inshore settlement areas.

Conclusions

Given the narrow thermal constraints of all life history phases of *H. americanus* (Fogarty 1995), and the sensitivity of lobster growth and reproductive dynamics to variations in temperature regimes (Waddy and Aiken 1995) it is not too hard to prognosticate how climatological changes could affect broodstock fecundity, size at maturity, egg development, and hatch, among others. For example, rising seawater temperatures would accelerate egg development and hatching, thereby shortening larval development. In some areas, offshore movements by lobsters seeking to avoid warm water could cause eggs to hatch too far offshore, setting up sub-optimal dispersal trajectories and possible larval wastage. Other climate-related scenarios are certainly possible; however, our data suggest that lobster eggs are flexible with respect to their ability to adjust their rates of development, and ovigerous lobsters can move to areas that allow hatch during favorable times of the year.

The role of temperature in lobster egg development and time to hatch has been explored in this study within a relatively small window (two full seasons) of thermal variability. Thus, the implications of our results in the context of longer-term climate change scenarios are uncertain. Changes in ocean temperatures will undoubtedly cause alterations to thermal profiles that would have cascading effects on the movement dynamics of ovigerous lobsters, which in turn, would influence egg development rates, timing of hatch, and ultimately, larval survivorship and dispersal. Continued and more detailed investigations of the physiological tolerances, thermal thresholds, and behaviors of ovigerous lobsters, their eggs and larvae would certainly contribute to current and changing oceanographic conditions for one of the most commercially important crustaceans in the North Atlantic.

CHAPTER 3

TRACKING LARVAL LOBSTER DISPERSAL ALONG THE NEW HAMPSHIRE COAST USING GPS-ENABLED DRIFTERS

Abstract

The distribution and abundance of marine decapod larvae are affected by the locations of spawning females in tandem with a host of abiotic factors (e.g., currents) that ultimately influence their final destination. Knowledge of these factors is imperative to our understanding of marine population connectivity and the management of commercially important species such as the American lobster, *Homarus americanus*. The goal of this study was to combine our knowledge of the location of ovigerous (egg-bearing) females along with predicted hatch times for their eggs, and data from ocean drifters released at appropriate times and locations, to predict the fate of larvae and the locations where they might settle. A total of 23 surface ocean drifters were released in three different areas where ovigerous lobsters were located when their eggs were hatching: inshore (< 5 km from the New Hampshire coast), offshore (5-10 km from the coast), and in the Great Bay estuary (GBE). Drifters released at inshore locations (n = 8, days-at-large, DAL_{avg} = 13.5 ± 2.1) showed a variety of patterns including: 1) alongshore transport; 2) retention in the vicinity of release; 3) movements further inshore; and; 4) in rare cases, movements offshore. Drifters released offshore (n = 7, DAL_{avg} = 28.1 ± 7.5) generally showed southerly movements towards coastal Massachusetts waters; however, some drifters exhibited movements back inshore, particularly in late summer. In the GBE all drifters

were retained (n = 8, DAL_{avg} = 5.8 ± 0.93), despite strong currents (> 30 cm/s) associated with tides. *In situ* temperature data indicates that larvae can complete the majority of their development within ~ two weeks. Overall, our data suggest that trajectories of drifters (larval dispersal) are, in large part, a function of where spawning occurs and may reflect a combination of transport away from local sources or partial retention over others.

Introduction

Whether larvae disperse widely or remain near their natal source, they must still somehow transit from the pelagic realm to coastal benthic nurseries where they settle, metamorphose, and become demersal. The amount of time spent in the water column, often referred to as pelagic larval duration (PLD), is probably the single biggest factor influencing dispersal and recruitment in marine populations (Sponaugle et al. 2002, Cowen and Sponaugle 2009). Physical factors such as tidal fronts, internal wave slicks, turbulence, and Ekman transport (among many others; Shanks 1995) work in tandem with larval behaviors (e.g., directional swimming, attraction to surface, depth regulation) to situate larvae in areas suitable for settlement and survival (Young 1995). The ability to couple both biological aspects of PLD with physical aspects of circulation makes it possible to evaluate the connectivity of populations (Cowen et al. 2000, 2006, Sale and Kritzer 2003, Butler et al. 2011, Incze et al. 2010) and is central to considering the population dynamics of commercially important marine species (Warner and Cowen 2002, Lubchenco et al. 2003, Palumbi 2003, Sale et al. 2005). Such data are vital for addressing critical management and conservation issues in marine populations.

Physical parameters at the time (and site) of hatching set the initial conditions for larval dispersal, and vary depending on the timing of this event. Larval release in many marine fishes and invertebrates is often synchronized to environmental cycles (e.g., tides, light-dark cycle), meteorological conditions, and seasonal factors such as the spring plankton bloom (Forward 1987, Edwards and Richards 2004). This is especially true in marine

decapods (crabs and lobsters) whose complex life histories and physiology are strongly modulated by their environment (Morgan 1995, Mente 2003, Jury et al. 2005).

Environmental variables are especially influential in the growth, reproduction, and distribution of American lobsters (*Homarus americanus*) (Waddy et al. 1995). A variety of environmental changes (e.g., temperature, salinity, storm events, etc.) initiate moderate to extensive seasonal movements (Cooper and Uzmann 1980, Lawton and Lavalli 1995, Jury et al. 2005, Chapter 1) and these movements, in turn, impact egg development and larval survival (Chapters 1 and 2). Ovigerous lobsters carry their eggs from 9-11 months, depending on temperature (Bumpus 1891), and typically release their larvae in small batches on successive nights during the summer (Ennis 1975).

The complex larval life-cycle of *H. americanus* includes four larval stages, three of which are known to drift passively under geostrophic and wind-forced currents (Katz et al. 1994), although studies have shown that early stages are positively phototactic, and later stages are capable of vertical migration (Harding et al. 1987). Stage IV (postlarval) lobsters are considered neustonic and exhibit strong swimming abilities, allowing them to search for optimal settlement habitat (Rooney and Cobb 1991, Annis et al. 2007).

Plankton sampling data suggest 65-96 % of lobster postlarvae reside in the top 0-0.8 m of the water column (Hudon and Fradette 1988, Annis 2005, Annis et al. 2007). The residence time for lobster larvae in the water column is controlled predominantly by surface water temperatures and, to a lesser extent, by food availability (Mackenzie 1988,

Annis 2005). The average total duration of passive and swimming stages (i.e., PLD) ranges from 15-30 days. A re-examination of historical lab data (Templeman 1936), coupled with more recent *in situ* field studies, suggests that PLD may be skewed toward the lower end (10-15 days). Thus, local retention of larvae may be more likely than previously predicted in at least some locations (Incze et al. 2006, Annis et. al. 2007).

In addition to PLD, the location of larval release can have profound implications for their fate. Mobile invertebrates, such as lobsters, generally reside in an environment favorable to their own survival; however this may not necessarily optimize the survival of eggs and larvae or the dispersal of larvae to suitable settlement habitats. It remains largely unknown if the seasonal inshore-to-offshore migrations by ovigerous lobsters (Lawton and Lavalli 1995, Cowan et al. 2006, Chapter 1) place their eggs in environments that maximize egg development, larval survival, or even dispersal trajectories that result in recruitment to optimal settlement habitat s (Byers and Pringle 2006, Goldstein and Watson in-prep.).

Lobsters may move into offshore waters to release their larvae in order to place them in a hydrodynamic environment that is more conducive to initial larval dispersal and survivorship (Goldstein and Watson in-prep.). Other ovigerous marine decapods migrate to specific areas for spawning and larval release and Booth (1997) documents a variety of brooding-related movements in a number of lobster species. For example, ovigerous Caribbean spiny lobster (*Panulirus argus*) appear to make directed movements from lagoonal or reef habitats to the reef tract to release larvae, presumably facilitating their

transport to seaward currents off the reef and to offshore nursery areas elsewhere (Bertelsen and Hornbeck 2009).

Other ovigerous lobsters such as the South African rock lobster (*Palinurus gilchristi*) and the slipper lobster (*Ibacus peronii*) make directed migrations that act to redress the downstream (contranatant) dispersal of phyllosomal larvae via currents (Stewart and Kenelly 1998, Groeneveld and Branch 2002). In addition, blue crabs (*Callinectes sapidus*) exhibit directed movements down-estuary and offshore using ebb-tide transport, an important determinant of larval dispersal back into settlement grounds in the estuary (Carr et al. 2004). Stone and O'Clair (2002) reported that the onshore movement of brooding female Dungeness crabs (*Cancer magister*) serves to situate these animals within appropriate brooding habitats during the spring phytoplankton bloom. Using a combination of archival tags and ultrasonic telemetry, Gonzalez et al. (2002) determined that female spider crabs (*Maja squinado*) off the coast of Spain routinely choose specific deep wintering habitats for mating and spawning before returning to shallower waters to hatch their eggs.

Given the strong evidence in other species that female movements may situate larvae in optimal areas for dispersal and survival, it is logical to assume the movements of ovigerous American lobsters serve the same purpose. The major goal of this study was to estimate the initial trajectories taken by lobster larvae by using oceanic surface drifters released at times and locations that corresponded to hatching events (Goldstein and

Watson submitted). Drifter data could then be used to determine how movements of female lobsters might influence the dispersal of their offspring.

Large-scale modeling of the origins, distribution, drift, and settlement of lobster larvae in the Gulf of Maine (GoM) has yielded several approaches to predicting their dispersal, degree of retention, and subsequent recruitment from disparate origins (Katz et al. 1994, Incze and Naimie 2000, Harding et al. 2005, Incze et al. 2006, Xue et al. 2008, Chassé and Miller 2010, Incze et al. 2010). These studies have incorporated a variety of components including hypothetical larval trajectories, mortality estimates, larval biochemical data and 3-D circulation models. The general consensus is that the GoM is a single interconnected lobster recruitment region (see Goldstein, Appendix C for review). However, very little is known about the fate of larvae on a much more localized scale (i.e., 10s of kms) and the physical oceanographic elements that shape initial larval advection (e.g., Lough and Manning 2001, Manning et al. 2009).

Oceanic drifters are an inexpensive tool for studying the dispersion of surface particles, such as fish or crustacean larvae, as well as other plankton (e.g., red tides) and buoyant pollutants such as oil (Levin 1983, Davis 1985, Tegner and Butler 1985, Thorpe et al. 2004, Keafer et al. 2005, Gawarkiewicz et al. 2007, Hare and Walsh 2007, Price et al. 2007, Caballero et al. 2008). Studies in the GoM involving these types of drifters have been used to observe Lagrangian flow along the Maine coastal current (Manning et al. 2009), estimate the rates of tidal-front entrainment and subsequent retention of fish larvae on Georges Bank (Lough and Manning 2001, Manning and Churchill 2006), and make

observations on a variety of local and regional current features (see: http://www.nefsc.noaa.gov/drifter/). We adopted this well-established technology for our study.

By combining our knowledge of the location of ovigerous females along the New Hampshire seacoast (Goldstein and Watson in prep.), along with their predicted hatch times (Goldstein and Watson submitted), we were able to deploy drifters when and where lobster larvae are released. Our goal was to follow the trajectories of the drifters for a period of time corresponding to larval development (2-3 weeks) so that we could estimate the locations where larvae might settle. We hypothesized that drifters deployed inshore would move in a pattern suggesting larval retention, while those released offshore would take paths suggesting recruitment of New Hampshire larvae to southern waters (e.g., Massachusetts). An additional set of drifters was released in an adjacent estuary (Great Bay, NH) to test the hypothesis that larvae hatched by resident ovigerous adults are retained there.

Overall, we found that drifters released at inshore locations predominantly moved parallel to the coast in a south to southeasterly direction although some released too close to shore became grounded on the coast nearby. Still, others were transited to offshore waters. In comparison, drifters released offshore generally showed movements to Georges bank and even to some drifters exhibited movements back inshore particularly in late summer. In a large estuarine system, all drifters were retained even over several tidal cycles.

Materials and Methods

Study Locations

Drifters were released in three separate locations: coastal (inshore) areas off New Hampshire (NH), offshore areas (2008-2009), and in an estuarine system (Great Bay, 2008-2009, 2011).

Coastal & Offshore

Coastal (inshore) locations for drifter deployments (~ 2-3 km from shore; Fig. 1) were near the mouth of the Piscataqua River (43°04 N; 70°42 W) and characterized by tidal influences and wind-driven currents. Offshore locations (8-15 km from shore) included an archipelago of islands, the Isles of Shoals (IOS) ~ 10 km from NH coast (42°59 N; 70°37 W; Fig. 1). The 20 m isobath was chosen as the boundary delineating inshore from offshore waters, to maintain consistency with previous studies of lobster movement in these areas (Scopel et al. 2009, Goldstein and Watson in prep., Chapter 1).

Great Bay Estuary

Drifters were also released in the Great Bay Estuary (GBE). The GBE is a large, tidally mixed, basin 15-25 km from the coast that comprises 23 km^2 of surface water and over 160 km of coastline. The GBE is linked to the ocean through the Piscataqua River

estuarine complex in New Hampshire and Maine, as well as through Little Bay (Brown and Arellano 1979, Fig. 1). The habitats of Great Bay and Little Bay are generally characterized by eelgrass beds, extensive mud flats, and oyster reefs (Short 1992), with freshwater input from several rivers that intermingle with tidal waters. Two locations with an abundance of egg-bearing lobsters were selected as drifter release sites (Langley et al. in prep.).

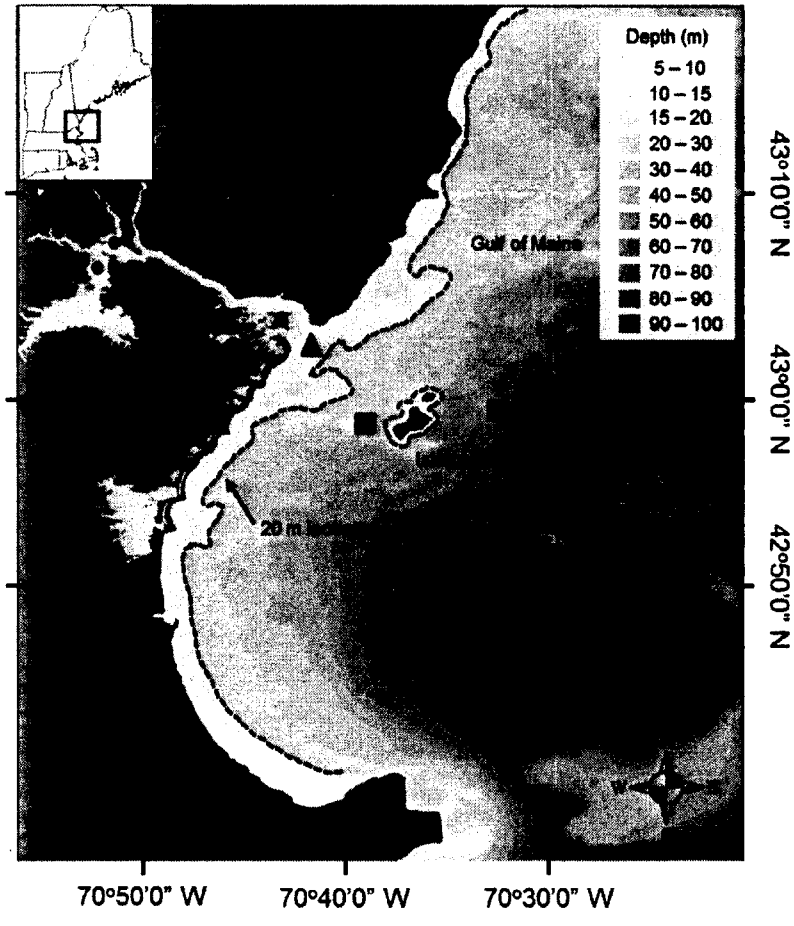

Fig. 1. Release areas for ocean drifters. Inshore (triangle, 2-3 km from shore) and offshore locations (square, 8-15 km from shore) were delineated by the 20 m isobath line (dashed line). Additional drifters were released in the Great Bay Estuary (circles) ~ 15-25 km from the inshore site (see text for detailed description). Drifters were released in all locations between 2008-2009. Additional drifters were also released in GBE in 2011.

Ovigerous Lobster Locations & Hatch Times

From 2006-2009 over 20 egg-bearing lobsters were fitted with ultrasonic tags and tracked using 1) hand-held and boat-towed hydrophones, and 2) fixed receiver stations (VR2s) in the Piscataqua River, NH seacoast (inshore) and around the IOS (offshore; > 30m depth). Additional information was provided by lobstermen who recaptured and reported information about tagged lobsters. These data were then used to determine the locations of ovigerous females throughout the year (Goldstein and Watson, in prep., Chapter 1). In addition, a series of lab-based studies generated empirical data on egg development that were then used to predicted hatch time (Goldstein and Watson submitted, Chapter 2). The combined data were then used to determine when and where most larvae hatched in NH coastal and estuarine waters.

Drifter Designs

Rachel drifters were modeled after the traditional 'Davis drifter' design widely used by physical oceanographers (Davis 1985). All components of the 'Rachel' drifters were designed to be below the waterline (~ 1 m) except for a small portion that contained the GPS unit and surface floats (Fig. 2). Drifters were constructed using a variety of materials including PVC for the main frame, a sash weight mounted at the bottom and foam floats at all four corners to maintain stability and neutral buoyancy.

A total of four sails were fabricated out of vinyl sail material and fastened along a series of fiberglass rods held together with stainless hardware (see Appendix D for details). Additional modifications to some of the drifters included a flashing light beacon (Guardian LED, Essential Gear Inc., Greenfield, MA) and reflective tape. A GPS mounted-unit (AXXON Tracker MMT, www.globalstart.com), contained in a clear water-proof case (Pelican model 1040, Pelican Products, Torrance, CA), was used to obtain regular positions in tandem with a GLOBALSTAT satellite communications system (accurate to within 300 m) at regular intervals, typically 30 minutes to 1 hour.

A second type of drifter used in this study was the 'Paul' drifter, commonly used to model tidal currents and wind-driven transport in confined waterways (bays and estuaries); this design has a more compact profile than its oceanic counterpart (Fig. 2). Each unit (modified 18 L bucket) contained a top-mounted GIS unit and a light beacon. This inverted bucket design was modified with floats and weights as ballast to achieve neutral buoyancy just below the surface. Full design details and building schematics for both drifter designs are available at: http://www.nefsc.noaa.gov/drifter/ and in Goldstein (Appendix D).

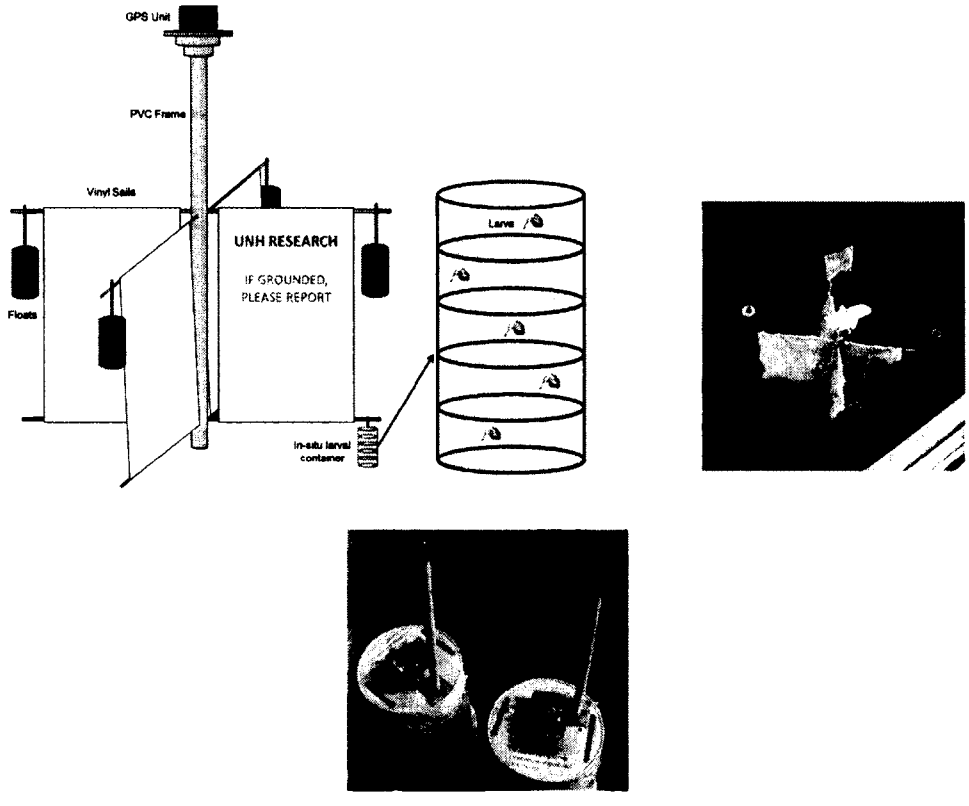

Fig. 2. Drifter units constructed and deployed in this study. (Top): A surface ocean 'Rachel' drifter standing design with 1.5 m PVC pole, sails, floats, and surface flag. Secured to some drifters were larval containment devices used to assess the final stage and survivorship of lobster larvae exposed to ambient seawater conditions during the residence time in some drifters. Inset: *In situ* deployment of Rachel drifter; GPS unit and flag are the only components that remain above the waterline. (Bottom): 'Paul' bucket drifters for use in estuarine trials. Each unit (modified inverted 18 L bucket) contained a GIS unit, and light beacon. This drifter was modified underneath with a series of floats and weights to achieve neutral buoyancy just below the surface.

Assays

Some drifters were fitted with additional components that were retrieved for analysis. Temperature loggers (n = 4) (HOBO temperature pendant loggers, model UA-002-64, Onset Computer Corp., Pocassett, MA) were fastened to the bottom pole to log subsurface water temperatures (~ 1 m) over the course of drifter residency to capture changes in seawater temperatures that would be experienced by larvae following the

same path. These loggers were downloaded on a PC-computer and software package (HOBOware Pro v. 3.0) to obtain average daily temperatures over the course of each drifter's spatial track.

In tandem with the temperature loggers, the same four drifters each carried a larval holding device containing five individual Stage I lobster larvae (n = 20 larvae total; Fig. 2). These devices provided a way to assess larval mortality and growth. The containment device was screened on both sides, allowing ample seawater and food exchange, and fastened to the bottom sail rod (Fig. 2). Upon retrieval of the drifters carrying these devices, larval activity (i.e., swimming and twitching movements) was assessed and animals were staged according to Hadley (1906). For each trial, we determined: 1) the proportion of larvae that survived; and 2) the number that was represented from each developmental Stage (1-4).

Time-lapse cameras (GardenCam, Brinno, Industry, CA) were mounted onto one inshore and one offshore drifter unit ~ 0.5 m from the top using metal hose clamps. This was designed to ascertain potential predators that might contribute to larval mortality while in the plankton. The camera was housed in a clear waterproof case (Pelican Products, Torrance, CA) and oriented to capture a 180° view. The camera captured a still digital photo every 30 sec. and stored these images on a 2 GB USB drive. Upon retrieval, images were downloaded and stitched together as a movie using iMovie 11 v. 9.0.4 software (Apple Computer, Co.). Observations of predators captured by camera were visually assessed and compared between the two drifter units over the first 24 hr.

Deployment Scenarios

A total of 15 'Rachel' drifters were deployed and tracked inshore (n = 8) and offshore (n = 7). These were launched at times when, and locations where, we predicted larvae would hatch (Fig. 2). An additional set of drifters was launched in the GBE (n = 4, 2008-2009; n = 4, 2011) in locations where most ovigerous females have been observed (see Appendix E for details). Drifters were tracked for 2-3 weeks (longer when possible), which is the typical duration of larval development in the field. In the GBE, drifters were released exclusively on outgoing tides. While many soon grounded due to the prevalence of shallow habitats, most drifter trials in the GBE continued through multiple tidal cycles and thus had several opportunities to leave the estuary.

Two additional types of drifter trials were conducted. First, we performed a preliminary calibration test (prior to the main drifter deployments) using two drifters at each of two separate inshore locations (n = 4). These tests were designed to determine if drifters released at the same location and at the same time would follow the same initial trajectory. These drifter pairs remained at large for 24-36 hours before retrieval. We analyzed their trajectories (as hourly compass headings in degrees) and compared these tracks using a Watson-Williams goodness-of-fit U-test (Oriana v. 3.0 software, Kovach Computing Services, UK). Paths did not differ significantly within each pair (Watson U-test; $U = 18.76$, $p > 0.05$). In the second trial, drifters (n = 4 total, two at each time period) were launched at times that were considered temporal extremes for larval hatch and did not coincide within predictable hatch times in our area of study. These releases

represented larval trajectories taken at inopportune hatching times (e.g., late fall and early spring; see Chapter 2). Drifter headings were compared at hourly intervals and early vs. late tracks were statistically compared using a Watson-Williams U-test to see if their mean angles differed. Comparisons of these two simulations indicated a significant difference between the two time periods (Watson U-test; $U = 1.662$, $p < 0.005$).

Data Analyses

Throughout the deployment of each drifter, positional (GPS, decimal degrees) data was received every 30-minutes (coastal and GBE) or 1 hour (offshore). Positional data were maintained and archived online using the AeroAstro database (Comtech AeroAstro, Inc., Ashburn, VA), and raw data from each drifter were downloaded and plotted using ArcGIS v. 9.3 software package (ESRI Inc., Redlands, CA) to map a trajectory. In some cases a series of filters were applied to those drifters that stalled or temporarily grounded themselves (as was the case in several estuarine drifters). Filters and corrected tracks were applied using a series of algorithms using MATLAB v. 7.1.3 (Natick, MA) (see Manning et al. 2009). The average continuous velocity for each drifter was also filtered using a series of looping routines and an average calculated for each group of drifters (inshore, offshore and estuarine) (Manning et al. 2009). For each drifter the days-at-large (DAL), total distance traveled (km), linear distance (km), and average velocity (cm/s) were calculated and compared between all three locations using non-parametric ANOVA (Kruskal-Wallis test).

Results

A total of 19 drifters were released and tracked in 2008-2009 in three general locations: inshore (n = 8), offshore (n = 7) and the Great Bay Estuary (GBE, n = 4). An additional four drifters were deployed in 2011 in the GBE (n_{total} = 8) for a total of 23 drifters for all years and locations (Table 1). Drifters traveled a combined total of 1,404 km (linear distance) and logged over 2,602 individual GPS positions. Drifters were released at times when eggs carried by ovigerous female lobsters were predicted to be hatching and followed for a time frame that included as much larval development time as possible. To meet these criteria, drifters were released in the GBE and inshore locations about a month earlier than in offshore areas. Because drifters released inshore and in the estuary grounded more often, offshore drifters spent more time at large compared with those deployed in the GBE (this was statistically valid) and their DAL were only significantly different from those drifters released in the GBE (ANOVA; Kruskal-Wallis test; KW = 13.34, p = 0.0013).

Likewise, linear distances between inshore and offshore drifters were not different from each other, but were both significantly different from those in the GBE (ANOVA; Kruskal-Wallis test; KW = 16.42, p = 0.0003). Drifter velocities between inshore and offshore drifters were not different but were both significantly different from those in GBE (ANOVA; Kruskal-Wallis test; KW = 18.42, p = 0.012).

	Inshore	Offshore	Great Bay Estuary
Drifters deployed (n)	8	7	8
Release period	Jun-Aug	Jul-Aug	Jun-Sep
DAL (avg)	13.5 ± 2.1	28.1 ± 7.5	5.8 ± 0.93
DAL (max)	26	68	10
Avg. mean speed (cm/s)	21 ± 12.1	18 ± 8.3	35 ± 18.7
Avg. total distance (km)	172.3 ± 95.8	485.3 ± 182.5	86.8 ± 16.4
Avg. linear distance (km)	26.1 ± 26.2	137.1 ± 48.0	6.8 ± 0.98

Table 1. Summary of all regular drifter deployments, June-August, 2008-2011 (n = 23 total). Days-at-large (DAL), average mean speeds (50 cm/s ~ 1 knot), and distance are given from the day of deployment to the last known position or active retrieval.

Inshore (coastal) Drifter Patterns

Eight drifters were released at inshore locations along the NH Seacoast between June and August. Most of these drifters moved parallel to the coast, in a south to southeasterly direction. However, those released too close to shore (e.g., mouth of the Piscataqua River), were carried partially up into the estuary, or onto a beach (Fig. 3). Some inshore drifters that were carried out to the IOS, were retained for 3-4 days in the vicinity of the IOS. One inshore drifter followed a counter-flow current northward, before taking a more southerly track back towards the IOS. Inshore drifters were at large for an average of 14 days and maintained an averge speed of 21 ± 12.1 cm/s (Table 1).

Fig. 3. Sample tracks of drifters released inshore (2008-2009) from June-August. Drifters (n = 6 pictured) were released (star) at locations where ovigerous lobsters were known to contain late-stage eggs (hatching events). Drifters exhibited a variety of movement patterns including transport southward along-shore, movements up into Portsmouth Harbor (NH), and offshore around the Isles of Shoals, IOS. Inshore drifters were at large for an average of 14 days (see Table 1).

Offshore Drifter Patterns

Seven drifters were released at offshore locations near the IOS (Fig. 4; stars indicate release locations). Some of these units (n = 3) moved in a southerly direction and approached the Massachusetts coastline after about 2-3 weeks. Other drifters (n = 3) continued in a more eastward pattern for ~ 1 month, eventually moving out to Georges

Bank (Figs. 4, 5). Offshore drifters were at large for an average of ~ 28 days and maintained an averge speed of 18 ± 8.3 cm/s (Table 1).

Fig. 4. Three sample tracks of drifters released offshore (2008-2009) from June-August of each year. Drifters were released at locations (stars) where ovigerous lobsters were known to contain late-stage eggs (hatching events). One drifter moved toward the coast of NH, another ended up along the coast of Massachusetts, and yet another moved to Georges Bank in about a month.

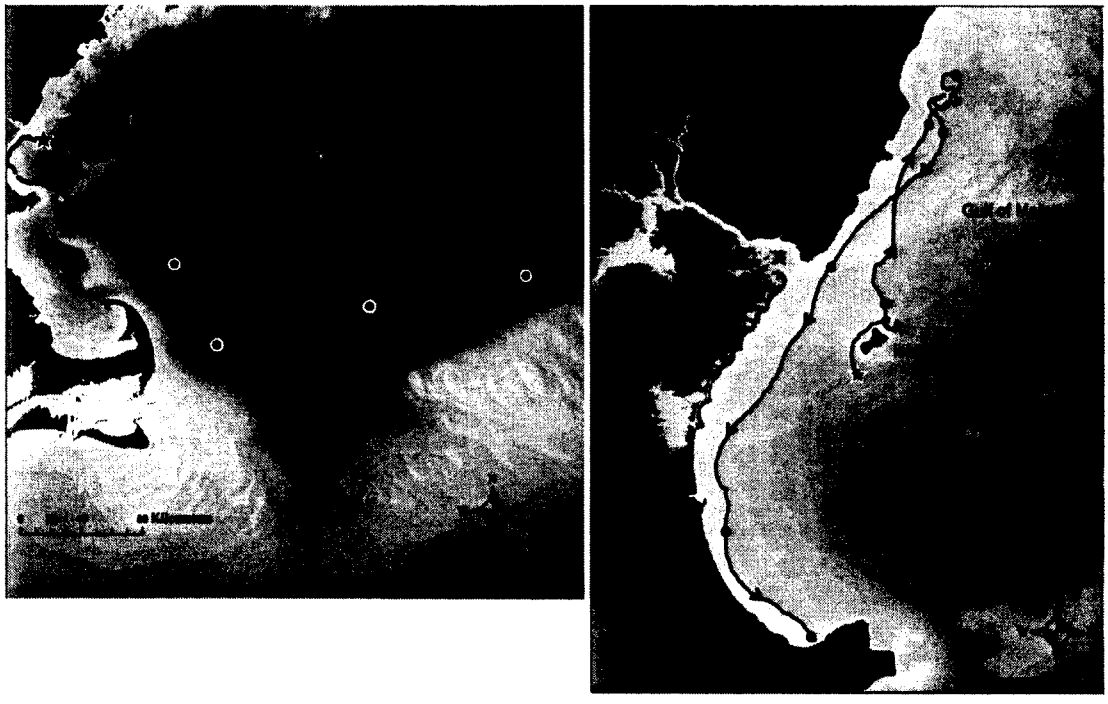

Fig. 5. Two other examples of offshore drifter tracks. (Left): This drifter was released (star) in July-09 (2008) near the IOS (offshore) at the same time that some ovigerous lobsters were hatching. It reached Georges Bank ~ 30 days later (Aug-7) and was recovered by an offshore fishing vessel. Dots indicate *weekly* locations. (Right): This drifter was also released near the IOS (June-18, black dots indicate *daily* locations). It was at large for 9 days, moving first in the northerly direction and then south, parallel to the coast. Eventualy it was grounded on Cape Ann, Massachusetts, near Gloucester.

