EFFECT OF SUBSTRATE ON SETTLEMENT BEHAVIOUR, DEVELOPMENT, GROWTH, AND SURVIVAL OF AMERICAN LOBSTER POSTLARVAE, AND EVIDENCE THAT MUD BOTTOM CAN SERVE AS SECONDARY NURSERY HABITAT

by

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ABSTRACT

Postlarval American lobsters, *Homarus americanus*, prefer settling onto a cobble substrate and delay settling onto other substrates. Using tanks lined with cobble, mud, or sand, I found that postlarvae settled first onto cobble, second onto mud, and last onto sand. Furthermore, postlarvae moulted sooner on cobble than on mud, and sooner on mud than on sand. The longest delay of settlement, over large, sand-lined tanks, resulted in reduced carapace length and mass at the next moult in comparison to postlarvae which settled earlier onto mud or cobble. The costs of delaying settlement could encourage settlement onto less-preferred substrates when cobble is unavailable. Accordingly, I deployed passive collectors onto mud habitat in Maces Bay, NB, Bay of Fundy. These collectors were colonized by juvenile lobsters ranging in size from young of the year up to adolescents. Consequently, I identify mud habitat as an overlooked nursery habitat for American lobster settlement and early life history.

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ANOVA	analysis of variance
CL	carapace length
CI	confidence interval
EBP	early benthic phase juvenile (< 40 mm CL)
SCUBA	self-contained underwater breathing apparatus
SD	standard deviation
SE	standard error
TAG	triacylglycerol
Tukey HSD	Tukey honest significant difference
YOY	young of the year

Introduction

The American lobster, *Homarus americanus*, is a commercially important crustacean found in the western Atlantic Ocean from North Carolina to Newfoundland (Cooper and Uzmann 1977). The species supports the single most valuable fishery in Atlantic Canada, grossing over $600 million in 2011 (DFO 2012). For the past 30 years, landings by the fishery, which are often used as a proxy for population size (DFO 2007, DFO 2012), have been increasing across many parts of the lobster's range in Canada and the United States of America (FAO n.d.). This has been attributed to various factors including increased juvenile survival owing to decreased populations of predators and decreased predator body sizes from overfishing, improved lobster conservation measures, and the benefits (in cold water areas) of increasing water temperature (Acheson and Steneck 1997, Boudreau and Worm 2010, Steneck and Wahle 2013). In addition to increased landings, there has been a concurrent steady recruitment of juveniles to some populations, through high settlement, although in recent years this may be slowing (Pershing *et al.* 2012).

Larval and juvenile early development, growth, and behaviour

Larval lobsters are released from a female as a stage I larva, and spend the early part of their life swimming in the upper water column (Scarratt 1973, Harding *et al.* 1987) where they develop through three additional moults. At each moult, the animal undergoes minor anatomical changes and increases in size (Charmantier *et al.* 1991). The duration of each stage (development rate), and the post-moult size increment (growth rate), are influenced by an interplay of environmental factors such as food,

temperature, salinity, light, and chemicals (Templeman 1936, Carlberg and Van Olst 1976, Aiken *et al.* 1981, Eagles *et al.* 1986, MacKenzie 1988) as well as biological factors such as injury (Cheng and Chang 1993) and conspecific density (Cobb 1970, Cobb and Tamm 1974, Aiken and Waddy 1978). The most striking morphological modifications occur at the fourth moult into stage IV, when the larva adopts the adult body form. Accordingly, the fourth moult represents metamorphosis of the larva and the resultant individual is termed a postlarva (Charmantier *et al.* 1991).

In addition to morphological changes, a stage IV postlarva begins to alter its behaviour in various ways (Charmantier *et al.* 1991). As with earlier stages, postlarvae initially swim at or near the water's surface (Ennis 1975, Cobb *et al.* 1983, Harding *et al.* 1987). However, after several days, postlarvae begin performing dives to the bottom and spend relatively more time swimming near the bottom of the water column than near the surface (Cobb *et al.* 1983, Cobb *et al.* 1989). When a postlarva encounters the seafloor on a dive, the animal will explore the seabed looking for shelter. Exploring involves probing among the crevices of hard substrates, or probing, fanning, and digging in sedimentary substrates (Pottle and Elner 1982, Wahle 1992). If the postlarva locates a suitable shelter, the individual will occupy it and begin its benthic existence, otherwise the lobster re-enters the water column and resumes swimming (Cobb *et al.* 1983). It is unknown how long postlarvae in the wild will delay settlement, but it is exceedingly uncommon to see stage V or older juveniles swimming in the water column (Herrick 1911; Templeman 1940, Templeman and Tibbo 1945, as cited by Lavalli and Lawton 1996); thus, stage IV of the lobster's life typically marks the animal's transition from a

planktonic life in the water column, to its final, settled life on the seafloor (Scarratt 1973, Cobb *et al.* 1989).

While leaving the water column and exploring the bottom, young lobsters are highly vulnerable to predation and may be attacked very quickly by predators (Wahle and Steneck 1992, Ball *et al.* 2001, Sigurdsson and Rochette 2013). Thus, shelter is essential to protect young lobsters from predation (Lavalli and Barshaw 1986, Johns and Mann 1987, Barshaw and Lavalli 1988, Wahle and Steneck 1992) and also helps to protect small lobsters against water currents (Howard and Nunny 1983, Johns and Mann 1987). Over a period of months to years, lobsters gradually outgrow many of their predators (Wahle and Steneck 1992) and eventually venture farther from shelter, probably to satisfy their growing nutritional demands and changing diet (Sainte-Marie and Chabot 2002). This has led Lavalli and Lawton (1996) to propose five broad life history phases for settled lobsters. These phases and approximate size ranges are based largely upon ontogenetic behavioural changes observed in the laboratory and to a lesser extent in the field. Shelter restricted juveniles are lobsters with a carapace length (CL) of 4-14 mm, which are believed to never leave shelter (Roach 1983, Barshaw and Bryant-Rich 1988); these animals are thought to initially consume plankton brought into the shelter on currents generated by the individual (Barshaw and Bryant-Rich 1988, Lavalli and Barshaw 1989), and subsequently they may ambush prey at the shelter entrance (Barshaw and Bryant-Rich 1988), and scavenge items from larger lobsters' prey (Saint Marie and Chabot 2002). Emergent juveniles (15-25 mm CL) spend most of their time sheltered but make brief, short forays outside shelter, presumably to forage. Vagile juveniles (25-40 mm CL) also continue to use shelter but venture farther abroad.

Collectively, these three shelter-associated phases (all < 40 mm CL) are referred to as early benthic phase (EBP) lobsters (Wahle and Steneck 1991). Adolescents and adults (approximately ≥ 40 mm CL) still use shelter, but are commonly found out in the open as well (Wahle and Steneck 1991). Thus, shelter is an important part of the lobster's life history, particularly early after settlement and probably for the first 1-5 years of life.

Preferences for different habitats

Postlarval and early benthic phase lobsters exhibit substrate preferences in selecting the habitat they occupy (Botero and Atema 1982, Pottle and Elner 1982, Wahle and Steneck 1992), and these preferences likely reflect the relative shelter-providing qualities of each substrate. Both laboratory experiments (Botero and Atema 1982, Wahle and Steneck 1992) and field observations (Wahle and Steneck 1991) confirm that postlarval and early benthic phase lobsters prefer rocks corresponding to cobble on the Wentworth Scale of rock grades (Wentworth 1922). Spaces between cobbles provide ready-made and relatively sturdy shelters (Botero and Atema 1982, Pottle and Elner 1982, Wahle and Steneck 1991), and lobsters can also remove sediment from under cobbles to produce a shelter underneath (Berrill and Stewart 1973). Both laboratory and field studies indicate cobbles produce a structurally complex, three-dimensional habitat, and offer the best protection against predators (Lavalli and Barshaw 1986, Barshaw and Lavalli 1988, Wahle and Steneck 1992, Barshaw *et al.* 1994). In soft, cohesive mud sediments, lobsters may dig U-shaped tunnels by loosening sediments with their legs and pushing, carrying, or fanning the mud away (Cobb 1971, Berrill and Stewart 1973, Botero and Atema 1982), but these tunnels offer less

protection (Barshaw and Lavalli 1988, Wahle and Steneck 1992), and they require more time to build, maintain, and repair (Barshaw and Bryant-Rich 1988) than shelters on cobble bottoms. In contrast to mud, bare sand is not cohesive enough to burrow into, and lobsters can only dig shallow depressions in this substrate (Botero and Atema 1982, this study), which offer poor protection against predators (Lavalli and Barshaw 1986, Barshaw *et al.* 1994). In accordance with these differences in shelter quality, lobsters in the laboratory settled fastest and preferentially onto cobble rather than mud, and were slowest settling onto sand (Botero and Atema 1982), although the consequences of delayed settlement were not examined. In field experiments, late stage IV lobsters released in the water column made dives to the bottom where they burrowed into crevices in rocky substrate or resumed swimming if they encountered flat mud or sand (Cobb *et al.* 1983), while in field surveys, newly settled postlarvae and early benthic phase juveniles have predominantly been found in cobble reefs or under rocks on sedimentary substrates (Cooper and Uzmann 1977, Hudon 1987, Wahle and Steneck 1991, Wahle 1993).

Energetic considerations in the search for shelter

The importance of shelter means that stage IV postlarval lobsters may continue swimming for several days while searching for an appropriate seafloor habitat (Botero and Atema 1982). However, the time which the lobster will spend diving and sampling the seafloor could ultimately be limited by the animal's available energy reserves (Sasaki *et al.* 1986). Stage IV lobsters begin to store more lipids (primarily of triacylglycerol, i.e. TAG) relative to previous stages, which may afford 3-5 days of

energy reserves providing time for a postlarva to find or construct a shelter where it will settle and eventually moult (Sasaki *et al.* 1986). In stage I larvae of the closely-related *Homarus gammarus* lobster, if starvation prevents the animals from accumulating enough reserves before a certain point in the moult cycle, they delay moult (Anger 1987). Similarly, in stage III American lobsters fed a diet with low levels of TAG, the time to develop to stage IV is lengthened, and growth and survival are reduced (Thériault and Pernet 2007). Hence, if prolonged swimming depletes energy stores before moulting, settling lobsters may exhibit delayed moult, reduced growth, and higher mortality rates. Therefore, when postlarvae are swimming over a "poor" substrate, they likely face a trade-off between the benefits of delaying settlement, which may lead to a subsequent encounter with a better substrate, and the cost of continued swimming in terms of consumption of energy stores.

Secondary habitats

If there are potential costs to delaying settlement, this could promote occupation of less-preferred, secondary habitats in nature. Laboratory experiments indicate that older postlarvae become less selective in choosing a shelter over time (Boudreau *et al.* 1993), and when cobble is unavailable, postlarvae do eventually settle onto other substrates (Botero and Atema 1982). While this has not yet been tested in the field, postlarvae in nature may similarly become less selective about where they settle after spending a prolonged period swimming in search of a quality settlement substrate. Although this question has received relatively little consideration, postlarvae may end up occupying secondary habitats as cobble habitat is relatively scarce and very patchily

distributed throughout much of the lobster's range (USGS 2005, Fader *et al.* 1977, Shaw *et al.* 2012, Schumacher *et al.* in preparation). This is particularly true in the Bay of Fundy as most regions of the Bay have extensive areas of mud or a sand-gravel mix (Fader *et al.* 1977, Shaw *et al.* 2012, Schumacher *et al.* in preparation). At the same time, the demand for cobble habitat is likely growing in regions experiencing increasing settlement. Much like adult lobsters, juvenile lobsters are territorial and dominant individuals will prevent others from using shelter in their territory (Paille *et al.* 2002), which could force some individuals that had previously settled onto cobble to move into secondary habitats. Furthermore, populations of many of the lobsters' large predators have declined, and those predators remaining tend to be less abundant and diverse outside of cobble habitats (Wahle and Steneck 1992), perhaps improving survival in secondary habitats. If secondary, non-cobble habitats are able to support postlarval settlers and juveniles, the carrying capacity of lobster nurseries may be greater than the availability of primary cobble habitat would suggest.

Previous field surveys that examined non-cobble habitats found postlarvae and early benthic phase (EBP) juveniles burrowed into structurally-complex peat reefs (Able *et al.* 1988) as well as low densities of EBP juveniles in eelgrass beds (Hudon 1987, Wahle and Steneck 1991). While there are poorly quantified accounts of very low densities of early benthic phase juveniles on bare mud bottom (MacKay 1929; Cooper and Uzmann 1977; Normandeau Associates 1999; Lawton *et al.* 2009; Peter Lawton, pers. comm.), to my knowledge only four peer-reviewed studies have attempted to quantify juvenile lobster densities on structurally simple substrates such as sand, mud, or bedrock. On bare sand at a site near the Îles de la Madeleine, Quebec, SCUBA divers

found no newly settled or EBP juveniles in 7 transects (2 m wide x 100 m long), although EBP juveniles were common in rocky habitat at the site (Hudon 1987). On mud bottom in the Annapolis Basin, Nova Scotia, SCUBA divers found only 4 juveniles < 50 mm CL in a transect 2 m wide x 150 m long, and in 129 minutes of SCUBA diving over 5 additional mud-bottom sites, observed only 9 juveniles < 50 mm CL (Lawton *et al.* 1995). SCUBA divers also visually surveyed and suction sampled 323 quadrats (each $0.25m^2$) of unvegetated seabed (mud, sand, or bedrock ledges) at five sites in mid-coast Maine where they found no EBP lobsters on unvegetated sedimentary bottom, and only two on bedrock ledges (sheltered amongst mussels) even though juveniles were common in cobble areas at the sites (Wahle and Steneck 1991). Similarly, SCUBA divers visually surveyed 6 transects (2 m wide x 30 m long) at six sites with sedimentary bottom in Narragansett Bay, Rhode Island (Wahle 1993), and found only a single EBP lobster (35 mm CL) on these structurally simple seabeds although EBP juveniles were again common on structurally complex cobble bottom. All four studies suggested that early benthic phase lobsters are largely restricted to cobble habitat, and only large adolescents and adults are commonly found upon structurally simple substrates. Despite the near absence of juvenile lobsters on structurally simple substrates, juvenile use of these habitats may now be increasing in some areas due to the increases in lobster abundances in many parts of the species' range. Given that after cobble, postlarvae and juveniles preferentially select mud habitat in the laboratory (Botero and Atema 1982, Wahle and Steneck 1992), and given the prevalence of mud across the lobster's range (USGS 2005, Fader *et al.* 1977, Shaw *et al.* 2012, Schumacher *et al.* in preparation), mud substrate in particular warrants further examination as nursery habitat.