Estuarine Drifter Patterns

Drifters released in the GBE were all retained within the estuary system (Little Bay, or the Piscataqua River; Fig. 6, Table 1). Estuarine drifters were at large for an average of ~ 6 days because they often ran aground due to the extensive shoal areas exposed during low tides. Due to the strong currents in the estuary, these estuarine drifters moved significantly faster than those released in coastal waters (35 ± 18.7 cm/s; Table 1). Interestingly, all of the drifters we released in Little Bay, near Goat Island (a very likely source of larvae in the estuary) remained within the GBE, even though they were released

on an outgoing tide. Even the drifters at large the longest (10 consecutive days for 2 drifters) maintained their positions in the GBE after more than 20 complete tidal cycles (Fig. 7).

Fig. 6. A compilation of positional fixes (colored dots indicate individual drifter units) for estuarine drifters released in each of two locations in GBE (stars; also see Fig. 1). Drifter residence times averaged 5.8 ± 0.93 days and included multiple tidal cycles; despite this, all drifters were retained in the GBE. This suggests that larvae are not exported out of the system and may settle and provide new recruits to this area.

Fig. 7. Spaghetti plots for two 'Paul' drifters released in Little Bay (GBE) in July, 2011. Both drifters were at-large over 10 days and were exposed to ~ 20 tidal cycles. This pattern was common in the other drifters we deployed regardless of the time we released them.

Assays

Temperatures & Larval Development

A total of three drifters (from the original four) were retrieved (one from each location, inshore, offshore, GBE) in 2009 with temperature data that could be downloaded. Average temperatures for each time period were as follows: 1) inshore (17.2 ± 0.31 °C, June 18 - 27); 2) offshore (19.6 ± 0.26 °C, July 16 - September 21; Fig. 8); and 3) the GBE (23.1 ± 0.25 °C, July 5 - 10).

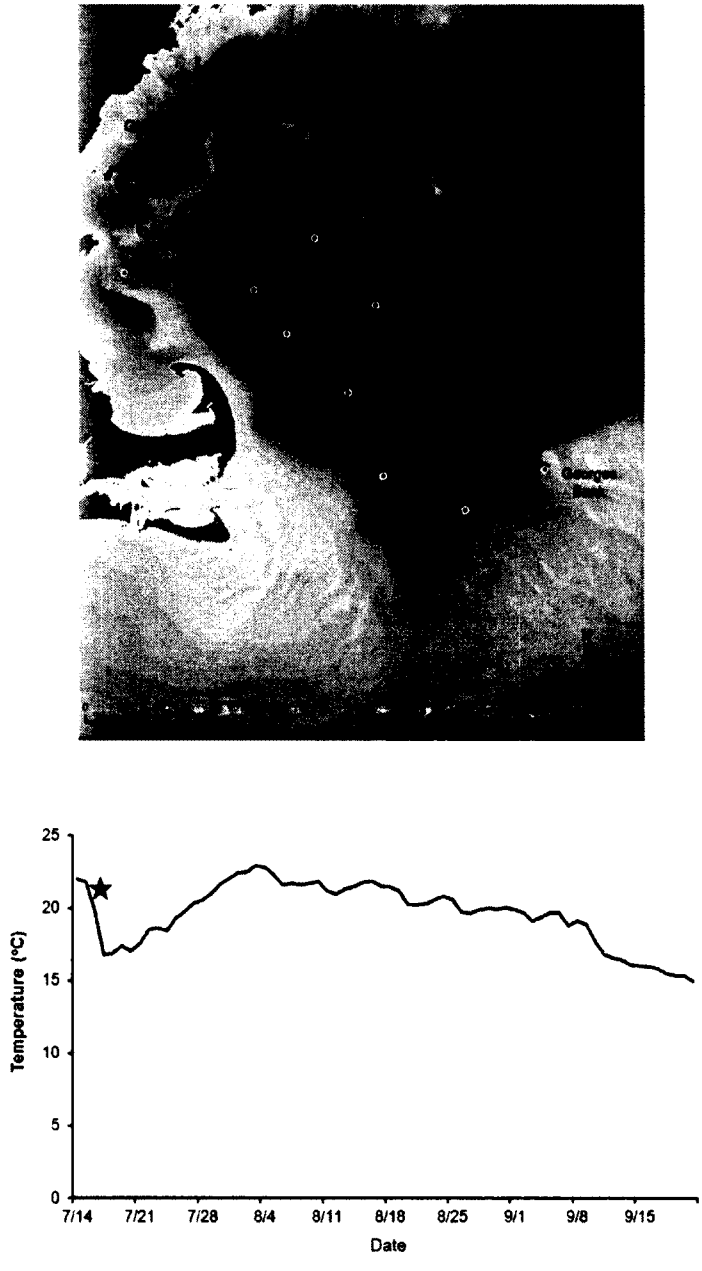

Fig. 8. (Top): Offshore drifter plot from Jul-16 (release date) through Sep-21, 2008 (closed circles indicate weekly positions). Drifter was released near the IOS and retrieved 68 days later by an offshore fishing vessel; this drifter track was similar to other offshore ones. (Bottom): Associated temperature profile (daily averages downloaded from *in situ* logger) for this drifter along its track. Temperatures averaged 19.6 ± 0.26 °C over the total track of this drifter.

A total of two larval collection devices (5 larvae/drifter) were successfully retrieved with live animals (one inshore and one offshore drifter). Larvae in the inshore drifter were at

large for ~ 12 days (July 5-17) and transported along the coast (avg. temp = 18.6 ± 0.75 °C), compared with those in the offshore drifter, that was at large for ~ 15 days and transported back inshore (avg. temp = 21.7 ± 0.50 °C). A total of three of the inshore larvae and two of the offshore larvae survived. All three larvae from the inshore drifter grew to developmental Stage III, compared with one Stage III and one Stage IV (postlarval) for the offshore larvae.

In situ Camera Assay

There were two kinds of predators that we were able readily identify from the camera video and almost always appeared in schools: Butterfish, *Poronotus tricanthus* and Atlantic striped bass, *Morone saxatilis*. These predator items for inshore drifters averaged 0.5 fish/hour (one every two hours) compared to 0.2 fish/hour (one every five hours) for the offshore drifter trial.

Discussion

The reproduction and movement dynamics in mobile marine decapods have profound effects on the location and timing in larval hatch and have been the subject of many empirical and theoretical studies (Thorrold et al. 2002, Pittman and McAlpine 2003, Carson et al. 2010). Those animals with planktotrophic larvae that are known to exhibit medium (1-2 weeks) to extended (> 3 weeks) pelagic larval durations are particularly vulnerable to the vagaries of physical oceanographic features (e.g., fronts, eddies, convergence zones). In addition, larvae are able to control their vertical and horizontal

positions thereby further altering their dispersal. In this study, we did not address these behavioral traits, instead, we were interested in the variability of the initial trajectories that ocean drifters would take given disparate locations (inshore vs. offshore) and times when ovigerous lobsters were spawning. Incze and Naime (2000) emphasize the critical importance in obtaining better resolution on the migrations and locations of females at the time of hatching in order '*to describe the average patterns of planktonic transport throughout the season*'. These kinds of empirical data are of paramount importance in further defining and manipulating existing and future biophysical models for the connectivity of *Homarus americanus* stocks in the Gulf of Maine (GoM) (Harding et al. 2005, Xue et al. 2008, Incze et al. 2010).

Our lobster tracking data has determined several locations where ovigerous females incubate their eggs; associated laboratory and field egg incubation trials have demonstrated when these eggs will hatch (Goldstein and Watson submitted). The next step was to determine where prevailing seasonal ocean currents carry larvae that hatch at these locations including specific times of the year. The observations and analysis of our tracks of drifters in this study that were released along coastal and offshore locations of New Hampshire in the southern GoM indicate that, at least initially, lobster larvae are exported to broad areas throughout the region that encompass southern coastal waters (primarily in Massachusetts), offshore locations (Georges Bank) and, rarely, northward towards Maine (Figs. 3, 4). In addition, drifters released in a large estuarine system were retained over a number of days and throughout multiple tidal cycles.

The drifters we used are designed so their movements are controlled by the currents in the top few meters of the water column where lobster larvae tend to reside throughout a large part of their development (Harding et al. 1987, Annis et al. 2007). Although lobsters are capable of exerting some degree of vertical control in the water column, most current larval transport studies consider the top 5 m for simulating their transport in the GoM (Wahle and Incze 1997, Incze and Naime 2000). The utilitarian application of such drifters is ideal in studying fundamental current patterns and such drifters have been used to track a variety of marine organisms with planktonic larvae including: green abalone, krill, polycheate worms, and lobster larvae (Leven 1983, Tegner and Butler 1985, Harding and Trites 1988, Thorpe et al. 2004).

Like other low-cost GPS-tracked drifters (e.g., George and Largier 1996, Austin and Atkinson 2004), the devices used in this study were capable of sampling a variety of water masses (over 10s of km) and operating in coastal, offshore, and estuarine systems. However drifters and drogues are limited in their ability to sample more than one circulation feature, and they are significantly larger than planktonic larvae and therefore do not experience the small-scale physical features (e.g., shear mixing) that would influence larval movements in the water column. One solution is the development of 'smart' drifters that are capable of adjusting their buoyancy to mimic larval behavior (Gawarkiewicz et al. 2007).

The drifters used in this study identified several important larval trajectories between inshore, offshore and estuarine waters and demonstrated patterns of dispersal that were

largely, in agreement with generalized circulation features of the GoM. For example, some drifters that were released inshore showed directed movements downstream along the coast, presumably a function of the GoM Coastal current. Some offshore drifters began in a similar fashion to those from inshore locations, but were entrained by cyclonic currents off Cape Cod and carried towards Georges Bank. Still, other drifters were retained near their release locations and were most likely subjected to tidally-influenced features (e.g., eddies). There is considerable variation in the circulation patterns of the GoM from year to year. Variations in temperature and volume of water flowing into the GoM (including freshwater input from rivers) along with atmospheric fluctuations (temperature and wind patterns) are all factors that significantly affect the scale and duration of GoM circulation features like water masses (different densities), gyres, and alongshore currents (Mountain and Manning 1994).

In particular, the role of cyclonic eddies seem to be especially effective at larval retention (Limouzy-Paris et al. 1997, Paris and Cowan 2004) and Largier (2003) asserts that nearshore larval drift is influenced more by eddy diffusion than advection. Several of our drifters were entrained in eddies over varying time periods and provides further support of cyclonic eddies in potential larval transport. Brooks (1994) showed that river plumes tend to form back eddies that can generate northeast flow in the nearshore areas of the GoM, but can be highly variable depending on river discharge periods. At least two drifters released close to the mouth of the Piscataqua River (inshore) were substantially influenced by back eddies and transported northward before returning to the same area a few days later (Fig. 5). Some drifter tracks also showed that even after being entrained in coastal or offshore current flows drifters could be detrained and move from inshore to

offshore waters (Fig. 3). However, for larval retention to occur, the temporal scales of the eddy must correspond to the length of larval development and the degree to which eddies truly retain larvae and contribute to self-recruitment (Sponaugle et al. 2002). We did not observe entrainment by drifters over a period long enough to correspond to the full course of larval development however, further drifter deployments could prove this.

Drifters released in the Great Bay Estuary (GBE) were all retained in the system even over extended periods (10 days) and throughout more than 20 tidal cycles (Fig. 7). Although larval transport and self-recruitment has been well studied in many estuarine species (e.g., crabs, barnacles, oysters), few studies of larval lobster transport and recruitment in estuarine systems have been conducted. Deep estuaries such as the GBE are characterized by two-layer circulation however strong tidal currents often mix the water column and break the existing stratification (Short 1992). Larvae that are capable of regulating their depth may utilize these currents to increase their retention in estuaries and thus exploit a wider range of areas. Although there are established lobster populations in the GBE (Watson et al. 1999), it is still unclear how seasonal movements by some of these lobsters (especially ovigerous females) may influence the degree to which larvae are retained in the estuary (Appendix D).

Islands (such as Isles of Shoals, IOS), feature circulation patterns and processes that limit larval dispersal away from an island source and include trapped eddies and other effects such as topographically steered currents (Paris and Cowen 2004). One of the best studies for the distribution and settlement of neustonic lobster postlarvae around islands was undertaken by Wahle and Incze (1997) in coastal Maine. This study determined that

wind-driven surface transport produces an asymmetric postlarval supply to the two sides of the island during the settlement season, favoring transport of postlarvae to the windward side. In addition, the topography of the island itself influences the retention of larvae in some areas more than others on a local scale. Wahle and Incze's results (1997) demonstrate the importance of wind-driven circulation to small-scale patterns of larval supply and benthic recruitment in *Homarus americanus* (Hudon and Fradette 1993) as well as other marine fishes and decapods (e.g., Roughgarden et al. 1988, Jones et al. 2009, Pringle et al. 2011). Because physical features and currents around the IOS influenced some of our drifters, we suspect that larvae in this area may be impacted by patterns of island-induced circulation.

Various sources and sinks have been suggested for lobster larvae (e.g., wind direction, nutrients, drift; Chassé and Miller 2010) in the GoM, although matching these with empirical data to predict hatch and settlement has yet to be fully determined. The most commonly used approach for quantifying larval transport are three-dimensional circulation models coupled with Lagrangian particle tracking algorithms (Werner et al. 2001); several key studies have looked at forecasting larval dispersal and settlement in the GoM. Incze and Naime (2000) reported on cross-shelf transport and the ability of larvae to utilize onshore sea breeze transport towards shore. Harding et al. (2005) showed that dispersal and retention on Georges and Browns Banks was possible based on measured wind fields (using drifters) and by measuring larval condition (lipid profiles). Recently, Xue et al. (2008) and Incze et al. (2010) identified sources and sinks for 15 coastal areas and modeled larval release and dispersal over a period of four months.

These models demonstrate cyclonic dispersal on a scale of 100s of kilometers and over a > 50-day drift. However, when daily larval mortality (values range; Chassé and Miller 2010) and realistic development times are factored into the model, residence time in the plankton is significantly less (20-30 days; Incze et al. 2010). In addition, it is now well known that larvae contain an extensive toolbox of behaviors, as well as morphological and physiological adaptations that allow them to overcome passive processes in large-scale ocean transport and exploit their environments. This is especially true in marine larval fishes (Jones et al. 2009) but has also been documented in one species of spiny lobster (*Panulirus argus*) where larval behavior (i.e., vertical migration) constrains the dispersal of even long-lived (> 4 months) larvae (phyllosomes), particularly in tandem with retentive oceanographic environments (Butler et al. 2011).

A variety of sources (e.g., predation, food quality; Morgan 1995) contribute to mortality in the field although being able to obtain accurate measurements remains challenging. Predation in the plankton may be a major cause of mortality and a primary factor controlling the ultimate survival of postlarvae. Established predators of postlarval lobsters include Cunner (*Tautogolabrus adspersus*) and Scombrid fishes (e.g., *Scomberesox saurus*) (Ennis 1995, Harding unpub. data). We found two dominant species of fishes in our predation survey: Butterfish (*Poronotus tricanthus*) and striped bass (*Morone saxatilis*). However, our results only indicate the presence of such potential predators since we did not confirm predation on actual larval lobsters and the presence of such fishes could be confounded with the presence of structure (drifter) that is known to attract fish. While predation rates on Stage I larvae are assumed to be the

highest (Chassé and Miller 2010), there is little direct laboratory or field evidence that addresses the causes of stage-specific predation over the full duration of their PLD.

Our study was not designed to model the dispersal and settlement of larvae over large regions and did not account for changes in larval dispersal over a vertical gradient of the water column. However, we did demonstrate that larvae were capable of completing the majority of their development over a period of ~ 10 days when they resided within a few meters of the surface. Larval development is not just affected by temperature but by a host of other factors (e.g., egg quality, food availability, genetics; Annis et al. 2007, Appendix A) that may protract or curtail PLD. Further, larval development times in the laboratory (Templeman 1936, MacKenzie 1988) are thought to over-estimate larval duration and studies that have been conducted *in situ* suggest development times in the field are significantly less (e.g., Annis et al. 2007).

While ocean currents and winds strongly influence their movements, lobster larvae (especially Stage IV, postlarvae) are also strong swimmers and exercise control over their ultimate settlement location (Ennis 1995). Several recent studies have attempted to estimate the pattern of recruitment of lobsters in the GoM by conducting broad trawl surveys of the locations of ovigerous females, (Incze and Naimie 2000, Incze et al. 2010, Chassé and Miller 2010). These studies provide a great deal of insight concerning the extent to which certain populations provide recruits for other areas of the fishery. However, the accuracy of these models depends a great deal on two variables that were investigated in our study: 1) the location of ovigerous females while they are carrying

late-stage eggs (Chapter 1) and; 2) the accuracy of laboratory models concerning the influence of temperature on egg development and hatching (Chapter 2). Most of the aforementioned studies are not currently adapted for the intricacies of local sources of ovigerous lobsters and their initial larval dispersal. Our goal was to ascertain how the timing of hatch in disparate locations affects larval dispersal by using drifter trajectories as a proxy.

Coupled biophysical models require well-grounded biological inputs (e.g., hatch location, PLD, mortality) as well as data sets with which to evaluate model performance. Our findings suggest that hatching farther inshore favors retention along the coast (via alongshore currents), and that seasonal movements of lobsters may vary sufficiently to cause differences. Furthermore, results from our offshore drifters suggest that larvae hatching in these locations are exposed to a wider array of currents that may offer a transport advantage in finding the best possibilities for settlement. However, comprehensive biophysical models of larval transport throughout the GoM (Xue et al. 2008, Incze et al. 2010) show that the predominant direction of larval transport (from all simulated hatching locations) is southwest and follows the cyclonic coastal current system. However, within-year and inter-annual variations substantially modify these expectations. These studies suggest that dispersal patterns would be further modified by spatial and temporal differences in hatching patterns. Finally, there is a significant amount of retention in most zones, indicating considerable potential for local recruitment in populations.

This study, along with existing biophysical coupled models, suggests that future work is needed to further illuminate the transport of lobster larvae in the GoM. First, additional studies will need to further address the transport of larvae closer to the mainland, taking into consideration a variety of topographic elements (bays, headlands, islands) that will inevitably influence larval dispersal and behavior. Second, further documentation of the distribution of lobsters hatching over a variety of depths, including deep offshore waters, will be beneficial to modeling larval dispersal over both large- and small-scale circulation features. We considered the release of our drifters during times and locations of hatch (Chapters 1 & 2) and over two general depth regimes, inshore (< 20 m) and offshore (> 20 m). However, data for lobsters that hatch in depths > 100 m is unknown, but we suspect that greater depths may impact the dispersal and transport of larvae to various regions. Finally, the ability to document (and model) the origins of larval hatch is vitally important: '*Migration and locations of females at the time of hatching remain critically important questions.*' (Incze and Naime 2000).

Our study assessed the fate of lobster larvae hatching in known locations due to the effects of hydrodynamic features (10s of km) coupled with temperature-based larval development. Modeling these attributes using individually-based models (IBMs) for lobsters in the GoM has since shown that a combination of outcomes are possible including downstream dispersal to adjacent areas, some long-distance dispersal, and local retention of larvae (Incze et al. 2010). Superimposed on these patterns are the potential changes to the GoM circulation regime and the timing of biological events due to climate

change, which would undoubtedly impact patterns of larval dispersal and subsequent settlement (Nye 2010, Appendix E).

Therefore, the use of ocean drifters, released at biologically relevant temporal and spatial scales, provides at least initial estimates of the dispersal, location, and destinations for larvae. Applying these biological correlates to other ongoing and future modeling studies will help to clarify the marine connectivity of lobster in the GoM and provide data for the future management of this important marine species.

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APPENDIX A

BIOCHEMICAL CHANGES THROUGHOUT EARLY AND MIDDLE STAGES OF EMBRYOGENESIS IN LOBSTERS (*HOMARUS AMERICANUS*) UNDER THREE THERMAL REGMES

Abstract

Most marine crustacean eggs contain a full complement of nutritional resources that fuel the growth and metabolic processes over the course of their development. In terms of biochemical constituents, lipids and proteins play pivotal and central roles in these processes and, accordingly, have been studied extensively in crustaceans. Given the propensity of some ovigerous (egg-bearing) American lobsters (*Homarus americanus*) to undergo seasonal inshore-to-offshore migrations, thereby exposing their eggs to varying thermal regimes, this study's goal was to assess egg quality over their course of development by documenting changes in total lipids, proteins, and egg size (volume) in lobsters subjected to one of three simulated thermal regimes (inshore, offshore, constant (12 °C), n = 5 / trt, 15 total) in the laboratory and sampled at five discrete time intervals. Total egg lipids showed a marked decrease over time ($r^2_{adj} = 0.85$, $p < 0.0001$), early in the fall (average = -26 %) and late spring (-62 %), compared with stark increases in proteins over the same period ($r^2_{adj} = 0.63$, $p < 0.0001$, averages = 60 %, 34 %, fall and spring). Although there were no significant differences in total lipid or protein values (or egg sizes) between eggs exposed to inshore and offshore temperatures ($p > 0.05$), differences occurred in eggs exposed to a constant temperature, and they hatched almost

three months sooner than inshore or offshore ones. Seasonal temperature fluctuations also appear to control the rates of biochemical processes in lobster eggs but may be confounded by other variables.

Introduction

Egg development for most marine crustaceans relies heavily on the production and sequestering of nutrients required for the development and maintenance over the entire process of embryogenesis. In terms of biochemical constituents, both lipids and proteins play pivotal and central roles throughout development, and, as a result, have been studied extensively in both crustaceans and fishes alike (Fraser 1989, Jaeckle 1995, Rosa et al. 2007). Lipids comprise the structural integrity of most cells and are responsible for the overall metabolism of growing crustacean embryos. Remarkably, these constituents have been reported to account for upwards of 60 % of the total energy expenditure for growth (Holland 1978, Amsler and George 1984). By contrast, the role of proteins as the basic building blocks of animal tissues are well known (Holland 1978), and function as alternative energy sources under certain conditions (Schmidt-Nielsen 1991, Heras et al. 2000).

Egg development in crustaceans is especially linked to temperature such that incubation periods can be extended (cold temps) or reduced (warm temps). Closely coupled metabolic rates increase with temperature thereby modulating yolk absorption, growth and ultimately, the survival of eggs (Pandian 1970, Schmidt-Nielsen 1991).

Development and metamorphosis of planktotrophic larvae, including decapod crustaceans, depends to a great extent on nutrition (Racotta and Ibarra 2003) from both exogenous (from feeding) and endogenous (yolk reserves) sources which are important metrics during early postembryonic development (Sasaki et al. 1986, Clarke et al. 1990). Together, the relationship between the primary biochemical components in crustacean eggs and their associated variability are considered central to the early-life history patterns for these organisms (Vance 1973, Jaeckle 1995).

This is especially true for American lobsters, *Homarus americanus* H. Milne-Edwards, 1837, characterized as large, highly mobile decapods whose habitats include coastal and continental shelf waters, bays and estuaries from Labrador, Canada to Cape Hatteras, U.S. (Fogarty 1995). Because the American lobster fishery garners such tremendous economic influence, fisheries scientists and managers focus much of their attention on many aspects of stock assessment including the fecundity, spawning stock biomass, and abundance of egg-bearing (ovigerous) females that are historically protected from being landed (ASMFC 2009). The life history of *H. americanus* includes a complex suite of embryonic, pelagic (larval), and benthic (juvenile and adult) developmental stages (see review in Lawton and Lavalli 1995), most notably, their yolk-laden eggs that are extruded and carried for 9-11 months over the full course of their development (Talbot and Helluy 1995); temperature is a key factor that determines the length of time the eggs are carried (Templeman 1940, Aiken and Waddy 1980). Mature lobster oocytes are large (1.4-1.6 mm diameter upon extrusion) and typically contain large amounts of high-density lipoproteins (> 40 %, lipovitellins) that are allocated as yolk material through a

complex suite of primary and secondary vitellogenesis (Nelson et al. 1988, Talbot and Helluy 1995).

Besides the often protracted egg development in *H. americanus*, one of the most interesting and sometimes dramatic features of some ovigerous lobsters is their propensity to migrate seasonally over an array of habitat types (including thermal ones) and distances (typically, 5-10 km, but sometimes much greater) throughout the development of their eggs (see reviews by Cooper and Uzmann 1980, Lawton and Lavalli 1995). The implications of such movement events in ovigerous lobsters has the potential to shape the developmental dynamics of the eggs they carry by subjecting them to differing thermal regimes whose rates of change can be quite different (Campbell and Stasko 1986, Cowan et al. 2006, Goldstein Chapter 2). For example, ovigerous lobsters subjected to inshore thermal regimes in the lab exhibited more rapid egg development and hatched sooner than their offshore counterparts (Goldstein Chapter 2). Therefore, the seasonal movements of ovigerous lobsters to thermally disparate waters may be strategies to both enhance egg development and the survival of larvae in the plankton.

Biochemical and energetics considerations in lobster eggs have been well studied and suggest the following key patterns: 1) differing thermal regimes influence the utilization of energy reserves in developing embryos and embryos raised at accelerated temperatures contain residual yolk reserves upon hatch (Sasaki et al. 1986); 2) the energy content of eggs tend to increase with female size (Attard and Hudon 1987); and 3) larval size at

hatch is independent of female size (Ouellet and Plante 2004). Despite some contradictory evidence between some of these studies, it is evident that egg resources influence their growth and development.

Although optimal temperatures for lobster egg growth are not fully known, naturally fluctuating temperatures result in disparate growth patterns and subsequently, differing hatch times (Sibert et al. 2004, Goldstein Chapter 2). In general, crustacean eggs subjected to either prolonged warm or cold temperatures can have a deleterious effect on the use of their yolk reserves (Garcia-Guerrero et al. 2003, Manush et al. 2006), and it has been suggested that prolonged cold temperatures (< 4 °C) negatively affect egg development in *H. americanus* (Waddy and Aiken 1995). Therefore, one way of assessing the effects of temperature on the overall development of lobster eggs is through the proximate analysis of their biochemical components, namely, lipids and proteins.

The goal of this study was to further elucidate the effects of lobster movements over varying thermal regimes (inshore and offshore) during the course of egg development, complementing existing work on egg development and hatch under differing thermal regimes in the laboratory, by quantifying two key biochemical descriptors (lipids and proteins) of egg resource utilization as well as changes in egg size. A constant, slightly elevated temperature was also used to compare egg development under non-fluctuating thermal conditions.

Materials and Methods

Lobster Source and Egg Assessment

Egg-bearing (ovigerous) lobsters were collected in late August and early September (2006) along the New Hampshire (NH) seacoast near Rye, NH and Gunboat Shoals (43°.0274 N; 70°.6938 W) by permitted commercial lobstermen using standard baited traps. Lobsters were transported to the University of New Hampshire (UNH) Coastal Marine Laboratory in Newcastle, NH and initially held in a large 1,200 L fiberglass tanks with shelters. Tanks were exposed to ambient light and sand-filtered seawater (average temp = 15.3 ± 0.5°C), and lobsters were fed a combination of fresh squid and crabs (*Cancer spp.*), twice weekly.

A subset of the eggs in each clutch were viewed under a dissecting scope and staged according to the methods outlined by Helluy and Beltz (1991). These samples also served as covariates for all subsequent statistical analyses. Only lobsters whose eye index was less than 18 % were used for this study (Perkins 1972, Chapter 2) in order to encompass as much of the early development process as possible. Lobster carapace lengths (CL) were measured to the nearest 1 mm using digital calipers (Mitutoyo IP 65, Mitutoyo Corp., Japan). A single, circular, laminated disc tag (diameter = 2.0 cm, Floy Tag Inc., Seattle, WA) was fastened to the claw knuckle of each animal for individual identification throughout the duration of the study.

Thermal Treatments and Sampling

The experimental setup and thermal treatments followed a companion study that served to concurrently quantify lobster egg development and hatch time in the same group of lobsters (see Goldstein Chapter 2). Briefly, a series of four 0.91 m diameter (600 L) tanks (2 tanks / treatment) were used to simulate either inshore, offshore, or constant (12 ± 0.4 °C) temperature regimes on a year-round basis (Fig. 1). For purposes of this study, inshore locations (shallow and coastal) were considered the same areas where animals were collected (2-5 km from shore, 8-10 m depth), while offshore ones were designated as 12-20 km from shore (20-30 m depth) to simulate those lobsters that might make seasonal, fall migrations offshore (see Chapter 1 methods). Constant temperatures were chosen to simulate a favorable growth temperature similar to eggs observed in Mackenzie (1988). Temperatures in all tanks were logged automatically every 30-minutes using HOBO pendant loggers (model UA-002-64, Onset Computer, Bourne, MA) and later downloaded into Microsoft Excel using Hoboware software (HOBOware Pro v. 3.0). Temperature profiles from the offshore tank treatment were adjusted semi-regularly to simulate seasonal temperature changes in the field and monitored from historical and real time data published on the Gulf of Maine Ocean Observing Systen (GOMOOS, www.gomoos.org; also see Chapter 2). A subset of five ovigerous females were sampled at each temperature treatment for a total of 15 lobsters. All lobsters were sampled for eggs at five discrete time periods: twice in the fall and spring (during periods of rapid growth; Sibert et al. 2004) and once in the winter.

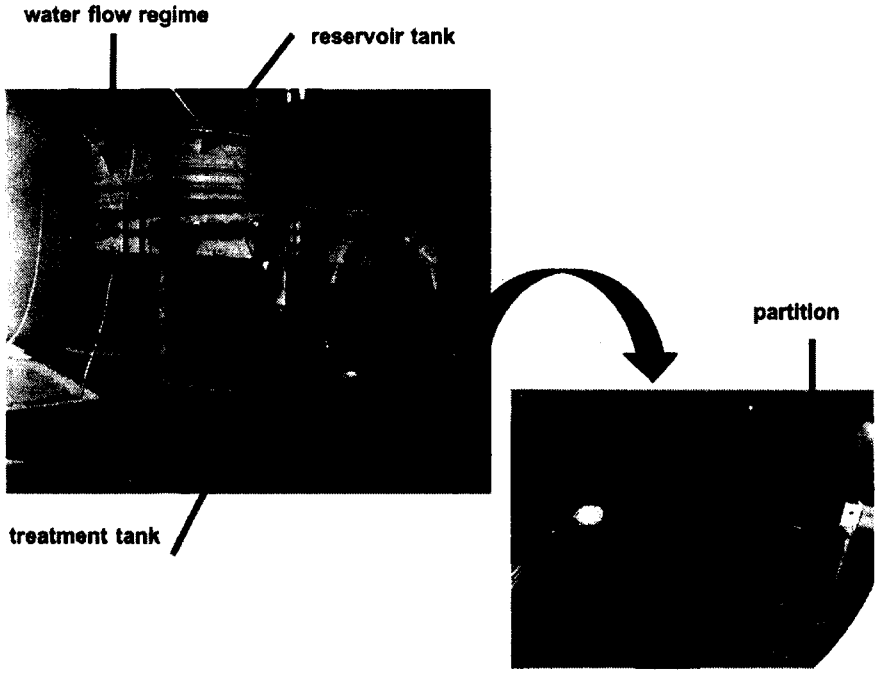

Fig. 1. Tank design for exposing lobsters to simulated inshore, offshore and constant temperature regimes. Inshore tanks received ambient seawater while offshore and constant tank treatments were manipulated using a series of heaters and chiller units (see Goldstein Chapter 2, for details). All tanks were maintained on a seasonal photoperiod using programmable timers. Tanks were partitioned to hold individual lobsters (n = 5 / tank).