The overarching goal of my thesis is to investigate the potential for mud to serve as settlement and nursery habitat for the American lobster (*Homarus americanus*). The first part of this thesis involves a series of laboratory experiments which assess how long lobsters will swim before settling onto various substrates (cobble, mud, sand, or a bare tank bottom), and which unlike previous studies, examines whether delayed settlement over some substrates affects survival, development, and growth of postlarva as they mature to stage V. This will establish whether there are costs to delayed settlement, which could explain why postlarvae do not delay settlement indefinitely and eventually settle onto less preferred substrates (at least in the lab). The second part of this thesis involves a field study to investigate whether juvenile lobsters are using mud bottom in Maces Bay (in the Bay of Fundy), which is largely comprised of mud seafloor with scattered cobble patches in the vicinity of reefs (Peter Lawton, pers. comm.). The study will describe for the first time, which life phases of juvenile lobsters are present on mud seafloor, will compare their relative abundances, and will examine how the juveniles are using the habitat. Determining whether mud seafloor is supporting juvenile lobsters, and describing which life stages may be using mud habitat, could greatly change our understanding of the diversity of substrate types that lobsters actually use for settlement and early survival. Taken together, the results of my thesis reveal new complexities in the settlement behaviour of postlarvae and challenge our current understanding of the importance of mud bottom to the demography of the American lobster.

Methods

This study involved three laboratory experiments to assess the effect of substrate on the behaviour (swimming and settlement), development, and growth of American lobster postlarvae, and one field survey to determine whether lobster postlarvae settle upon, and young juveniles use, less-preferred mud bottom at a site containing large stretches of mud where cobble is patchily distributed.

Postlarvae and general laboratory setup

All lobsters used in the laboratory experiments were stage IV postlarvae, mass-reared at 20°C in a hatchery by Homarus Inc. and the Coastal Zone Research Institute in Shippagan, New Brunswick. Lobsters used in the three laboratory experiments were from three separate batches of larvae produced between mid-June and mid-August 2012. All animals in each of these batches had moulted to the fourth stage within the past few hours (maximum 24 hours) before transportation from the hatchery to our laboratory facilities where the experiments were conducted; hence they were all one day into their fourth stage when received. In each of the experiments, animals were transported in an aerated cooler from Shippagan, N.B., to the University of New Brunswick, Saint John (experiments 1 and 2), or to the St. Andrews Biological Station in St. Andrews, NB (experiment 3). Lobsters were placed in either 1-litre glass jars (Experiment 1: Individually monitored settlement), 30-litre rectangular tanks (Experiment 2: Group monitored settlement), or 600-litre round tanks (Experiment 3: Group monitored settlement). Jars and tanks were either lined with cobble (irregularly shaped, intertidal stones), mud (taken from a nearby mudflat in Pocologan, N.B.), sand (commercial play

sand), or were left bare. All animals were maintained under a 14:10 hour, day:night cycle, and were fed frozen brine shrimp *ad libidum* throughout the experiment. Recently-moulted lobsters were identified visually as they changed from a bright green or blue colour to a dull olive green or yellow, and antennae of the stage V lobsters were noticeably longer than the antennae of the unmoulted stage IV lobsters. In each experiment, approximately 4% of the individuals exhibited an extremely small size increase after moulting and appeared no larger than the unmoulted stage IV individuals. These individuals occurred on each of the substrates and were assumed to have moulted normally as in many cases an exuvia was seen, the individuals changed colour, and they did not possess the dorsal spines or long telson spines characteristic of a stress-induced or damage-induced moult to an intermediate V′ stage (Charmantier and Aiken 1987). Accordingly, the swimming behaviour, time until moult, and post-moult morphometrics of these individuals were included in all analyses, and it should be noted that removing these data points did not alter any of the conclusions drawn. Morphometrics involved measuring the carapace length (CL) as the distance from back of the eye socket to the end of the thorax, under a dissecting microscope with an ocular micrometer (± 0.1 mm), and blotting the animal dry for five minutes before weighing mass on a digital balance (± 0.001 g). At the end of each experiment, to measure lobsters after their moult to stage V, carapace length and mass were measured three days after an individual had moulted to allow the animal time to absorb water and fill out its new, larger carapace. Only lobsters retaining both claws after moulting were included in analyses of time until moult and post-moult morphometrics, as limb regeneration can alter time until moult and post-moult size increment (Cheng and Chang 1993). The incidence of claw loss was

independent of substrate in each experiment (Fisher's Exact Test; experiment 1: $p = 0.52$, $df = 3$; experiment 2: $p = 1$, $df = 3$; experiment 3: $p = 0.89$, $df = 2$) and resulted in 3-11% of lobsters being excluded from analyses. All statistical analyses were performed using R statistical software (R Core Team, 2013).

Experiment 1: Individually monitored settlement in 1-L jars

To examine the effects of substrate on lobster development and growth while reducing any potentially confounding effects of swimming, as well as to track individual postlarvae, lobsters were held individually in small 1-L glass jars (8 cm diameter x 16.5 cm height) which constrained swimming. Jars were lined with either 3 small cobbles (1 cm thick, longest dimension 3 cm) on a 1 cm-deep sand base, 3 cm of mud (dried for 48 hours at 80°C, sieved through a 1-mm mesh), 3 cm of sand, or were left bare. Each jar was supplied with filtered seawater (20 μm) transported by truck from Brandy Cove, St. Andrews, N.B., and recirculated through a closed system in the laboratory. Seawater entered via the top of each jar at a rate of approximately 4 L/min, while excess water overflowed through a mesh screen covering the top of each jar. Larvae for this experiment were transported to the University of New Brunswick on July 21, 2012 and were held overnight in an aerated cooler where they acclimated from the 20°C hatchery temperature to the 18°C laboratory temperature. The initial carapace length and mass were measured for each animal, and individuals were randomly distributed among the substrate treatments. Each treatment's lobsters began with a similar mean carapace length (ANOVA, $F_{3,71} = 0.96$, $p = 0.42$) and mass (ANOVA, $F_{3,71} = 1.27$, $p = 0.29$). This batch of lobsters exhibited high post-transport mortality and claw loss but allowed the

following number of lobsters retaining both claws to be used in each treatment: $n_{cobble} = 19$, $n_{mud} = 20$, $n_{sand} = 19$, $n_{bare} = 17$. Lobsters were checked twice daily for mortality or moults. Upon moulting, the time until moult (days between moult to stage IV and moult to stage V), post-moult carapace length and wet mass were recorded for lobsters that had retained both claws (assessment of mortality included all lobsters regardless of claw loss). Holding lobsters in separate jars allowed individuals to be tracked, thus it was possible in this experiment to analyze length and mass increment for each lobster (i.e. the amount by which length or mass increased after moult to stage V) rather than being limited to comparing final length or mass. The experiment began on July 22, 2012 (when the postlarvae were into their second day as stage IV lobsters) and ended August 11, 2012.

Statistical analyses

Proportion of individuals surviving the 21 day experiment was compared among substrates using a G-Test, while time until moult and mass increment were compared among substrates using non-parametric Kruskal Wallis tests (Cochran's Tests and Shapiro-Wilk Tests indicated ANOVA assumptions were not met even after transforming the data). Significant global results were further investigated with Wilcoxon Rank-Sum post-hoc tests with a Bonferroni correction. Mean post-moult increment in carapace length was compared amongst substrates using a One Way ANOVA on untransformed data (Cochran's Tests and Shapiro-Wilk Tests indicated homoscedasticity and normality assumptions were met). Significance was tested at $\alpha = 0.05$ for all analyses.

Experiment 2: Group monitored settlement in 30-L tanks

To examine the effects of substrate on lobster swimming, development, and growth, the postlarvae were held at 18°C in clear-sided, 30-litre acrylic tanks (length x width x depth = 48.5 x 23 x 26 cm) of non-recirculating (static) seawater filtered to 20 μm and transported by truck from Brandy Cove, St. Andrews, N.B, and aerated with an air stone. Each tank was lined with 3 cm of the same cobble used in Experiment 1 (1 cm thick, longest dimension 3 cm), mud (dried for 48 hours at 80°C, sieved through a 1-mm mesh), sand, or was left bare. Five replicate tanks were used for each substrate treatment, and five lobsters were introduced into each tank. Two of the five lobsters in every tank were first measured with respect to carapace length and wet mass before beginning each experiment to ensure all treatments began with similarly-sized animals (carapace length: ANOVA, $F_{3,36} = 0.98$, $p = 0.41$; mass: ANOVA, $F_{3,36} = 0.18$, $p = 0.91$). A trial experiment run in 1-L jars found no significant difference between handled ($n = 10$) and unhandled ($n = 10$) lobsters with respect to post-moult carapace length (t-test, $t = -0.85$, $df = 16.4$, $p = 0.41$), post-moult mass (t test, $t = -1.02$, $df = 15.3$, $p = 0.32$), or survival (Chi Square test, $X^2 = 0.5$, 1df, $p = 0.48$). Time until moult was only marginally non-significant (Welch's t-test, $t = -2.02$, $df = 12.1$, $p = 0.066$) but if handling did introduce error here, this would have been equally distributed across all experimental treatments as two out of five lobsters were handled (measured) in each tank. Larvae for this experiment were transported to the University of New Brunswick on June 15, 2012. The experiment began the same day (the postlarvae's first day as a stage IV lobster) and ended on July 8, 2012.

Lobsters were allowed to swim in the tanks, and the proportion of the five animals in the water column was observed for 30 seconds for each tank every fifteen minutes for 13 days using a team of observers. Night observations in the dark were performed using a red flashlight. Swimming proportions were eventually averaged across observation periods to produce twice-daily estimates, a daylight estimate (06h00 - 19h45), and a night-time estimate (20h00 - 05h45), to simplify analyses and graphical representations of swimming trends over time. Observations of swimming behaviour ceased after 13 days even though not all postlarvae had settled, because some individuals had begun moulting and it was impossible to remove them without disturbing the last few individuals still swimming in each tank. Lobsters in cobble-lined tanks left the water column after 1-3 days to shelter under their cobbles where they could not be checked for moult; therefore, three days after the last individual left a cobble tank's water column, all lobsters of that tank were removed and placed into individual, gravel-lined 1-L jars to await moult. Individuals in the mud-lined, sand-lined, or bare tanks were visible through the clear tank walls and moulted individuals were readily identified; accordingly, these lobsters were allowed to moult in their 30-L tank. All tanks and jars were checked twice daily, and the time until moult (days between moult to stage IV and moult to stage V), the post-moult carapace length, and the post-moult wet mass were measured for surviving lobsters that had retained both claws (assessment of survival included all lobsters regardless of claw loss).

Statistical analyses

The proportion of lobsters swimming over each substrate over time was compared via a Repeated Measures ANOVA using the "aov" command in R (repeated factor = day of experiment, non-repeated factor = substrate, error term = tank). For the factor "day of experiment", the proportions of lobsters swimming during daylight and dark were averaged into a single daily proportion as paired t-tests performed for each substrate showed that the two periods did not differ (cobble: $t = -1.05$, $df = 3$, $p = 0.37$; mud: $t = -1.22$, $df = 12$, $p = 0.25$; sand: $t = -1.16$, $df = 12$, $p = 0.27$; bare: $t = -0.02$, $df = 12$, $p = 0.99$). The repeated measures sphericity assumption was not upheld (epsilon = 0.17) so Greenhouse-Geisser - adjusted degrees of freedom were used. Significant global results (using $\alpha = 0.05$) were further explored with post-hoc pairwise comparisons with a Bonferroni correction.

Mean percent survival, carapace length, and mass were compared amongst substrates using One Way ANOVAs on untransformed data (Cochran's Tests and Shapiro-Wilk Tests indicated homoscedasticity and normality assumptions were met). For all tests, each of the five tanks was used as a replicate in each of the substrate treatments. Significance was tested for at $\alpha = 0.05$ and significant global results were further explored with Tukey HSD post hoc comparisons to determine which substrates differed from one another. Mean time until moult was compared amongst substrates using a Welch's ANOVA to accommodate for unequal variances (Brown-Forsythe Test indicated heteroscedasticity) followed by pairwise comparisons with a Bonferroni correction to identify which substrates differed.

Experiment 3: Group monitored settlement in 600-L tanks

Postlarvae in 30-L tanks consistently encountered tank walls while swimming. To examine the effects of substrate on lobster swimming, development, and growth, in larger tanks with more swimming room, particularly in the vertical axis, the postlarvae were held at 16°C in round 600-litre fibreglass tanks (dimensions 100 cm diameter x 75 cm depth) at the St. Andrews Biological Station. Each tank was lined with 5 cm of cobble (ca. 5 cm thick, longest dimension 5 cm), 5 cm of mud, or 5 cm of sand. It was impractical to dry the quantities of mud used in this experiment, so fresh, unsieved intertidal mud was used (from the same Pocologan mudflat as the dried mud used in the other experiments). Water was pumped from Brandy Cove outside the station, and filtered through sand before entering into each individual tank at a rate of ca. 10 L/min. Water entered via a pipe mounted over the side of the tank and exited through a central standpipe. Four replicate tanks were used for each substrate treatment, and 13 lobsters were introduced into each tank. Three of the 13 lobsters in each tank were first measured with respect to carapace length and wet mass to confirm all treatments began with similarly-sized animals (carapace length: ANOVA, $F_{2,33} = 0.84$, $p = 0.44$; mass: ANOVA, $F_{2,33} = 0.86$, $p = 0.43$). Larvae for this experiment were transported to the St. Andrews Biological Station in St. Andrews, N.B. on August 17, 2012. The experiment ran from August 18, 2012 (when the lobsters were into their second day as a stage IV lobster) until September 18, 2012.

Lobsters were allowed to swim in the tanks and the proportion of individuals in the water column was recorded once during daylight hours and once in the evening in the dark using a small white flashlight as red light did not illuminate the entire tank or

its depths. Swimming lobsters were caught in a hand net to facilitate counting all the lobsters in the large tank; a trial run simultaneously with this experiment demonstrated there was no significant difference in mean time to moult, post-moult carapace length, or post-moult mass of lobsters caught twice a day in the net compared to unhandled lobsters (Nested ANOVAs, time until moult: $F_{1,6} = 0.23$, $p = 0.65$; carapace length: $F_{1,6} = 0.07$, $p = 0.80$; mass: $F_{1,6} = 0.0003$, $p = 0.99$). Unlike the previous experiment in 30-L tanks, swimming observations in the 600-L tanks continued until all individuals in all tanks had left the water column as the tanks were sufficiently large to remove and examine individuals for moulting without disturbing postlarvae still swimming in the tank.

Lobsters that had settled onto the bottom were allowed to remain in their tank until moulting. If not clearly visible from the surface of the tank, suspected moulted lobsters were caught in the hand net for closer inspection. To facilitate moult checks in the cobble substrate, the uniform layer of cobble was removed after 13 days and replaced by small clumps of 4-5 cobbles arranged around the tank, which the settled lobsters immediately occupied. These cobble clumps were lifted twice daily to inspect the lobsters underneath. Upon moulting, the time until moult (days between moult to stage IV and moult to stage V), the post-moult carapace length, and post-moult wet mass of each surviving individual that had retained both claws were measured for lobsters in all treatments (assessment of survival included all lobsters regardless of claw loss).

Statistical analyses

The proportion of lobsters swimming over each substrate over time was compared via a Repeated Measures ANOVA using the "aov" command in R (repeated factor = day of experiment, non-repeated factor = substrate, error term = tank). For the factor "day of experiment", the proportions of lobsters swimming during daylight and dark were averaged into a single daily proportion as paired t-tests performed for each substrate showed that the two periods did not differ significantly (cobble: $t = 0.46$, $df = 7$, $p = 0.66$; mud: $t = 2.11$, $df = 7$, $p = 0.073$; sand: $t = -0.54$, $df = 7$, $p = 0.61$). The difference between the proportion of lobsters swimming over mud in daylight versus night almost reached significance but the difference was only 8% more swimming during daylight. Moreover, the main interest was how long postlarvae swam over each substrate over the course of the experiment, and whether that swimming was performed largely in daylight or night was irrelevant as swimming observations were made during both times. Accordingly, the proportion of postlarvae swimming over mud was also reported as a single daily averaged proportion. The repeated measures sphericity assumption was not upheld (epsilon = 0.13) so Greenhouse-Geisser - adjusted degrees of freedom were used. An interaction between substrate and day of experiment ($F_{58,261} = 14.8$, $p < 0.00001$) precluded analyses of main effects so two additional Repeated Measures ANOVAs were performed. Since postlarvae swimming over cobble clearly settled much sooner than those over mud or sand, the two additional Repeated Measures ANOVAs only examined the proportion of postlarvae swimming over mud or sand. Furthermore, the experiment was divided into two time periods based on visual examination of changes in swimming behaviour over time, the first 14 days, during

which time the proportion of lobsters swimming over mud or sand appeared fairly constant, and the last 16 days of the experiment, during which time the proportion of lobsters swimming over mud or sand decreased rapidly. Accordingly, a Repeated Measures ANOVA was performed on the proportion of lobsters swimming over mud or sand during the first 14 days of the experiment (repeated factor = day of experiment, non-repeated factor = substrate, error term = tank), and another was performed for the final 16 days of the experiment (repeated factor = day of experiment, non-repeated factor = substrate, error term = tank). Again, Greenhouse-Geisser - adjusted degrees of freedom were used ($epsilon_{first\ 14\ days} = 0.29$, $epsilon_{final\ 16\ days} = 0.14$).

To examine whether settlement time influenced development and growth, the mean number of days until 80% and 100% of lobsters in each tank had settled was compared via a regression to each tank's average time for lobsters to moult, as well as to their post-moult carapace length and mass. Mean percent survival, time until moult, carapace length, and mass were compared amongst substrates via one-way ANOVAs on untransformed data (Cochran's Tests and Shapiro-Wilk Tests indicated homoscedasticity and normality assumptions were met). Each of the four tanks was used as a replicate in each of the substrate treatments. Significance was tested using $\alpha = 0.05$ and significant global analyses were further explored with Tukey HSD post hoc comparisons to determine which substrates differed from one another.

Maces Bay field experiment

Description of Maces Bay study site

To investigate the possibility that lobster postlarvae and juveniles use mud seafloor for settlement and early survival, a field study was conducted in Maces Bay, New Brunswick, which is a large bay on the north shore of the southwestern Bay of Fundy. Maces Bay was chosen for this study because multibeam backscatter imaging (UNB Ocean Mapping Group) and extensive SCUBA diving-based surveys by the Department of Fisheries and Oceans Canada indicates that it contains extensive stretches of mud and sand-mud seafloor as well as several islands and tidally-exposed reefs containing fringing subtidal gravel, cobble and boulder habitats (Peter Lawton, pers. comm.). As well, past surveys of these cobble patches by Fisheries and Oceans Canada found good densities and a wide size range of juvenile and adult lobsters in Maces Bay (Lawton *et al.* 2001), and recent surveys indicate cobble patches continue to support strong settlement of lobsters and relatively high juvenile densities (Gudjon Mar Sigurdsson, pers. comm.). The study area in Maces Bay was located just west of New River Island on the western side of the bay as this area has an extensive stretch of mud with two rocky reefs at its southern end.

To explore whether early benthic phase (EBP) lobsters found on mud had originally settled there or had recently wandered in from cobble reefs, the muddy section of the study area was subdivided into three separate mud sites: a North site, a Centre site, and a South site (Fig. 1). The north and south sites, hereafter referred to as North Mud and South Mud, were designed as sites of intermediate distance between the Centre Mud section, and respectively, the rocky coastline to the north, and the rocky reefs to the

south. If juveniles found in the three mud-bottom sites had initially settled in rocky reef areas and had walked into all three mud sites rather than having settled on the mud itself, we would expect high catches of EBP lobsters in collectors on rock, moderate catches in collectors at the North Mud and South Mud sites (~ 400 m from known cobble reefs or rocky shore), and few in the Centre Mud collectors (~ 400 m from North or South mud sites and > 800 m from known cobble reefs or rocky shore). Since the smaller shelter-restricted and emergent juveniles are thought to remain closely associated with their shelter (Lavalli and Lawton 1996) and are unlikely to have walked 400-800 m while unsheltered, we would expect to find very few of these lobsters in North Mud, South Mud, and Centre Mud collectors unless they had settled there as postlarvae.

The bottom composition at each of the three sites was characterized using a combination of benthic grabs, video footage taken by an ROV towed by a boat, and the boat's bottom sounder. The North Mud site (14,870 m^2) was approximately 400 m from shore, and its bottom was predominantly mud with an isolated patch of sand and a patch of rock (6 benthic grabs, 9 video transects; Fig. 1). The Centre Mud site was a stretch of mud (55,700 m^2) lying approximately 400 m south of North Mud, and was at least 800 m from shore in all directions. Within the Centre Mud site, the bottom appeared to be entirely comprised of mud (5 benthic grabs, 12 video transects; Fig. 1); similarly, mud seabed separated the Centre Mud site from the North Mud and South Mud sites (16 benthic grabs around the periphery of the Centre Mud site all revealed only mud, Fig. 1). The South Mud site (24,140 m^2) lay ~ 480 m south of Centre Mud, and its bottom was a

mix of mud and rock (4 benthic grabs, 9 video transects, 18 bottom soundings; Fig. 1). This site had a very small rock island about 125 m from its southeastern edge.

Two cobble reef sites in the Maces Bay study area, known lobster nurseries (Gudjon Mar Sigurdsson, pers. comm.), were also examined. Both sites were located northeast of Pocologan Island, within 100 m of an unnamed island. The first site, "No Name Rock", lay 400 m south of South Mud, while the second cobble site, "Pocologan Rock", lay approximately 200 m southwest of No Name Rock.

Collector deployment

The fine sediment bottom of Maces Bay rendered sampling quadrats by SCUBA diving impractical as the sediments were easily disturbed and obscured visibility. Instead, artificial lobster habitats, hereafter referred to as collectors, were deployed at each of the five sites to attract juvenile lobsters for collection. The collectors were wire cages measuring 61.0 x 91.5 x 15.0 cm (Wahle *et al.* 2009) that were filled with cobbles ranging from 9 - 22 cm (longest dimension) and lined with 2-mm mesh to retain specimens during retrieval onto the boat. When deployed on the seafloor, collectors mimic the natural habitat of settling and young EBP lobsters and passively attract postlarval lobsters as they settle out of the water column, as well as young juveniles that crawl in from the benthos (Wahle *et al.* 2009). Collectors have been previously used to describe the presence and sizes of juvenile lobsters present in an area and are largely comparable to more active sampling techniques such as suction sampling quadrats, in terms of the size range and densities of lobsters caught (at least in cobble habitats where the two techniques have been compared) (Wahle *et al.* 2009, Wahle *et al.* 2013).

It is unknown how large an area a collector samples, whether areas of equal size are sampled on cobble reefs and mud bottom, and whether catchability of juvenile lobsters is equal in collectors deployed on cobble and mud bottom. As a result, the density of lobsters retrieved in a collector does not necessarily reflect the true density of juveniles in the surrounding habitat. As well, the frequency and distance of juvenile movements increase with increasing body size, which could introduce a size bias to sampling with collectors relying on colonization by lobsters. Collectors do, however, allow us to note the presence or absence of various size classes of juvenile lobsters at a site, and allow general comparisons of abundances between sites, and general comparisons of relative abundances of different size classes within and between sites. Most importantly, collectors can readily sample a fine-sediment bottom, and can be deployed over a wide area, for a long period of time.

Collectors were deployed on July 4 and 9, 2012, before postlarval settlement began, in lines running roughly from WNW to ESE at the North Mud site (42 collectors in 2 parallel lines, mean depth = 8.6 ± 1.0 m SD relative to 0 Chart Datum, Canadian Hydrographic Services), at the Centre Mud site (100 collectors in 5 parallel lines, mean depth = 9.0 ± 1.0 m SD), at the South Mud site (42 collectors in 2 parallel lines, mean depth = 7.4 ± 0.4 m SD), and at the No Name Rock site (20 collectors in 1 straight line, mean depth = 9.3 ± 1.4 m SD) (Fig 1). Collectors were deployed in an inverted "V" at Pocologan Rock to follow the reef (20 collectors in 1 line, mean depth = 9.1 ± 0.7 m SD) (Fig 1). Temperature loggers attached to two different collectors at each site indicated that the mean temperature during the study (all sites combined) was 13.3°C, and each site's mean temperature varied from this by no more than 0.15°C. After

approximately four months, the collectors were retrieved in two sessions: October 26 and November 7, 2012. Retrieval of all collectors on a single day was not possible so approximately half of the collectors at each of the five sites in Maces Bay were retrieved on each date. The collectors were brought to the Huntsman Marine Science Centre, St. Andrews, NB, where it took 2-3 days to sort through their cobbles and count and remove all lobsters. All lobsters were rinsed, blotted dry and weighed on a digital scale (± 0.0001 g), and had their carapace length measured with digital Vernier callipers (± 0.1 mm). Only lobsters ≥ 13 mm carapace length were used in most analyses because these were benthic juveniles that had settled to the seafloor in a previous year and colonized the collectors from the surrounding area. In contrast, individuals < 13 mm carapace length likely represented newly-settled young of the year (Gudjon Mar Sigurdsson, pers. comm.) that settled onto the cobble in the collectors from the water column and therefore would not represent occupation of the surrounding cobble or mud substrate upon which the collectors were deployed.

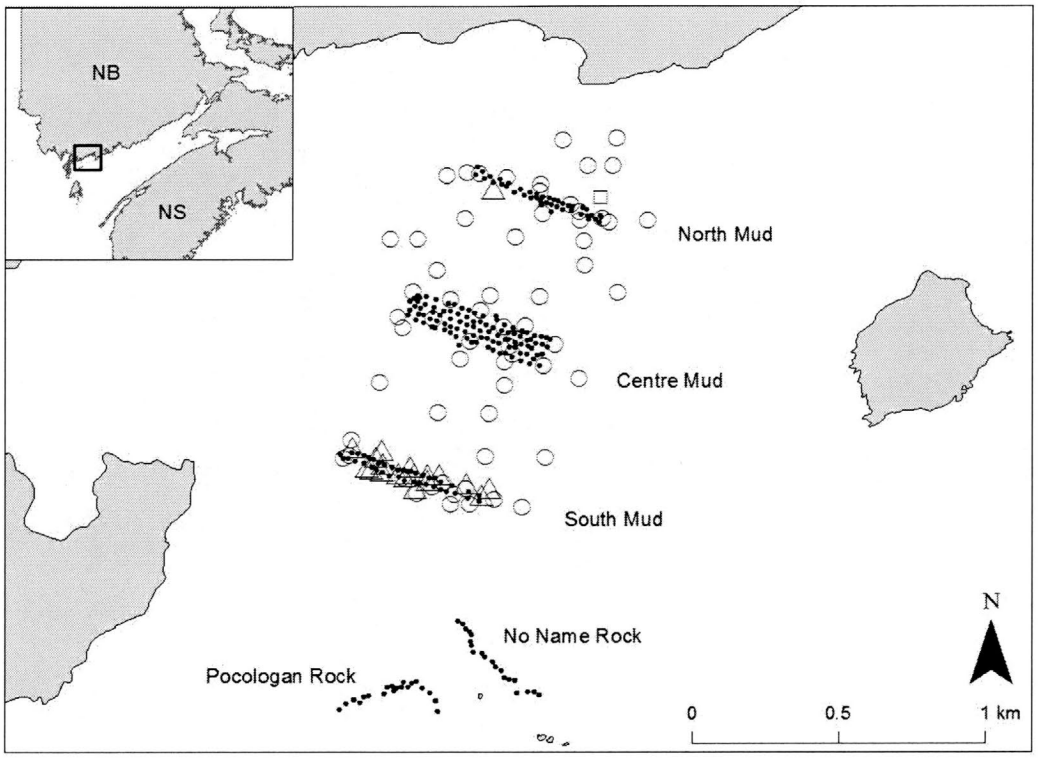

Figure 1: Locations of the five study sites in Maces Bay, Bay of Fundy, New Brunswick. Black dots indicate individual collectors, while open symbols indicate seafloor substrate identified by sediment grabs or video survey: mud ○, rock △, and sand □.

Statistical analyses

To examine the relative abundance of various size classes at each site, the size frequency distributions of juveniles were compared by Komolgorov-Smirnov Tests. Since the size frequency distributions of lobsters found inside collectors at the two rocky sites were similar to one another ($D = 0.14$, $p = 0.73$) they were pooled into a "Pooled

Rock" area. Likewise, the two edge mud sites were similar to one another ($D = 0.095$, $p = 0.91$) and were pooled into a "Pooled Edge Mud" area. The Pooled Rock and Pooled Edge Mud areas were compared to one another and to the Centre Mud site by further Komolgorov-Smirnov Tests.

To further investigate potential differences in use of cobble and mud areas by different sized/aged juveniles, the juveniles > 13 mm CL caught in collectors were divided into Lavalli and Lawton's (1996) life history phases (13-25 mm CL: shelter restricted and emergent; 25-40 mm CL: vagile; ≥40 mm CL: adolescent), and each phase's abundance inside collectors was compared among sites (with collectors as the unit of replication) using Kruskal Wallis Tests (Cochran's Tests and Shapiro-Wilk Tests indicated homoscedasticity and normality ANOVA assumptions were not met). This test was also used to compare the abundance of recent settlers (≤ 13 mm CL) among sites. Significant global tests were followed by Wilcoxon Rank Sum Tests (with Bonferroni correction) to determine which of the 5 sites differed. Once again, the two rocky sites were pooled because they did not differ from one another with respect to abundance of juveniles in any of the life history phases (all p values > 0.50), and the two edge mud were pooled because abundance of juveniles in each life history phases did not differ from one another (all p values > 0.25). This facilitated comparing abundances of juveniles in each life history phase between rocky and muddy habitats via additional Kruskal Wallis Tests (Cochran's Tests and Shapiro-Wilk Tests indicated homoscedasticity and normality ANOVA assumptions were not met) followed by Wilcoxon Rank Sum Tests (with Bonferroni correction) to investigate significant differences.