Lobster eggs (~ 100 / sample) were removed from the center of each clutch with a pair of fine forceps and placed in labeled plastic sample trays. All egg samples were rinsed and gently agitated with a 0.5 % sodium hypochlorite and distilled water solution for ~ 1 min., after which they were rinsed with 100 % distilled water and blotted dry to remove the cement matrix holding the eggs together (P. Talbot pers. comm.). Rather than mechanically separate eggs, this technique was chosen for its efficacy. Preliminary studies that were conducted indicated that this chemical separation technique was non-invasive and did not compromise the biochemical integrity of the egg due to their complex and thickened membranes (Johnson et al. 2011).

For biochemical analyses, egg samples (~ 30 / sample) were frozen at -80 °C prior to processing and freeze-dried at -40 °C for 24 hr (Labconco Freeze Dryer 5, Kansas City, MO). Dried egg samples were then ground down into a fine power using an industrial-grade milling machine (Wiley Mill #4, 40 μm mesh screen, Thomas Scientific, Swedesboro, NJ) and samples were stored in labeled polyethelene scintillation storage vials for subsequent analyses (Fig. 2).

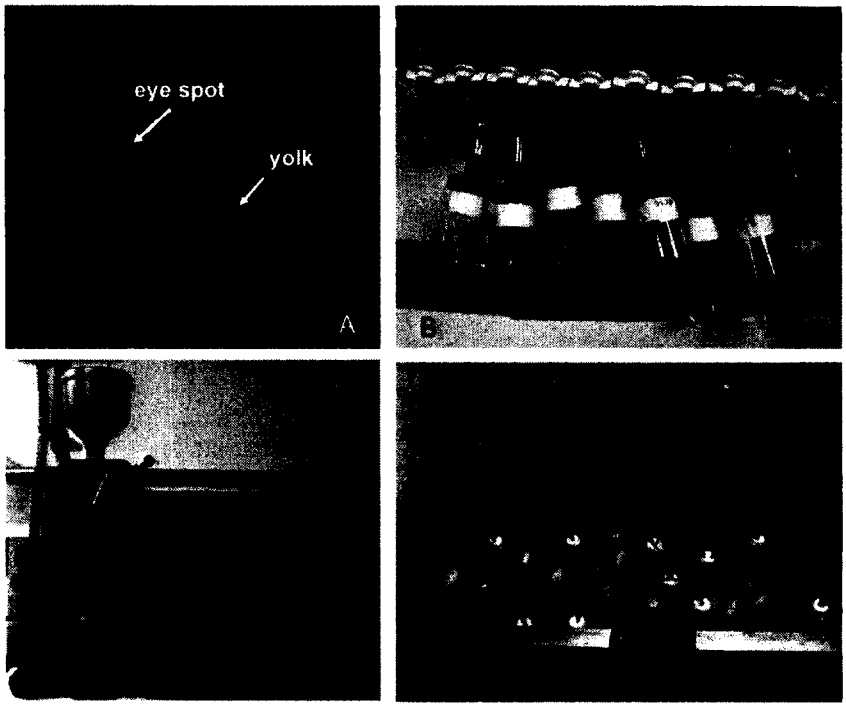

Fig. 2. Overview of methods used for some lobster egg analyses: A) image of lobster egg depicting eyespot and yolk mass; B) freeze-drying egg samples in preparation for biochemical analysis; C) grinding and milling egg samples after freeze-drying and; D) lipid extraction of egg samples using a shaker tray and water bath (also see Goldstein Appendix B).

Biochemical Analyses

Over each sampling interval, a total of three replicate egg samples/female were pooled for lipid and protein values. Total protein levels were determined using a modified Lowry method (Lowry et al. 1951) using a BioRad protein assay kit with Coomassie

Brilliant Blue G-250 (reagent) and bovine serum albumin as a standard (Biorad Laboratories, Hercules, CA). Egg samples were digested in 1N NaOH, filtered and read on a spectrophotometer (Beckman DU-250; λ= 595). Total lipid was quantified gravimetrically using the general protocol detailed in Bligh and Dyer (1959). The procedure was modified in a ratio of 1:2:2.5 chloroform-methanol-water extraction, respectively. Samples were dried for 24 hr. at 37 °C and stored in a glass dessicator, before being weighed on an analytic balance (Fig. 2). Detailed protocols for both total lipids and proteins can be found in Appendix B.

Egg Volumes

For calculating egg volumes, 10-15 eggs were removed at each of the aforementioned five time periods and placed in plastic 2.0 mL storage tubes, preserved in a 4 % formalin and sterile seawater solution and stored at 4 °C. For each egg, a digital picture was taken under a dissecting microsope (Nikon SMZ-2T, Nikon USA Inc., Melville, NY) using a scope-mounted Nikon Coolpix 995 digital camera. All egg images were imported into an image processing software (Image J v.1.35, see http://rsb.info.nih.gov/ij/) and a digital measuring tool was used to make calculations of each egg's longest length. All calculations were measured to the nearest 0.01 mm (then converted to μm) and values for each sample were averaged (± se). Egg volumes were then calculated using the formula: $V = 4/3*(\pi r^3)$, where r is the radius for spheroid-shaped embryos (García-Guerrero and Hendrickx 2004).

Data Analysis

Analysis of variance (ANOVA) was used to investigate potential differences in egg protein and lipid content between the three thermal regimes (fixed factor 1) at each of the five sampling intervals (fixed factor 2). A 3x5 full factorial design was used and analyzed as a split-plot (SP) ANOVA (whole-plot = temperature, sub-plot = month, df_{total} = 15) using a PROC MIXED model in SAS v. 9.3 (SAS Institute Inc., Cary, NC). Differences between groups were compared using the PDIFF function in SAS. Regression analyses were carried out using JMP v. 9.3 (SAS Institute Inc., Cary, NC) statistical software. All means are expressed ± se.

Results

Water Temperatures

Seawater temperatures over the course of this study (October-May) averaged 7.1 ± 0.24 °C (range = 2.1-11.2) for inshore laboratory simulations, compared with 6.0 ± 0.19 °C (range = 2.8-10.1) for the offshore thermal regime, and 12.2 ± 0.21 °C for the constant treatment tank (see Chapter 2). There was an overall significant difference in water temperatures between the constant tank treatment and both inshore and offshore ones (ANOVA; $F_{2,7} = 10.32$, $p < 0.0001$) but not between inshore and offshore.

Lipid and Protein Content

Total egg lipid levels from inshore and offshore thermal regimes were very different from their constant temperature counterpart (SPANOVA; $F_{2,44} = 10.3$, $p = 0.0002$) and also differed by month ($F_{4,44} = 302.9$, $p < 0.0001$; Fig. 3). Likewise, total protein levels in lobster eggs between inshore and offshore thermal regimes also differed from eggs exposed to constant temperatures (SPANOVA; $F_{2,44} = 67.17$, $p = 0.0002$) as well as by month ($F_{4,44} = 350.3$, $p < 0.0001$, Fig. 3). The interactive effect of temperature and month was significant for both lipid ($F_{7,44} = 2.27$, $p < 0.045$) and protein levels ($F_{7,44} = 46.5$, $p < 0.0001$) and are summarized in Tables 1 & 2.

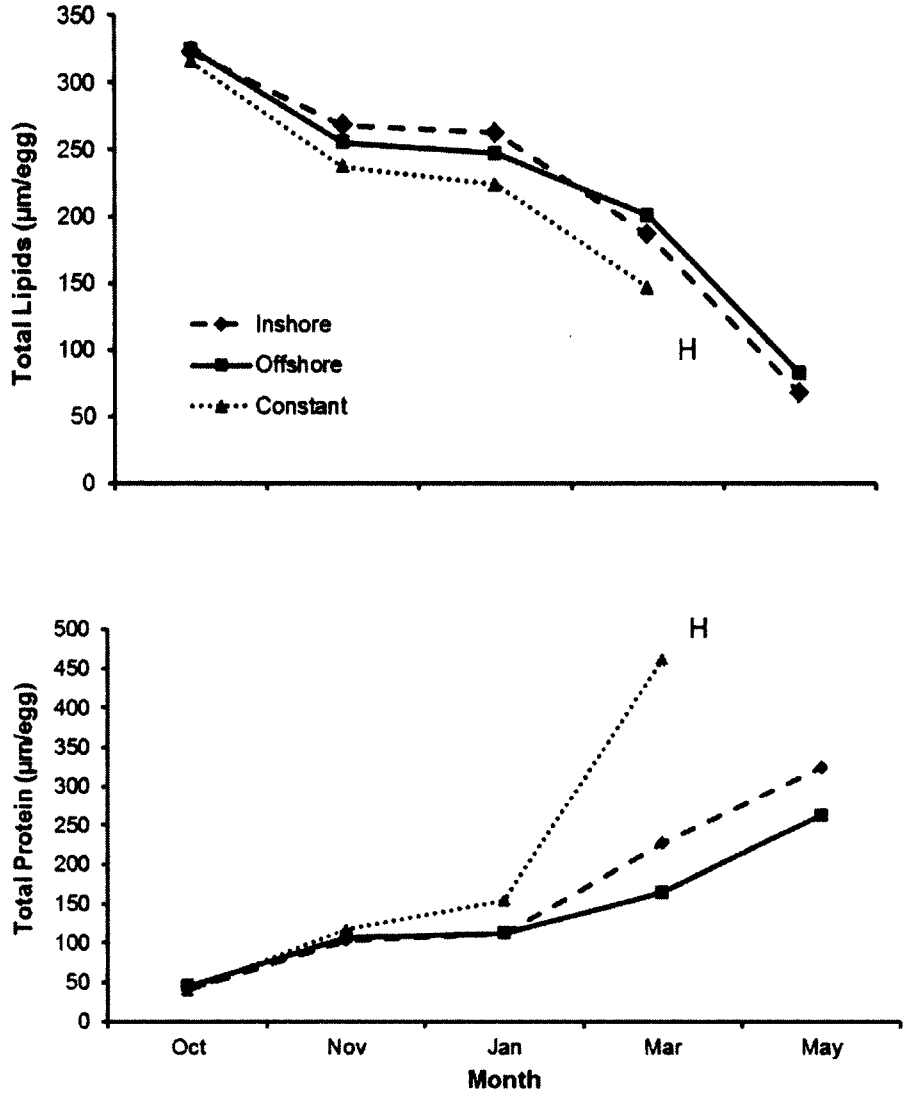

Fig. 3. Change in lipids (top) and protein (bottom) levels through the course of seven months of egg development for all lobsters sampled (n = 5 / trt). Lobsters subjected to inshore and offshore thermal treatments did not hatch their eggs until after May, unlike eggs from the constant treatment, where eggs hatched (H) in April. Points for each treatment represent the means for each treatment group, standard errors are shown in Table 2, below.

	Lipids				
	October	November	January	March	May
Inshore	322.2 ± 7.5	268.2 ± 9.6	262.6 ± 12.2	186.4 ± 7.3	67.6 ± 3.6
Offshore	324.6 ± 7.4	255.2 ± 11.7	247.0 ± 12.5	200.8 ± 3.8	82.4 ± 7.3
Constant	315.7 ± 8.7	237.3 ± 4.8	224.0 ± 7.2	146.4 ± 12	

	Proteins				
	October	November	January	March	May
Inshore	322.2 ± 7.5	268.2 ± 9.6	262.6 ± 12.2	186.4 ± 7.3	67.6 ± 3.6
Offshore	324.6 ± 7.4	255.2 ± 11.7	247.0 ± 12.5	200.8 ± 3.8	82.4 ± 7.3
Constant	315.7 ± 8.7	237.3 ± 4.8	224.0 ± 7.2	146.4 ± 12	

	Post-hoc PDIFF Results ($\alpha = 0.05$)		
Treatment group:	Constant[a]	Inshore[b]	Offshore[b]

Table 1.
A summary of means (± se) for lobster egg total lipids and total proteins over five months. Post-hoc differences (from SAS) for both variables are given below; groups with different superscripts denote treatment differences ($p < 0.001$).

Treatment	October	November	January	March	May
inshore * offshore	0.85	0.30	0.21	0.25	0.24
inshore * constant	0.89	**0.03**	**0.002**	**0.002**	-
constant * offshore	0.72	0.22	**0.04**	**< 0.0001**	-

Table 2.
Pairwise comparisons between temperature treatment and month for both lipids and protein values. Shaded p-values (< 0.05) denote significant differences between temperatures for a specific month.

Overall egg lipid values showed a marked decrease over time (equation: lipids = 381.76 - 55.00*month, $r^2_{adj} = 0.85$, $p < 0.0001$; Fig. 4), falling most dramatically early in the fall (-16.8 % inshore, -21.4 % offshore, -24.8 % constant) and late spring (-63.7 % inshore, -59.0 % offshore). By contrast, total lobster egg protein values increased over the same time frame (equation: proteins = -35.53 + 69.11*month, $r^2_{adj} = 0.63$, $p < 0.0001$; Fig. 4), but exhibited large increases in the fall (60.4 % inshore, 57.7 % offshore, 66.5 % constant) and spring (30.1 % inshore, 37.1 % offshore) and much more modest ones in the winter, typically 10-15 %.

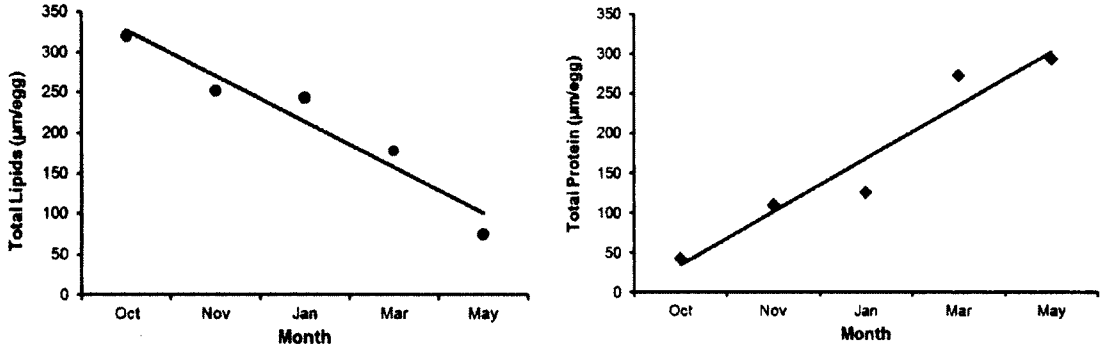

Fig. 4. Relationship between lipids (left) and protein (right) over the course of seven months of egg development for all lobsters sampled (n = 5 / trt). Total lobster egg lipid values showed a marked decrease over time (equation: lipids = 381.76 - 55.00*month, $r^2_{adj} = 0.85$, $p < 0.0001$). By contrast, total lobster egg protein values increased over the same time frame (equation: proteins = -35.53 + 69.11*month, $r^2_{adj} = 0.63$, $p < 0.0001$).

Egg Volumes

Overall, there was a significant increase in egg volume over time for all eggs over all treatments ($r^2_{adj} = 0.413$, $p < 0.001$). Although there were no significant changes with respect to egg volume by treatment ($F = 0.73$, $df = 2$, $p = 0.513$) (overall means: inshore = 3226 ± 163 μm^3, offshore = 3254 ± 167 μm^3, constant = 3476 ± 152 μm^3) differences from month-to-month did exist ($F = 2.25$, $df = 3$, $p < 0.001$; Fig. 5). Gains in egg volume (for all treatments) accounted for ~ 52 % between September and February, although there was a slight decrease (-13.5 %) in egg volume for the constant treatment between November and January.

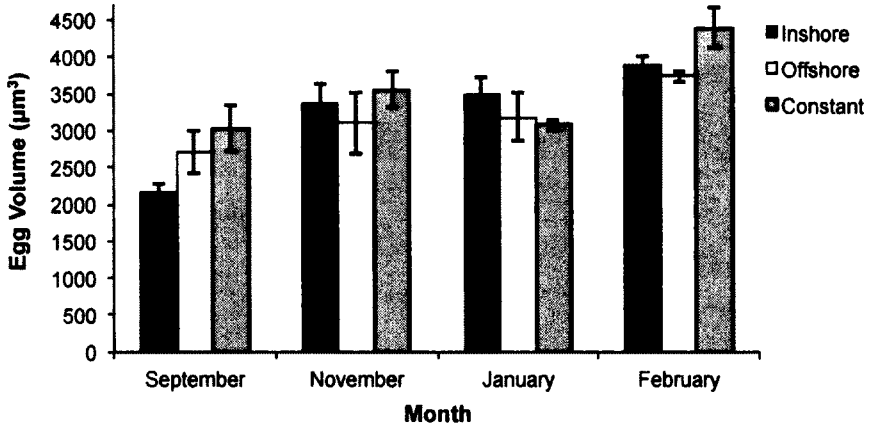

Fig. 5. A summary of means (± se) for changes in lobster egg volumes (given in μm^3) over a six month period. There were no significant differences in egg volume by treatment (Tukey's HSD; $q = 2.40$, $p > 0.05$), but differences did exist from month-to-month ($F = 2.25$, $df = 3$, $p < 0.001$)

Discussion

The main goal of this study was to document the changes in lipids and proteins in lobster eggs over three disparate thermal regimes and the effect that temperature has on these important biochemical processes. In general, the trends during embryogenesis in *H. americanus* were typical of other decapods: lipid reserves were catabolized while proteins were utilized to make tissues (Holland 1978, Sasaki et al. 1986, Jacobs et al. 2003, Brillon et al. 2005). In tandem with these patterns, eggs were also shown to absorb water during development with a resultant increase in egg diameter. Not surprisingly, lobster eggs exposed to an elevated, constant temperature elicited dramatic changes compared with inshore and offshore ones and, as a result, hatched sooner. Furthermore, the methods that were employed in this study were able to replicate those of other studies that tracked similar metrics in lobster eggs over time (Pandian 1970, Sasaki et al. 1986, Sibert et al. 2004).

This study did not obtain data for biochemical changes that occurred in eggs that were approaching hatch (~ 30 days prior) or the effects of such changes on larval survivorship or condition. As a result, there were no apparent biochemical differences in lobster eggs between inshore and offshore temperature treatments. Despite this, it has been shown that large changes in egg yolk lipids and protein levels occur within the last few weeks of development (Sibert et al. 2004), suggesting a large influence in the rate of temperature change between inshore and offshore locations. Concurrent with this are the associated (but different) rates of temperature increase that occur between inshore and offshore waters especially in the late spring and early summer that impact when lobsters hatch (Chapter 2). As a result, this could change how energetic reserves are allocated near the end of development more intensively, compared to the beginning.

Other studies have shown the influence of such thermal exposures on larval condition (Sasaki et al. 1986, Ouellet and Plante 2004), and it was very clear that significant changes to lobster egg biochemistry are apparent in the first couple months of development (this study) as well as leading up to the month before hatching (Sasaki et al. 1986). The effect of temperature on metabolic and developmental rates is expressed through changes in the consumption rates of metabolic reserves that are affected by changing temperatures (Sasaki et al. 1986). Thus, the seasonal aspects of fluctuating temperature have a 'real' impact on the rates and course of development in lobster eggs. It is suggested that fluctuating seasonal temperatures help to accelerate egg development during some time frames while depressing it at others, providing temporal windows

where hatching generally takes place (Helluy and Beltz 1991,Waddy and Aiken 1995, Chapter 2).

Seasonal movements by ovigerous lobsters provide one potential strategy for exposing their eggs to variable seawater temperatures and locations where the timing of hatch could be favorable. These movements influence overall egg incubation time and may affect how internal egg resources are utilized (Sasaki et al. 1986, Chapter 2). This was seen most clearly in eggs that were exposed to constant, elevated temperatures. In this case, egg lipid and protein levels changed dramatically and eggs hatched almost three months prior to inshore and offshore egg treatments (Fig. 3). It is presumed that egg hatching in March or April would be detrimental to survival in the plankton due to suboptimal levels in temperature and food across most areas (e.g., match-mismatch hypothesis). Seasonally changing temperatures, including a refractory period of cold seawater temperatures (< 5 °C), are important to conserving egg resources for more rapid increases in temperature (> 10 °C) that typically occur later on (Waddy and Aiken 1995). These thermal conditions were simulated in both inshore and offshore treatments and resulted in egg development that extended well into the spring and early summer (offshore). Although eggs exposed to a constant temperature, hatched much sooner, they also contained residual yolk reserves upon hatch; this was also documented by Sasaki et al. (1986).

Lipids and Proteins

Most studies conducted on crustacean eggs show that lipids are the major energy reserve (Holland 1978, Fraser 1989, Clarke et al. 1990, Heras et al. 2000). Egg yolk lipids were rapidly consumed in all thermal treatments and throughout all months, although much more modestly in winter (Fig. 3; Table 1). This pattern is seen consistently in other crustaceans at similar rates. For example, the egg lipid content of fiddler crab (*Uca rapax)* decreases significantly (78.4 %) through embryogenesis, confirming that lipids constitute an important energy source for embryonic development. In addition, lipids are also used as structural components of cell membranes that are being formed as they grow (Rosa and Nunes 2003). Thus, the catabolism of lipids is a classic feature of crustacean eggs and many other crustaceans produce eggs with large lipid reserves that are used throughout embryogenesis (Rosa et al. 2007). Lipid depletion rates are directly related to incubation temperature, and it has been observed in other crustaceans that the energy consumption per day, mostly provided by lipids, slightly intensified 3 or 4 days before hatching (esp. with higher temps), could be related to a higher energy production need at this time (Heras et al. 2000). Yolk lipids tend to become catabolized first followed by yolk proteins. These ratios change and can be used to estimate the cost of egg development at differing temperatures (Sasaki et al. 1986). In the field, lipid profiles (e.g., fatty acids) have been used to identify offshore from inshore lobster eggs (Castell et al. 1995); therefore, it is possible that these constituents are utilized differently across different geographic regions that correspond to disparate thermal regimes.

For proteins, the consumption rate during embryogenesis may increase as temperature rises (Conceicao et al. 1998). Proteins not only function as building blocks for tissue and organs but more so, may act as intermediates in carbohydrate and lipid metabolism (Schmidt-Nielsen 1991). Thus, trying to quantify protein levels may be masked by their intricate link to other biochemical components. Over prolonged cold temperatures or those conditions in which temperatures are too high for even short periods of time, some crustacean embryos may instead utilize proteins as an energy source if lipids are low due to thermally-induced demands (Conceicao et al. 1998).

At elevated temperatures (constant), increases in protein levels were clearly detected. At sub-optimal temperatures tissue synthesis tends to be inefficient and more protein might be used as energy instead (Garcia-Guerrero et al. 2003). Therefore, the duration and rates of differing thermal profiles would most certainly affect these biochemical changes and allocations of resource components over time. How this translates to larval survivorship remains poorly understood. However, Sasaki et al. (1986) showed that up until Stage IV (post-larval), lobsters depended upon stored capacities of lipids and that these residual lipids maybe favorable to settlement processes.

Egg Volume

The increase of water in the eggs (egg volume) as seen in this study and others is directly related to water uptake during new cell formation in the embryo and has been noted to increase by more than 50 % over the course of development (Pandian 1970). Increases in

egg volume are primarily due to water uptake by the embryo as well as from the retention of metabolic water resulting from respiration (Pandian 1970, Petersen and Anger 1997). The associated osmotic changes that ensue during egg development can be an important component to hatching and have also been implicated in mechanically aiding the breakage of the chorion near the time of hatch (Pandian 1970). Slight changes in lobster egg volume have been previously explained as a function of a plastic response to variations in salinity (Charmantier and Aiken 1987), and for later eggs, a consequence of physiological factors during development (Pinheiro and Hattori 2003). In this case, the movements or residency of lobsters in certain locations where seawater salinities can vary dramatically during certain times of the year (e.g., estuaries; Watson et al. 1999) may have an impact on aspects of development or hatch, especially near the latter part of egg development (Charmantier and Aiken 1987).

Female Size and Condition

In this study we did not specifically address the influence of maternal size or nutritional condition on egg quality. However, other related studies have showed that caloric energy content per egg increases with female size (Attard and Hudon 1987). Sibert et al. (2004) described this relationship by creating a growth index model for egg development and found that bigger eggs used yolk lipids more efficiently and sustained faster embryonic growth compared with smaller eggs. In addition, Ouellet and Plante (2004) reported that first-time (primiparous) spawners produced compromised larvae compared to larger, multiple ones (although larval size was independent of female size). Results from these

key studies point out the need to more clearly investigate these factors in more depth. Since female size and reproductive history may play a role in the allocation of metabolic egg reserves.

Large invertebrate eggs often have greater organic content than small eggs (Clarke et al. 1990, Clarke 1992) but egg size is not always an accurate predictor of organic content in decapods. Jacobs et al. (2003) for example, found that the larger size of blue crab (*Callinectes sapidus*) embryos in the spring is due, for the most part, to increased water uptake and the concomitant increase in inorganic salts (ash) commonly seen in crustacean embryos (Pandian 1970). An effect of female size on egg reserve allocation has been reported in other decapods including snow crab (*Chionoecetes opilio*), giant crab (*Pseudocarcius gigas*) and lobster (*Homarus americanus*) (Attard and Hudon 1987, Sainte-Marie 1993, Gardner 2001). In lobsters it has been speculated that the effect of female size may mean that larger females make a greater contribution towards egg reserves (Attard and Hudon 1987). However, the added effect of temperature on egg 'quality' may, in some cases override this effect and more work is needed to address this.

In addition to female size are potential effects that maternal nutrition has on enhancing or deterring egg quality (Appendix F). The lecithotrophic nature of lobster eggs is determined largely through the sequestering of maternal nutrients throughout the processes of primary and secondary vitellogenesis during oocyte formation, the latter of which is highly dependent on the female's organic energy reserves (e.g., lipoprotein; Dehn et al. 1983). Therefore, the biochemical composition of eggs is directly related to

the physiological and nutritional status of the female (Sasaki et al. 1986, Racotta and Ibarra 2003), and has an influence on the success of embryonic and larval development (Holland 1978).

The rates of biochemical processes and incubation time for developing lobster embryos are temperature dependent. Although the changes in biochemical components (lipids and proteins) were not dramatically different from inshore and offshore thermal regimes, there is still the potential for variations in the energetics of embryogenesis based on the seasonal movements of some lobsters to and from differing geographic regions.

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APPENDIX B

BIOCHEMICAL PROTOCOLS

Modified Total Lipid Procedure

I. PRINCIPLE: Quantify total lipids using a modified Bligh and Dyer extraction procedure

II. REAGENTS & STANDARDS:
1. Chloroform
2. Methanol
3. dH_2O

Table 1. Lipid chemical ratios to add

	CHCl3	Methanol MeOH	dH_2O
	10	20	8
For example*:	500 mL	1000 mL	400 mL

***this gives a total of 1900 mL based on above ratios**

III. PROCEDURE:

1. Weigh out 30 eggs to obtain an average weight; ratios of chloroform, methanol, and water can then be determined
2. Grind egg samples and add to 50 mL plastic centrifuge tubes
3. Cap and/or wrap tubes sufficiently with Durseal® sealing film (Diversified Biotech, Boston)
4. Place tubes on their sides in a shaking water bath (mdoel Precision, Precision Scientific Group, Chicago, IL) with enough water to cover bottom of tubes
5. Let tubes shake for 24 hr. at an RPM = 186 oscillations/min (OPM)
6. Remove egg tubes from shaker
7. Filter solution using Whatman #41 (9.0 cm) ashless filters into glass (acid-washed), pre-weighed 25 mL Erlenmeyer flasks
8. Add 1ml chloroform first to rinse and then finally 2ml dH_2O solution as needed (make this fresh) to rinse tubes and enhance phase formation in flasks
9. Let egg slurry sit at room temp. for ~ 30-45 minutes to allow layering to occur
10. Pipette out (glass pipettes) methanol- dH_2O layer and place in clean (also acid-washed) culture tube

11. Place rubber sleeve stoppers and Duraseal® sealing film over tube and insert two 19 gauge needles into stopper for gas setup
12. Place flasks into warm water bath (37 °C) shaking at 186 OPM and start N_2 gas flow.
13. Leave flasks until dry (~ 20-30 minutes)
14. Place all flasks in drying oven (VWR model 1380 FM) at 37 °C for 24 hr.
15. Move all flasks to dessicator and allow them to cool to room temp. (~ 30 min).
16. Clean outside of flasks with lens paper and weigh to 0.0001 g using an analytical balance (model A-200 DS, Denver Instruments Co.)
17. Do a subtraction to calculate total lipid

IV. TUBE PREPARATION

1. Acid-wash (1M HCl) and dry tubes at 60 °C in drying oven (VWR model 1380 FM) for 24 hr.
2. Label tubes accordingly
3. Place in dessicator for storage
4. Remove from dessicator with tongs and clean tubes thoroughly with lens paper
5. Weigh tubes to 0.0001 g (model A-200 DS, Denver Instruments Co.)
6. Place back in dessicator until needed

V. EQUIPMENT: (see procedure section)

VI. REFERENCES:

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Modified Protein Procedure

I. PRINCIPLE: Quantify total protein using a modified Lowry procedure and a Biorad Protein Assay Kit
II. REAGENTS & STANDARDS:

1. Biorad Protein Reagent: Coomassie Brilliant Blue G-250

   Filter (Nalgene 160 filter unit, pore size= 0.45 um) 1 part reagent to 4 parts dH_20. This solution can be made in advance and stored for 1-2 weeks.

2. Standards: Bovine serum albumin (BSA)

   Reconstitute serum with 20ml dH_2O and mix to dissolve

%	ug/ml	Stock (BSA)	dH_20
0	0	0	1
12.5	187.5	0.125	0.875
25.0	375.0	0.250	0.750
37.5	562.5	0.375	0.625
50.0	750.0	0.500	0.500
62.5	937.5	0.625	0.375
75.0	1125.0	0.750	0.250
87.5	1312.5	0.875	0.125
100.0	1500.0	1	0

Table 2. BSA Standard dilutions for Biorad Protein Assay (green highlighted values represent standards used).

III. PROCEDURE:

1. Count out, and grind 10 eggs/larvae per sample x 2
2. Add ground egg sample to 5 mL 1N NaOH in glass tubes
3. Vigorously vortex each tube
4. Count out, and grind 10 eggs/larvae per sample x 2
5. Let samples sit (digest) for 24 hr.
6. Filter sample using Whatman #1 (110mm)
7. Pipette 100 µL of sample or standard into 16 x 125 culture glass tubes.
8. Add 5 ml of reagent to all tubes
9. Let samples sit (react) for 5 min.
10. Transfer to disposable cuvettes
11. Read samples ($\lambda = 595$) using the spectophotometer

IV. EQUIPMENT:

1. Beckman DU 520 Spectrophotometer
2. Suction Apparatus (for filtering reagent and samples) using a Nalgene 160 filter unit
3. Disposable glass tubes, cuvettes, pipettes, tips, etc.

APPENDIX C

AN OVERVIEW OF GULF OF MAINE OCEANOGRAPHY AND ASSOCIATED MECHANISMS FOR LARVAL LOBSTER DISPERSAL

Abstract

The Gulf of Maine (GoM) contains one of the most biologically productive ecosystems in the world and, as a result, has supported some of the most successful and historically lucrative commercial fisheries including cod, tuna, herring, and lobster. However, this high level of biological productivity and ecological diversity would not be so without the unique physical make-up of the GoM basin and its associated circulation system. This overview serves as background for putting larval lobster dispersal in the GoM into a bio-physical contextual framework as well as to describe some of the major features. Applying a variety of biological correlates to other ongoing and future modeling studies will help to clarify the marine connectivity of lobster in the GoM and provides data for the future management of this important marine species. In addition, some of the relevant links to my thesis are also emphasized.