Results

Experiment 1: Individually monitored settlement in 1-L jars

Swimming behaviour

Handling lobsters while introducing them to 1-L jars often triggered an escape response with the animal tail-flipping away from the handler, which caused the animal to quickly encounter the bottom of the jar. Many individuals re-entered the water column and swam around the circumference of the jar for a few minutes, but swimming soon became sporadic and ceased amongst most lobsters, particularly those in the cobble treatment which sheltered under their cobbles. After the first 4 days, most postlarvae occupied the jar bottom, except for a few postlarvae in mud-lined or bare glass jars which sometimes hung from the mesh covering the jar's mouth. After the first week, all postlarvae occupied the bottom of their jar. No complex tunnels were observed in any of the sedimentary substrates, although 9 of 19 lobsters in mud-lined jars, and 13 of 20 lobsters in sand-lined jars sheltered in shallow depressions they had excavated along the side of their jar.

Post-moult survival and morphometrics

Survival of lobsters to stage V was similar across all substrates (G = 1.83, df = 3, p = 0.62) and averaged 62.2 ± 4.4% (SE). However, substrate treatment had a significant effect on mean time until moult (X^2 = 20.0, df = 3, p = 0.00017), with moulting taking significantly longer (37%) in the unlined glass jars than in all three substrate-lined jars, in which lobsters moulted at approximately the same time (Fig. 2). After moulting to the fifth stage, the mean increment in carapace length did not differ significantly among

substrate treatments ($F_{3,36}$ = 1.65, p = 0.195, Fig. 3). The mass increment of lobsters, however, was dependent upon substrate (X^2 = 9.38, df = 3, p = 0.025, Fig. 3), with lobsters moulting over mud showing only 79% of the mass increment that lobsters moulting over cobble showed. Lobsters over mud were also lighter than those in bare jars (only 78% of the mass) although the difference was marginally non-significant (Fig. 3).

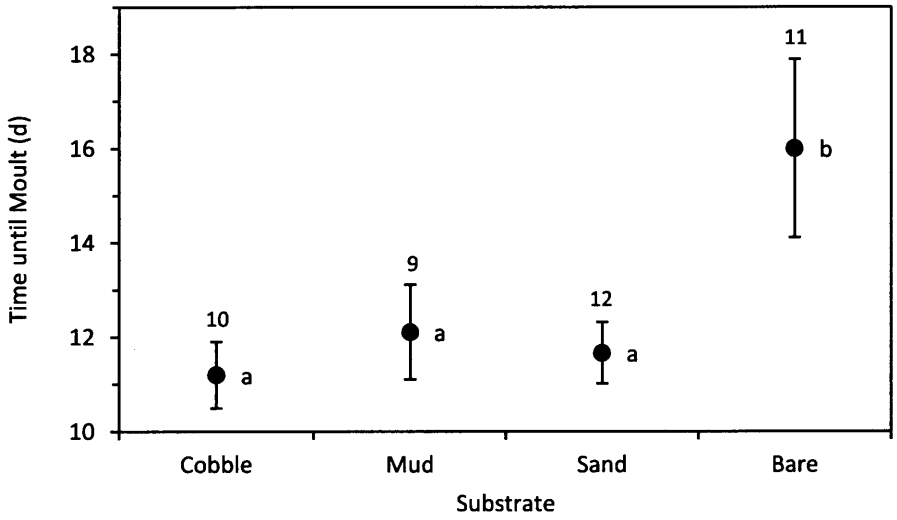

Figure 2: Mean (± 95% CI) time lobster postlarvae took to moult to stage V in cobble-lined, mud-lined, sand-lined, or bare 1-L jars (sample sizes shown above error bars). Different letters indicate significantly different means ($p < 0.05$) identified by Wilcoxon Rank-Sum post hoc tests with Bonferroni adjustment of p-values.

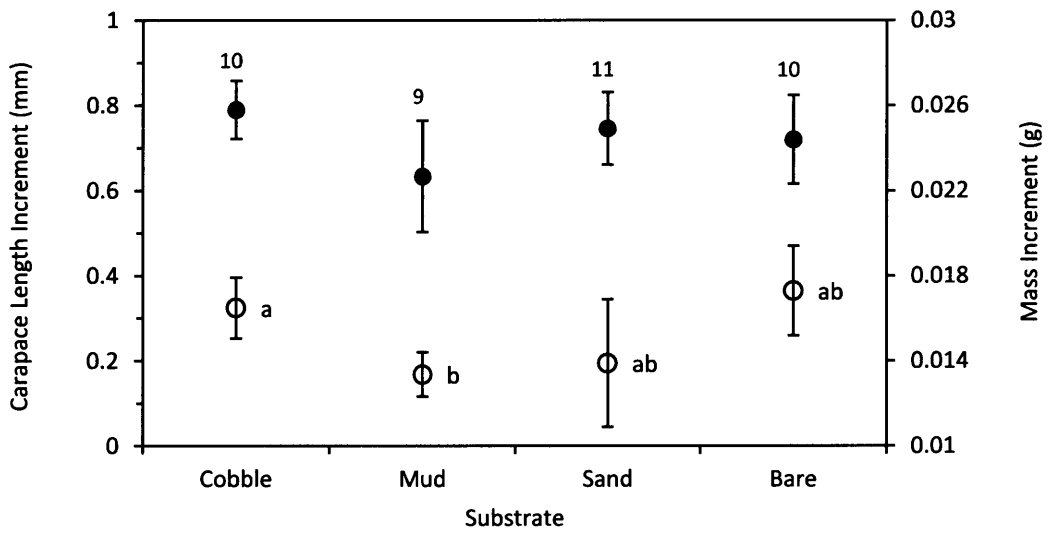

Figure 3: Mean increment (± 95% CI) in carapace length ● and mass ○ of lobster postlarvae after moult to stage V over cobble-lined, mud-lined, sand-lined, or bare 1-L jars (sample sizes shown above error bars). Different letters indicate significantly different mean mass increments ($p < 0.05$) identified by Wilcoxon Rank-Sum post hoc tests with Bonferroni adjusted p-values.

Experiment 2: Group monitored settlement in 30-L tanks

Swimming behaviour

Upon introduction to a 30-L tank, postlarvae often retreated from their handler to the tank bottom. With the exception of lobsters in cobble-lined tanks, most individuals soon re-entered the water column and resumed swimming. Unlike lobsters in 1-L jars, the five lobsters in each of the 30-L tanks could swim longer before encountering a wall, although some were still occasionally seen swimming along a tank wall. Early in the

experiment, the red light used during dark observation periods attracted postlarvae as they swam at the surface. Over time, fewer individuals responded to the light, especially those observed on the tank bottom; these individuals remained motionless or walked into shaded areas when illuminated.

The average proportion of lobsters swimming during the experiment declined somewhat the first two days over all substrates (except cobble where lobsters settled immediately upon introduction to the tank) and then remained relatively stable on each substrate for the remainder of the 13 days of observations (Fig. 4). Despite the small decline in swimming during the first two days there was no significant interaction between day of experiment and substrate ($F_{36,192} = 0.90$, $p = 0.50$), so the main effects of each factor were interpreted separately. The proportion of lobsters swimming was independent of the day of the experiment ($F_{12,192} = 1.48$, $p = 0.23$), but varied significantly by substrate ($F_{3,16} = 47.9$, $p < 0.00001$) with cobble-lined tanks having significantly fewer swimming lobsters than all other treatments (all comparisons $p < 0.00001$), mud-lined tanks having significantly fewer swimming larvae than bare and sand-lined tanks (both $p < 0.00001$), and finally, bare and sand-lined tanks having similar numbers of swimming postlarvae ($p = 1$) (Fig. 4). It was not possible to track individual lobsters and determine whether the same few lobsters remained swimming or whether all the lobsters rotated between the tank bottom and the water column, although lobsters on the bottom very rarely started swimming during observations.

Lobsters in cobble tanks were always the first to leave the water column and 100% of the survivors hid under cobbles and made no further forays into the water column. Among the other substrates, only a single mud-lined tank had all of its lobsters

leave the water column within the 13-day experimental period. The other four mud-lined tanks and all sand-lined and bare tanks always had at least one lobster swimming over the 13-day experimental period. Lobsters on the bottom of mud-lined or sand-lined tanks sometimes occupied small depressions dug into the sediment along the sides of the walls but no tunnels were constructed under the sediment's surface. Lobsters on the bottom of bare tanks usually remained along the sides of the tank walls or in corners.

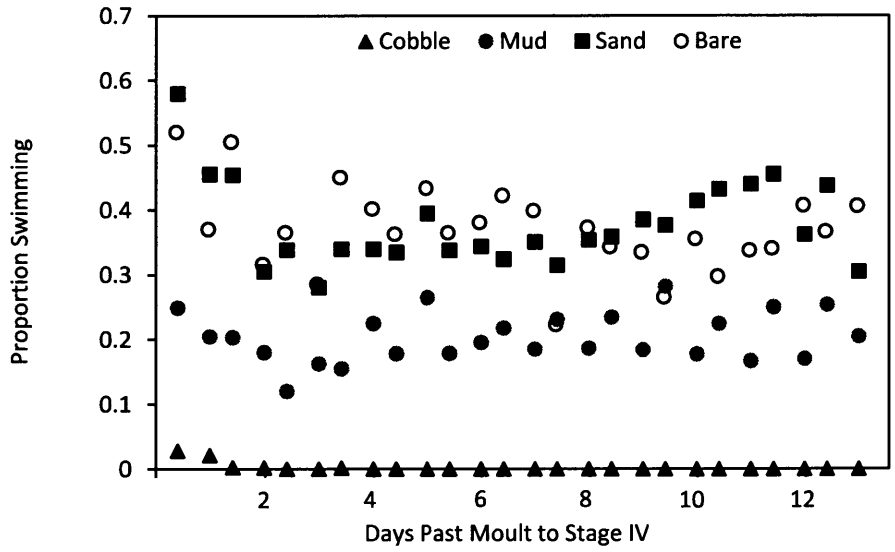

Figure 4: Mean proportion of lobster postlarvae swimming over cobble-lined, mud-lined, sand-lined, or bare 30-L tanks (n = 5 tanks per treatment); observations were made every 15 minutes in each tank, averaged twice daily and then these values were averaged across replicate tanks.

Post-moult survival and morphometrics

Survival of lobsters to stage V averaged 68.0 ± 4.1% SE and did not differ among substrates ($F_{3,16} = 0.64$, $p = 0.60$, Fig. 5). While substrate significantly affected mean time until moult ($F_{3,7.4} = 8.50$, $p = 0.0086$, Fig. 6), with a shorter time until moult on cobble than on sand (all other comparisons were not significantly different), neither mean post-moult carapace length ($F_{3,16} = 0.59$, $p = 0.63$, Fig. 7), nor mean post-moult mass ($F_{3,16} = 2.89$, $p = 0.068$; Fig. 7) varied significantly by substrate.

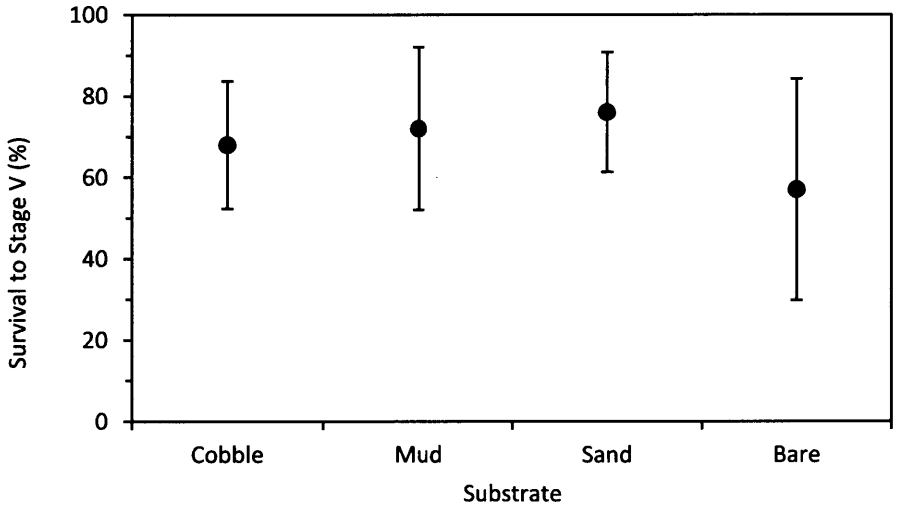

Figure 5: Mean percent survival (± 95% CI) of lobster postlarvae to stage V over cobble-lined, mud-lined, sand-lined, or bare 30-L tanks (n = 5 tanks per treatment).

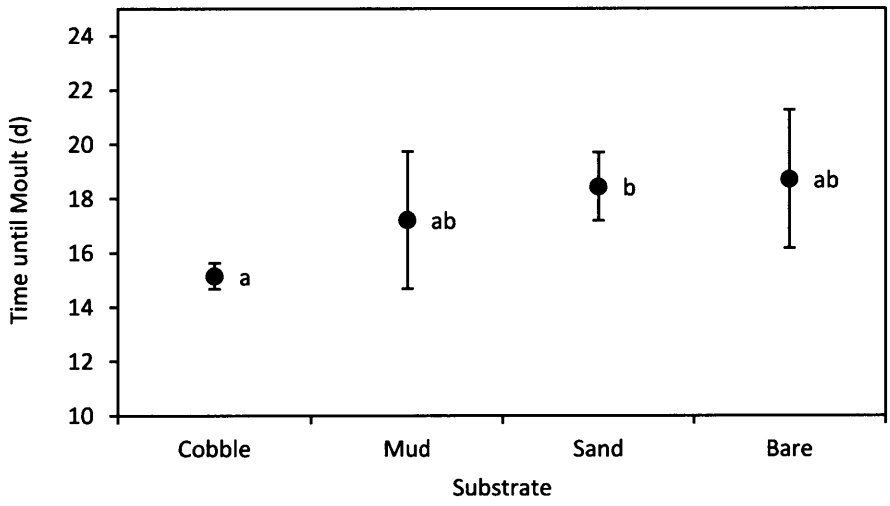

Figure 6: Mean number of days (± 95% CI) before lobster postlarvae moulted to stage V over cobble-lined, mud-lined, sand-lined, or bare 30-L tanks (n = 5 tanks per treatment). Different letters indicate significantly different means ($p < 0.05$) identified by Tukey HSD post hoc tests.

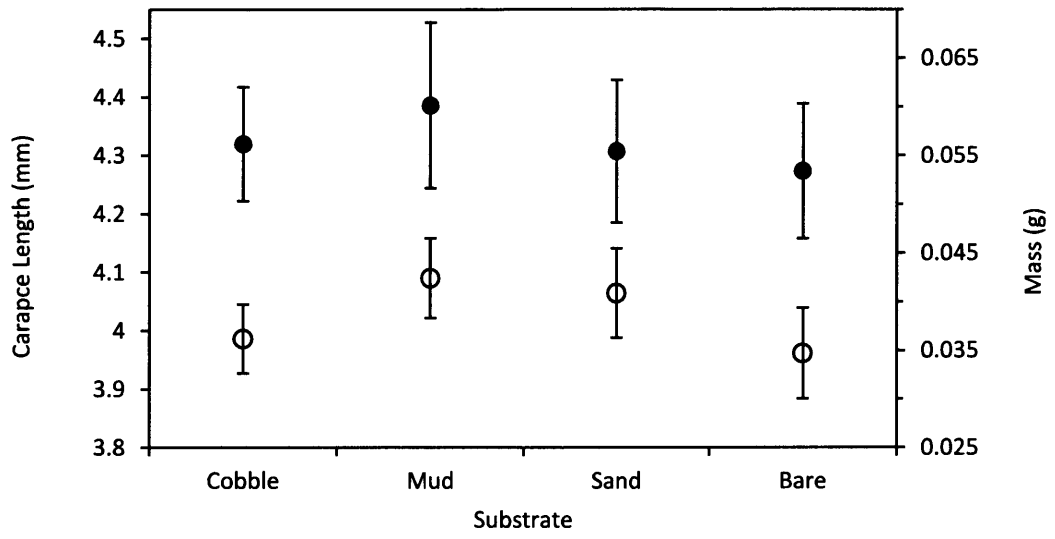

Figure 7: Mean (± 95% CI) carapace length ● and mass ○ after lobster postlarvae moulted to stage V over cobble-lined, mud-lined, sand-lined, or bare 30-L tanks (n = 5 tanks per treatment).

Experiment 3: Group monitored settlement in 600-L tanks

Swimming behaviour

Unlike the 1-L jars and 30-L tanks, the 600-L tanks provided much more room for swimming. When first introduced to a tank, a lobster's retreat from a handler did not immediately bring the animal into contact with the bottom as generally happened in the 30-L tanks and 1-L jars. Instead, lobsters remained within the top few centimeters of the water column and began swimming in the centre of their tank and along the tank walls. A white flashlight was used during night observations as the red light could not illuminate the entire tank or its depths. Early in the experiment, postlarvae swimming at the surface were attracted to the light, but towards the end of the first week, fewer

swimming individuals moved towards the light, and postlarvae on the tank bottom either ignored the light or walked away from it into shaded areas.

In contrast to experiments in the 1-L jars and 30-L tanks, the proportion of lobsters swimming in each 600-L tank was observed until all lobsters in the experiment had settled. There was a highly significant interaction between day of experiment and substrate ($F_{7.6,261} = 14.8$, $p < 0.00001$), which precluded interpretation of the main effects of these factors via the Repeated Measures ANOVA. The interpretation of this interaction was, however, both straightforward and informative when separately examining the first 14 days of the experiment and the last 16 days. Whereas the proportion of postlarvae swimming over cobble decreased abruptly and reached 0% after only 8 days, the proportion swimming over mud and sand remained similar between these two substrates and declined with time as shown by performing a Repeated Measures Anova on just the mud and sand treatments for the first 14 days of the experiment (day of experiment by substrate interaction: $F_{3.7,78} = 1.0$, $p = 0.41$; substrate: $F_{0.29,6} = 0.066$, $p = 0.47$; day of experiment: $F_{3.7,78} = 2.9$, $p = 0.030$). About 80% of lobsters were still swimming over mud or sand by day 14. For the remaining 16 days of the experiment, the proportion of postlarvae swimming over mud and sand varied by time and between the two substrates as indicated by an interaction between the two factors when performing a Repeated Measures Anova on just the mud and sand treatments for the last 16 days of the experiment (day of experiment by substrate interaction: $F_{2.1,90} = 3.9$, $p = 0.022$). The proportion of postlarvae in the water column finally reached 0% after 25 days over mud and after 30 days over sand (Fig. 8).

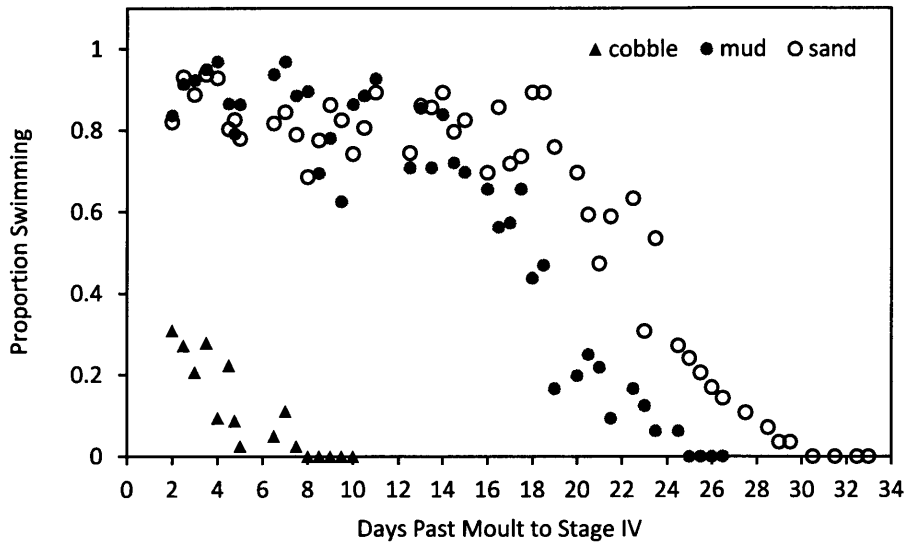

Figure 8: Mean proportion of lobster postlarvae swimming over cobble-lined, mud-lined, or sand-lined 600-L tanks (n = 4 tanks per treatment) observed twice daily.

When each of the replicate tanks was plotted separately to examine how the time until settlement affected time to moult and post-moult morphometrics, the time until 100% of lobsters exited the water column showed a strong positive exponential relationship to time until moult ($R^2 = 0.92$, df = 2,9, $p < 0.0001$, Fig. 9A). In contrast, time for 100% settlement showed a negative and non-significant relationship to post-moult carapace length ($R^2 = 0.28$, df = 2,9; $p = 0.23$, Fig. 9B) and post-moult mass ($R^2 = 0.12$, df = 2,9; $p = 0.56$, Fig. 9C), with most of the variability in lobster size seeming to occur between the sand treatment and the other two substrates. The time for 80% of lobsters to leave the water column was also regressed against time until moult, post-moult carapace length, and post-moult mass to exclude individuals taking

disproportionately longer to settle, however, the conclusions were unchanged (time until moult: $p < 0.0001$, carapace length: $p = 0.11$, mass: $p = 0.45$).

It was again impossible to track individual lobsters and determine whether the same few lobsters remained swimming or whether all the lobsters rotated between the tank bottom and the water column. In the 600-L tanks, however, all of the lobsters in all of the tanks eventually left the water column and descended permanently to the bottom (Fig. 8). Lobsters in cobble-lined tanks hid under and amongst cobbles while in mud-lined tanks, some lobsters remained on the surface of the sediment and others occupied depressions in the mud or short tunnels under the mud. Tunnels were rare and generally had 2-3 openings approximately 3-5 cm apart. All depressions and tunnels were constructed along the sides of the tank or against the central standpipe. In sand-lined tanks, most lobsters remained on the surface of the sediment although a few did occupy shallow depressions in the sand against the tank wall.

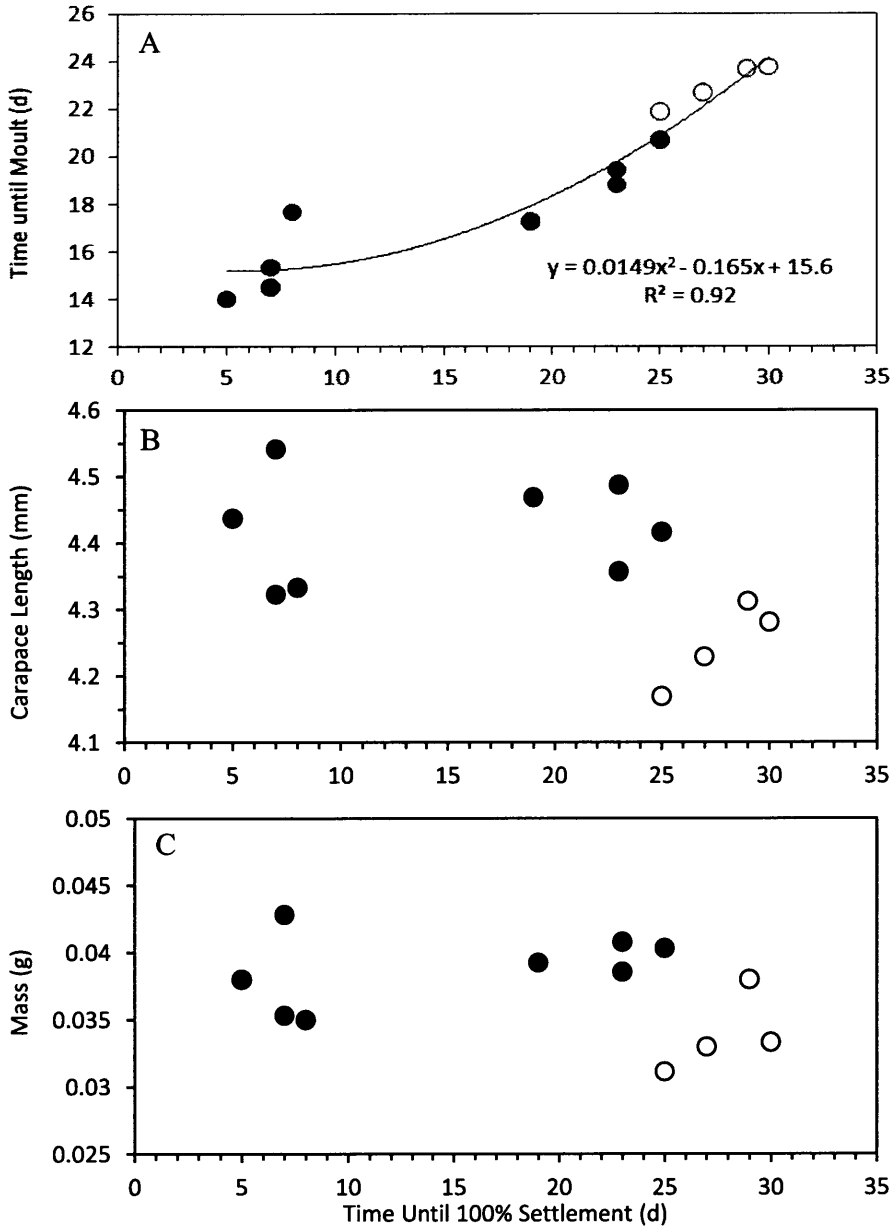

Figure 9: Relationship between time for 100% of lobster postlarvae in a tank to leave the water column and their (A) mean time until moult, (B) mean post-moult carapace length, and (C) mean post-moult mass. The 600-L tanks (n = 4 per substrate treatment) were lined with cobble ●, mud ●, or sand ○.

Post-moult survival and morphometrics

Survival of lobsters to stage V averaged 52.2 ± 2.5% (SE) and did not differ amongst substrates ($F_{2,9} = 0.31$, $p = 0.74$, Fig. 10). In contrast, mean time until moult varied significantly among substrates ($F_{2,9} = 32.0$, $p < 0.0001$) with time until moult on Cobble < Mud < Sand (Fig. 11). On average, lobsters took 22% longer to moult over mud than over cobble, and 49% longer to moult over sand than over cobble. One individual in a sand-lined tank took 45 days to moult (compared to the average of 28 days over sand). It is possible this individual had moulted unnoticed to stage V and remained swimming until moulting to stage VI, when its moult was recorded. Consequently, this animal's time until moult and morphometrics were excluded from all analyses. This animal was missing an eye and due to eyestalk damage may have prematurely moulted (Trider *et al.* 1979, Cheng and Chang 1993) into a stage V lobster superficially resembling a stage IV, except lacking settling behaviour (Charmantier and Aiken 1987). For the remaining lobsters, post-moult mean carapace length varied by substrate ($F_{2,9} = 7.83$ $p = 0.011$) and was significantly smaller (by 3.9%) in lobsters from sand-lined tanks than from either cobble-lined or mud-lined tanks (Fig. 12). Post-moult mean mass also varied by substrate ($F_{2,9} = 5.52$, $p = 0.027$) and exhibited a similar pattern to carapace length, with lobsters from sand-lined tanks significantly lighter (by 16%) than those from mud-lined tanks and 10% lighter than those from cobble-lined tanks, although this second difference was not significant (Fig. 12).

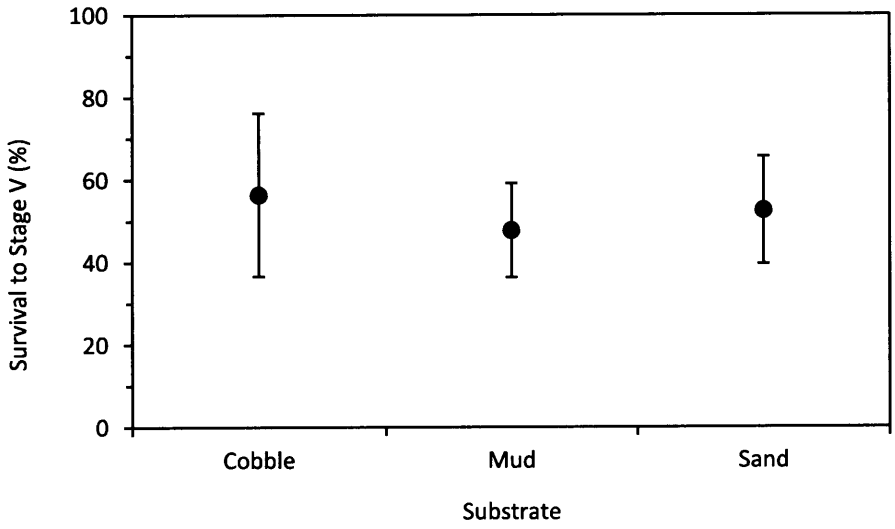

Figure 10: Mean percent survival (± 95% CI) of lobster postlarvae to stage V over cobble-lined, mud-lined, or sand-lined 600-L tanks (n = 4 tanks per treatment).

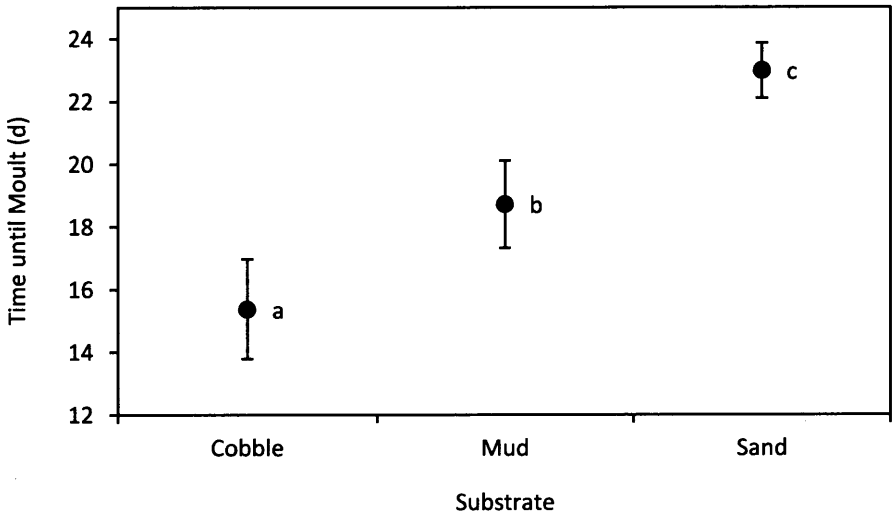

Figure 11: Mean (± 95% CI) time lobster postlarvae took to moult to stage V over cobble-lined, mud-lined, or sand-lined 600-L tanks (n = 4 tanks per treatment). Different letters indicate significantly different means ($p < 0.05$) identified by Tukey HSD post hoc tests.