Gulf of Maine Oceanography

Geography

The Gulf of Maine (GoM) is a large, semi-enclosed sea adjacent to the northeast corner of the United States and includes parts of maritime Canada as well (Fig. 1). The GoM is delineated by Cape Cod to the southwest and Cape Sable located to the northeast and includes the coastlines of New Hampshire, Maine, and Massachusetts (north of Cape Cod) as well as the southern and western coastlines of New Brunswick and Nova Scotia (Pringle 2006).

The GoM encompasses over 93,000 km^2 of ocean and has an average depth of only 150 m (492 ft). Undersea valleys in the central basin can reach depths of 500 m while undersea mountains rise over 200 m from the sea floor, almost reaching the surface in some locations and creating islands in others (see Beardsley et al. 1997, for review). The GoM is also bounded offshore by large shallow banks and shelves including Georges Bank (GB), Brown's Bank, Nantucket Shoals, and the Scotian Shelf (SS). There are also three major basins contained within the Gulf of Maine: Wilkinson Basin to the west, Jordan Basin in the northeast, and Georges Basin in the south, which are isolated from each other beneath the 200 m isobath. The Northeast Channel is the major channel between the Gulf and the rest of the Northwest Atlantic although a secondary, shallower connection to the rest of the Atlantic is the Great South Channel, located between GB and Nantucket Shoals (Bigelow 1927, Brooks 1985). The watershed of the GoM contains an

area roughly 180,00 km^2 (69,000 m^2) including several prominent large river systems that empty into the GoM: St. Croix, St. John, Kennebec-Androscoggin, Penobscot, Saco, Piscataqua, and Merrimack rivers. Taken together these riverine systems greatly influence seasonal freshwater input into the GoM system (Keafer et al. 2005, Pettigrew et al. 2005, Pringle 2006).

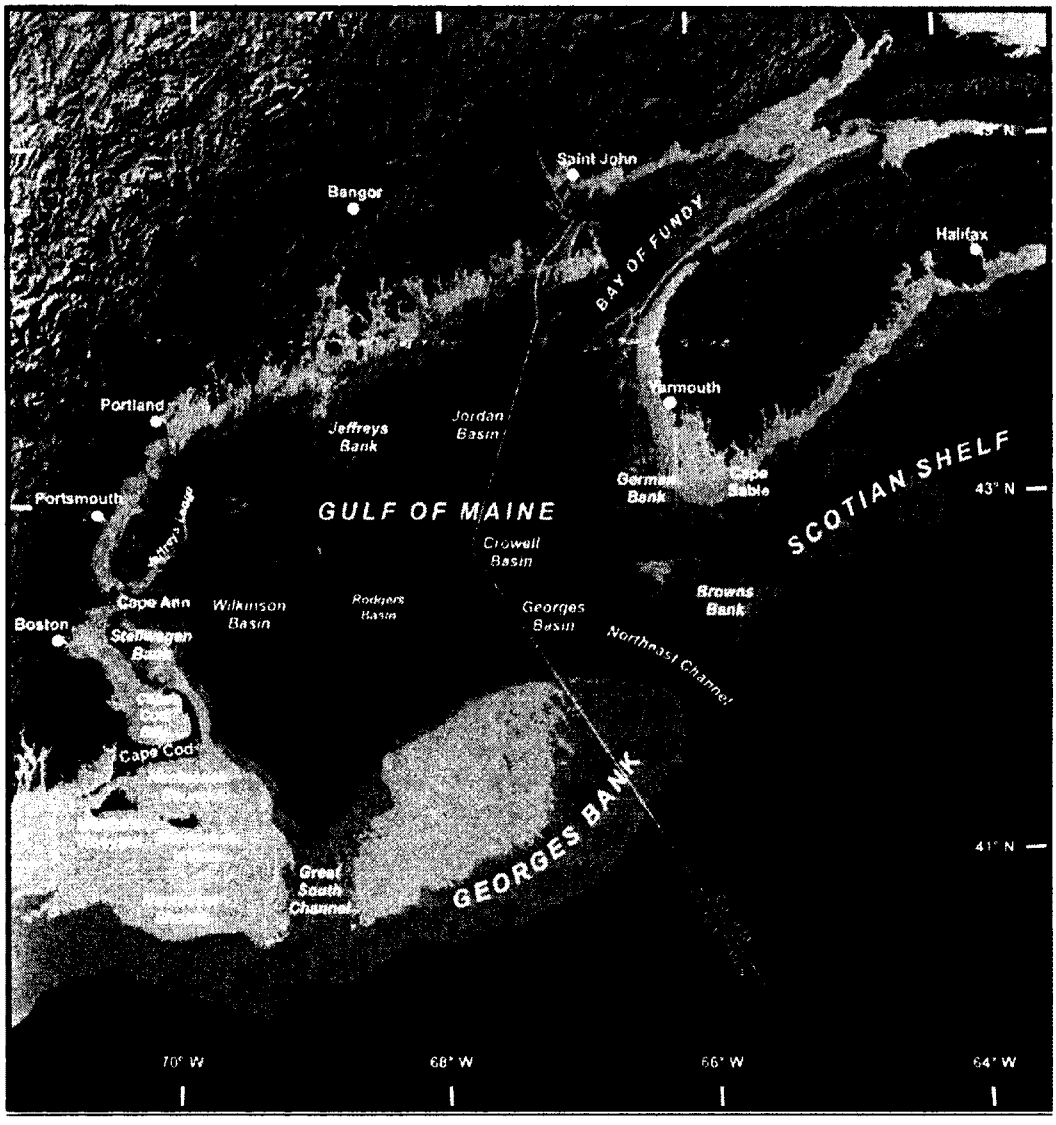

Fig.1. Geography of the Gulf of Maine, its coastal boundaries, and some of its features. (Source: USGS, Woods Hole, MA).

Circulation Overview

Overall circulation in the Gulf of Maine consists of a counterclockwise (cyclonic) gyre that rotates around the coast from Nova Scotia to Cape Cod (Bigelow 1927; Fig. 2). Jordan Basin is considered the central cyclonic circulation cell located in the eastern GoM (Pettigrew et al. 1998). Circulation patterns in the GoM are highly complex, featuring elements of varying degrees of scale that include river plumes, coastal currents, wind-driven flows, strong tides, thermohaline influences and deep gyres (Xue et al. 2000, Keafer et al. 2005, Pettigrew et al. 2005, Pringle 2006). Although flow is seasonally variable, it is driven, in large part, by buoyancy fluxes (i.e., freshwater input from large rivers); this is especially the case around the perimeter of the GoM (Xue et al. 2000, Pringle 2006). A mixture of waters from the St. Lawrence River system (and other large rivers) along with Labrador Current water over the SS and into the GoM regulates an upstream buoyancy current (Xue et al. 2000, Brown and Irish 1992). It has been suggested that the circulation in the GoM is related to its evolving density structure (Xue et al. 2000, Brown and Irish 1992). As the water in the interior GoM warms, it expands, (thermal expansion) becoming less dense. The difference in density between these more buoyant waters and cooler offshore waters contributes to a pressure gradient (Beardsley et al. 1997, Pringle 2006), and the force of this gradient presumably creates a downward slope of water towards offshore (Coriolis forces this water to the right; Brooks 1985). The resulting westerly flowing coastal current helps to draw water into the GoM. Factors that influence the density distribution inside the GoM include wind, winter cooling, river runoff, the inflow from the SS, the deep inflow of the slope water, and tidal mixing. In addition, wind changes from predominantly northwesterly in winter to predominantly

southwesterly in summer provide summer upwelling along the Maine coast. Winter cooling erodes the stratification in the upper water column, whereas the warming in summer reestablishes the stratification.

Relatively fresh, cold water enters the GoM over the SS and tends to flow northeastward around the tip of Nova Scotia (Brooks 1985). After a turn through the Bay of Fundy, this current flows southwestward, setting up the coastal current that dominates circulation along the coast of Maine (Keafer et al. 2005, Pettigrew et al. 2005). Riverine discharge (seasonally significant at times) and tidal flows contribute to the coastal current and give the GoM its distinct estuarine character (Mountain and Manning 1994). Dense, warmer and more saline water from the continental slope (outside of the GoM) enters through the Northeast Channel, a deep valley between GB and Browns Bank. This water mass flows into Georges Basin and characterizes the cyclonic gyres (Georges and Jordan). The outflow of seawater from the GoM occurs primarily at two points: the Great South Channel and upper layers of the Northeast Channel.

The dynamics of the coastal current that flows southwestward along the coast of Maine divides the central Gulf of Maine into two oceanographically distinct areas. The eastern portion of this current (also known as the eastern Maine Coastal Current (EMCC), Keafer et al. 2005, Pettigrew et al. 2005) is relatively fast moving and somewhat colder than the western portion of the current (i.e., western Maine Coastal Current, WMCC). Where the two meet in the vicinity of the Penobscot Bay area, the faster moving eastern current is, to a large degree, deflected offshore. These waters are, in turn, entrained in the cyclonic

gyres that flow over Georges and Jordan Basins. This process divides the interior Gulf into eastern and western portions that are relatively isolated from one another.

Circulation Patterns

The circulation of the GoM has a distinct seasonal pattern (Xue et al. 2000). The counterclockwise gyre takes shape in the early spring. As the season proceeds, the discharge of freshwater from over 60 rivers in the GoM watershed contributes to the currents, and the action of the tides strengthens the summer circulation (Mountain and Manning 1994). In addition, the warming of the surface of the ocean results in stratification; a warmer layer floats on top of a mid-depth layer that preserves winter temperatures and salinities. It, in turn, is underlain by more saline bottom water. Stratification is most pronounced in the deeper areas of the western GoM. The counterclockwise gyre is established in the top layer, and the current picks up speed as the top layer slides over the middle layer. These currents reach their broadest extent and greatest speeds by the end of December (Pettigrew et al. 2005). Then, cooling of the atmosphere results in cooling of the ocean surface. As it cools, surface waters sink, replacing the stratified layers with well-mixed waters. As the currents mix downward they are slowed by the friction encountered when they reach the bottom. By February, the counterclockwise circulation pattern is generally diminished.

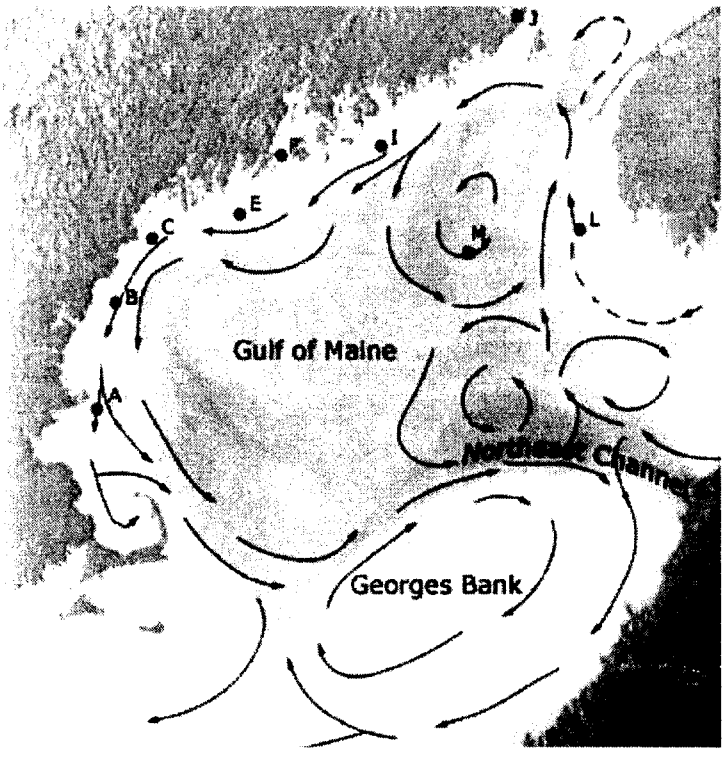

Fig. 2. The overall circulation patterns in the GoM. Notice the combination of both cylonic and anticyclonic flow regimes as well at the inflow and outflow of water from the system. Letters dots (A-L) indicate locations of ocean-observing buoys. (Source: www.GOMOOS.org).

There are several physical features of the GoM that contribute to its overall circulation and inter-annual variability (Fig. 2) that include, but are not limited to:

1. A large estuarine component emanating from a large amount of freshwater entering from the SS in addition to local runoff from coastal riverine systems that mix with salty slope water from the Northeast Channel. This produces water of intermediate salinities that flow westward along the shelf and off the shelf (Brooks 1985, Manning et al. 2009).
2. Strong influence by bottom topography, with clockwise (anticyclonic) flow over Browns and GB, and Nantucket Shoals compared with counterclockwise (cyclonic) flow over Jordan, Wilkinson, and Georges Basins. The Northeast

Channel is the only partial barrier to the cross-over of SS water from Browns to GB (Brown and Irish 1992, Brooks 1985, Fig. 3).

3. Circulation patterns that vary on a wide range of time and space scales, but strong tidal currents over regions such as GB, generating strong turbulent mixing in bottom layers and large amplitude internal waves on the flanks of the bank especially in Wilkinson Basin (Loder et al. 1992, Werner 1996, Manning et al. 2001). Additionally, other periodic events such as shelf-slope interactions and the formation of warm-core rings that operate on seasonal levels with a high degree of variability (Lough and Manning 2001, Mountain et al. 1996), thereby setting up various scenarios for off-bank (or shelf) dispersal by marine larvae.
4. A variety of meso-scale type circulation patterns, especially in the western Gulf, that include a complex and highly variable coastal current system that flows from Nova Scotia down to Massachusetts – The GoM Coastal Current or GMCC contains two distinct branches whose waters tend to bifurcate to offshore locations at specific temporal and spatial locations, and has implications for flow and dispersal of particles (Brooks and Townsend 1989, Pettigrew et al. 2005).

Georges Bank

The presence and features of GB, a shallow, sediment-covered plateau, greatly impacts the characteristics and productivity of the GoM. This immense underwater bank creates a situation where the GoM is more greatly influenced by the colder waters of the Labrador Current from the north than the Gulf Stream waters to the south. Therefore, the waters of

the GoM are more nutrient-rich than more southern waters, an important factor that has helped sustain this area as a historical fishing ground for centuries (Bigelow 1927, Kurlansky 1997).

Flow over GB is dominated by strong M_2 tidal currents that exhibit a rotary-like flow over the bank that increases as the water becomes shallower (Lough and Manning 2001). An interesting oceanographic event occurs on banks such as Georges when long, barotropic, tidal waves propagate from the deep ocean onto the shallow bank and its topography. The resultant effect, a clockwise (anticyclonic) flow is generated over the bank due to the 'nonlinear transfer of vorticity and momentum from tidal currents' (Loder 1980). This phenomenon has the effect of then moving current in an eastward direction (current jets of 20 cm/s on the northern flank) and, at the same time, re-circulates westward as a relatively broad and weaker flow (1-3 cm/s) on the southern flank of GB (Lough and Manning 2001, Manning et al. 2001, Manning and Churchill 2006). Another feature around GB occurs when waters stratify thereby causing various levels of tidally-induced vertical mixing and the formation of tidally-mixed fronts (TMFs) (Shanks 1995), especially in shallow areas of GB (Loder and Wright 1985). Throughout the summer months, the TMF is well established (especially on the southern bank of GB) but tends to disappear in the winter due to strong mixing from wind coupled with surface cooling. Therefore, TMFs influence on the circulation patterns over GB can often vary quite a bit seasonally (strongest in the summer) (Loder and Wright 1985, Chen et al. 1995) and impact the advection and retention of certain fish larvae such as cod and haddock on the bank (Manning et al. 2001). The unique physical processes operating

around GB that include clockwise gyral circulation, and tidal mixing make it one of the most productive shelf ecosystems in the world (Horne et al. 1989, Wiebe and Beardsley et al. 1997).

Aspects of Larval Lobster Dispersal

Overview of Larval Lobster Life-History

Ovigerous lobsters (*Homarus americanus*) carry and incubate their eggs for 9-12 months prior to hatching, and these prelarvae are released over hatching events that occur over the course of several days to a couple of weeks, typically in the spring and summer (Herrick 1909, see introduction). These larvae quickly molt into positively buoyant, Stage I zoeal larvae and, along with Stage II and III larvae, remain planktonic mostly in the top 10 m of the water column (Harding et al. 1987, Ennis 1995). At Stage IV, larvae metamorphose into a specialized, strongly swimming, postlarval stage that swims close to the surface for 10-30 days (Cobb et al. 1989) before making the transition from a pelagic to a benthic realm. At any of these stages, larvae can be transported considerable distances (e.g., Katz et al. 1994). Although the complete cycle of stages is normally completed in 20-30 days (Herrick 1895, Templeman 1940), larval duration in the plankton is highly temperature dependent, and it has recently been argued that it is markedly shorter than previously thought (Annis et al. 2007). The distribution and abundance of larvae are affected by the locations of spawning females in tandem with a host of abiotic factors (e.g., temperature, salinity, light intensity, surface current and

velocity, etc., Phillips and Sastry 1980) that ultimately help to influence their final destination along with intrinsic larval behaviors (e.g., vertical migration and swimming, Harding et al. 1987, Ennis 1995).

Larval Lobster Transport

Larvae may be present in the water column for longer periods of time (summarized above) because their rate of development is a function of water temperature (MacKenzie 1988, Hudon and Fradette 1988). While ocean currents and winds strongly influence their movements, they are also strong swimmers and thus exercise some control over their ultimate settlement location (Ennis 1995). By taking a number of these factors into account, several recent studies have attempted to estimate the pattern of recruitment of lobsters in the GoM, given the distribution of ovigerous females (Incze and Naimie 2000, Incze et al. 2003, Incze et al. 2010, Chassé and Miller 2010). All of these modeling studies have provided a great deal of insight concerning lobster stocks and the extent to which certain lobster populations provide recruits for other areas of the fishery. However, the accuracy of these models depends a great deal on two variables that have been investigated in-depth in this thesis: 1) the location of ovigerous females while they are carrying late-stage eggs and; 2) the accuracy of laboratory models concerning the influence of temperature on egg development and hatching.

Selected Physical Elements that Impact Larval Distribution

There is considerable variation in the circulation patterns of the GoM from year to year. Variations in temperature and volume of water flowing into the GoM (including freshwater input) for example, along with atmospheric fluctuations in temperature and wind patterns are all factors that significantly affect the scale and duration of GoM circulation features like water masses (different densities), gyres, and alongshore currents. As a result, the cross-shelf or offshore to onshore dispersal of marine invertebrate larvae is often accomplished through a combination of behavioral traits in tandem with the oceanographic features they encounter. The goal of this section is to outline some of the physical elements that might facilitate larval advection, retention or dispersal. Examples of how larval behavior can be exploited to help utilize these flow features to maximize their transport for settlement (see Shanks 1995 for full review) is also discussed.

To a large part, the cross-shelf transport of larvae depends heavily on the physical geometry of the shelf (e.g., Georges Bank, continental shelf) that sets up a variety of features important to larval dispersal and transport (Incze et al. 1997). Additionally, coastal complexity (i.e., the interaction of flow fields along variable topographic relief) can be characterized over a length of spatial and temporal scales by the presence and duration of fronts, eddies, convergence zones, and upwelling events, among others (Wolanski and Hamner 1988, reviewed in Sponaugle et al. 2002). Flow variability

(advective or diffusive) and water column structure of ocean realms are influenced by many factors. Below are descriptions and overviews of some of the most prominent:

Winds

The major types of wind-derived water movements include wind drift currents, surface waves, and Langmuir circulation (LC, Langmuir 1938). LC typically operates at ~ 1.5 m/s, oriented into the direction of the wind. Wind drift current and surface waves then alternate in a clock and counter-clockwise direction (Coriolis effect), creating rotating currents. This movement forms jets that flow downward, then angle upward in a more diffuse pattern, often represented as surface slicks or 'foam lines' that delineate convergence zones. Based on plankton tows conducted in LC areas, Stommel (1949) concluded that negatively-buoyant larvae could be retained in these zones (i.e., Stommel Retention Zones, SRZ). STZs exist where 'upwelling currents are roughly equal and opposite to their downward sinking or swimming' (Stommel 1949). More recent work has built on this by looking at the distribution and buoyancy of particles at varying degrees (e.g., Buranathanitt et al. 1982). There are few studies investigating the association of marine larvae with LC however, Jillett and Zeldis (1985; crab larvae) and Kingsford et al. (1991; jellyfish) both found evidence for larval convergence in these types of oceanographic features. Harding et al. (2005) observed that stage IV lobster larvae were found in flotsam lines and speculated that perhaps these larvae collected in convergence zones.

Breezes are another important wind-type that can help facilitate the surface transport of larvae, especially at certain times of the day. Diurnal sea breezes (reviewed in Atkinson 1981) set up in the morning when the land warms faster than the ocean, creating low pressure thereby 'sucking' air from the ocean. Alternatively, land breezes form in the afternoons and evenings when the land is cooling faster than the ocean thereby creating a low pressure air flow from land. Both sea and land breezes thus produce surface currents directed on- and offshore, respectively. Therefore, larvae that may be near the surface during the day (positively phototactic) for example, would be exposed to onshore transport.

Wind Drift Currents

Winds affect water motion by setting up an Ekman spiral, in which the wind sets surface water in motion. Due to the Coriolis effect, these waters are deflected to the right (about 45 °; over 20-30 m depth) in the northern hemisphere and, as depth increases, the deflection angle is increased. If this flow is integrated throughout all depths, the net result is Ekman transport. The exceptions to this include situations where low wind speeds would yield little deflection, and if near land, water would move more downward (downwelling). The recruitment of cod larvae in the GoM is a particularly good example of the role of downwelling and its ability to send larvae closer to shore (Churchill et al. 2011). Along with larval behavior (diel vertical migration), Churchill et al. 2011 determined that cod larval retention is favored during downwelling events within the western GoM. Alternatively, given situations where an offshore wind persists, sea levels

would drop near the coast as subsurface flows move shoreward causing upwelling events. The overall effect of wind drift on larval transport depends on the depth where larvae reside. When this is known, it is possible to model the direction and extent of transport, based on average wind speeds over several months (Johnson and Hess 1990). Studies with crab larvae have found a positive relationship between the strength of onshore wind and recruitment of crab megalopae to adult (benthic) populations (e.g., Johnson and Hess 1990, McConnaughey et al. 1992). Despite these modeling efforts, two important limitations remain: 1) the inter-annual variability in oceanographic features (e.g., downwelling) that can affect larval dispersal, transport and mortality; and 2) behavioral attributes of larvae (e.g., vertical migration in the water column) that can result in larvae that choose disparate ocean depths and thus, different transport scenarios, resulting from Ekman spiral.

Internal Waves and Tidal Bores

Internal waves are derived from a combination of tidal currents and features of the bottom topography. Typically, a tide ebbs off a sharp relief (bank or reef), a lee wave is formed, and then at flood tide, a wave is propagated and evolves into a set of waves. Internal waves are capable of transporting plankton, especially those found in the convergence zone (i.e., slicks), in a shoreward direction. The best evidence of this comes from studies with flotsam and surface drifters. Shanks (1988) set out drifters that were carried by internal waves and showed that barnacle larvae had 10 times better settlement when utilizing internal waves. Internal waves that have been shown to originate from

neap to spring tidal cycles have been implicated in the roughly two-week pulsing of large numbers of larvae to some coastal areas. These larvae include crab, fishes, and spiny lobster (Shanks 1983, Robertson et al. 1988). Like surface waves, internal waves are refracted by bottom topography and therefore deposited unevenly along the shore, providing spatial variability in larval settlement. Internal tidal bores result when an internal wave gets too big, and the wave breaks forming a bore; the tail end of the bore is made up of large amplitude waves. Internal tidal bores can propagate into very shallow water and, as a result, the amplitude of these waves is affected by ebbing tides. Larval transport by tidal bores is reviewed and described in detail in Pineda (1991).

Fronts and Eddies

These features typically occur at sharp surface boundaries between two water masses. The flow at fronts is convergent and sets up strong vertical water masses. This is important in the horizontal transport of larvae since the convergence of fronts can act to concentrate larvae (LeFevre 1986, Kingsford 1990). However, the retention of larvae in fronts is only as good as the depth-dependent behaviors of the larvae that reside there. Fronts are often barriers to horizontal transport except when waters mix. Changes in larval behavior may occur at frontal boundaries where low-density water (e.g., estuary plume) meets denser shelf water. There is little data suggesting the behavioral responses of larvae when contact frontal boundaries except that depth regulation (Sulkin 1984) and swimming ability probably play a large role.

Eddies are characterized as rotary currents that range in scale from centimeters to hundreds of kilometers and play a major role in the cross-shelf transport. Eddies are also commonly formed from currents flowing around an obstruction (e.g., island, shelf or bank), and the directions of eddy rotation on the lee side are governed by flow regimes such as von Karmen vortex streets that provide a back-spinning of currents. Eddies usually reside in the upper 100 m of the ocean and begin to attenuate after several days, traveling downstream of the general flow. Eddies produce convergences at their center and therefore can concentrate larvae (Boehlert et al. 1992). The retention of larvae in these features is based on the length of time in the eddy, which can be advected over large distances. Some have suggested that eddies may, in fact act as retention mechanisms keeping some larvae trapped and close to shoreline features like coastlines and islands (e.g., Crawford et al. 1990). The key to how retentive eddies can be for larvae is more dependent on eddy residence time (i.e., eddies with short retentive time may be beneficial to larvae with short development times). Other studies suggest that eddies forming far offshore are capable of transporting marine plankton (especially larval fishes) closer inshore, and those generated close to shore can transport larvae out towards the continental shelf (e.g., Hare and Cowen 1991).

Selected Biological Elements that Impact Larval Distributions

Along with the influence of physical oceanographic features (described above), pelagic marine larvae are capable of controlling both their horizontal and vertical distribution in the water column using a variety of behaviorally-derived traits. Below, I have

summarized some mechanisms by which larvae respond to biotic and abiotic attributes while swimming, thereby influencing their distributions. A full review with detailed examples can be found in Young (1995).

Buoyancy and Swimming

Buoyancy and drag are used by larvae to slow their sinking by increasing their drag. Morphological features such as feathery appendages, neutrally buoyant shells and lipid accumulation help to facilitate these functions (Sulkin 1984). Swimming speeds and their associated trajectories involve movements that are often a function of temperature and viscosity. For example, sand dollar larvae (*Dendraster excentricus*) swimming speeds are reduced by over 40 % over a 10 °C drop (Podolsky and Emlet 1993). Directed movements by copepods give them fine control over their vertical movements while crustacean and fish larvae possess a variety of features (e.g., pleopods, fusiform shapes) that reflect significant horizontal swimming capabilities (Leis 2007, 2010). Some crustacean larvae propel themselves by contracting muscles in their tails (e.g., postlarval lobsters). In turn, the larva creates an effective forward stroke and maximum thrust by taking advantage of the large surface area of water that the abdominal section comes in contact with. Likewise, backward thrusts are shortened by 'reducing the area on the recovery stroke' by folding it up under the abdomen (Young 1995). These kinds of movements are particularly important especially for the postlarval stage of both clawed and spiny lobsters that are typically found in the upper few meters of the water column (Rooney and Cobb 1991, Acosta and Butler 1999). To a large part, these animals use

their strong swimming capabilities to propel themselves towards suitable settlement habitats.

Movement of many other kinds of larvae through the use of ciliary bands is very common in sponges, cnidarians, trematodes, and the larvae of nemerteans (see Emlet 1991 for review). The complex patterns of ciliary arrangements and their associated ontogentic changes are typical in some larvae such as echinoderms. More dramatic is the condition in some larvae referred to as metachrony or the oscillations of bands of cilia that result in the spinning while swimming phenomenon seen commonly in echinoderms.

Depth Regulation

Larvae often use depth regulation to position themselves in very specific water masses for food, transport, or to avoid predators. The behavioral basis for depth regulation usually include –tropism (an organism turning toward or away from a stimulus), -taxes (directional movements to a particular cue), or –kinesis (changes in the speed or rate). Scalar cues often are described as barokinesis, halokinesis, thermokinesis, photokinesis, thigmokinesis while vector cues typically encompass geotaxis, phototaxis, rheotaxis, or polarotaxis. Geotaxis behaviors for example occur in species that possess statocysts that allow them to sense gravitational pull, and most larvae possess a high center of gravity that makes them fall head first. Crustacean larvae have been studied extensively in this area and are known to demonstrate complex larval behaviors associated with vertical migration and depth regulation. Theoretically, a larva with sensory structures on opposite

sides of its body (e.g., spiny lobster phyllosoma) contains chemoreceptors at the ends of its appendages that can discriminate sharp gradients (e.g., salinity) on small scales (Phillips and Macmillan 1987). Alaskan king crab larvae are positively rheotactic, however they swim so slowly that they cannot resist most currents (Shirley and Shirley 1988). *H. americanus* larvae are also positively rheotactic. Interestingly, many invertebrate marine larvae exhibit barokinesis but the mechanisms are mostly unknown. Forward (1990) found that pressure is a graded response in crab larvae and that these animals are capable of very precise depth regulation.

Defensive behaviors and intraspecific aggregation are also often associated with control over pelagic transport and movement in the water column. In some larvae these include ciliary reversal, velums that are drawn in, and swarming events (which have been observed in mysid shrimp and portunid crabs (e.g., Gonor and Gonor 1973). Interestingly, some crustacean larvae (crab larvae) are known to also use photokinetic responses in abrupt shading to help drive their transport (Forward 1986).

Many marine larvae are also adept at responding to specific oceanic features that, in turn, help to influence their ultimate location. For example, discontinuities in water masses (i.e., pycnoclines) are often places where larvae can accumulate as the discrete density gradients can act as physical barriers. Larvae will also commonly respond behaviorally to thermal or salinity changes to the pycnocline by temporarily halting their swimming and sinking for some time. This has been reported in several kinds of marine larvae including hermit crabs and lobsters (Scarratt and Raine 1967). In particular, post-larval

lobsters are able to detect these features and discriminate among depths and will not settle on bottom habitats where the thermal gradient is too steep (Boudreau et al. 1992).

Feeding and Turbulence

The effects of turbulence, although ubiquitous in the marine environment, are much harder to quantify as a physical parameter affecting marine larvae (see Kiorboe 1993 for review). Turbulence is caused by a combination of wind, currents, and tides both at the surface and the bottom and, as a consequence, increases the encounter rates between larvae and their food (reviewed in Rothschild and Osborn 1988). However, there is also an optimal window for turbulence such that levels too high or low are not conducive to feeding (e.g., larval cod and haddock; Lough and Mountain 1996, MacKenzie and Kiorboe 2000). The benefits of increased feeding efficiency with turbulence have been shown in studies with cod larvae and their predation on copepod (*Calanus*) nauplii (MacKenzie and Leggett 1991). In one other study, Sundby (1997) looked at predation rates of cod larvae on plankton and found feeding rates that were 7-10 times greater as wind speeds increased. Some invertebrate larvae can detect and respond to turbulent conditions. Conch (*Strombus gigas*) veligers respond by stopping their swimming and drawing in their velums (Young 1995). Specialized structures (statocysts and setal hairs) in some spiny lobster phyllosomes (e.g., *Jasus edwardsii*) are capable of detecting turbulence and changes in water motion (Nishida and Kittaka 1992). Most of the information on turbulence related to marine crustacean larvae is very speculative and warrants a great deal more research.

Tidally-Driven Larval Transport

Early-stage larvae tend to show diel vertical migrations (DVM) whereby they are found at depth during the day and near the surface at night. These tend to be regular occurrences but can change with ontogeny. For example, postlarvae of several species are known to make reverse DVM, perhaps as an evolutionary strategy, to exploit cross-shelf transport in tandem with a diurnal sea breeze (described above). Blue crab (*Callinectes sapidus*) larvae are known to utilize DVM to migrate to the surface on nighttime flood tides (prior to sitting on the bottom during ebb flow), as a way to be transported into or out of a bay or estuary (selective tidal stream transport). Most recently, spiny lobster (*Panulirus argus*) larvae have been shown to exhibit clear changes in their vertical position in the water column using a combination of ontogenetic vertical migration, and DVM controlled by an endogenous circadian rhythm (Ziegler et al. 2010, Butler et al. 2011). At present, there is limited evidence of these kinds of behaviors in *H. americanus* (see Cobb and Wahle 1994 for review).