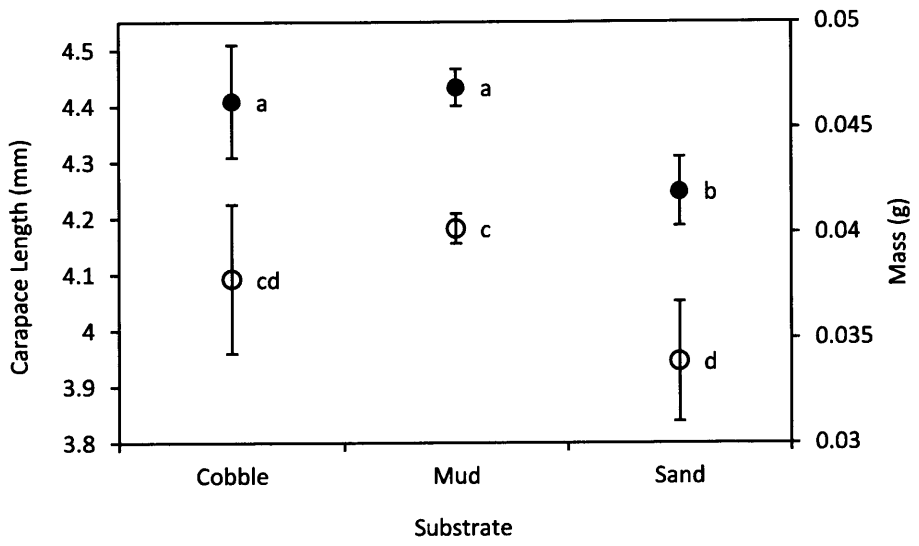

Figure 12: Mean (± 95% CI) carapace length ● and mass ○ of lobster postlarvae after moult to stage V over cobble-lined, mud-lined, or sand-lined 600-L tanks (n = 4 tanks per treatment). Different letters indicate significantly different means ($p < 0.05$) identified by Tukey HSD post hoc tests.

Maces Bay field experiment

All collectors were successfully retrieved from Maces Bay except for two from the Centre Mud site and one from the No Name Rock site. Collectors deployed onto mud substrate occasionally had small amounts of mud inside which, while not quantified, seemed to have filled no more than 10% of the depth of the few affected collectors. Lobsters colonized collectors at all sites, and ranged in size from 7.1 mm to 51.0 mm carapace length (CL), representing both recent settlers of the year (< 13 mm CL, Gudjon Mar Sigurdsson, unpublished data) and older juveniles (Fig. 13A). The

number of lobsters, excluding recent young of the year (YOY) settlers, that colonized a collector from the benthos ranged from 0 to 6 individuals.

Size frequency distribution of lobsters in collectors

After excluding the YOY (< 13 mm CL), the cumulative size frequency distribution of lobsters in the Centre Mud site was significantly different from that at the Pooled Rock area ($D = 0.32$, $p < 0.00001$, Fig. 13B) and the Pooled Edge Mud area ($D = 0.25$, $p < 0.001$, Fig. 13B), and the latter two were similar to each other ($D = 0.15$, $p = 0.14$, Fig. 13B). The most pronounced differences were between the size frequency distributions at the Centre Mud and Pooled Rock areas, with the Pooled Edge Mud areas showing an intermediate pattern (Fig. 13A). Focusing on the Centre Mud site and Pooled Rock areas and visually comparing the cumulative size frequency distributions, the most striking difference seen was in juveniles ranging from 25-36 mm CL, which were much more relatively abundant (Chi Square Test: $X^2 = 10.5$, df = 1, $p = 0.0012$) at Pooled Rock (44.7%) than at Centre Mud (23.1%) while larger juveniles between ~ 37-51 mm CL were relatively more abundant (Chi Square Test: $X^2 = 22.9$, df = 1, $p < 0.00001$) on Centre Mud (38.9%) than on Pooled Rock (9.6%). The 25-36 mm juveniles were probably 2-4 years old and the 37-51 mm juveniles were probably 3-5 years old (Taryn Minch, unpublished data). A less pronounced but significant difference (Chi Square Test: $X^2 = 3.9$, df = 1, $p = 0.049$) in relative abundance was also observed for smaller lobsters measuring 13-16 mm CL, which somewhat surprisingly were relatively more abundant on Centre Mud (22.2%) than on Pooled Rock (11.7%). These animals were probably 1 year of age with a few 2-year-olds (Taryn Minch, unpublished data).

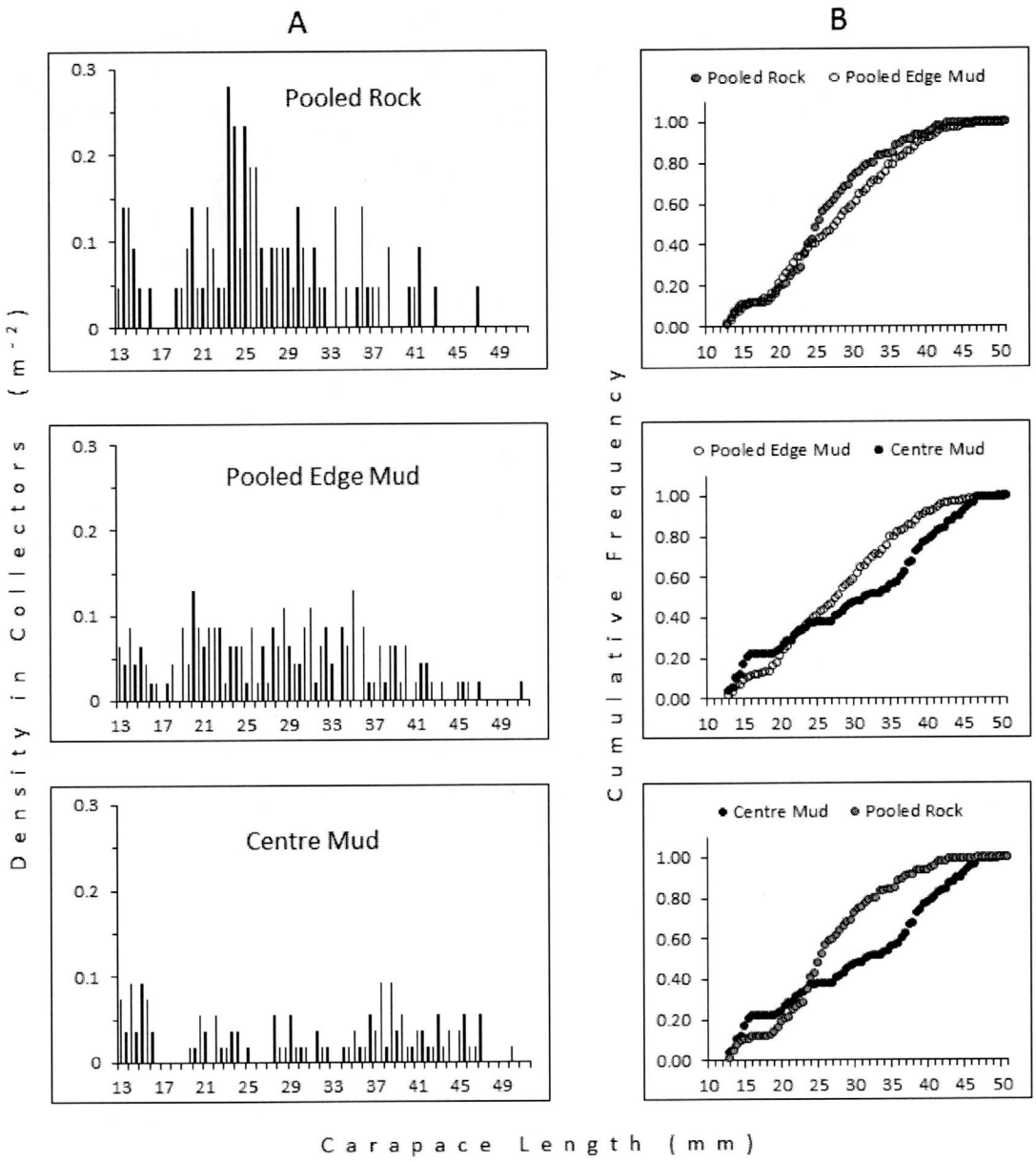

Figure 13: Plots showing (A) the size frequency distributions and (B) the cumulative size frequency distributions of lobsters caught in collectors in Maces Bay. No Name Rock and Pocologan Rock were pooled into an area named "Pooled Rock", while North Mud and South Mud were pooled into an area named "Pooled Edge Mud". To exclude recent settlers, only individuals with ≥13 mm carapace length were analyzed.

Mean number of lobsters caught in collectors

Lobsters < 13 mm CL, representing juveniles that settled that year, were not equally abundant among the pooled areas (Kruskal Wallis Test, $X^2 = 22.7$, df = 2, $p < 0.0001$), being significantly more numerous in collectors at the Pooled Rock areas than those at the Centre Mud or Pooled Edge Mud areas, which in turn supported similar numbers to one another (Fig. 14). Similarly, lobsters measuring 13-24.9 mm CL, representing Lavalli and Lawton's (1996) shelter-restricted and emergent life history phases, were not uniformly abundant across areas (Kruskal Wallis Test, $X^2 = 21.4$, df = 2, $p < 0.0001$), nor were lobsters 25-40 mm CL, representing juveniles at the vagile phase (Lavalli and Lawton 1996) uniformly abundant across collectors sampled in the different areas (Kruskal Wallis Test, $X^2 = 30.7$, df = 2, $p < 0.00001$). For these life history phases, juveniles were most abundant in collectors at the Pooled Rock areas, less abundant in collectors on the Pooled Edge Mud areas, and least abundant in collectors on Centre Mud, although only the differences between Centre Mud and the two other areas were significant (Fig. 14). In contrast, adolescent lobsters >40 mm CL (Lavalli and Lawton 1996) appeared somewhat more abundant in collectors on Centre Mud than in the other two areas, although the differences were not significant (Kruskal Wallis Test, $X^2 = 3.4$, df = 2, $p = 0.18$, Fig. 14).

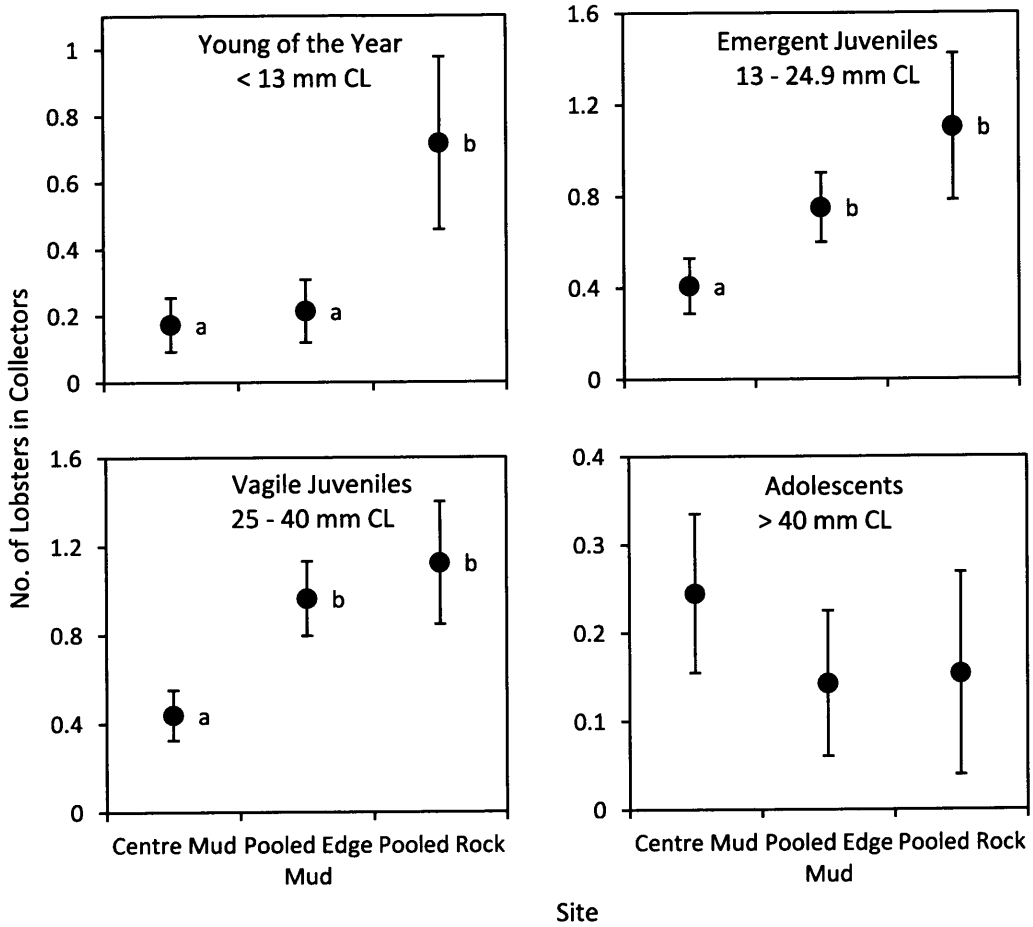

Figure 14: Mean (± 95% CI) number of lobsters caught per collector in Maces Bay. Size groupings are based upon Lavalli and Lawton's (1996) behavioural life history phases with the exception of their shelter-restricted (5-14 mm CL) phase. Juveniles < 13 mm CL, representing young of the year, were analyzed separately and juveniles 13-14 mm CL were combined into the emergent juveniles group (13-25 mm CL). No Name Rock and Pocologan Rock were pooled into an area named "Pooled Rock", while North Mud and South Mud were pooled into an area named "Pooled Edge Mud". Different letters indicate significantly different means identified by Wilcoxon Rank-Sum post hoc tests with Bonferroni correction.

Discussion

The laboratory results presented in this thesis verify that cobble is the preferred settlement substrate for postlarvae, and that postlarvae will delay settlement over other substrates. The results also reveal that postlarvae delaying settlement, and prolonging swimming, risk incurring reductions to both development and growth, although there is no evidence that survival is compromised. A trade-off between finding suitable shelter and avoiding a reduction in developmental and growth rates could explain why postlarvae will eventually settle upon less preferred substrates such as mud bottom in Maces Bay. Indeed, I found evidence of postlarval settlement on mud bottom in Maces Bay as well as conclusive evidence that this habitat is used by juvenile lobsters ranging from shelter-restricted juveniles up to adolescents. These findings challenge our understanding of what habitats juvenile American lobsters are currently using as nursery.

Settlement delay in the laboratory

Settling postlarvae and early benthic phase lobsters exhibit substrate preferences

In all four components of this study (one field and three lab experiments), lobster postlarvae and early benthic phase (EBP) juveniles showed a strong and consistent preference to settle, and ultimately remain upon, structurally complex cobble bottoms versus more homogeneous substrates (i.e. mud, sand, or tank bottom). In the three lab experiments, substrate preference was assessed on the basis of how rapidly postlarvae left the water column to establish residence on the bottom. It was not possible to track individual lobsters in the 30-L and 600-L tanks to distinguish whether the dwindling number of lobsters in the water column over time represented (i) the increasing

settlement of individuals permanently onto the bottom, or (ii) individuals spending more time on the bottom before periodically re-entering the water column. However, the first scenario is most likely to be true for two reasons. First, after the first few days of the experiment, lobsters on the bottom were usually stationary, sheltering under cobble, or (in tanks without cobble) against the tank wall, the central standpipe, or in a depression. Individuals occasionally walked about but were rarely seen swimming from the bottom back into the water column despite observation periods totalling 13 days in the 30-L tanks, and 33 days in the 600-L tanks. Second, individuals in 1-L jars could be individually monitored and once on the jar bottom, they almost never re-entered the water column. The disappearance of postlarvae from the water column almost certainly represented permanent settlement onto the bottom. It is also possible that the structurally complex cobble substrate separated individual lobsters, reduced agonistic interactions, and accordingly promoted faster settlement compared to the other homogenous treatments where aggressive interactions, which can alter a subdominant lobster's behaviour (Lawton 1987), may have forced subdominant individuals to constantly retreat into the water column. However, even though lobsters in mud, sand, and bare treatments were not separated from one another or prevented from interacting, the proportion of individuals swimming in the water column in 30-L tanks and the settlement time of individuals in 600-L tanks still differed by substrate. Thus, settlement time was unlikely to have varied by substrate due to differences in agonistic interactions. Accordingly, the proportion of postlarvae swimming in the water column and postlarval settlement time likely represents, and serves as a good proxy for, substrate preference.

Although the three laboratory experiments differed markedly in their design, particularly in terms of space afforded for postlarval swimming, they all suggest that postlarvae prefer cobble substrate. In all experiments, postlarvae left the water column much more rapidly over cobble bottom than over less complex substrates. Also, the faster settlement upon mud compared to sand or bare tank bottom in both the 30-L and 600-L tanks indicates that mud is a preferred settlement substrate when cobble is absent. We can conclude that postlarval settlement substrate preferences are: cobble > mud > sand and bare. These substrate preferences are similar to previous findings in the lab (Botero and Atema 1982), and field (Cobb et al. 1983, Hudon 1987, Wahle and Steneck 1991). For example, postlarvae given a choice of substrates in the same tank settled preferentially and most rapidly upon cobbles, followed by mud, and last upon sand (Botero and Atema 1982). Similarly, early benthic phase lobsters given a choice of substrates in the same tank sheltered on cobble more often than mud, and on mud more often than on sand (Wahle and Steneck 1992), while in a tank with exposed cobbles at one end and cobbles buried by silt-clay (i.e. mud substrate) at the other, significantly more EBP lobsters sheltered in the exposed-cobbles end of the tank (Pottle and Elner 1982). In a field experiment, swimming postlarvae settled and sheltered when encountering rock crevices on the bottom but kept swimming when encountering a sand-mud substrate (Cobb *et al.* 1983). In a field survey, newly-settled and small EBP lobsters were seen in cobble but not on sand or flat bedrock (Hudon 1987, Wahle and Steneck 1991). Similarly my field study in Maces Bay indicated that both YOY and juvenile EBP lobsters prefer cobble to mud (see below). Thus, there is considerable evidence that postlarval and early benthic phase lobsters prefer cobble substrate, or in the absence of cobble, they prefer mud.

Lobster preference for a substrate reflects the habitat quality of the substrate

There is good evidence that substrate preference demonstrated by lobster postlarvae and juveniles in this and previous studies is a good indicator of each substrate's suitability as juvenile habitat. Given that settling postlarvae and small juvenile lobsters are vulnerable to predation (Wahle and Steneck 1992, Ball *et al.* 2001, Sigurdsson and Rochette 2013), a good habitat should provide ready access to a shelter and should protect well against predators and be easy to maintain. Rapid settlement onto cobble, a delay before settlement onto mud, and a longer delay before settlement onto sand or a bare substrate suggests cobble is the most suitable habitat followed by mud followed by flat sand and bare exposed surfaces (such as flat bedrock).

Cobble does indeed protect juvenile lobsters from some predators better than either mud (Barshaw and Lavalli 1988, Wahle and Steneck 1992) or sand does (Lavalli and Barshaw 1986). Cobble provides ready-made, permanent shelters in the interstices of the cobbles, or underneath if lobsters remove sediment from underneath (Cobb 1971, Berrill and Stewart 1973, Botero and Atema 1982). On bare mud with no rocks, lobsters must excavate a U-shaped tunnel into the sediment (Botero and Atema 1982, this study), which takes considerable time and energy as the tunnel must be continuously maintained or rebuilt when it collapses (Barshaw and Bryant-Rich 1988). Also, and perhaps most importantly, mud does not protect against predation as well as cobble (Barshaw and Lavalli 1988, Wahle and Steneck 1992), although presumably an underground tunnel would afford better protection than being exposed on open sand or bedrock. Sand is not cohesive enough to support burrowing, and on bare sand with no rocks, lobsters can only scoop out a shallow depression to occupy (Botero and Atema 1982, this study). Even

simple manipulations are impossible on bedrock; thus, on bare sand as on exposed bare rock, lobsters cannot produce a shelter. Clearly, a structurally complex, three-dimensional environment (such as that produced by cobble or tunnels in mud) provides better shelter than a simpler, structurally homogenous environment (such as that provided by flat sand or bare bedrock). This varying shelter quality probably explains why postlarvae rapidly settle upon cobble, settle upon mud after a short delay, and delay settling the longest when encountering sand or a hard tank bottom.

The costs of delayed settlement

The experiments reported here suggest that postlarvae delaying settlement may incur three costs: (i) greater risk of predation, (ii) decreased development rate by delaying moult to stage V, and (iii) decreased growth rate due to a smaller size increment in carapace length and mass after moulting. First, delaying settlement increases the duration that swimming postlarvae are exposed to predation while they are in the water column (Hudon 1987), as they dive to the bottom to explore (Roach 1983, Sigurdsson and Rochette 2013), and while they remain exposed on the bottom while seeking shelter (Lavalli and Barshaw 1986, Wahle and Steneck 1992). The risk of predation presumably drops once a lobster leaves open water or open ground and occupies a protective shelter.

The second potential cost to delaying settlement is a delay in the moult to stage V, and its associated size increase, which would render an individual competitively inferior to larger conspecifics that had already moulted, and extend the amount of time small lobsters are susceptible to some predators. It is notable that in the 30-L tanks, in which swimming (particularly vertically) was more constrained and only monitored over 13

days, time until moult showed fewer significant differences between substrate treatments than in the 600-L tanks, although the pattern was similar. More specifically, in both the 30-L and 600-L tanks, lobsters settled more rapidly onto cobble bottom than onto all other substrates. However, whereas in the 600-L tanks this resulted in lobsters moulting significantly faster over cobble than over mud, and significantly faster over mud than over sand, in the 30-L tanks the only significant difference in time until moult was between postlarvae over cobble and sand. I believe the reason why patterns were not as clear in the 30-L as in the 600-L tanks is related to constraints to swimming behaviour imposed by the smaller tanks. Interestingly, differences in swimming time and time until moult on different substrates in these two experiments support this interpretation. More specifically, over cobble, a substrate that consistently led to rapid settlement in both experiments, the average time until moult was similar in the 30-L and 600-L tank experiments (15 d). However, over the less-preferred substrates, where postlarvae delayed settlement, lobsters did not swim as long in the smaller 30-L tanks (after 13 days, only 20% still swam over mud, 30% over sand, and 39% over bare) as they swam in the 600-L tanks (after 13 days, 85% still swam over mud, and 86% over sand). Neither did lobsters delay moult as long in the 30-L tanks (mud = 17 d; sand = 18 d; bare = 19 d) as they did in the 600-L tanks (mud = 19 d; sand = 23 d). I cannot directly investigate the relation between settlement time and time until moult in the 1-L jar experiment, given that swimming behaviour was not quantified in this experiment as the small containers offered little space for swimming and indeed very few postlarvae were seen swimming during daily observations, even after only 4 days (estimated at less than 5-10%). However, moult patterns in this experiment also suggest that constraining swimming

behaviour affects time until moult, as postlarvae in 1-L jars consistently moulted more rapidly on all four substrates (cobble = 11 d; mud = 12 d; sand = 12 d; bare = 16 d) than in larger tanks as outlined above. Thus, delayed settlement over less-preferred substrates was associated with delayed moult, although this effect became less clear when swimming behaviour was constrained by reduced tank size.

The third potential cost to delaying settlement is a decreased growth rate, manifested as a smaller size increment in carapace length and mass upon moulting to stage V. In the 600-L tanks, the postlarvae taking longest to settle (those over sand) exhibited, on average, a smaller increase in carapace length and mass after moulting than postlarvae that settled sooner (those over cobble and mud). Substrate had comparatively little effect on growth of postlarvae in the 1-L jar and 30-L tank experiments, again probably because these offered little space for swimming, as just discussed in regards to variation in time until moult. Interestingly, when using each of the 12 individual tanks of the 600-L experiment as replicates, settlement time showed no continuous relationship with post-moult carapace length or mass as seen for time until moult. Instead, increasing settlement time resulted in no differences in post-moult growth except at the longest delay times (over sand) where post-moult growth suddenly dropped. This result was surprising, and in particular the observation that growth of lobsters over mud was not significantly different from those over cobble, despite the fact that they delayed settlement markedly longer relative to conspecifics over cobble (100% settlement took 8 days over cobble, and 25 days over mud). In fact, the mass of stage V lobsters over mud in the 600-L tanks tended to be less variable and somewhat greater (5-17% heavier) than those over cobble, although not significantly so. Given our attempts to provide food *ad*

libidum to lobsters in all treatments, these results suggest that there may exist a complex relationship between settlement time and growth, where increments in time to settle have little effect on postlarval growth up until a point beyond which marked effects on growth occur. More specifically, the extra days spent swimming over mud (versus cobble) did not reduce post-moult growth, or at least reduced it insufficiently to be detected by the morphometric indicators I used (length and mass), whereas the few days extra spent swimming over sand (versus mud) strongly reduced growth. This result suggests that, in the laboratory, lobster postlarvae over mud do not delay settlement to the point of negatively impacting growth, as those over sand do, and suggests that juveniles regard mud as a better settlement habitat than sand when cobble is not available.

An alternative explanation for the similar growth of lobsters over cobble and mud, despite the markedly different swimming time over the two substrates, is that unequal feeding opportunities between the cobble and mud treatments may have compensated for differences in energetic status arising from the different settlement times on these two substrates. Under this scenario, postlarvae delaying settlement over mud would have been expected to experience lower growth relative to postlarvae over cobble, but in my experiments these differences may have been compensated for in one of two ways. First, if lobsters on mud acquired more food from their substrate after settlement this could have boosted their growth to a level similar to lobsters on cobble. The mud used in the 600L tanks was not dried or sieved and contained some macroscopic organisms such as small worms and crustaceans. Lobsters on mud could thus have supplemented their brine shrimp diet with these items. Although a possibility, I do not believe this food compensation occurred. If lobsters on mud were supplementing growth by consuming

live organisms from the mud, then growth in the 600-L tanks lined with fresh mud should have been greater than growth in the 30-L tanks lined with mud that had been sieved and dried to remove most of these additional food sources. Instead, the mean mass of stage V on mud was similar in 600-L (0.040 g) and 30-L (0.042 g) tanks, and mean carapace length was identical (4.4 mm). Furthermore, if lobsters were feeding on small pieces of organic matter present in either fresh or dried mud, then growth over mud should have been enhanced in all the laboratory experiments. Instead, in the 1-L jars, lobsters on mud had a smaller mean carapace length (4% shorter) and significantly lower mean mass (11% lower) than those on cobble. It is clear that the use of intertidal mud was not consistently linked to increased growth in the mud treatment and it therefore seems unlikely this could account for the similar post-moult size of individuals in the mud and cobble treatments in the 600-L tank experiment.

A second potential food-related explanation for the similar growth of lobsters over cobble and mud, despite the markedly different swimming time over the two substrates, is that lobsters in cobble tanks may have tended to remain sheltered and as such, encountered food less frequently than conspecifics on mud. In the 600-L and 30-L cobble-lined tanks, postlarvae quickly left the water column to shelter under cobbles and were not seen foraging outside their shelter. Recent settlers are believed to remain in their shelter, feeding by capturing organisms in the water using pleopod fanning or by ambushing prey at the shelter entrance (Barshaw and Bryant-Rich 1988, Lavalli and Barshaw 1989). It is possible that this cryptic behavior limited access to food as brine shrimp that fell to the bottom would not always have landed immediately in front of the cobble shelters. In contrast, in the 600-L and 30-L tanks, postlarvae delaying settlement

over mud, sand, or a bare tank bottom were observed feeding readily on food floating in the water column. Once these postlarvae settled to the bottom of their tank, they may have been more likely to detect and encounter brine shrimp falling to the bottom than postlarvae hidden under a cobble shelter would have. Accordingly, postlarvae over mud, sand, or a bare tank may have had better access to food than those over cobble. This interpretation is supported by the fact that the mean mass of stage V lobsters in cobble in the small 1-L jars, where food was confined in the immediate area of settled postlarvae, was heavier (0.044 g) than the mean mass of lobsters in the larger cobble-lined 30-L tanks (0.036 g) or 600-L tanks (0.038 g). The design of my study does not enable me to assess the effect of food acquisition (in the plankton and on the benthos) on the relationship between settlement delay and growth, but it does conclusively demonstrate that delaying settlement can negatively affect lobster development and growth. Future studies should investigate the separate and interactive effects of food availability (planktonic and benthic) on settlement time and growth.