Considerations and Applications for Modeling Larval Lobster Dispersal

Connectivity

Levins (1969) formulated and described the term metapopulation as *a population of populations*. More specifically, a metapopulation can be thought of as a group of populations (of the same species) that are separated spatially but can interact at some

level (Fig. 3). The theory of metapopulations extends to other ideas about the levels of independence or connectivity between sub-populations based on the structure or size of each population.

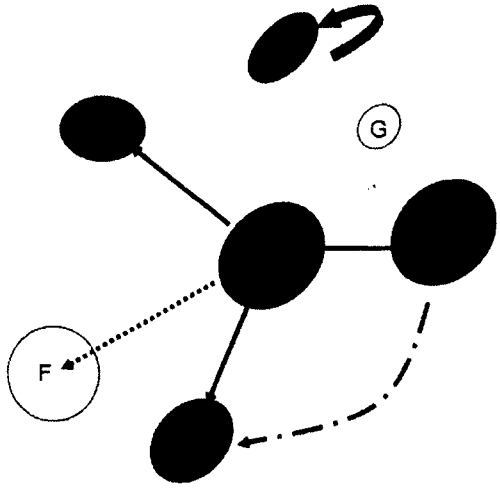

Fig. 3. A representation of metapopulations and their connectivity. Source **A** contributes to **B** and **C**, both sink populations. Population **D** has some exchange with **A**. Population **E** is an example of a self-recruiting/sustaining population. Populations **F** and **G** are going extinct (F more recently) due to some environmental forcing event and are not being supported by any others. Also, **D** may contribute propagules to **C** but not consistently. This hypothetical model represents the dispersal and retention of many kinds of marine larval fishes and invertebrates.

Connectivity is *the demographic linking of local populations through the exchange of individuals among them as larvae, juveniles, or adults* (Jones et al. 2009). If no connectivity exists, then populations are isolated and referred to as closed populations. On the other hand, if there is high connectivity due to numerous exchanges, then the populations are considered to be open populations. As such, population connectivity plays a fundamental role in local and metapopulation dynamics, genetic diversity, and the resiliency of populations (Cowen and Sponaugle 2009). It establishes the spatial scales over which a population is connected, as well as the primary scale over which population interactions and ecosystem dynamics occur (Cowen et al. 2006).

Connectivity between populations in the marine environment is maintained primarily through larval dispersal, the spread of larvae away from a source to settlement site at the

end of the larval stage (Pineda et al. 2007). The extent of this larval movement is important for determining the natural processes that limit population growth and resilience to disturbances (Cowen et al. 2006). Factors that can influence the scale of dispersal include pelagic larval duration (PLD), water currents, larval behavior, and the availability of suitable habitat for settling (Munday et al. 2009, Bulter et al. 2011). Larval dispersal is often described and quantified as dispersal kernels. Dispersal kernels relate the probability that a larva released from a particular location will disperse to another specific location with suitable habitat and settle successfully (Largier 2003, Pelc et al. 2010). These kernels are continuous functions that represent the spatial distribution of dispersed larvae, and can vary in magnitude, width and displacement symmetry at a variety of spatial and temporal scales (Botsford et al. 2009).

Understanding the scale of connectivity becomes important for designing effective networks of Marine Protected Areas (MPAs) for example and for effectively managing fishery stocks (Munday et al. 2009, Gaines et al. 2010). Connectivity helps to determine the optimal size and spacing of the areas for conservation and the potential for larval dispersal and recruitment to non-reserve areas. Connectivity can also have implications for how MPAs are managed (Cowen et al. 2006), especially as it helps to exhibit the importance of adopting a metapopulation perspective in which the subpopulations, linked through dispersal, can serve as the management unit (Botsford et al. 2009).

Biophysical Modeling

The process of larval dispersal is intrinsically a biophysical problem. It involves interactions between biological traits of the larvae and physical properties of the environment operating on multiple scales (Cowen and Sponaugle 2009). Although the horizontal transport of larvae has traditionally been attributed solely to advection in the direction of the flow, larval behavior along with other related biological factors (e.g., origin of larvae and PLD) have recently emerged as having a considerable influence on the outcome of dispersal (Kingsford et al. 2002, Levin 2006, Metaxas and Saunders 2009, Leis et al 2010, Butler et al. 2011). Therefore, a full understanding of population connectivity within the marine environment requires an adequate comprehension of both the biological and physical process involved in dispersal and transport of larval propagules.

Biophysical models are increasingly being used as predictive tools for larval dispersal and for the general evaluation of the various factors responsible for larval transport. By coupling general circulation models with particle tracking models that can simulate larval transport, these models can be used as methods to quantify larval transport, assess the role of transport in regulating population connectivity, and evaluate the role of different biological and physical factors on dispersal (Metaxas and Saunders 2009). Development of these models requires an interdisciplinary approach that combines larval biology with the physical oceanography of the study area of focus (Incze et al. 2010). The physical processes that affect larval dispersion involve both advection and diffusion properties of

water circulation; the advection comes from the mean current velocity and direction, while the turbulent diffusion comes from instabilities generated by stochastic motion of the mean current. These physical factors are determined by hydrodynamic processes that include: tidal currents, Ekman transport, density gradients, frontal structures, and vertical stratification (described above). It is also important to note that these physical processes have the ability to limit larval transport at all spatial scales (Cowen et al. 2006, Pineda et al. 2007, Werner et al. 2007).

Alternatively, the biological processes within the biophysical model include those that influence offspring production, growth, development, and survival. Biological parameters that influence dispersion include spawning behavior, larval duration, larval mortality, and larval behavior such as vertical migrations, settlement behavior, and navigation (Werner et al. 2007). Two models are commonly used to simulate larval dispersion in marine organisms: eulerian and lagrangian models. Eulerian models are used to solve an advection-diffusion equation while providing the spatial and temporal changes in larval concentrations. This model type is used primarily when knowledge of the biological parameters are limited. Lagrangian models, also known as individual-based models (IBMs), are used to capture individual particle pathways (Paris et al. 2007). The latter model is widely used to simulate dispersal by following the trajectories of a large number of particles with specific parameters. The use of IBMs allow for the parameterization of the biological variables that are specific to a particular area, thereby reflecting the most accurate scenario (Paris et al. 2007, Cowen and Sponaugle 2009).

Larval Connectivity in the GoM

Larval transport is one mechanism that presumably links inshore (coastal) and offshore (basin) lobster populations (Incze et al. 2010). With hatching occurring over a period of two months, beginning generally in late June in southern areas and a month later in northern areas, conditions experienced by developing larvae can be very different. Favorable conditions for larvae can greatly increase development rate and when coupled with the typical physical forcing factors observed within the GoM, as described above, create a delivery mechanism of competent larvae to nearshore nursery grounds (Incze and Naimie 2000). As larvae develop in the summer on Georges Bank, a strong cyclonic gyre tightens and increases residence time to 50 days inside the 100 m isobath (GLOBEC 1997). By contrast, in Southern New England, Fogarty (1983) observed peak larval densities following periods of onshore winds in the days preceding sampling in Block Island Sound (Rhode Island) and identified offshore areas and Long Island Sound as larval sources. Lund and Stewart (1970) suggest that relatively high concentrations of larvae in western Long Island Sound are a result of surface currents creating a larval retention area. This notion of oceanographic forcing is confirmed in a review by Epifanio and Garvine (2001) who suggest that larval transport is primarily influenced by onshore wind stress and water density differences along the Atlantic continental shelf.

Superimposed over these existing patterns and processes are the potential changes to the current circulation regime in the GoM, and the timing of biological events due to future climate change. Predicted forecasts suggest increases in overall coastal sea surface

temperatures, a freshening (decreased salinity) of water from the SS from Arctic sea ice melt, increased acidification of ocean waters due to the stressed buffering capacity of the sea to absorb CO_2, an overall rise in mean sea level and an increase in the frequency and intensity of storm events (Nye 2010). There are a variety of changes that might ensue resulting in marked changes in the survival of larval lobsters and the alterations to their physical transport. A better understanding of these biological and physical factors is important to identifying and managing these changes as they occur.

Summary & Conclusions

There should be little doubt that a discussion of larval dispersal is only one-dimensional if only considering the physical characteristics by which larvae are subjected to or the inherent behaviors that help to influence and orchestrate their ultimate locations. Instead, this recently new paradigm of bio-physically coupled larval dispersal has been solidified with examples from both larval fishes and invertebrates in both temperate and tropical oceans. The Gulf of Maine is no exception and has been the subject of several good studies including lobster. These findings have quantified the effects of larval lobster fate from the effects of mostly large-scale current fields and associated hydrodynamic features (100s of kilometers) along with temperature-based larval development and their distribution in the water column. Modeling these attributes using individually-based models (IBMs) for lobster in the GoM has since shown that a combination of outcomes are possible including downstream dispersal to adjacent areas, some long-distance dispersal, and still other areas that retain larvae. Superimposed on these patterns are the

potential changes to the GoM circulation regime and the timing of biological events due to climate change.

My work seeks to complement these studies and the existing knowledge base of GoM oceanography by focusing on the use of empirical, biological data to help calibrate existing dispersal models especially at local (10s of kilometers) scales for which there is a paucity of data. Specifically, my work is aimed at tracking the seasonal movements of egg-bearing lobsters and their location when they are close to hatching (Chapter 1) and to quantify the development of lobster eggs at naturally fluctuating temperatures (Chapter 2), both inshore and offshore to look at changes in hatching based on location. Together, these studies lend credence to the timing and location of larval hatch, two parameters that are vital biological inputs for future modeling studies. Additionally, the use of surface-ocean drifters (Chapter 3), released at biologically relevant temporal and spatial scales, provides at least initial estimates of the dispersal, location, and destinations for larvae that are released there. Applying these biological correlates to other ongoing and future modeling studies will help to clarify the marine connectivity of lobster in the GoM and provides data for the future management of this important marine species.

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APPENDIX D

CONSTRUCTION OF THE RACHEL DRIFTER

1. Starting with a 50" length of 2" PVC drill a ½" spar hole 2" from the bottom of the pipe. Used jig supplied. (Note: if your shower drain includes a coupling, you should start with a 49" length of pipe)

2. Drill a second spar hole 36" (on center) above the first.

3. Rotate pipe 90^0 and drill a spar hole 2" from the bottom and a second 36" OC above. (Note: To ensure the holes are perpendicular to each other, we often use a jig wrapped around the pipe which has holes equi-distant from each other.)

4. Measure the window weight, add four inches to the length and drill a hole for the 3/8" bolt at that distance from the bottom.

5. Pad window weight with pipe insulation and duct tape and insert weight into pipe. Insert bolt to hold the weight in position and tighten the locknut.

6. Heat plastic sleeve and position in the center of the 55" spars.

7. Drill two cotterpin holes in both ends of the 55" spar ¼" and 7/8" from the end.

8. Drill one cotterpin hole in each end of the 48" spar 1/4" from the end (Note: This step is optional since these cotterpins are used to hold washers that prevent sails from sliding off the spars at the bottom but one might alternatively use several wraps of electric tape)

9. Cut sail material to 41"x 19". Fold and glue edges to create a sleeve for the spars to a finish length of 36". (Note: If the material has a shiny side and a dull side, you want to glue the dull side. So, makes lines on the shiny side 2.5 inches from the edges to depict where the fold is made and make lines on the dull side 4.75" from the edge to mark where the fold should reach. Apply glue on both sides but make sure there is no excess glue at the fold that would prevent the spar from sliding into place. After glue has setup, insert spars temporarily to test the sleeve.)

10. Drill two 3/8" holes (¼" and 1 ¼") from the bottom of the buoy sticks.

11. Secure two toggles Secure two toggles (brown net buoys) to each buoy stick – a rubber mallet works well for this (Put a piece of scrap PVC pipe between the toggle and the mallet). Keep hammering the pair of toggles until there is a few inches of buoy stick poking through.

12. Add buoyancy to PVC (optional but recommended in winter conditions)
    - Cut a 3" disk of 3" diameter "floatie" material
    - Shove it down the top of the PVC pipe as far as the topmost spar hole
    - Pour in approximated 4oz of 2-part marine urethane foam

13. Attach GPS transmitter:
    - Drill ½" hole in the bottom of the shower drain so that water doesn't collect in it
    - Clean pipe & shower drain w/PVC cleaner & primer and PVC glue together
    - Protect GPS transmitter with either 4200 or 5200 marine caulking
    - Double bag the transmitter with bags made of extra sail cloth (Note; Put a note inside the bag describing the project, your contact info, and ask finders to mail transmitter back.)
    - Drill two side-by-side ½ holes through both sides of the PVC pipe approximately ½" from the shower drain or, if a coupling is used, through the coupling
    - Place transmitter on top and secure with 6½" hose clamp (Note: To prevent the hose clamp from chaffing through the bag material, pad with rubber or similar material)

Materials List

Item	Quantity
• PVC pipe - Sched40 – 2" by 50"	1
• Cesspool or Shower drain*	1
• Fiberglass Spars 48"	2
• Fiberglass Spars 55"	2
• Vinyl sails 39x19 (material supplied)	4
• Plastic sleeves for spars – 3.5"	4
• Buoy sticks	4
• Toggles	8
• GPS transmitter	1
• 5/8" hose clamp	8
• 6 1/2" hose clamp	1
• Cotter pins 3/32x1 1/4	12
• Washers 3/8"	14
• Hex head bolt - 3/8" x 3 1/4"	1

• Lock nut for hex-head bolt	1
• Window weight 4.5 – 5.5 lb.	1
• Pourable 2-part Marine Urethane Foam	4oz
• 3" length of 3" diameter "floatie"	1
• Extra vinyl cloth to protect GPS units	1
• PVC cleaner, primer, and glue	cans
• tube of Marine caulking (5200 best)	1
• Jig for marking hole positions	1
• pipe insulation (wraps window weight)	10 inches
• electric tape for securing the insulation	1
• strips of rubber-like chaffing guard	12" by 2"
• HH66 Cement	

*All Hardware should be Stainless Steel.

Tools List:

- Hack saw
- Tape measure
- Drill and drill bits: 1/2", 3/8", 3/32", 13/32"
- Files: flat and round
- Scissors
- Heat gun
- Vise
- Rubber mallet
- Screw driver
- Wrench: 3/8"

APPENDIX E

A COMPARISON OF HABITAT COMPOSITION AND CORRELATES TO SEASONAL LOBSTER MOVEMENTS IN A NEW ENGLAND ESTUARY

Abstract

Estuarine habitats provide interesting insights into the population dynamics and physiological tolerances of lobsters and may serve an adaptive significance in lobsters (*Homarus americanus*) residing and utilizing estuarine habitats like Great Bay Estuary (GBE). While many lobsters in GBE show directed seasonal movements into and out of GBE, a proportion remain resident year-round within the estuary, including ovigerous (egg-bearing) females. Few studies consider the actual utilization of estuarine habitats among mature, adult lobsters especially those that are egg-bearing and are potentially able to contribute future recruits to the fishery. The goal of this study was to correlate known positions of tagged lobsters in GBE by characterizing the habitats where these animals resided or moved to over a two-year tagging study. Underwater habitat mapping (videography) in tandem with diver surveys were conducted in four locations where lobsters were released. Habitat types, compiled and analyzed with a random-point count software package revealed significant differences between some sites (sandy, soft sediment) and others that comprised habitats that were characteristically more coastal marine habitats (estuarine tidal rapids) where lobsters preferentially exhibited long resident times. The strong relationship between complex,

rocky habitats and lobster residency suggest that habitat quality, marine landscape, and movements of lobsters (both transient and resident) are key criteria for lobster management and potential larval recruitment in some estuarine systems.

Introduction

Although the American lobster (*Homarus americanus*) supports one of the most important and successful fisheries in the Gulf of Maine (ASMFC 2009), lobster also comprises ecologically consequential populations in estuaries, such as the Great Bay estuary (GBE) in New Hampshire. GBE is a large, tidally mixed basin that comprises 23 km^2 of surface water and over 160 km of coastline intimately linked to the ocean through the Piscataqua River estuarine complex in New Hampshire and Maine as well as through Little Bay at a distance of 15 - 25 km from the coast (Brown and Arellano 1979; Fig. 1). Both Great Bay (GB) and Little Bay (LB) possess habitats that are generally characterized by eelgrass beds, extensive mud flats, and oyster reefs (Short 1992) with freshwater input from seven rivers that intermingle with tidal waters, influencing salinity levels, especially after severe episodic events.

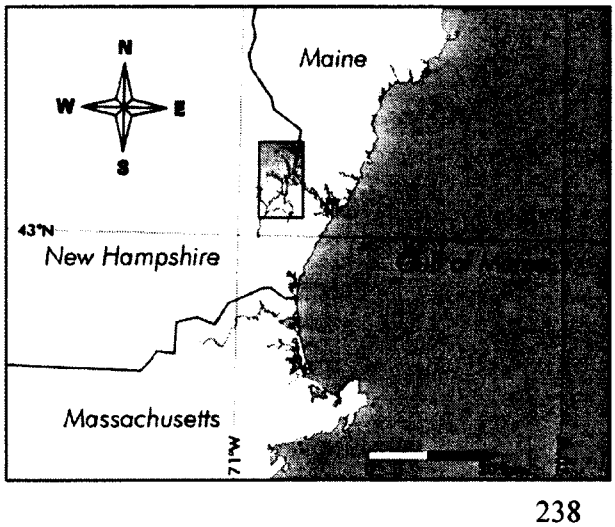

Fig. 1. Great Bay Estuary (GBE) is a large, tidally-mixed esutary with freshwater input from seven river systems. The upper reaches of GB are ~ 25 km from the ocean making it highly insulated. Historical and present habitats including eelgrass beds and oyster reefs make this system biologically diverse and ecologically significant. (map courtesy of UNH CCOM-JHC; http://ccom.unh.edu/).

Over the past decade commercial lobstering in GBE has generated over 100,000 pounds of lobsters at a value in excess of $500,000 (NHFG 2009). Estuarine habitats provide interesting insights into the population dynamics, behavioral patterns, and physiological tolerances of lobsters (Jury et al. 1994, Howell et al. 1999) and there may be an adaptive significance in lobsters residing and utilizing estuarine habitats that includes accelerated growth and molting cycles (due to warmer temperatures), protection from predation, reproduction, and the utilization of nursery habitats (Lawton and Lavalli 1995, Moriysu et al. 1999, Short et al. 2001). Both lab and field studies all indicate that lobsters in GBE can detect small changes in both temperature and salinity and also show predictable patterns of seasonal movements, highlighted most dramatically in the fall and spring (Vetrovs 1990, Jury et al. 1994, Crossin et al. 1998, Watson et al. 1999). While many lobsters in GBE show directed seasonal migrations into and out of the estuary (Watson et al. 1999), some remain year-round including ovigerous females (Chapter 2). These animals carry their eggs over the winter and spring months, and they subsequently hatch in the summer after a 9-11 month incubation period (Waddy and Aiken 1992).

Although lobsters are also regularly found in estuaries from Canada to New England, few studies consider the actual utilization of estuarine habitats among mature, adult lobsters especially those that are egg-bearing and are potentially able to contribute to future recruits to the fishery. Limited historical data from neuston tows in GBE indicate that lobster larvae (Stages I-IV) are present in the water column during the summer months (NHFG 2009). More recently, small young-of-year (YOY) lobsters were captured during juvenile lobster surveys in the Piscataqua River, just outside the Great Bay reserve area

(NHFG 2008). Taken together, these data suggest that lobster larvae are released and settle in the GBE and that some proportion of the population is derived from resident lobster reproduction.

It is more than likely that adult lobsters utilize benthic habitats in estuaries that are conducive as shelters for overwintering. These marine landscapes, often composed of a mosaic of habitat types, can often delineate the ecological conditions for that area influencing things like resource utilization and survival (Saunders et al. 1991). Changes in habitat use can be influenced by contrasting habitat types, the presence of other species, and the age and size of organisms, among others. The movements by animals such as lobsters in fragmented landscapes (e.g., estuaries) may involve trade-offs between foraging, reproduction, or predation risk (Werner and Gilliam 1984). Lobsters utilize a variety of habitat types (e.g., nearshore rocky areas, offshore canyons, enclosed embayments, estuaries) that differ in their abiotic and biotic features over spatial and temporal scales (Selgrath et al. 2007). Habitat types and usage can also change over the course of lobster development (i.e., ontogenetic shifts; Lawton and Lavalli 1995). For example, during the early benthic settlement phase, small lobsters have been reported to preferentially select habitats for shelter that include cobble beds or salt-marsh peet reefs (Able et al. 1988, Wahle and Steneck 1992, Hovel and Wahle 2010). In addition, Short et al. (2001) found evidence of adolescent lobsters and their preference for eelgrass beds in the lower portion of the GBE.

The GBE contains a diverse cross-section of potentially conducive lobster settlement and juvenile nursery habitats including eelgrass beds and rocky intertidal habitat, among

others (Short 1992). Recruitment studies of lobsters to estuaries are rare although in one study, Wahle (1993) used a combination of population censuses and underwater surveys to quantify lobster recruits and found a gradient of settlement occurring up into Narragansett Bay. One goal of this study was to ascertain if there are characteristics of GBE habitats that are distinctly advantageous to both early-benthic-phase lobsters as well as adults.

Despite some of the aforementioned studies of habitat preference and utilization by early benthic and juvenile-stage lobsters, almost no information exists for habitat preference and utilization by sub-legal and adult-sized lobsters in estuaries. In fact, most papers that involve studies of seasonal movements by adult crustaceans (primary crabs and lobsters) neglect to single out habitat type as a correlate to explain movements or residency in an area. Stone and O'Clair (2002) used ultrasonic telemetry to track female Dungeness crabs (*Cancer magister*) over the course of a season in an Alaskan estuary and relate seasonal movements to habitat use. These findings suggest that female crabs utilized benthic sediments for brooding their eggs over the winter and then moved into more shallow rocky habitats in the spring where dissolved oxygen levels were higher to release their larvae. Geraldi et al. (2009) used a combination of geo-referenced lobster-trap arrays to measure catch and describe associated benthic habitats (using side-scan sonar) to determine that lobster movements depend on the quality of habitat which, in turn, affect their movements (i.e., habitat-related behavior).

The goal of this study was to correlate known positions of tagged lobsters in GBE with habitat use by characterizing local habitats where these animals resided or moved to over

a two-year tagging study. Correlates of these habitat types may be beneficial to egg development, shelter, or foraging activities.

Materials and Methods

Study Site

Underwater video mapping was conducted in the fall of 2009 at four selected areas within the Little Bay-Piscataqua River complex in areas where lobsters were tagged and followed over two successive seasons from 2007-2009. Specifically, these areas consisted of sections of benthic habitats around the peripheries of Goat Island (GI) (north and south), Fox Point (FP), and Little Bay (LB) with an average depth of 5 m (Fig. 2). Movement studies and analyses using ultrasonic telemetry revealed that a large proportion of lobsters of all sizes (including ovigerous lobsters) stayed within the confines of the areas that were mapped (see Langley et al. in prep.).

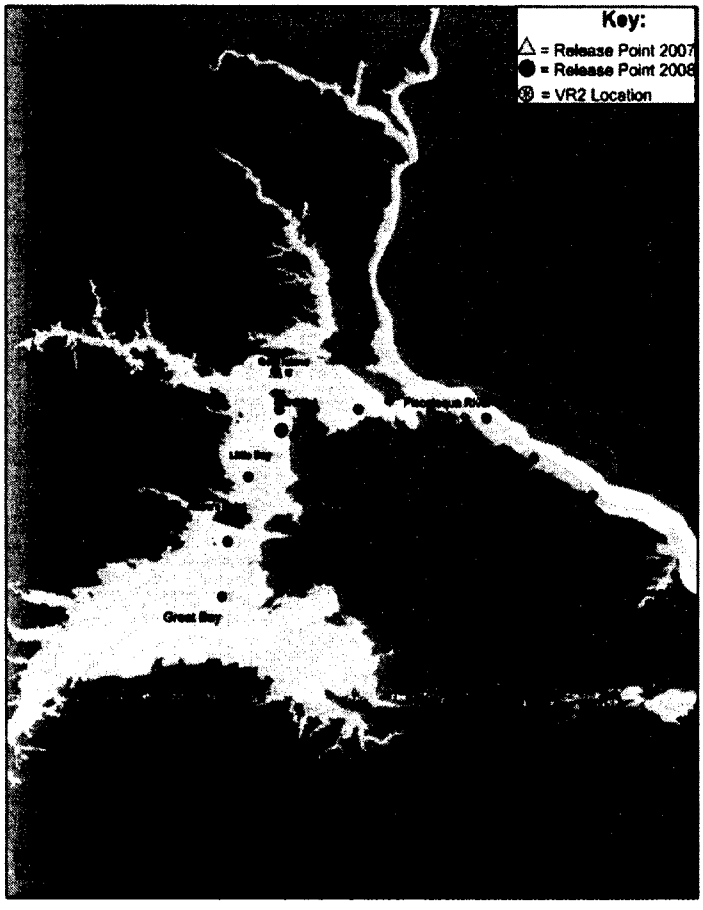

Fig. 2. Map of the Great Bay (GBE) estuary complex including the Piscataqua River, leading to Portsmouth, NH. Lobsters were tagged with ultrasonic tags and released at two separate sites from 2007-2008. All animals were tracked both manually and using a fixed array of logging devices (VRs) set out within GB. The majority of lobsters tagged in 2007 stayed within the shaded area compared with lobsters that tended to move down-estuary at the 2008 release site.

Videography System

Underwater video mapping was conducted using a custom-made underwater videography system consisting of a Sea-Drop 650 underwater color camera complete with 45 m of fabricated cable mounted on a stainless-steel benthic sled (see Grizzle et al. 2008 for details). Video was viewed live on an onboard LCD screen and recorded to an 80 GB hard-drive using a SEA-DVR, mini digital video recorder and a SEA-TRAK™ GPS video overlay which was selected for later spatial analysis using ArcGIS v. 9.3 (ESRI Corp. Redlands, CA). All video components were purchased and customized from an underwater video specialty manufacturer (SeaViewer Inc., Tampa, FL). The camera-sled

system was typically deployed into the water with a steel cable on a manually operated winch from a boat (Fig. 3). After positioning the camera at a height suitable for obtaining adequate image quality and swath width (typically about 0.5 m), the unit was slowly towed (~ 1.5 knots) alongside the boat so that it remained directly below the winch. Video images were viewed in real-time to allow for quick adjustments of the camera throughout the survey. For purposes of this study, continuous video imagery was acquired from 3-5 boat transects parallel across each study area, and an additional set of 3-5 transects set perpendicular. A total of 40-55 minutes of video was recorded for each location.

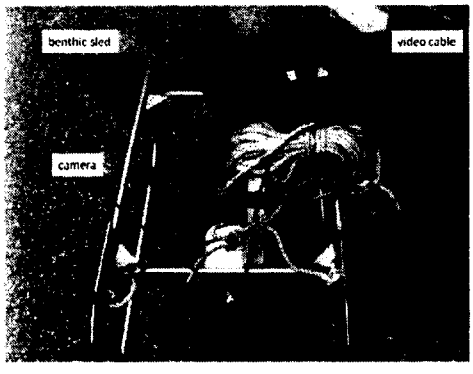

Fig. 3. Benthic sled design and setup for video-mapping selected nearshore areas of Great Bay. Stainless steel sled included a color camera connected to 45 m of video cable fed topside to a small research boat pulling the sled at ~ 1.5 knots. Video was viewed live onboard, recorded to a DVR, and over-layed with GPS coordinates for further analysis (see results).

Video and Habitat Analysis

All digital video was uploaded from its hard-drive to a Mac-mini computer (Apple Inc., Cupertino, CA). Still images from video recordings were digitally captured at 30-second intervals using QuickTime Pro v.7.0 (Apple Inc.) and saved as individual JPEG files for a total of 50-60 images per site. The compilation of all still images were then imported into a random-point count software program (Coral Point Count with Excel extensions, CPCe v. 3.6; see: http://www.nova.edu/ocean/cpce/) that assigns points to prominent

bottom habitat features (e.g., coral, rubble, algae, sand, etc.) to visually identify and quantify them (see Kohler and Gill 2006 for details). CPCe uses a matrix of randomly distributed points overlaid on digital images to quantify the proportion of substrate types and then statistically compiles these values to estimate the proportion of biota present. For this analysis, a stratified random design (5 rows, 5 columns, and 1 point per cell) was chosen with a total of 25 points for each image (Fig. 4).

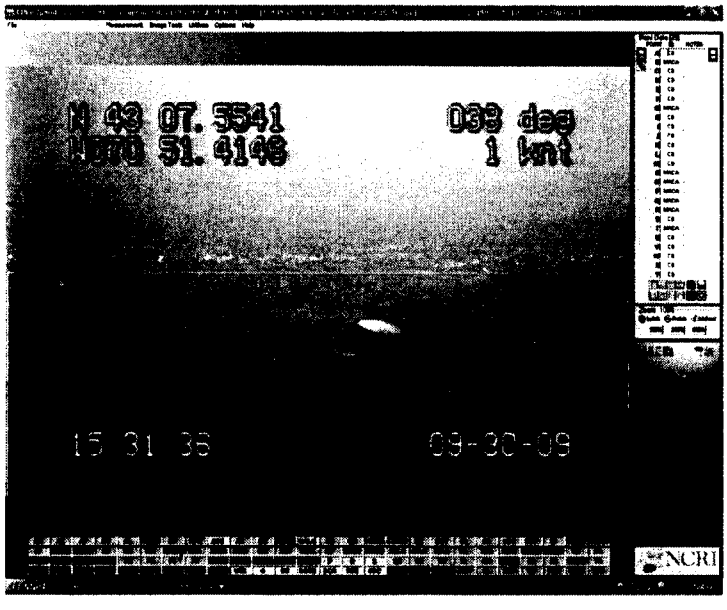

Fig. 4. Example of image processing in Coral Point Count with Excel extensions, CPCe v. 3.6; www.nova.edu/ocean/cpce/). Habitat features are assigned based on pre-defined features and quantified using a stratified random design (side and bottom graphics bars). Points are assigned as letters and black horizontal line designates the cutoff of benthic habitat that is being analyzed.

Preset coral categories were customized to six categories representative of local benthic habitats (verified by SCUBA) and included: cobble, rubble, boulder, sand, macroalgae, and other. Habitat features were categorized according methods and descriptions in both Wahle and Steneck (1991) and Wahle (1993). CPCe analysis gave the average % cover (± se) for each image as well as ascribing a Shannon-Weiner diversity index based on comparisons of each of the 6 habitat types defined for each image. A Shannon-Weiner

index (H) in this case, takes into account the proportion of each habitat (evenness) and the amount of each habitat feature (richness) represented by the following function:

$$H = -\sum_{i=1}^{s} p_i \ln(p_i)$$

Where H is the diversity index, s is the number of species, and p_i is the proportion of individuals of the total sample belonging to the i^{th} species (Smith and Smith 2001).

Statistics

Data compiled by the CPCe algorithm from each of the four sites were pooled into two sites demarcated by Fox Point (FP): 1) Goat Island and downstream of FP; and 2) FP and upstream to Little Bay (Fig. 2). Overall differences between sites were analyzed as a 2-factor nested ANOVA (model I) using SPSS v. 18.0 (SPSS Inc., Chicago, Illinois). A GLM (univariate) model was fit for 2 factors: location (2 levels), and habitat types (6 levels) with the dependent variable, %-cover. Raw data were arc-sin transformed to meet the parametric assumptions of homogeneity of variance and normality. Differences among habitat features in the interaction term (location*habitat type) were assessed using a series of post-hoc Tukey's HSD tests at an $\alpha = 0.05$. Differences in diversity indicies (H) were tested using a one-way ANOVA between each of the two sites. All graphical output is represented as the mean ± se.

Diver Surveys

For each of the four areas a total of two SCUBA surveys were conducted with the goals of 1) confirming and comparing major habitat types seen by the video analysis; and 2) visually censusing lobsters. For the first goal, two divers surveyed a 2 m swath along 25 . m transects placed out from a center point in each of the four cardinal compass directions. Habitat types (mentioned above) were quantified as % cover calculated as a proportion of habitat along each transect. For the second goal, divers conducted 30-mintue visual surveys in each area to count all lobsters encountered. Coverages for each habitat type were compared to similar data from the video surveys using a series of one-way ANOVA analyses at an $\alpha = 0.05$

Results

Overall, a total of 114 images and 121 images were extracted and analyzed from videos at site 1 (GI - FP downstream) and site 2 (LB – FP upstream), respectively. In general, habitat composition was different with respect to habitat type and coverage between the two locations (Fig. 5). LB can be typically characterized by large, sand-covered sections and mud bottoms interspersed with small patches of cobble and some boulder compared with GI, a predominantly rocky cover containing complex macroalgal patches and some sandy areas. A photomontage (Fig. 6) also shows details of habitat differences among survey sites.

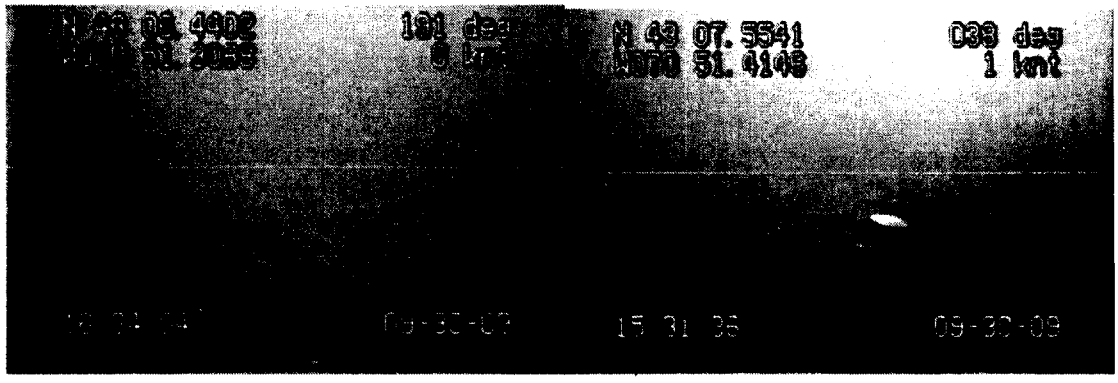

Fig. 5. A comparison of typical bottom habitat between A) Little Bay and B) Goat Island. Lobsters were tagged at each site however, animals that were tagged in Little Bay had a propensity to move toward Fox Point and Goat Island, especially to over-winter in such habitats. All images were taken as still frame JPEGs from video. Pictures were imported into the CPCe software and analyzed below the yellow line. GPS overlays for all video (and images) provide georeferencing for mapping areas covered using ArcGIS v. 9.3.

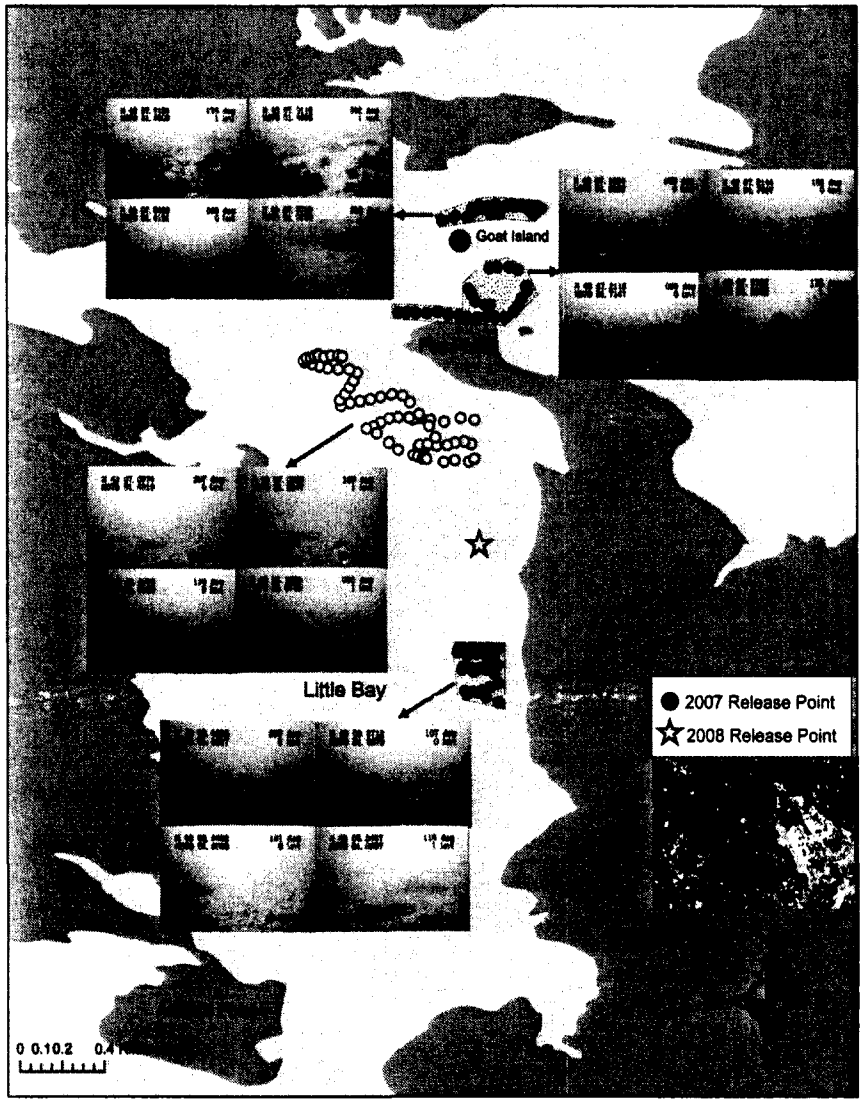

Fig. 6. A photomontage of habitat composition between sites: 1) Little Bay and upriver of Fox Point (blue and yellow) 256,947 m^2, and 2) Goat Island and downriver of Fox Point and Downriver (green and red) 177,465 m^2. Digital images are representative of habitat features for each area and averaged from a compilation of still images extracted from video. Points for each location were georeferenced to images and mapped using ArcGIS v. 9.3.

Habitat Analysis and Diversity Indices

Total %-cover of all habitat types suggests a predominant mix of cobble, boulder, and sand downstream from Fox Point to Goat Island, compared with a shift more towards

sand and cobble upstream from Fox Point into Little Bay (Table 1). Pooled data between GI and FP (downriver) and LB and FP (upriver), indicated a significant difference was seen between sites with respect to habitat coverage ($F = 49.04$, $df = 5, 304$; $p = 0.001$, $1\text{-}\beta = 1.00$) and the interaction of location with habitat ($F = 4.720$, $df = 5, 304$; $p = 0.001$, $1\text{-}\beta = 0.98$) (Table 2). Post-hoc comparisons of each of the 6 habitat types examined showed that three of the habitat types: boulder, sand, and macroalgae, were markedly different in overall %-cover at GI compared with LB (Tukey's HSD; $p = 0.001$; Fig. 7). Average sand and macroalgal coverages in LB were 74.4 and 14.1 % compared with 11.5 and 23.6 % for GI (Fig. 6).

Location:	Goat Island	Fox Point (downriver)	Fox Point (upriver)	Little Bay
Habitat:				
cobble	38.63 ± 6.63	62.35 ± 6.24	49.17 ± 6.81	30.25 ± 7.33
rubble	5.38 ± 1.98	3.53 ± 1.44	1.08 ± 0.57	7.00 ± 2.44
boulder	8.63 ± 3.07	18.59 ± 4.66	1.17 ± 1.01	4.5 ± 2.43
macroalgae	15.5 ± 2.86	6.24 ± 2.82	3.42 ± 1.08	8.5 ± 2.02
sand	30.38 ± 5.78	8.12 ± 3.91	42.92 ± 6.11	49.63 ± 7.17
other	1.5 ± 0.67	1.18 ± 0.52	2.25 ± 0.75	0.13 ± 0.13
TOTAL	100.00	100.00	100.00	100.00

Table 1. Averages (± sem) for total %-coverage between areas that were mapped using videography. Also see Fig. 6 for spatial references to each specific location.

Source of variation	**df**	**MS**	**F**	**p**	**1-β**
location	1	0.570	3.83	0.051	0.50
habitat type	5	7.311	49.04	0.001	1.00
location*habitat type	5	0.704	4.720	0.001	0.98
within Groups (Error)	304	0.149			
Total	315	8.734			

Table 2. ANOVA summary table for the analysis of habitat type differences between upstream of Fox Point (including a portion of Little Bay) and downstream of Fox Point, including Goat Island.

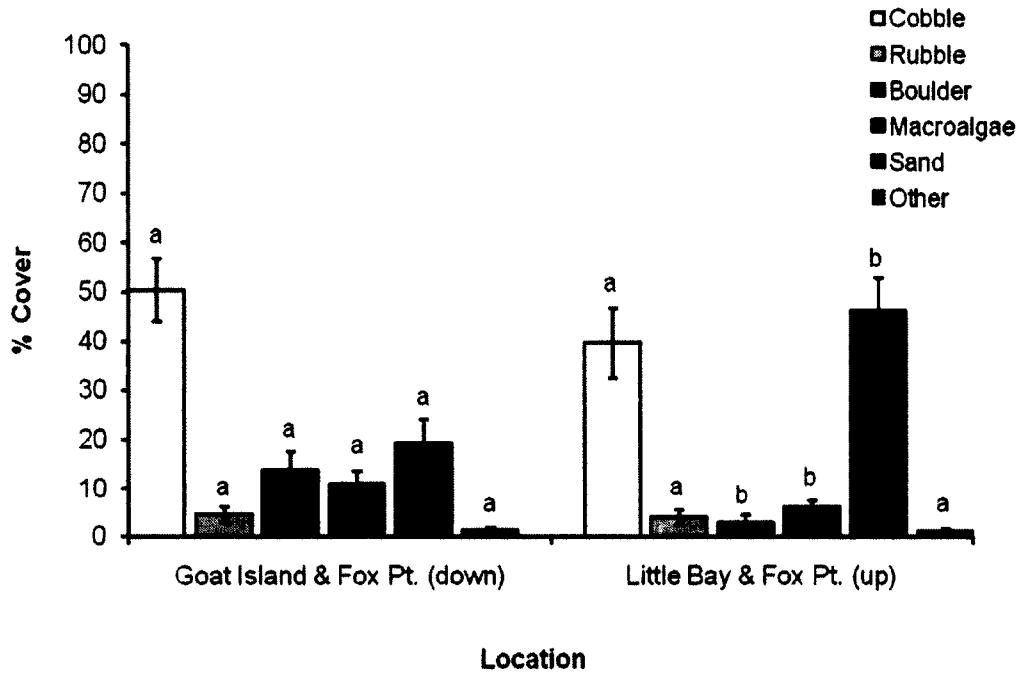

Fig. 7. Habitat coverage for Goat Island and Little Bay (pooled with Fox Pt data from either upriver or downriver). Habitat types were compiled from images derived from video surveys at each location and analyzed with a random-point count software package (CPCe). Data are presented as means ± se. Different letters denote a significant difference between habitat types (ANOVA, $p < 0.05$).

Overall, Shannon-Weiner indices (H) were higher and significantly different at GI (H_{AVG} = 0.75 ± 0.041; n = 55) compared with LB (H_{AVG} = 0.56 ± 0.49; n = 51) (1-way ANOVA; F = 8.312, df = 1, 102; p = 0.005) indicating a more diverse and even habitat composition around GI than LB.

Diver Surveys

SCUBA surveys from GI and LB verified that habitats were representative of what was captured by underwater video surveys and also not significantly different from any of the six habitat types measured (one-way ANOVAs; $p > 0.05$; Fig 8). A total of 22 lobsters

were counted at LB dive sites compared with 53 lobsters at GI. Divers confirmed a variety of sizes (sublegal and legal) at each site but individual lobster measurements were not conducted due to logistical constraints with the dives.

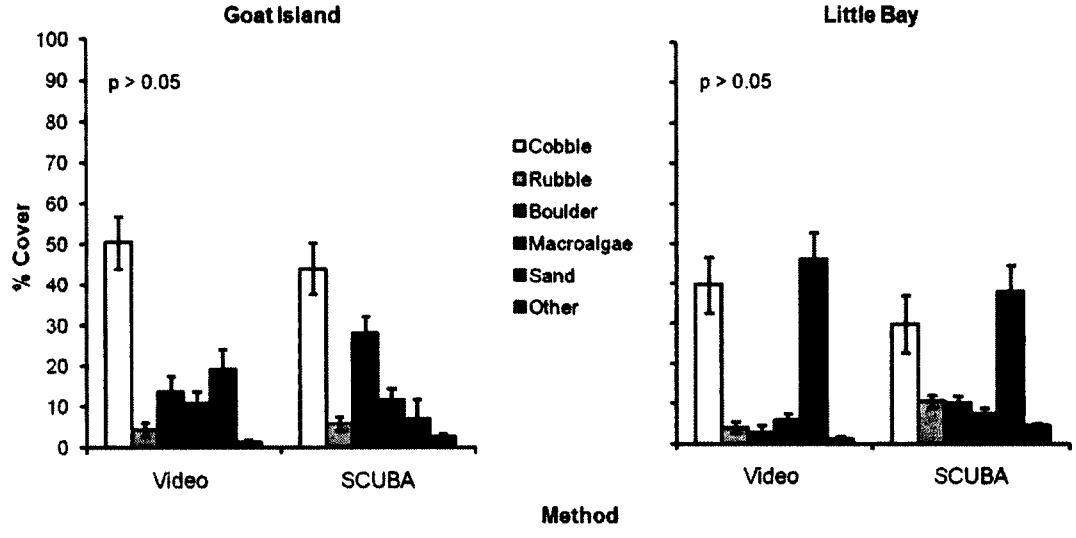

Fig. 8. Habitat coverage for Goat Island and Little Bay (pooled with Fox Pt data from either upriver or downriver) between video and SCUBA methodologies. Data are presented as means ± se. There were no significant differences between any of the habitat types among both methodologies that were used (ANOVA, $p > 0.05$).

Discussion

It is generally considered that elements of habitat quality and its associated spatial distribution are factors that can significantly shape the distribution, movements, and population structure of local species (Pittman and McAlpine 2003). The impetus for this study was to determine if differences in habitat composition could be used to help characterize the movements or residency of lobsters that were tagged in areas of the GBE over a two-year period. Although the bathymetry and generalized habitat features of GBE have recently been mapped (CCOM-JHC 2002), this study was aimed at mapping and quantifying habitat features that were associated with known areas of lobster

movement and residency at a much smaller, but at a more detailed biological resolution (i.e., microhabitat). Overall, these findings suggest that habitat composition was markedly different between upstream and downstream locations of Fox Point (FP). Areas downstream of FP are complex and often described as 'ocean-like' with high, rapid flows and patches of kelp interspersed with cobble and boulder fields (NHFG 1990, Becker 1994, Grizzle 2005, this study). Mathieson et al. (1981, 1983) described a variety of flora including Irish moss (*Chondrus crispus*) and subtidal kelp (*Laminaria digitata*) beds in the lower reaches of the GBE (downstream from FP) that are more typical of coastal areas (i.e., estuarine tidal rapids) than estuaries. Although LB contained some areas of cobble (closer to FP), most of the area that was surveyed encompassed extensive sand and mud flats.

Becker (1994) conducted a more extensive SCUBA survey of habitat composition in LB and an adjacent cove just downstream of our GI survey site that also corroborates a measureable difference in bottom cover between similar locations. Becker (1994) quantified over 15 types of bottom cover including 6 types of cobble and boulder size compositions to find that > 80 % of coverage in the lower reaches of the GBE estuary were composed of cobble-boulder complexes compared with 80 % soft-sediment coverage upstream of FP. It was somewhat surprising that cobble habitat was not statistically different between the sites examined, however this particular study was designed to examine habitats in areas only specific to lobster release sites and areas where animals resided (Figs. 2, 6) whereas larger, macro-scale surveys were more comprehensive in their study area. Additionally, what is generally termed 'rocky habitat'

(boulder and cobble) were analyzed as separate categories in this study (Table 1); Selgrath et al. (2007) defined cobble as *a mixture of unconsolidated pebbles, cobbles, and boulders, 1-400 cm.* When conducting an additional ANOVA analysis combining both cobble and boulder between sites, we found that they are significantly different (1-way ANOVA; $p = 0.018$) with a total coverage of 66 % and 48 % for GI and FP, respectively.

Use of Complex Habitats by Lobsters

What was not surprising was the affinity of lobsters for complex habitats as was the case in an order of magnitude difference of lobsters found downstream from FP compared with LB. Although lobster size frequencies were not amassed for this study, Becker (1994) found that > 50 % of all lobsters found in their dive surveys were between 40-60 mm CL downstream of FP including a proportion of very small, newly recruited, lobsters (10-30 mm CL). The ecological ontogeny of small lobsters to complex habitats such as cobble, boulder, and algal beds affords them advantages in shelter and foraging (Wahle and Steneck 1991, see Lawton and Lavalli 1995 for review) and some studies suggest a positive correlation between complex habitats and a representation of a variety of lobster sizes (Selgrath et al. 2007). Often referred to as microhabitat features, such areas are capable of supporting a variety of flora and fauna influencing the distribution of organisms in particular areas (Saunders et al. 1991). The higher diversity index (H) in habitat coverage at GI compared with LI further supports the complexity of habitat variety in these areas.

Lobster Movements and Distributions

Movement to and residency of lobsters in complex habitats may be related to behavioral traits and requirements for both juvenile and adult lobsters (Lawton and Lavalli 1995). For example, it has been well documented that some lobsters from the coast more up into GBE during the summer months (Watson et al. 1999), however it remains unclear how many stay as densities of animals that do remain resident may be in proportion to the total number of available shelters or suitable habitat that is available (Cobb 1971, Wahle and Incze 1997). There are few, if any, reports of year-round resident adult lobster populations in estuarine systems (e.g., Wahle 1993) however, given that selected areas from this survey support appropriate lobster habitat, it is reasonable to speculate that a proportion of animals reside there. In 2007, over 30 lobsters (70-90 mm CL) were tagged at GI and although some moved downstream to Great Bay Marina, an overall distance of about 0.5-0.75 km, most remained near their original tagging location for a full season.

By comparison, lobsters tagged and released in 2008 in LB showed a net cumulative movement downstream towards FP and GI (Langley et al. in-prep.). Although environmental cues (e.g., temperature, salinity, photoperiod) are most likely punctuating these movements (Watson et al. 1999), the presence of functional habitat may also elicit a significant influence on residency once it is encountered or becomes available. In one study using seabed mapping and lobster trap and tagging techniques, Geraldi et al. (2009) found that lobsters caught in rocky, complex substrates moved far less than those caught and released in soft sediment habitats. Specifically, 82 % of lobsters caught on rocky

substrate were caught again in the same habitat. Furthermore, it is suggested that some areas of sediment between bedrock outcroppings or deep channels serve as corridors for lobsters engaged in short- or long-term movements to finding sheltering habitats (Geraldi et al. 2009). Other crustacean (spider and king crabs) movements have been attributed to ontogenetic movements in response to habitat selection on a seasonal basis and have been characterized by temperature and substrate type (Stone et al. 1992, Gonzalez-Gurriaran et al. 2002). Clearly, more work is needed to accurately determine the relationship between habitat quality, marine landscape, and movements of lobsters (both transient and resident) as a key consideration for lobster management and for the consideration of no-take areas.

Implications of Habitat and Lobster Movements

Lobsters that may concentrate and reside in smaller regions of preferred habitat in locations like the GBE suggest that these areas provide direct benefits to local lobster populations and the fishery. Rowe (2002) for example found that no-take reserves in Bonavista Bay, Newfoundland in suitable lobster habitat increased lobster density and suggested this to offer shelter for ovigerous females. Similarly, Selgrath et al. (2007) reported that patchy environments (particularly edges), that included cobble and seagrass, were integral to the survival and distribution of lobsters among a range of sizes. Fragmented habitats also are known to hold significant refuge value for crustaceans as well as influence predator-prey dynamics (Micheli and Peterson 1999, Hovel and Lipcius 2001, Grabowski et al. 2008, Hovel and Wahle 2010). Other studies have looked extensively at lobster densities in eelgrass beds (Short et al. 2001) but more studies are

needed to determine potential populations in other kinds of habitats. Wind-drive surface drifters released around FP and GI tended to show that larvae released from ovigerous females are largely retained locally in the system (Chapter 3) suggesting that settlement by at least a proportion of these larvae, would be most favorable to survival in complex marine habitats such as GI where cobble fields and macroalgal beds afford the most conducive habitat type for shelter (Wahle and Steneck 1991, Wahle and Steneck 1992).

The endpoint of larval transport (i.e., settlement) could also fill in other knowledge gaps in local settlement patterns for important estuarine shellfish species including oyster as well as aid in modeling potential larval retention (connectivity) as has been done for a variety of marine larvae including lobster in the Gulf of Maine (Incze et al. 2010). Special habitats such as these have potential management implications for lobsters in the GBE as it relates to essential fish habitat (EFH; Steneck 1995) and may provide evidence for habitat correlates for all life-stages of lobsters that are distinct traits of EFH over both spatial and temporal scales and would be highly unique to estuarine systems.

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APPENDIX F

BAIT-SUBSIDIZED DIETS AND THEIR EFFECTS ON OVARY AND EGG QUALITY IN OVIGEROUS AMERICAN LOBSTERS, *HOMARUS AMERICANUS*

Abstract

Ovigerous (egg-bearing) American lobsters (*Homarus americanus*) exhibit a protracted period of ovary maturation and maternal care while incubating their eggs thereby influencing offspring fitness. Lobsters consume a wide and flexible range of food items; however, trap bait may comprise a large proportion of the diet in some fished areas, and the long-term consequences of a bait-based diet remain largely unexplored. We tested the hypothesis that disproportionate amounts of bait in the diets of pre-ovigerous females affect the quality of their ovaries and eggs. A total of 15 pre-ovigerous lobsters were collected and held over a period of ~ 300 days (range = 270-378) while being fed diets of herring bait, natural foods, or a combination. Nutritional status, measured as biweekly blood indices and total glucose levels, indicated differences between lobsters fed a natural or combination diet and lobsters fed a bait-based diet (ANOVA; $p < 0.05$). Bait diets contained more protein (58.5 %) and lipids (31.6 %) compared to natural diets (34.5 % and 13.2 %, respectively). Lipid levels in ovaries and eggs were significantly correlated for all treatments ($r = 0.76$, $n = 15$, $p = 0.028$). Finally, histopathological analyses suggest that ovary tissue was compromised in lobsters that were starved or fed with bait. Our findings suggest that a varied diet of food constituents promotes the

overall fitness of ovigerous lobsters and the associated reserves that are used for ovary development and subsequent oocyte formation

Introduction

Ovigerous (egg-bearing) American lobsters (*Homarus americanus*) exhibit a protracted period of ovary maturation and maternal care in comparison to other marine crustaceans, carrying their eggs (externally) in excess of nine months (Bumpus 1891, Talbot and Helluy 1995). Such maternal effects, defined broadly as *nongenetic traits that are acquired from the mother*, elicit potentially significant impacts on the ecological and evolutionary life histories of marine organisms (reviewed in Bernardo 1996). Within marine populations, environmental stressors (e.g., salinity, temperature, pollution) and food availability can all negatively affect egg size and offspring fitness (reviewed in Marshall and Keough 2008). In particular, maternal nutrition can have pronounced 'carry over effects' on offspring fitness as is the case in a variety of invertebrate species including beetles, polycheate worms, bryozoans, and crabs (Bernardo 1996, Qian and Chia 1991, Fox 2000, Marshall and Keough 2004, Sato and Suzuki 2010), although there are exceptions to this (see Lewis and Choat 1993). Based on these complex relationships, theoretical models that build on the linkages between maternal size and offspring fitness incorporate the influence of maternal nutritional state among other factors including environmental changes and fishing pressure, among others (Marshall and Keough 2008, Brander 2010, Moland et al. 2010).

American lobsters are fished intensively throughout their range in the U.S. and Canadian maritimes, and the fishery relies heavily on baited-traps (ASMFC 2009). It is estimated that coastal Maine alone fishes in excess of 650,000 traps per season (Maine DMR 2010) resulting in almost 70 % of Atlantic herring (*Clupea harengus*) landings (70,000-75,000 metric tons) being reintroduced back as lobster bait into coastal benthic habitats (Salia et al. 2002, Grabowski et al. 2010). Consequently, it is strongly believed that herring bait subsidizes lobster growth and population increases in some areas of its range (Salia et al. 2002, Grabowski et al. 2009). The mechanisms by which this happens are debated and largely unproven, but may be enhanced by: 1) inefficiencies of the traps themselves, creating a disproportionate number of sub-legal lobsters that enter traps and escape (Karnofsky and Price 1989, Jury et al. 2001, Watson et al. 2009); 2) changes in trophic level dynamics (e.g., decline in large predatory fishes, Steneck and Wilson 2001); 3) changes in long-term management practices (e.g., protection of egg-bearing females, Miller 1995); and 4) alterations to physical oceanographic conditions (e.g., increased seawater temperatures, Drinkwater et al. 1996).

It is proposed that bait may comprise a large proportion of a lobster's diet (upwards of 34-55 %), which could substantially impact overall lobster health (Myers and Tlusty 2009). A recent survey of bait use by Nova Scotian lobstermen indicated an average of 860 g (1.9 lbs.) of bait was used each time a trap was set, translating to over 5,216 kg (11,500 lbs.) of bait/year/lobsterman (Harnish and Willison 2009). With such large volumes of bait being used in some areas, the ecological and economical implications of bait subsidies are a concern to both scientists and industry (Thunberg 2007).

Nonetheless, the biological impacts resulting from a disproportionate amount of bait input into the lobster fishery are largely uninvestigated but could very likely act as potential nutritional stressors increasing the susceptibility of lobsters to a number of aquatic diseases (Sindermann 1990, Tlusty et al. 2000, 2008, Myers and Tlusty 2009).

Lobster bait in the Gulf of Maine predominantly comprises salted or fresh herring or mackerel (*Scomber scombrus*) that, if not included among a more diverse diet, is depleted of certain nutritional constituents (e.g., amino acids, minerals, Gendron et al. 2001). The nutritional requirements of adult lobsters have yet to be fully resolved (reviewed in Conklin 1995); however, the best growth results from larval and juvenile culture operations have been obtained from a varied diet (Conklin 1995, Tlusty et al. 2005a, b). In the absence of fishing, lobsters forage among a wide spectrum of plants and animals that include crustaceans, mollusks, echinoderms, polycheates, and macroalgae. Lobsters are also known to temporally shift their diet depending on season or habitat (Elner and Campbell 1987, Conklin 1995) and are considered 'keystone' predators, capable of driving the trophic dynamics in many benthic communities (Mann and Breen 1972). In addition, ovigerous lobsters are known to undertake sometimes long and dramatic seasonal movements, allowing them to encounter a variety of benthic habitat types as well as fishing areas of varying trap densities (Cooper and Uzmann 1980, Campbell and Stasko 1985, Cowan et al. 2006). These patterns of behavior and activity potentially affect the kinds and quantities of food items that they encounter.

Alternatively, the prevalence of bait and natural foraging items for lobsters depends heavily on the habitats they occupy, trap densities being fished, and the distribution and abundance of lobsters in a particular area (Geraldi et al. 2009, Watson et al. 2009, Grabowski et al. 2010). Laboratory studies (using juvenile lobsters) have shown that a diet composed primarily of fish can cause phenotypic changes in shell color, but it remains unknown if an exclusive diet of fish induces deleterious effects on maturation, egg production, or shell integrity in adults (Tlusty et al. 2008). There is some evidence to suggest negative, short-term consequences associated with lobsters consuming bait. Prince et al. (1995) for example observed a reduction in the overall incidence of shell disease in a lobster pound fed with an artificial diet compared to one that used salted cod racks. Gendron et al. (2001) reported that a varied natural diet, augmented by rock crab (*Cancer irroratus*) rations over a 3-week period had favorable effects on ovary condition throughout the maturation process. Using stable isotope ratio analysis, Grabowski et al. (2010) showed that bait-subsidized lobsters grew faster than those animals in seasonally closed fishing areas. However, the long-term consequences of lobsters fed primarily on bait, particularly the effects on ovary and egg condition in mature adult lobsters, remains unexplored.

Pre-ovigerous female lobsters presumably enter lobster traps as frequently as other lobsters, possibly more often during periods of seasonal movement events (Herrick 1895, Campbell 1986). If these animals feed primarily on the bait from traps, nutritionally-mediated maternal effects could result in carry over effects on ovary condition, leading to poor egg quality (e.g., low lipid content) – this is the working hypothesis for this study.

Sexually mature females may spend most of the year prior to egg extrusion foraging to build energy reserves in preparation for ovary maturation and oviposition (Waddy and Aiken 1992). It is not unreasonable then, that food items that are readily consumed (trap bait and natural foods) would have an impact on ovary, and in turn, egg constitution. Newly extruded eggs are high in lipid content presumably a reflection of maternal provisioning that originates from the ovary maturation process and the sequestering of yolk reserves during vitellogenesis (Sasaki et al. 1986, Talbot and Helluy 1995). If pre-ovigerous lobsters are consuming disproportionate amounts of low quality bait in their diets, their health could be compromised or altered at many levels (e.g., decreased disease resistance, altered maturation schedules, egg quality) and may ultimately translate into variable egg quality and larval survival. Nutritional provisioning in lobsters from mother to offspring may have direct carry-over effects manifested at any level of their life-history and has been documented for other marine invertebrates (e.g., sea urchins, Bertram and Strathmann 1998).

The impetus for this study came from many of our recent studies involving egg development, seasonal movements, and mating dynamics of ovigerous lobsters (Johnson et al. 2011, Goldstein and Watson submitted, Pugh et al. in prep., Goldstein et al. in prep.). In light of the potential disparity in quality between natural food sources and trap bait, the goal of this study was to ascertain the potential nutritional effects that bait-subsidized diets have on lobster health by focusing on ovigerous lobsters and their ovary tissue and egg quality. The hypothesis that was tested was that pre-ovigerous females that consume disproportionate amounts of bait could compromise their ovary condition

and egg quality (i.e., egg reserves). To begin to address this, pre-ovigerous lobsters were collected and held long-term while being fed diets of herring bait, natural foods, or a combination of the two.

Materials and Methods

Animal Collection and Assessment

Pre-ovigerous female lobsters were collected by permitted lobstermen in Massachusetts (Cape Ann) and New Hampshire coastal waters in the spring of 2009. All lobsters were held onboard commercial fishing boats in live-wells with running ambient seawater. All animals were transported to the University of New Hampshire's Coastal Marine Laboratory (CML) in New Castle, New Hampshire, USA where all subsequent work was conducted. A total of 15 lobsters (size range = 85-96 mm carapace length, CL, average = 94 ± 2.2 sem) were measured using digital calipers to 1 mm (Mitutoyo IP 65, Mitutoyo Corp., Japan) and selected for this study. A preliminary analysis was conducted to confirm that there were no significant differences in the sizes of lobsters used between treatment groups (1-way ANOVA; $F_{3,14} = 0.62$, $p = 0.62$).

The reproductive histories of all female lobsters were assessed in the laboratory as they were used in a previous mating study (duration ~ 30 days) and fed a combination of fresh and frozen fish, squid and shrimp. In these cases, animals were visually confirmed (by video; Pugh unpub. data) as having mated and were sampled noninvasively for the presence of a small amount of fresh sperm plug from individual spermatophores

(Goldstein et al. in prep.). At the start of the study (July-2009), all lobsters were tagged with small circular laminated disc tags (diameter = 2.0 cm; Floy Tag and Mfg., Inc. Seattle, WA) fastened to the knuckle of their claw and held individually in floating totes (81.3 cm × 50.8 cm × 38 cm, LxWxH) subjected to ambient seawater flow (16.3 ± 1.6 °C; mean ± se) and light in an outside impoundment until they extruded their egg clutches (Fig. 1).

Diet Treatments

Lobsters were divided randomly among four diet treatment groups: 1) lobsters (n = 3) which received no food items (control); 2) a mixed fish (bait) diet (n = 4) consisting of frozen herring (*Clupea harengus*) and American shad (*Alosa sapidissima*); 3) natural diets (n = 4) consisting of fresh local food items including fish (same as bait diet), urchin (*Strongylocentrotus droebachiensis*), rock- (*Cancer irroratus*) and Jonah crab (*Cancer borealis*), blue mussel (*Mytilus edulis*), and a mix of red, green, and brown macroalgae (*Ulva lactuca, Laminaria agardhii, Chondrus crispus, Rhodymenia palmata*) and; 4) a mixed diet (n = 4) composed of a ~ 50/50 combination of bait and natural feed items. Fish was purchased in bulk 18 kg frozen flat trays from a single source (The Bait Lady, Newington, NH).

All natural food items were collected locally on a periodic basis by SCUBA divers and held in separate holding totes from the lobsters. Lobsters were fed to satiation twice/week over the summer and fall months (July-November) and once/week during the

rest of the year. Uneaten food items were removed upon each feeding event, and totes were cleaned periodically. Prior to starting any of the treatments, all lobsters were starved for a total of two weeks to insure the absence of any previous food items.

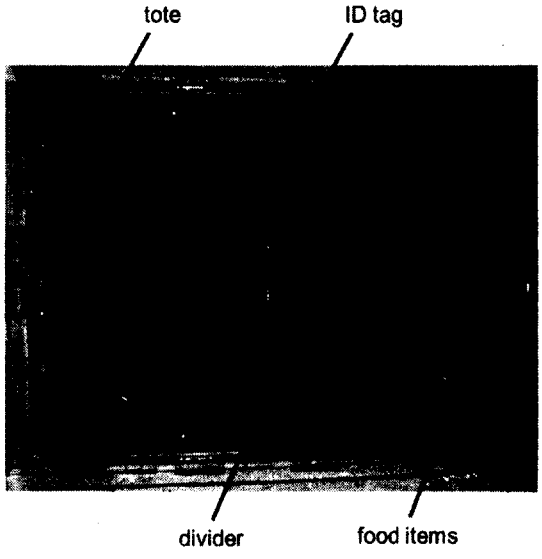

Fig. 1. Lobster diet treatment setup. Animals were kept in floating totes (dimensions: 81.3 cm × 50.8 cm × 38 cm) and held at ambient light and temperature in an outside impoundment subject to natural flowing seawater.

Nutritional Status

Female lobster nutritional status was monitored by collecting a small sample of hemolymph on a biweekly basis, similar to other lobster studies (Leavitt and Bayer 1977, Oliver and MacDiarmid 2001). A total of 30 µL of hemolymph was collected from the base (sinus) of the fifth walking leg of each animal using a 3.0 mL 22-gauge syringe. Samples were then placed on an analog clinical protein refractometer (model CLX-1, resolution = ± 0.2, VEE GEE Scientific, Inc. Kirkland, WA) and a blood refractive index (BRI) was recorded (g/100 mL). The refractometer was calibrated with deionized water

prior to each use and a bovine albumin serum (Sigma-Aldrich, St. Louis, MO) was used to create a standardized curve by which raw data values could be compared.

On three occasions (start, middle and end of trials), larger samples (1.0 mL) of whole lobster blood were drawn and frozen at -80 °C for later total glucose analysis. Total glucose was quantified from total blood serum using a standardized glucose *in-vitro* assay (kit # 439-90901; Wako Chemicals USA Inc., Richmond, VA). Both hemolymph and total glucose values were averaged across all individuals for each of the four treatments and compared using a repeated-measures ANOVA using JMP v. 9.0.3 statistical software package (SAS Institute, Cary, NC).

Diet and Egg Analysis

Representative feed samples from each diet treatment were collected at three separate time intervals (the outset of the trials, mid-January, and June, day chosen randomly) and temporarily frozen at -80 °C, before being freeze dried at -40 °C for 24 hr. (Labconco Freeze Dryer 5, Kansas City, MO). Samples for each diet treatment were combined to obtain enough sample (15-20 g) for analysis and were ground down into a fine powder using an industrial-grade milling machine (Wiley Mill #4, 40 μm mesh screen, Thomas Scientific, Swedesboro, NJ) and stored in polyethelene storage vials. Samples were then sent out for analysis (Forage Testing Laboratory, Dairy One, Inc., Ithaca, New York), and three major components (total protein, lipid, and ash) were analyzed and reported on a % - dry weight basis.

Small samples of ovary tissue were extracted from each lobster ~ 1 month prior to estimated egg extrusion (see Johnson et al. 2011 for details). Briefly, a small square was cut into the lateral side of the carapace (behind the eye), and a section of ovarian tissue was removed with blunt forceps. The incision was sealed with the use of cyanoacrylate glue, gauze, and adhesive tape; all lobsters survived. Upon egg extrusion, ~ 500 eggs/lobster were gently removed in clumps, washed with cold, sterile seawater and placed in clean, labeled storage vials. Both egg and ovary samples were processed similarly to feed samples.

Histolopathological Analysis

Once egg extrusion was completed, each lobster was sacrificed so that tissue samples could be extracted and analyzed. Samples of ovary, hepatopancreas, claw muscle, shell cuticle, and midgut were removed, stored in individual tissue cassettes (Fisher Tissue Path cassettes IV), and preserved in a solution of 10 % neutral buffered formalin. After 36 hours, tissue samples were transferred into 70 % ethanol and shipped to the Virginia Institute of Marine Science (VIMS, Gloucester Point, VA). All tissue samples were processed using paraffin histological techniques and stained with Mayer's hematoxylin and eosin (see Wheeler et al. 2007).

Prepared slides were viewed and photographed using an Olympus BX51 compound microscope and a Nikon DXM1200 digital camera, respectively.

Results

Hemolymph Indices

Overall, nutritional status (measured as blood refractive index, BRI) in ovigerous lobsters was highest in the 50/50 diet treatment (BRI = 5.8 ± 0.1) compared with the natural (BRI = 5.4 ± 0.2), bait (BRI = 4.9 ± 0.1), and starved (BRI = 2.3 ± 0.3) lobsters (Fig. 2; Table 1). With the exception of the starved treatment, all other treatments indicated an increasing trend, especially in the last few weeks (Fig. 2). The largest increase in nutritional condition over the course of the study was seen in the natural diet treatment (+ 54 %); likewise the biggest loss occurred in animals that were starved (-79 %). BRI values were significantly different between treatments (ANOVA; Kruskal-Wallis test, $\chi^2 = 128.91$, $p < 0.0001$). Post-hoc follow-up tests (Dunn's Multiple Comparison tests) revealed differences in diets (Table 1) although the starved treatment differed very significantly from all other treatments ($p < 0.001$).

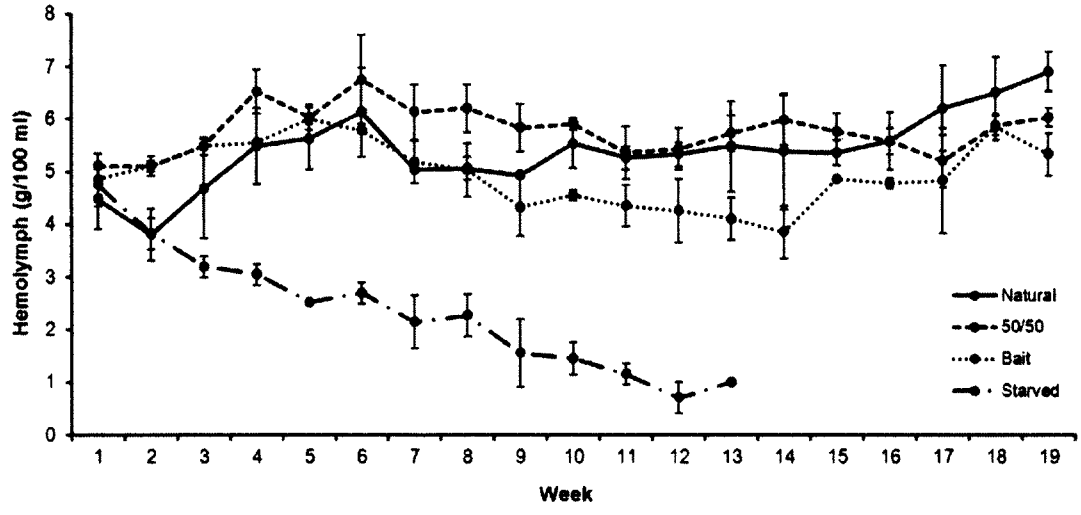

Fig. 2. Mean (± se) hemolymph values (blood refractive index, BRI) for lobsters held in one of four diet treatments. Values were averaged for all lobsters in each treatment (n = 4, n = 3 starved). BRI values in the starved treatment were only measured up until week 13, as there was 100 % mortality thereafter.

Diet	Average ± sem	Range
natural	5.4 ± 0.2	3.8 – 7.7
50/50	5.8 ± 0.1	0.7 – 4.8
bait	4.9 ± 0.1	3.9 – 6.0
starved	2.3 ± 0.3	5.1 – 6.8

Dunn's MC test:

natural	50/50	bait	starved

Table 1. Average BRI values for each diet treatment with range. A nonparametric Kruskal-Wallis test indicated differences among some diets with a Dunn's multiple comparison test indicating those differences. Treatments that share a line are considered different ($p < 0.001$) at the $\alpha = 0.05$ level.

Glucose Levels

Glucose values (means ± se) are given in Table 1. Total glucose values significantly changed over time (RMANOVA; $F_{2,15} = 19.28$, $p = 0.0014$), between treatments ($F_{3,4} =$

7.80, $p = 0.0379$) and the interaction (time*trt) ($F_{5,15} = 6.20$, $p = 0.017$). At the outset of the trial period glucose values averaged 8.9 mg/dL, decreased to 4.1 mg/dL in winter and increased significantly over the last sampling period (average = 15.6 mg/dL; Fig. 3). In addition, the relationship between glucose values and absorbance (standard curve values) were fit using logistical regression and were significant ($r^2 = 0.99$, $p < 0.001$; Fig. 4).

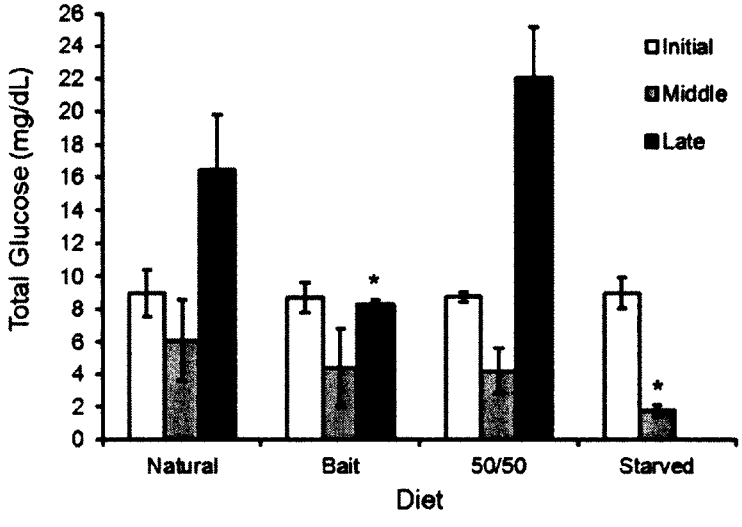

Fig. 3. Total blood glucose values (means ± se) for lobsters held in each of four diet treatments and sampled at three time intervals: 1) initial (fall); 2) middle (winter); and 3) late (spring). Values were averaged for all lobsters in each treatment (n = 4, n = 3 starved). Values in the starved treatment were unavailable in the spring due to 100 % mortality. Significant differences ($p < 0.05$) between groups over each time period are indicated by an (*).

	Natural	Bait	50/50	Starved
Initial	9.0 ± 1.45	8.7 ± 0.9	8.8 ± 0.28	9.0 ± 0.95
Middle	6.1 ± 2.5	4.4 ± 2.4	4.2 ± 1.4	1.8 ± 0.4
Late	16.4 ± 3.4	8.3 ± 0.3	22.0 ± 3.2	--

Table 2. Total glucose levels (means ± se) for lobsters sampled at three time intervals.

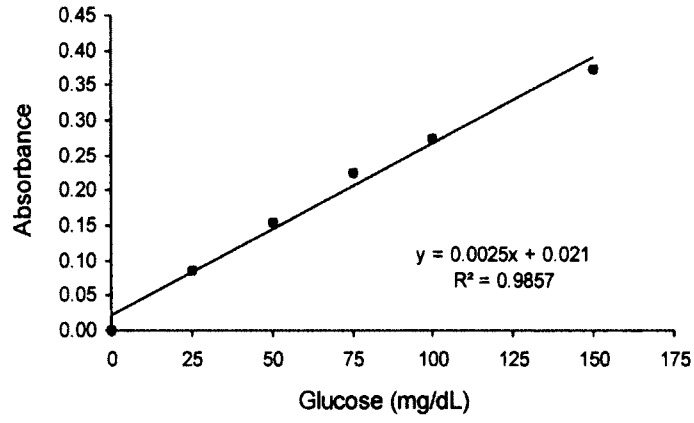

Fig. 4. The relationship between glucose values and absorbance (standard curve values). Values were fit using logistical regression and were significant ($p < 0.001$). This model used bovine albumin serum as a protein standard.

Diet and Nutritional Analysis

Bait-fed diets contained higher amounts of both protein (58.5 %) and lipids (31.6 %), compared with natural diets (34.5 % and 13.2 %, respectively) (Fig. 5). In addition, natural diets comprised > 50 % of its constituents from ash (inorganic materials), including large amounts of calcium.

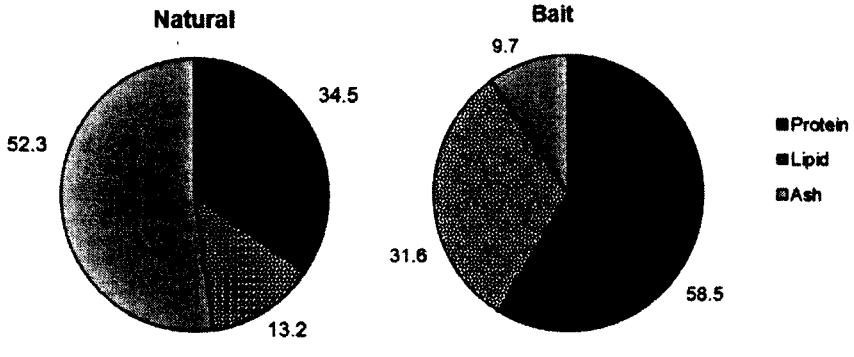

Fig. 5. Nutritional components for bait and natural diets expressed on a % - dry-weight basis. The breakdown of diet components was obtained from processed diet materials that were pooled from three separate time periods.

We did not see statistically significant differences in the proportion of total lipids between ovary samples of diet treatments with the exception of lobsters in the starved treatment (ANOVA; $F_{3,15}$, $p < 0.05$; Fig. 6). A similar outcome was also seen in lobster eggs ($p = 0.081$; Fig. 6). There was a significant correlation between lipid levels in ovaries and eggs for all treatments ($r = 0.76$, $n = 15$, $p = 0.028$).

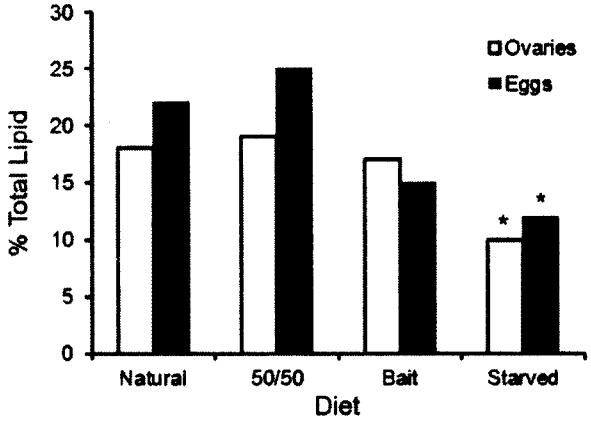

Fig. 6. Comparison of total lipid (as %) for both egg and ovary samples from lobsters of all diet treatments. Correlation between egg and ovary values ($r = 0.76$, $n = 15$) was significant ($p = 0.028$).

Histolopathological Analysis

Although other tissues were examined in this study, both hepatopancreas and ovary allowed for the best comparisons between individual lobsters and their diet treatments. Lobsters that were starved typically displayed hepatopancreas tissue that was devoid of reserve inclusion (RI) cells (Figs. 7-9).

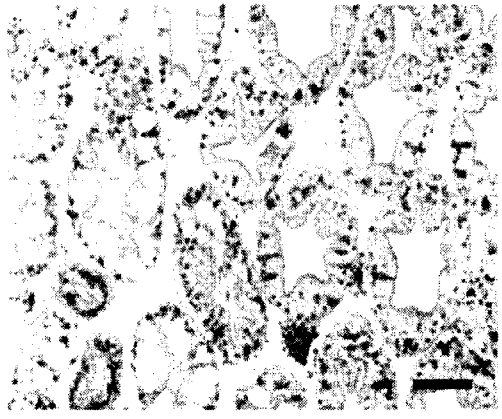

Fig. 7. Hepatopancreas with sparse clumps of RI (reserve inclusion) cells that are granular in appearance (10x) – example of a starved lobster. (Scale bar = 100 μm).

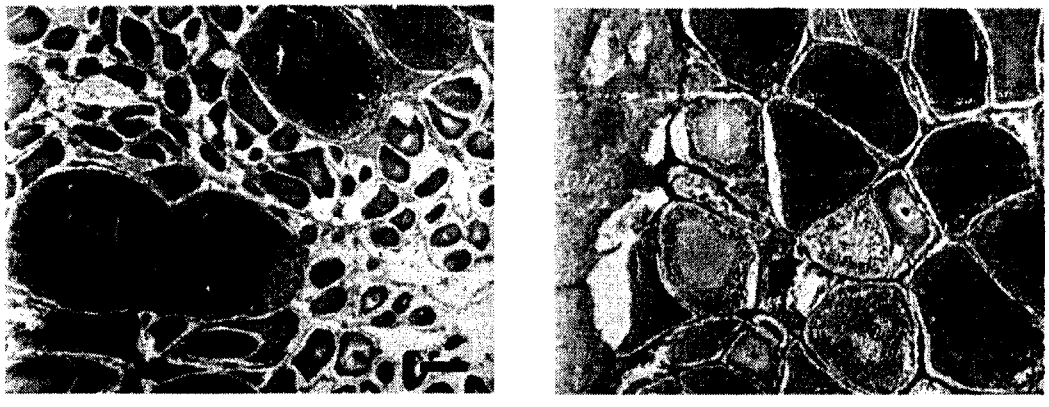

Fig. 8. Ovary sections from two lobsters. Left: lobster (CL = 92) fed a 50/50 diet of bait and natural food items. Right: lobster (CL = 88) fed herring bait diet only. Individual ova in the 50/50 diet were characterized as highly variable in size (range =) and patchy (indicative of active ova production and vitellogenesis). More comments on RI cells. While ova from the bait diet were not as prevalent in the smaller sizes. Also no apparent RI cells or lipid mobilization in tissues. Mag = 4x, (Scale bar = 200 μm).

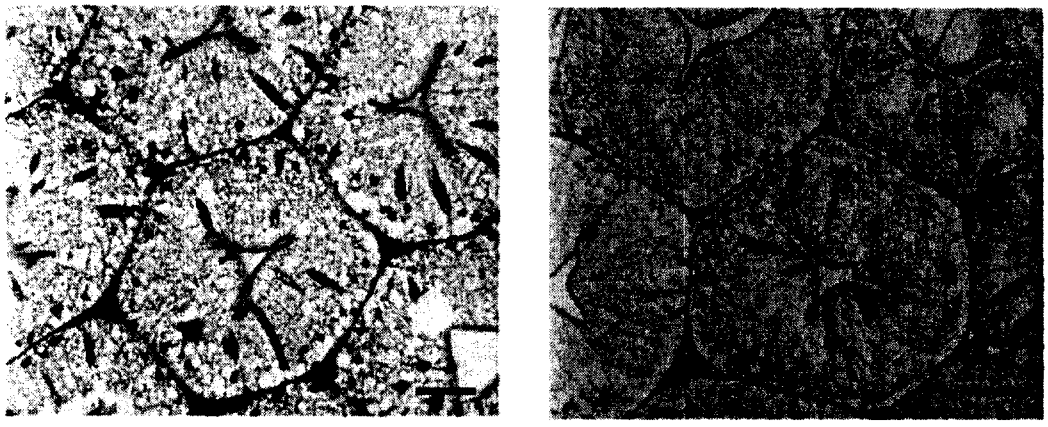

Fig. 9. Hepatopancreas sections from two lobsters. Left: lobster (CL = 95) from starved (control) treatment. Right: lobster (CL = 85) fed a natural diet. The hepatopancreas in the starved lobster contained no reserve inclusion (RI) cells and was characterized as having tubules with little B cell activation, tightly packed, few arterioles with fixed phagocytes present. Additionally, there were few hemocytes in the hemal sinuses in the arterioles around the organ. Comparatively, the lobster in the natural diet (some notes/comments here). Mag = 20 x, (Scale bar = 50 μm).

Discussion

The extended ovary maturation process followed by equally long maternal care that lobsters provide to their egg clutches affords the opportunity to allocate significant amounts of nutritional reserves even before egg extrusion through the complex biochemical pathways of vitellogenesis. The nutritional aspects of adult broodstock often translate into egg quality and larval success in other marine invertebrate species (Sasaki et al. 1986, Jaeckle 1995, Sibert et al. 2004). To our knowledge, this is the first study to address the effects of a trap-based bait diet on the long-term aspects of ovigerous lobster health, ovary condition, and egg quality in mature adult lobsters, compared with other diets. We were able to follow the health and egg quality in female lobsters over a period of ~ 300 days (range = 270-378) that were subjected to a variety of diet types. Our findings suggest that a varied diet of food constituents is probably integral to the overall

fitness in adult lobsters, in particular ovigerous females and the reserves that are used for ovary maturation and subsequent oocyte development.

It has been proposed that lobsters in many areas of the commercial fishery are actively being 'farmed' due to their propensity to frequent traps, consume bait, and revisit other traps (Grabowski et al. 2010) and this was a major impetus for this study. We sought to quantify the potential effects in lobster ovaries and eggs from lobsters fed disproportionate amounts of bait. Overall, we did see some deleterious effects on lobsters, their ovary condition and their eggs when compared to those animals that were allowed to feed exclusively on natural foraging items. However, we did not allow eggs to continue to develop and hatch. Under this scenario, we might expect to see true carry-over effects with respect to larval competency and survivorship.

Other studies have shown a link between maternal nutrition and the fecundity and hatchability in marine crustaceans, particularly with dietary lipids (Castell and Kean 1986). Even though it has been suggested that in some areas of the fishery herring bait may augment the growth rates in some lobsters (Saila et al. 2002, Grabowski et al. 2010), three factors may diminish this effect: changes in lobster density, variable fishing effort and the availability and abundance of a natural prey base. In addition, both historic and recent analyses of *in situ* dynamics of lobsters in traps suggest that their bait consumption is highly variable, as lobsters are often out-competed from by-catch (e.g., crabs and fishes) and bait washout (Jury et al. 2001, Watson et al. unpub. data). Clearly, there needs to be more effort aimed at ascertaining the actual consumption of bait by different

kinds of lobsters and the effects of this on a number of different levels including the storage of nutrients by adults for subsequent biological processes (e.g., growth, maturation).

In addition, recent evidence suggests that the timing and sequestering of energy stores can impact reproductive schedules. As a result of low-quality food sources, some fishes can manipulate oocyte development, adjust the number of eggs they produce, or forego egg production (i.e., skipped spawning) altogether (Rideout and Tomkiewicz 2011). Some of these disruptions have been documented in American lobsters and include changes to inter-annual molting schedules and spawning during sub-optimal times but little conclusive evidence has been made (Waddy et al. 1995). Thus, alterations in these events could influence not only the survivorship and hatchability of larvae in this species it could also alter the timeframe of hatch that is critical to survival in the plankton (e.g., match-mismatch, Cushing 1990).

Although lobsters are described as opportunistic foragers consuming prey over a variety of taxa (Conklin 1995, Sainte-Marie and Chabot 2002), there is evidence to suggest that they have preferences to certain food items, namely crab. Rock crab provides a disproportionate amount of nutritional constituents (e.g., amino acids, proteins) to lobster diets compared to other fauna (Boghen et al. 1982, Gendron et al. 2001). In addition, lobsters are known to be chemically attracted to rock crabs, and they may contain mechanisms for stimulating their ingestion of such tissue and associated metabolites (Hirtle and Mann 1978). Because rock crabs are common and dominant decapods in

many coastal communities (Palma et al. 1998), interactions of these two species could easily provide a large and readily available food base for lobsters. We incorporated rock crab into our natural diet treatments and surmise that this component is integral in the overall health of lobsters (Gendron et al. 2001). Although crab lipid is known to be an important diet component for lobsters, especially to the growth and health of lab-cultured juvenile lobsters (Kean et al. 1985, Conklin 1995), bait diets registered the highest overall total lipid content which was not too surprising.

Lipids play a major role in embryo growth and are a vital source of metabolic reserves (Holland 1978). Lobster ovary tissue sequesters the majority of lipids allocated for egg reserves, comprising over 30 % of wet tissue weight (Castell, unpub. data). For most crustaceans, lipids significantly affect ovarian development, fecundity, and the hatchability of eggs (Amsler and George 1984, Sasaki et al. 1986). Obtaining sufficient kinds of lipids are also pivotal to some biochemical processes. For example, triglyceride reserves at the time of hatch can impact the initial food resources for planktotrophic lobster larvae (Castell and Kean 1986, Sasaki et al. 1986, Sibert et al. 2004). Although we did not differentiate between lipid classes in this study, we surmise that lipids derived from a variety of dietary sources are best for embryo growth and development, including the requisitioning of other kinds of dietary lipids.

One such lipid class, carotenoids (lipochromes), are natural, fat-soluble pigments derived from plant-based pigments (reviewed in Myers and Latscha 1997, Linan-Cabello et al. 2002). Dietary carotenoids are purported to be involved in aspects of egg production and

facilitate hatchability in some crustaceans and fishes (Miki et al. 1982, Latscha 1990). The characteristic dark green color in newly extruded lobster eggs is characteristic of the carotenoid astaxanthin. The dietary role of astaxanthin in lobsters remains largely unknown, however there is evidence in other animals that astaxanthin plays a physiological role as an antioxidant and as a hormone that promotes fertilization in some animals (e.g., trout and shrimp, Myers and Latscha 1997). Castel (unpub. data) noticed a negative correlation between lighter-colored lobster eggs and the viability of larvae. It is likely that astaxanthin plays an important role in embryonic development. In some fishes, carotenoids are important in the coloration and protection of eggs against environmental factors (e.g., light, temperature) during respiration (Castell and Kean 1986).

Most lobster diets (especially bait) are devoid of carotenoids. However, these pigments likely play a key role in lobster reproduction (e.g., vitellin production, embryo development), as is the case in other marine crustaceans (Bordner et al. 1983, Conklin 1995, Linan-Cabello et al. 2002). The natural diets used in this study included a variety of animal and plant constituents that contain components that are beneficial to the ovary and egg maturation process in some lobsters. The ingestion of plant matter by some lobsters would offer one mechanism by which carotenoids could be obtained under a scenario of natural foraging. The common occurrence of macroalgal material found in lobster stomachs seem to suggest that plants are not just ingested by chance (along with epiphytic invertebrates) and are actively sought as a viable nutritional component (Scarratt 1980, Elner and Campbell 1987).

Herrick (1909) typically observed seaweed in the stomachs of wild-caught lobsters and suggested that the mineral constituents in these materials were beneficial to overall lobster health. In a lab-based formulated diet study, Gallagher et al (1982) reported that optimal ratios of C:P in juvenile lobsters were also beneficial to adult lobsters. In this study, natural feed diets contained proportionately more inorganic materials compared to other diet treatments and, as a result, may provide a variety of trace minerals for overall biological and physiological function.

While there is a paucity of detailed dietary requirements and nutritional deficiencies in adult lobsters, more information exists for juvenile lobsters, largely stemming from hatchery and aquaculture-related studies (Conklin 1995, Tlusty et al. 2005a,b). Therefore, more directed efforts investigating the nutrient requirements for adult lobsters and the foods they most commonly consume are paramount to understanding the potential changes to larval mortality and recruitment.

One of the most important considerations with sub-optimal diets is their link to disease. This is especially true in specific areas of the fishery where environmental stressors (e.g., increased temperatures) have been implicated. Most notably are lobsters that acquire shell disease (Glenn and Pugh 2006). Although there are a variety of factors that may increase lobster susceptibility to aquatic diseases, nutritional stress has been a prime candidate. For example, lobsters that show signs of nutritional stress may show clinical signs of compromised hepatopancreas function (e.g., Figs.7, 9) and altered lipid

metabolism. Myers and Tlusty (2009) were able to show the cuticles of juvenile lobsters fed a bait diet were thinner and weaker compared with lobsters fed other diets.

Laboratory-cultured lobsters fed a diverse array of food constituents more often confers the greatest overall benefits to overall health (e.g., growth, molting cycles, Conklin 1995, Tlusty et al. 2005a,b, Tlusty et al. 2008). The susceptibility of lobsters to some diseases have not yet been conclusively shown to be affected by varying or sub-optimal levels of food and in some cases the reverse was found (see Stewart et al. 1972, Bethoney et al. 2011). However, the degree to which diet contributes to disease in lobsters is probably augmented by more than one environmental stressor and is difficult to quantify.

This study's goal was to ascertain potential carry over effects of a sub-optimal diet on the ovary and egg condition in mature female lobsters. Our results suggest that lobsters fed on an exclusive bait diet are compromised nutritionally. However, without coupling this to other exacerbating effects, it is difficult to truly document this in wild populations over a sufficient time frame. We speculate that in the field, lobsters are feeding on a large variety of taxa including trap-based bait. With the increase of bait input into some areas of the lobster fishery, it would be important to address the variability and quality of herring being used (Melvin and Stephenson 2007) over temporal scales, as well as the consumption of bait by lobsters compared with natural foraging items. Understanding these kinds of relationships would allow fisheries managers to address more accurately the impacts of lobster bait (along with changing environmental conditions) on the health of the fishery throughout its range.

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APPENDIX G

TWO METHODS FOR DETERMINING THE FERTILITY STATUS OF EARLY-STAGE AMERICAN LOBSTER, HOMARUS AMERICANUS, EGGS

Abstract

The American lobster (*Homarus americanus* Milne Edwards, 1837) is the focus of the most important commercial fishery in New England and relies on a variety of biological monitoring programs and surveys to guide the development of appropriate management plans. One key piece of information provided by these surveys is the number of females that are carrying eggs (ovigerous) that will subsequently contribute new recruits to the fishery. A major assumption is that all eggs carried by ovigerous females are fertilized and will thus result in viable recruits. However, because some lobsters extrude, and briefly carry, unfertilized eggs, this assumption needs to be re-evaluated. In particular, it is important to determine the approximate proportion of newly extruded eggs that are either fertilized, or not. The major goal of this project was to develop reliable methods for determining if early-stage lobster eggs (live and preserved) are in fact fertilized. One method involved using a nucleic acid stain to visualize egg DNA, after pretreatment of eggs with a proteolytic and collagenolytic enzyme solution to facilitate stain penetration through the egg membrane. With this method multi-nucleated (fertilized) eggs could be clearly distinguished from unfertilized eggs. A total of 20 egg clutches were tested to determine their fertility status using this method. Of these, 16 clutches (80 %) were fertilized while 4 were unfertilized (20 %). Of the 16 clutches with fertilized eggs, two

had a mix of both fertilized and unfertilized eggs. A second method, using fluorometry to obtain measurements of total egg DNA, was also developed. There was a significant difference between the total DNAconcentration in unfertilized control oöcytes and early-stage fertilized eggs ($p < 0.001$), and the total amount of DNA gradually increased as eggs developed ($r = 0.961$, $p < 0.0001$). Both of these methods will make it possible to make a more accurate assessment of the proportion of female lobsters that will actually contribute new recruits to the fishery.

KEY WORDS: American lobster, DNA, egg development, Hoechst stain, *Homarus americanus*, lobster eggs, proteolytic and collagenolytic enzymes, ovigerous

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Introduction

The American lobster, *Homarus americanus* H. Milne-Edwards, 1837, is one of the most valuable commercial fisheries in the North Atlantic and supports the economy of many New England coastal communities ($372 million in 2007; FAO Stat 2009). As a result, fisheries scientists and managers spend a considerable amount of time and effort monitoring the fishery so they can make informed decisions and effectively manage this resource. Along with data on growth, mortality, and reproduction (fecundity, spawning stock biomass), some surveys assess the abundance of egg-bearing (ovigerous) females that will contribute new recruits to the fishery. Estimates of the reproductive capacity and future recruitment rates of the stock are then based, in part, on the number of ovigerous females caught during these surveys (ASMFC 2009).

There are a number of models and indices used in American lobster stock assessment, two of which are the spawning stock biomass (SSB) index of abundance, and the egg per recruit (EPR) model (Fogarty 1995, ASMFC 2009). SSB indices are used to estimate the total reproductive potential of a population and can be done on a statewide or regional basis (ASMFC 2009). EPR models (modified from finfish models) generally use the number of mature lobsters on the bottom, their carapace length, and the probability of surviving and producing eggs, as a means of calculating the number of eggs that will be produced in the near future. In the case of the EPR model used in a recent lobster stock assessment (ASMFC 2009), the model assumes that sexually mature females, provided they survive, will mate and extrude a quantity of eggs based on their total fecundity, which is dependent on their carapace length (CL) (Herrick 1909). The assumption of

these EPR models is that female lobsters fertilize 100 % of the eggs that they carry. While some crustacean fisheries (spiny lobster and crab) tend to be quite resilient to heavy exploitation (Pollock 1993, Hankin et al. 1997), it is unclear how fishing pressure might influence the reproductive dynamics of American lobsters. For example, slight shifts in size-at-maturity schedules and sex ratios (Landers et al. 2001, Little and Watson 2005) could reduce mating success in some areas, and this might be manifested in a decline in fertilization rates and eventually, new recruits.

Although the length and timing has been debated (see Waddy and Aiken 2005), the female American lobster reproductive cycle typically involves molting and mating in the summer, the storage of sperm in the spermatophore, the extrusion and presumed fertilization of the egg clutch, and the incubation of eggs for 9-12 months until they hatch into larvae 1-2 summers later (Bumpus 1891, Herrick 1895). Throughout egg development, growth measurements of the size of the prominent eyespot, along with egg color and other morphological and physiological features, are often used to stage eggs and determine if they are fertilized (Bumpus 1891, Herrick 1909, Templeman 1940, Perkins 1972, Helluy and Beltz 1991). However, with newly extruded egg clutches, it is virtually impossible to visually determine if eggs are fertilized, especially in the field. Early-stage eggs (> 2 months old) are characterized as featureless, solid, and dark green, with no discernible differences from eggs that are unfertilized (Fig. 1).

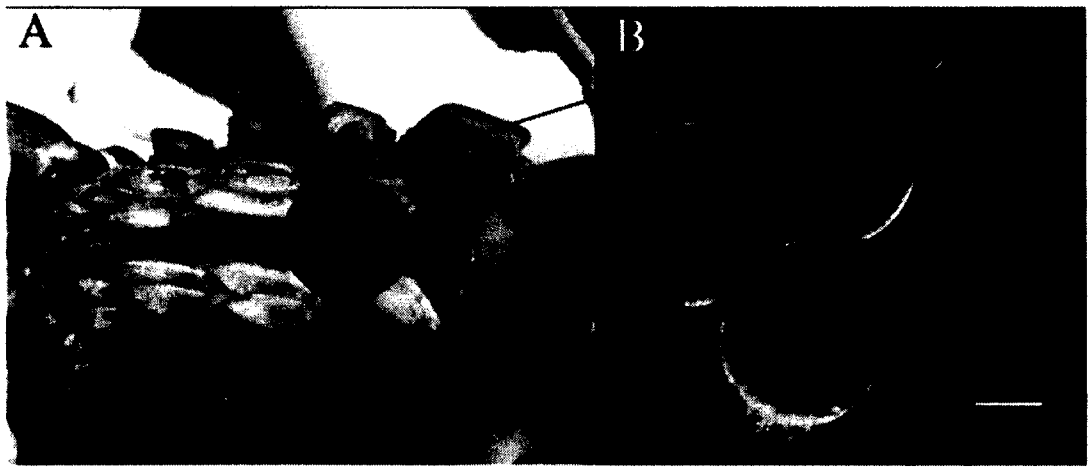

Fig. 1. A: Typical clutch of lobster eggs. At 20 days through at least one month old, (green eggs, size range = 1.6-1.7 mm ± 0.4 mm in diameter), there are no discernible features that indicate fertilization status. B: Photograph of eggs taken under a dissecting microscope at a total magnification of 40X (scale bar = 50 μm).

Growing evidence from both lab and field studies suggest that female lobsters may extrude unfertilized egg masses that are a result of unsuccessful mating attempts or perhaps inadequate sperm stores (i.e., sperm limitation; Knight 1918, Talbot and Harper 1984, Aiken and Waddy 1982, MacDiarmid and Butler 1999, Gosselin et al. 2003, Pugh et. al. unpub. data). In at least two studies, females of *H. americanus* have been observed extruding unfertilized eggs that they subsequently carried for varying amounts of time (Talbot and Harper 1984, Talbot et al. 1984). This has also been observed in spiny lobsters (e.g., *Panulirus cygnus,* Chittleborough 1976).

Other studies suggest significant egg attrition (early on) from lobsters in both lab and field studies originating from a variety of causes including disease, trap handling, faulty egg-attachment, and sub-optimal environmental conditions (e.g., increased temperatures; Perkins 1971, Aiken and Waddy 1980, Hedgecock 1983, Talbot and Harper 1984).

However, discerning the origin of these losses early on, especially for those egg clutches that could be unfertile, remains elusive and largely uninvestigated. Therefore, if some ovigerous females observed during assessments are carrying unfertilized eggs, managers could be overestimating the number of new recruits to the fishery. A major goal of this project was to develop a technique for determining if early stage eggs are fertilized. Eventually, we hope to use the methods developed to estimate the percentage of ovigerous females in a given population that are carrying unfertilized eggs and thus not contributing recruits to the population that year.

Lab-based methods that have been developed to assess fertilization of early developing eggs using nucleic acid stains (e.g., DAPI and Hoechst) have become common among a diverse range of terrestrial and aquatic invertebrates (Buttino et al. 2003, Masci and Monteiro 2005, Zirbel et al. 2007). Because of the strong affinity of DNA-binding proteins and their specificity to the major groove of DNA and its A-T rich region, nuclei can be readily visualized with these DNA specific stains, especially if there are multiple nuclei and cells that are actively dividing (Dervan 1983). However, staining techniques for lobster eggs have not been very successful due, in part, to their complex morphology and the nature of their fertilization membranes compared with other decapod crustaceans (Cheung 1966, Talbot and Goudeau 1988). For example, during the extrusion process, lobster eggs develop two prominent, thick, outer envelopes that help protect the developing embryo. The mechanism by which these membranes are formed has been debated, however in a study by Talbot and Goudeau (1988), it was concluded that the outer coat of the oöcyte is formed in the ovary and the inner coat originates from a

complex cortical reaction that occurs during fertilization. Together, these tightly bonded coats comprise the fertilization envelope of the developing egg and, due to its impermeable nature, make typical DNA staining extremely challenging.

In this study, we modified existing nuclear staining methods so they would work consistently with American lobster eggs. Specifically, we used an enzyme solution to breakdown the outer egg membranes so that a nuclear staining agent was able to penetrate into the egg and bind with the DNA. We also demonstrated that this method will work with fixed eggs, making it possible to obtain numerous egg samples from field sampling surveys and then store them prior to subsequent analyses in the laboratory. In addition, a secondary method of fertility testing was utilized to quantify the amount of DNA within individual lobster eggs using fluorescence spectroscopy (i.e., fluorometry). This method allowed us to quantify and compare the amount of DNA present in unfertilized control oöcytes and early- and late-staged fertilized eggs. Like the DNA staining method, this technique made it possible to reliably determine if eggs were fertilized and contained a large amount of DNA due to multiple nuclei, or were unfertilized. In the future either method will make it possible to obtain data that could improve the accuracy of programs designed to predict the number of new recruits that will be added to the lobster fishery in a given year.

Materials and Methods

Animals

A total of 14 female lobsters were caught in standard traps along the New Hampshire seacoast near Rye, New Hampshire, USA, by permitted commercial lobstermen and transported to the University of New Hampshire (UNH) Coastal Marine Laboratory in Newcastle, New Hampshire. All animals were held in floating totes (81.3 cm X 50.8 cm X 38 cm) at ambient light and temperature levels (16.3 ± 1.6 °C; mean ± SD) in an outside impoundment until they extruded their egg clutches. Lobsters were fed twice weekly with fresh herring and rock crabs (*Cancer* spp.) and checked for egg extrusion three times per week.

In order to monitor the appearance of eyespot formation (and confirm fertilization status), after egg clutches were extruded, small batches of eggs (n = 10/lobster) were removed from each lobster's clutch with forceps at weekly intervals (from 1 June to 15 August) and photographed. All sampled eggs were disinfected for 5-10 minutes by dipping them in a 10 % solution of medical-grade iodine and sterile seawater at a concentration of ~ 150 mgL^{-1} (Uglem et al. 1996) to clean them (externally) of epibiotic bacteria. Digital images of a subset of eggs from each clutch were taken with an Olympus SZH-5 stereomicroscope equipped with a color digital Olympus DP-20 camera system (Olympus America, Center Valley, Pennsylvania) to monitor the appearance of eyespot formation. In some cases (later-developed eggs), developmental stage was determined using staging

tables described in Helluy and Beltz (1991).

Egg samples were collected from an additional six ovigerous females during sea sampling efforts in the same area (n = 10 eggs/lobster). However, these eggs were first photographed and then placed into 1.5 mL sample vials containing the following fixative: 97 % glucamine-acetate buffer, 2 % formalin, and 1 % Triton-X (Sainte-Marie and Carriere 1995). Therefore, a total of 20 clutches of eggs (4 fixed and 16 live) were used for testing the two methods developed for this study.

Method I: Nuclear Staining of Lobster Eggs

Fertilization status was determined by sampling a single subset of eggs from each lobster within 1-2 weeks after egg extrusion. Nuclear staining was performed on both the fixed and live eggs that had been photographed earlier. For live eggs, a total of five eggs were removed from each clutch (one time) and stained, for a total of 70 eggs (n = 5 eggs X 14 clutches). For fixed eggs, a total of five eggs/clutch were stained from the samples collected from the six lobsters sampled at sea, yielding a total of 30 fixed eggs. Both fixed and live eggs (5 eggs/sample) were first placed into 1.5 mL plastic conical tubes and rinsed 3X in a PTA buffer solution (phosphate buffered saline, 0.4 % Triton X-100, 0.1 % sodium azide). Eggs were then set in 100 µL of Accutase™ enzyme solution (A6964, Sigma-Aldrich, Inc., St. Louis, Missouri) and left on a rotating plate (Nutator model 421105) at room temperature for 24 h. Samples were then rinsed 3X in PTA, placed in 100 µL of Hoechst nucleic stain (H6024, Sigma-Aldrich, Inc.) and placed back

on the rotating plate for 24 h. Finally, eggs were rinsed 3X in PTA and placed on silica glass depression slides with a few drops of sterile seawater (32 psu) for viewing.

Stained eggs (live and fixed) were observed and photographed using a Zeiss Axioplan-2 imaging compound microscope (Carl Zeiss IMT Corp., Thornwood, New York) using a DAPI filter cube (excitation = 358 nm; emission = 463 nm). These filter cubes are typically inserted into the fluorescence filter revolver of the microscope and reflect UV excitation while transmitting DAPI emission (see http://www.zeiss.com, for details).

Successive digital images were taken using AxioVision v.4.7 software and the multi-dimensional acquisition routine (z-stacking) through an Axiocam MRm/MRc5 camera (Carl Zeiss IMT Corp.) connected to a PC-based computer (Dell Optiplex G2410T). Eggs with multiple stained nuclei were considered fertilized, while those with either one or no nuclei visible, were considered not fertilized.

Extraction of Unfertilized Oöcytes (control)

Pre-extruded oöcytes were removed from the intact lobster ovaries of eight females (CL range: 86-98 mm; n = 5 eggs/lobster) (n_{total} = 40 eggs) according to methods described in Little and Watson (2005). Briefly, a small square was cut in the carapace behind the eyes, and a section of ovarian tissue was removed with blunt forceps. Pressure was then applied to the wound to stop the blood flow and the incision was sealed with the use of cyanoacrylate glue, gauze, and adhesive tape (lobsters typically survived this procedure).

The dissected ovaries were then placed in a small glass petri dish with sterile seawater, and the ova were gently teased away and separated from their connective tissue (Talbot 1981). The same nucleic acid staining protocol used for fertilized eggs was then followed. Typically, staining of one nucleus was observed under UV excitation as opposed to the visualization of multiple nuclei in fertilized eggs.

Method II: Egg DNA Measurements (fluorometry)

In tandem with staining, total DNA concentration was measured in unfertilized and fertilized eggs (each measurement was made using a total of 5 eggs), as well as some that were at more advanced stages of egg development. Samples (n = 10 eggs/female) from two control females (oöcytes extracted from the ovaries) yielded a total of 20 unfertilized ova. Samples of fertilized eggs (n = 10 eggs/lobster) were obtained from three females (n_{total} = 30 eggs) when eggs were 5-10 days old and again when their eggs were 20-30 days old. An additional subset of eggs (n = 10 eggs/female) was removed from two additional lobsters (n_{total} = 20 eggs) that were developmentally staged as advanced (50-60 % developed; Perkins 1972). All egg samples were set in 200 mL of SDS buffer solution and carefully homogenized in separate 1.5 mL plastic conical tubes. Next, the tubes were vortexed (Vortex-Genie 2, model G-560) for 5 seconds and placed onto a rotating plate (Nutator model 421105) at room temperature for 24 h. Egg samples were again homogenized and vortexed and then allowed to sit and settle for 30 min before measurements were obtained.

The fluorometer unit (Hoefer DyNA Quant 200, Hoefer Inc., Holliston, MA) was calibrated with a known 100 µL standard before each trial (calf thymus DNA; D3664, Sigma-Aldrich, Inc.). A 2 mL aliquot of reagent solution (100 µL Hoechst with 100 mL 1XTNE) was mixed with 2 µL of homogenized egg solution in a 5 mL cuvette and gently shaken. The cuvette was then placed into the fluorometer and the concentration of DNA in the sample was measured in ng/mL. Each sample was measured in triplicate and the values averaged. A correlation analysis was conducted using JMP v. 8.0.2 (SAS Institute, Cary, NC, USA) to examine the relationship between days after egg extrusion and the total amount of DNA per egg, with the expectation that DNA content increased as eggs developed.

Results

Method I: Nucleic Acid Staining

A total of 20 lobster egg clutches were tested to determine their fertility status (14 from females kept in holding tanks and 6 from lobsters captured while sea sampling); a total of 16 clutches (80 %) were fertilized and 4 were not (20 %). Of the 16 clutches with fertilized eggs two had a mix of both fertile and unfertilized eggs (Table 1).

D	CL (mm)	Extrusion date	Staining result (fertilized)?	Egg development (eyespot formed)?
05	86	28-Jun	yes	yes
17	98	30-Jun	yes	yes
01	85	10-Jul	mixed	some
26	87	11-Jul	yes	yes
14	87	17-Jul	yes	yes
43	86	17-Jul	yes	yes
48	85	17-Jul	yes	yes
53	93	17-Jul	yes	yes
66	92	17-Jul	yes	yes
89	88	21-Jul	yes	yes
04	100	28-Jul	no	no
10	82	28-Jul	mixed	some
12	83	2-Aug	yes	yes
42	86	2-Aug	no	no
100	88	unknown	yes	N/A
101	78	unknown	no	N/A
102	91	unknown	yes	N/A
103	86	unknown	yes	N/A
104	82	unknown	no	N/A
105	87	unknown	yes	N/A

Table 1. Lobsters that served as a source of eggs for this study. A total of 14 egg-bearing lobsters (two-digit ID numbers) were held in totes at ambient seawater temperatures (16.3 ± 1.6 °C; mean ± SD) and photoperiod until they extruded their clutches from 28 June-2 August 2010. We continued to hold these lobsters, remove eggs weekly (n = 10/lobster), and examine the eggs to determine if they developed normally or not. An additional 6 egg-bearing lobsters were collected during seasampling trips (ID: 100-105) and their eggs were fixed and also examined (n = 20 lobsters total). For staining purposes, we collected a total of 70 live eggs (n = 5 eggs X 14 clutches) and 30 fixed eggs (n = 5 eggs X 6 clutches). Lobsters were sized (carapace length, CL), and in some cases egg extrusion date was noted.

Eggs that were fertile typically displayed multiple nuclei that were readily visualized when exposed to UV illumination (Fig. 2). Conversely, stained unfertile eggs emitted a hazy blue halo around the outer egg membrane and occasionally a single nucleus was also visible. Thus, it was simple to distinguish between fertilized and unfertilized eggs using this staining procedure. While fertilized eggs of all different developmental stages were successfully stained, the most important finding was that this method made it

possible to determine if eggs as early as 7 days old, expressing no other discernable biological indicators of their fertility status, were fertilized or not (Fig. 3).

In order to confirm that DNA staining was yielding an accurate assessment of egg fertilization status, after we removed eggs for staining, we retained the 14 ovigerous females and held them at ambient conditions so that their eggs could continue to develop. The eggs carried by these females were observed weekly to determine if they were fertilized or not. Appearance of an eyespot after ~ 25 days indicated they were fertilized, while unfertilized eggs either fell off the females, turned a yellowish-orange color, or did not develop an eyespot after ~ 30 days. In all cases, if the staining method indicated that a female was carrying fertile eggs, these eggs continued to grow until the eyespot stage of development (Perkins 1972; Table 1). In addition, in the few cases where staining yielded mixed results, a female was carrying a clutch of eggs that contained some eggs that developed eyespots and some infertile eggs that never developed, started to deteriorate or changed color. Thus, while only 14 clutches (lobsters in holding) were tested, this method indicated their fertilization status with an accuracy of 100 %.

Method II: DNA Fluorometric Measurements

Measurements of total DNA in unfertilized eggs and eggs at various stages of development were obtained to determine if, as cells divided, DNA levels would increase and thus serve as another proxy of fertilization status. There were significant differences in DNA concentrations between eggs that were: 1) unfertilized (n = 4, 5 eggs/sample, 20

eggs total); 2) 5-10 days old (n = 6); 3) 20-30 days old (n = 6) and; 4) 60-80 days old (50-60 % developed) (n = 4) ($r = 0.961$, $p < 0.0001$; Fig. 4). Importantly, for our purposes, there was also a difference in the total DNA concentration between unfertilized control oöcytes and early stage fertilized eggs that did not have eyespots (unpaired t test, $t = 8.581$, $p < 0.001$). The average concentration of DNA in unfertilized oöcytes was 28.6 ± 16.1 ng/mL (range = 10 - 50 ng/mL), compared with 80.5 ± 5.86 ng/mL (range = 55 - 131 ng/mL) in fertilized eggs (Fig. 4). Therefore, it appears as if this fluorometric assay could also be used effectively to determine if young eggs had been fertilized or not.

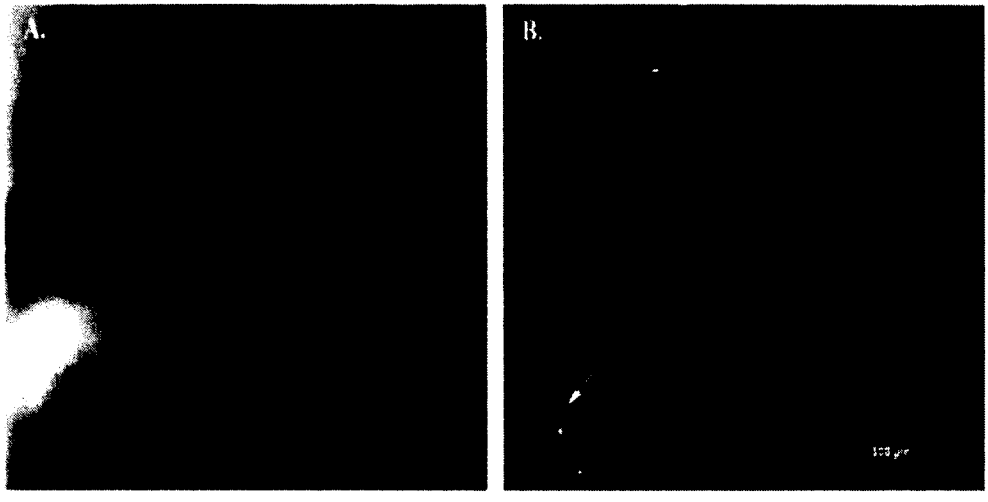

Fig. 2. A: Dividing cells in a fertilized lobster egg visible under bright field illumination; B: The same dividing cells with nuclei visible (arrow) after being treated with Accutase™ and Hoechst stain and viewed under UV light ($\lambda = 463$ nm). Note the appearance of dividing nuclei resulting in the visualization of two clusters of DNA present within some cells, (total magnification = 100X).

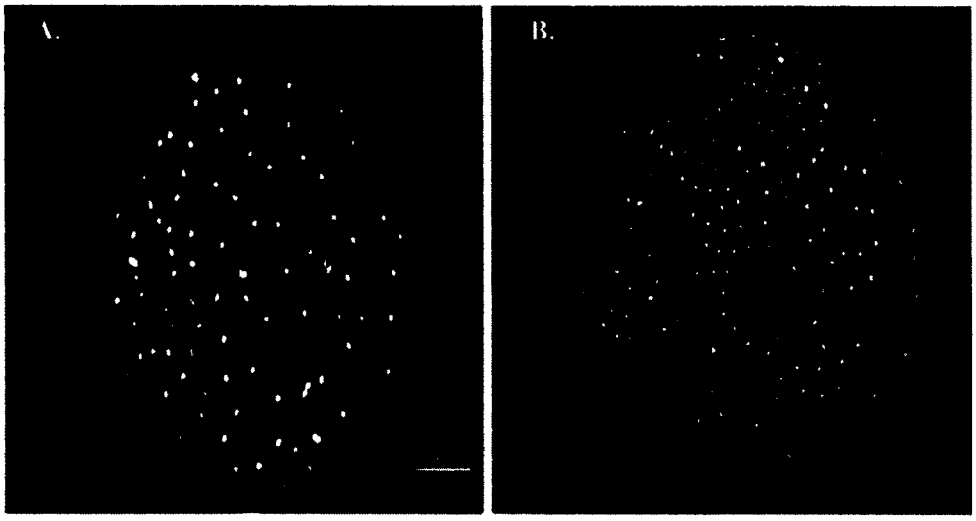

Fig. 3. Appearance of lobster eggs following DNA staining. A: Stained nuclei are visible in an early-stage egg (7 days after extrusion, DAE) exposed to fluorescent excitation and created as a z-stack image; B: Stained nuclei in an egg taken from the same clutch 14 DAE. Notice the increased number of nuclei present due to continued mitotic divisions, (total magnification = 100X). (scale bar = 100 μm).

Discussion

We have described two simple procedures for determining if early-stage lobster eggs have been fertilized. The first method, nucleic staining of DNA with the use of the enzyme Accutase™ and Hoechst stain, enabled us to visualize nuclei and distinguish fertilized eggs with multiple nuclei from unfertilized, haploid eggs. Fluorometry was also used to demonstrate that the amount of DNA differed in a predictable manner between unfertilized, early fertilized, and more advanced-staged eggs. Although our results from both egg nuclear staining and fluorometric methods complement each other, nucleic acid staining should be considered the preferred method to test for lobster egg fertility status because it is: 1) more cost-effective ($ US 50.00/~ 300 eggs); 2) less time consuming; and 3) a more consistent and reliable procedure that allows for a clear-cut determination based on the presence or absence of multiple stained nuclei.

The major modification that made it possible to attain consistent results with Hoechst stain was the use of the Accutase™ enzyme solution to degrade egg membranes enough so that the stain could penetrate into the egg. Talbot (1981) reported that hydrolytic enzymes, such as collagenase, appear to weaken lobster egg cell membranes and allow *in vitro* fertilization to occur. While our initial attempts with collagenase and other enzymes were only moderately successful, we found that Accutase™, a cell detachment solution consisting of a mixture of proteolytic and collagenolytic enzymes, was the most successful at degrading egg membranes and allowing the Hoechst stain to penetrate and bind to egg DNA. Eggs that were first preserved with fixative and then set in Accutase™ and Hoechst stain also showed successful staining of nuclei. Fixing eggs makes it possible to collect eggs in the field and store them for subsequent examination in the laboratory. Therefore, for example, eggs could be collected and preserved by offshore lobstermen who are often at sea for up to 10 consecutive days.

In early development, following fertilization, lobster eggs go through superficial cleavage and rapid cellular division before reaching the 16-cell morula stage. The nuclei of dividing cells, each surrounded by an amoeboid mass of protoplasm, divide within the yolk and approach the periphery (Bumpus 1891). As development continues, constant cellular division results in the formation of a blastula and eventually leads to gastrulation. This growth and increase in cellular density can be visualized as eggs develop based on the amount or concentration of stained nuclei present in the egg. Thus, while very young eggs can be seen dividing using a light microscope, eggs that are days to weeks old are difficult to stage, especially due to the high degree of yolk reserves present that often

occlude developmental features (Sasaki et al. 1986). These are the kinds of eggs that this method was designed to examine.

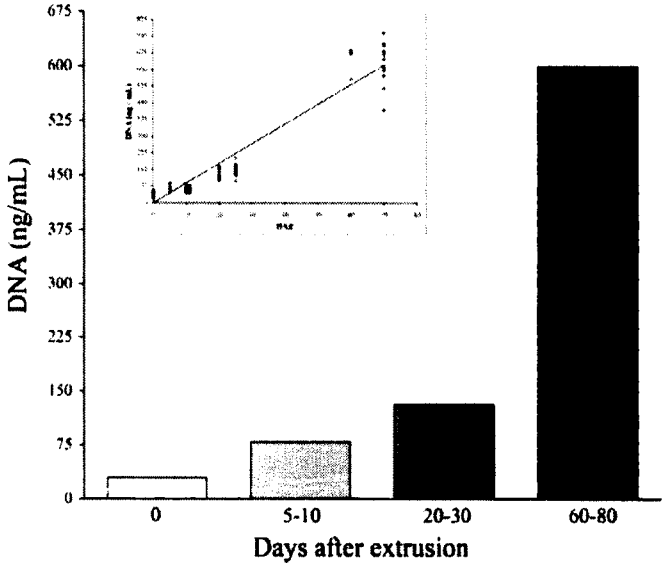

Fig. 4. Total DNA (ng/mL) per egg determined by fluorometry (see text for sampling details). Eggs: unfertilized (5 eggs/sample; n = 20 eggs, 2 females X 10 eggs each), early-stage (5-10 days old, n = 30 eggs, 3 females X 10 eggs each), early-stage (20-30 days old, n = 30 eggs, 3 females X 10 eggs each), and advanced-stage (60-80 days old, n = 20 eggs, 2 females X 10 eggs each). Inset: correlation between DAE and total DNA concentration ($r = 0.961$, $p < 0.0001$).

Unfertilized haploid oöcytes contain one nucleus and one set of DNA within that nucleus. Ideally, upon staining, one strand of DNA should be visible. However, we found that the single haploid nucleus was difficult to locate within the oöcyte. Rather, stained unfertilized eggs contained a hazy-blue overall stain and no evidence of the individually stained nuclei seen in fertilized eggs. Therefore, while it is not easy to identify one haploid nucleus in an unfertilized egg, it is easy to determine if an egg is fertilized or not.

Our staining assay allowed us to collect data on the fertility status of 20 different clutches of lobster eggs (Table 1). Egg clutches that had not been fertilized (n = 4 lobsters, size range = 78-100 mm CL) were likely the result of females that molted and then failed to mate (e.g., lobster 04; Table 1). At the present time, it is not clear how common this phenomenon is in natural populations. In a separate study, female lobsters (n = 6) were held in isolation after they had molted, so that they did not have a chance to mate. Four of these lobsters did not extrude eggs and the remaining two extruded eggs that were unfertilized; if a spermatophore is present, it is lost when they molt (Aiken and Waddy 1980). Sato et al. (2006) reported that on occasion, female king crabs, *Paralithodes brevipes* (Milne Edwards and Lucas 1841), extrude eggs that are not fertile due to insufficient sperm allocation from males. We also found two cases where lobsters (CL = 82, 85 mm) had a mixed clutch of eggs. This situation is likely caused by a female attempting to fertilize a clutch of eggs using a spermatophore that does not contain sufficient sperm for all the eggs.

Both of the aforementioned situations suggest that some sexually mature females are not obtaining sufficient sperm to fertilize all their eggs. Possible causes for this situation may include: 1) females molting and failing to find a mate during the time period when they are most receptive; 2) females mating with a male that produces a smaller than normal spermatophore due, for example, to an effort to allocate sperm to many different females; or 3) females using a single spermatophore to fertilize more than one clutch of eggs (but see Gosselin et al. 2005). Male sperm depletion has been confirmed in both spiny and clawed lobsters (Gosselin et al. 2003, MacDiarmid and Stewart 2005), and sperm supply

is now considered a potential factor limiting the reproductive output in some lobster and crab populations (MacDiarmid and Butler 1999, Rondeau and Sainte-Marie 2001, Kendall et al. 2002, Gosselin et al. 2003, Hines et al. 2003, Sato and Goshima 2007). Although sperm limitation has not been formally documented in American lobster populations, several observations suggest that in some heavily exploited areas where there is a highly skewed sex ratio, the reproductive dynamics could be altered (e.g., evidence for multiple paternity in some females; Gosselin et al. 2005, ASMFC 2009). Sperm limitation has not been well documented or quantified in American lobsters because either pre-extruded, unfertilized eggs are resorbed (Waddy et al. 1995) or extruded, unfertilized eggs tend to fall off females within a month and, during this first month, before they develop eyespots, unfertilized eggs look very similar to fertilized eggs. We anticipate that the methods reported in this paper will make it possible to examine these early eggs and develop a much better understanding of the reproductive dynamics of different American lobster populations. In particular, it will be useful to know if some type of sperm limitation is occurring and, if so, why?

The potential now exists for fishery biologists and managers alike to use these methods when conducting biological surveys to help determine the fertilization status of early-staged eggs before the development of eyespots. Combined, these methods alongside seasonally-timed surveys (e.g., fall lobster surveys) would improve measurements of the reproductive potential of ovigerous populations of lobsters, especially in areas where differences in reproductive dynamics may exist (e.g., sex ratios, mating structure, male size differential). The ability to quantify those females that fall into a potential 'sperm

limitation' category will make it possible to improve models that predict the number of new recruits to the fishery and also better understand how the fishery may, or may not, be influencing the reproductive dynamics of this very valuable marine resource.

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