While several studies have looked at substrate preference by lobster postlarvae and early benthic phase juveniles (Botero and Atema 1982, Pottle and Elner 1982, Wahle and Steneck 1992), and one has quantified postlarval settlement time on different substrates (Botero and Atema 1982), my study is the first to demonstrate that delaying settlement can negatively affect lobster development and growth. Growth in lobsters is only possible when the old carapace is moulted to free the new, larger carapace that has developed underneath. Thus, frequency of moulting, time to each moult, and size increment after moult all determine the rate at which a lobster develops and grows. Although it is unknown if postlarval lobsters delaying settlement incur costs only to their

first benthic moult or to subsequent moults as well, in some species the costs of delaying settlement persist through subsequent developmental stages (de Jesus de Brito Simith *et al.* 2013), or may even manifest in later juvenile or adult stages (Pechenik 2006). However, even if development and growth rates soon resume levels exhibited by non-delaying individuals, those that delayed settlement may still be smaller than average due to their first reduced growth increment (Gebauer *et al.* 1999). For juvenile lobsters, any reduction of development and growth is problematic, given that smaller individuals are at a disadvantage when competing with larger conspecifics for food and shelter (O'Neill and Cobb 1979), and are at greater risk of predation with a smaller body size (Wahle and Steneck 1992). Any delay in moult and any reduction in carapace length and mass increment would extend the amount of time an individual is at higher risk.

It is likely because of these costs of delayed settlement that larvae do not indefinitely delay settlement in search of high quality cobble habitat. Instead, postlarvae are known to become less selective about the quality of their shelter with time (Boudreau *et al.* 1993), and in my study they eventually settled on all substrates before survival to stage V was affected. This behaviour would limit the expenditure of energy and its associated costs to development and growth, while still allowing some time to seek high-quality shelter. Ultimately, to maximize growth and survival postlarvae must balance the hazards of settling into a poor quality habitat (risking higher exposure to predation by pelagic and benthic predators), against the hazards of spending more time swimming in the water column (risking predation by pelagic predators as well as energy depletion and the associated reduced development and growth rates).

Mechanisms underlying developmental and growth costs of delayed settlement

Delayed settlement could delay moult and reduce post-moult size increment in several different ways, but it seems most probable that the energetic demand associated with the additional swimming while delaying settlement is responsible. Postlarval lobsters store more lipids than earlier larval stages, and these stores are believed to serve as an energy reserve allowing postlarvae a few days to find or construct shelter as they transition to the benthos before they must begin feeding (Sasaki *et al.* 1986). The growth increment between stage IV and V is extremely low relative to earlier larval stages as well as older juveniles up to stage XIV (Hudon 1987, James-Pirri and Cobb 1987), which has been interpreted to mean that new settlers are spending more time seeking shelter or remaining sheltered than they are feeding (Hudon 1987, James-Pirri and Cobb 1997). Shelter-restricted behaviour may increase the reliance of newly-settled postlarvae on their energy stores (Sasaki *et al.* 1986), which extended swimming may have depleted. Lobsters that are poorly nourished or starved (i.e. have reduced energy reserves) have been shown to delay moult or exhibit smaller growth increment upon moulting (Templeman 1936, Carlberg and Van Olst 1976, Anger 1987, Thériault and Pernet 2007). In a similar fashion, reduction of energy reserves by extended swimming may also delay moult and reduce the post-moult size increment. In my experiments, the amount of swimming room in the tanks influenced how strongly swimming time (i.e. settlement delay) affected time until moult as well as post-moult increase in carapace length and mass. Only in the large 600-L tanks, which had ample room for postlarvae to swim extensively, and where more swimming activity was indeed observed, did we consistently see significantly different times to moult and significantly smaller size

increments in lobsters taking the longest to settle (i.e. swimming the longest). These results suggest that in nature, where swimming is not constrained, delayed settlement reduces development and growth by way of the energy consumed during prolonged swimming.

Energy consumed while swimming during delay of settlement is a more plausible explanation for the reductions in development and growth seen in these experiments than other factors such as establishment of a dominance hierarchy, effects of high conspecific density, or energy consumed while manipulating a substrate. While a dominance hierarchy may delay moult (Cobb 1970, Cobb and Tamm 1974), and high conspecific density may lower post-moult size increments (Aiken and Waddy 1978, Roach 1983), I saw marked growth differences in the 600-L and not the 30-L tanks, despite the fact that lobsters were stocked at lower densities, and presumably interacted less, in the 600-L tanks than in the 30-L tanks. Bottom substrates themselves do not appear to directly affect time until moult or growth at moult (Cobb 1970, Roach 1983), although substrate manipulation could consume energy normally put into moulting and growth. However, the length and mass of stage V lobsters in 30-L and 600-L mud-lined tanks were similar to, or even greater than, the length and mass of lobsters on cobble or sand, even though lobsters on mud had manipulated the substrate the most by scooping out depressions and digging tunnels. In contrast, individuals on cobble or sand performed no or few substrate manipulations as lobsters on cobble were too small to move their cobbles, and lobsters on sand made very few depressions and no complex shelters, due to low cohesiveness of this substrate. Thus, extended swimming time and depletion of energy reserves seem to best

explain the differences among substrates in time until moult and post-moult carapace length and mass.

In summary, it is advantageous for postlarvae to delay settlement for some time over substrates which provide only poor shelter. However, there can be a trade-off in terms of the energy consumed and the resulting effects on development and growth if an individual delays too long. The decisions made by individual postlarvae are important as they can influence the demography of lobster populations. The amount of time spent swimming in the water column over various substrates will influence dispersal distances and patterns and will shape connectivity within and between populations, while the substrate choices postlarvae make when settling will result in settlers being patchily distributed in nature. In particular, we can expect higher densities on high quality cobble habitat, and this is indeed observed in nature (Hudon 1987, Wahle and Steneck 1991), but my lab findings also suggest that postlarvae may also be settling on mud bottom to avoid development and growth costs associated with delaying settlement when preferred cobble bottom is not encountered.

Mud substrate as juvenile habitat in Maces Bay

The field survey in Maces Bay, in the Bay of Fundy, an area containing cobble reefs known to support lobster settlers and EBP juveniles (Gudjon Mar Sigurdsson, pers. comm.) was performed to assess whether settlers and juvenile lobsters are also present on mud substrate in Maces Bay and to examine how the juveniles are using mud habitat. Our current understanding is that only cobbles, or cobbles scattered on sedimentary substrates, play a significant role as settlement habitat for American lobsters (Hudon

1987, Wahle and Steneck 1991). However, in nature, cobble habitat may not always be available and my laboratory experiments (see also Botero and Atema 1982) have shown that postlarvae prefer mud substrate over sand or glass bottom and will settle upon non-cobble substrates rather than die. Mud, in particular, can be modified to produce a structurally complex shelter in the form of tunnels (Berrill and Stewart 1973, Botero and Atema 1982). Given that mud is present across the lobster's range (USGS 2005, Fader *et al.* 1977, Shaw *et al.* 2012, Schumacher *et al.* in preparation) and abundant in many parts of the Bay of Fundy (Fader *et al.* 1977, Shaw *et al.* 2012, Schumacher *et al.* in preparation) such as Maces Bay, and given the previous observations of juvenile lobsters on mud seafloor (see Introduction), mud habitat has the potential to serve as settler and juvenile habitat in the wild.

In this field study, I found evidence of newly-settled lobsters and early benthic phase juveniles using mud bottom as habitat in Maces Bay. Juvenile lobsters measuring 7.1 - 51 mm in carapace length (CL) were found in the collectors, encompassing animals only a couple of months old, up to probably 5 years of age (Taryn Minch, pers. comm.). This represents all juvenile life history phases, including shelter-restricted juveniles (4-14 mm CL), emergent juveniles (15-25 mm CL), vagile juveniles (25-40 mm CL), and adolescents (>40 mm CL) (Lavalli and Lawton 1996). While this size range has been previously seen in collectors on cobble reefs (Wahle *et al.* 2009, Wahle *et al.* 2013, Gudjon Mar Sigurdsson, pers. comm.), this is the first time collectors were deployed on mud bottom and my findings strongly suggest that mud habitat in Maces Bay is supporting juvenile lobsters encompassing all of the Lavalli and Lawton (1996) life history phases. Whereas animals < 13 mm CL were probably young of the year (YOY)

that settled on cobble inside the collectors, and thus never lived on mud bottom, the older individuals ≥ 13 mm CL had likely spent some or all of their lives on mud bottom (see below).

Settlement onto cobble and mud bottom

The densities of newly-settled young of the year (YOY) in the collectors support our current understanding that postlarval settlers preferentially settle in cobble habitat. There were more YOY settlers found in collectors on cobble than in collectors on mud (centre and edge), despite both types of collectors containing identical cobble substrate. This is most likely due to postlarval preference of the surrounding cobble substrate over mud substrate (Botero and Atema 1982, Wahle and Steneck 1992, this study) leading to individuals spending more time testing the bottom over their preferred cobble substrate and less often over mud, leading to fewer encounters with collectors on mud.

The greater abundance of YOY settlers in collectors on cobble is also far more likely to reflect higher initial settlement into collectors on cobble rather than factors such as oceanography or differential mortality. While the numbers of settlers at a site can be influenced by site-specific differences in oceanographic properties such current strength and direction, water depth, and temperature (Boudreau *et al.* 1992, Wahle and Incze 1997, Xue *et al.* 2008, Chassé and Miller 2010), this is unlikely to be the case here. My five sites in Maces Bay were in close proximity (in an area of roughly 1.8 km^2), the mean depth among sites varied at most by 1.8 m, whereas depth could vary by 1.4-5.3 m within a site, and mean bottom temperature only varied by 0.15°C among sites, whereas temperature could vary by 7-16 °C within a site over the study period. Higher mortality

in collectors on mud could also have lowered counts of YOY settlers compared to collectors on cobble, but it is unclear why mortality by lobster predators would differ for postlarvae in collectors on the two substrates. Lobsters would have been sheltering in identical cobble-filled cages placed on either mud or cobble seafloor and predators of small lobsters, such as fish and crabs, exist at higher densities in cobble habitat (Wahle and Steneck 1992). Thus, it seems most likely that the different abundance of YOY settlers between the cobble and mud environments was due to higher settlement into collectors on cobble, possibly due to increased sampling of the preferred cobble seabed, leading to more encounters with the collectors on cobble.

Although postlarvae prefer settling onto prime cobble habitat, my study provides evidence that they are also settling onto mud bottom. It is important to stress that the presence of settlers of the year (YOY: carapace length 7.1 - 13 mm) (Gudjon Mar Sigurdsson, unpublished data) in collectors on mud does not actually provide evidence of settlement on mud bottom, as these postlarvae settled into a small patch of cobble habitat I provided that was surrounded by mud, but they did not settle onto mud itself. However, the presence of YOY does indicate larvae are present in the water over the mud sites and are sampling the benthos in muddy areas. Instead, the best evidence for settlement onto mud comes from the presence of the small juveniles measuring 13-16 mm CL in collectors on mud bottom at the Centre Mud site, which includes both shelter-restricted and small emergent juveniles according to the Lavalli and Lawton (1996) scheme, and probably comprises individuals that are mostly 1 year old, and possibly some that are 2 years old (Taryn Minch, pers. comm.). Shelter-restricted juveniles are thought to be largely confined to their shelters, acquiring food by suspension feeding or ambushing

prey at the entrance of their shelter (Barshaw and Bryant-Rich 1988, Lavalli and Barshaw 1989), while emergent juveniles make only short forays outside shelter (Lavalli and Lawton 1996). It thus seems unlikely these individuals would have left patches of prime cobble habitat, which I estimate would have been a minimum of 400 m away from the centre of the mud patch where I deployed collectors, and walked unsheltered and exposed into an area of mud habitat with poorer prospects for shelter. It is also interesting that the relative abundance of these small individuals (13-16 mm CL), compared to other-size lobsters, was significantly greater in collectors on mud than in collectors on cobble, which may reflect a greater tendency for these individuals to move in mud habitat, where shelters may periodically degrade, than in structurally complex cobble habitat. Thus, there is evidence for both settlement and retention of postlarval lobsters on mud habitat.

Use of mud bottom by early benthic phase juveniles

The presence of lobsters measuring 17-51 mm CL in collectors provides further evidence that juvenile lobsters occupy mud bottom. I believe variation in the abundance and relative abundance of lobsters 37-51 mm CL, which are estimated to be 3-5 years of age (Taryn Minch, pers. comm.), is best interpreted as resulting from emigration of larger individuals into mud-bottom areas. Whereas settlement was greater in collectors on cobble bottom than on mud, and densities of previously settled juveniles in my study were markedly greater in collectors deployed on cobble than those on mud, the relative abundance of individuals 37-51 mm CL compared to other sizes was actually greater in collectors on mud (38.9%) than on cobble (9.6%). Similarly, the absolute abundance of the larger adolescent juveniles measuring more than approximately 40 mm CL was as

high inside collectors on mud as inside those on cobble. I believe these patterns reflect larger lobsters moving into the Centre Mud site from outlying areas. The Pooled Edge Mud areas had an abundance and size structure of juvenile lobsters intermediate to those at the Centre Mud site and Pooled Rock areas, between which it lay. This observation could reflect movement of juveniles from rocky areas into the Pooled Edge Mud areas where they influenced the abundances and size composition of EBP juveniles therein (although the substrate composition of the Pooled Edge Mud areas, which was intermediate to that at the purely mud Centre Mud site and the rocky Pooled Rock area, may also explain the observed patterns). Nonetheless, given the mobility of larger juveniles, and their patterns of abundance, I believe their presence in collectors on mud is at least partly the result of these individuals moving broadly among different substrates. A recent ultrasonic telemetry study has shown that large juveniles 37-51mm CL are capable of crossing the ~ 400 m separating the cobble patches from the Pooled Edge Mud areas and the ~ 400 m distance from the Pooled Edge Mud areas to the Centre Mud site (Bryan Morse, unpublished data). These individuals correspond to the largest vagile juveniles and adolescents (Lavalli and Lawton 1996), which are thought to be less strictly associated with shelter and to range farther abroad (Lavalli and Lawton 1996), since risk of predation decreases with larger body size (Wahle and Steneck 1992) and the diet of larger lobsters changes from items foraged or scavenged near the shelter, to one more reliant on larger, captured prey (Sainte-Marie and Chabot 2002). Furthermore, there is evidence that larger lobsters move away from areas of high conspecific density into areas of lower density, and reduced competition (Steneck 2006). As a result of these

ontogenetic changes in movement patterns, larger juveniles may move out from cobble reef and onto mud.

The abundance and relative abundance of smaller juveniles, including emergent and vagile individuals measuring 17-36 mm CL, which are estimated to be 1-4 years of age (Taryn Minch, pers. comm.), were markedly greater in collectors on cobble than in collectors on mud. The greater relative abundance of 17-36 mm CL juveniles in comparison to other size individuals in collectors on cobble than in collectors on mud is probably a result of the movement of large adolescents discussed above. As individuals over 37 mm CL leave cobble habitat and move into mud, this would increase the relative abundance of the 17-36 mm CL juveniles remaining on cobble, and decrease the relative abundance of 17-36 mm CL juveniles on mud as larger adolescents migrate in. The greater absolute abundance of the 17-36 mm CL juveniles inside collectors on cobble is not surprising, and undoubtedly is the result of much greater settlement, possibly combined with greater survival, on cobble than on mud bottom (Barshaw and Lavalli 1988, Wahle and Steneck 1992). As well, perhaps the small 17-36 mm CL juvenile lobsters move more on cobble bottom than on mud bottom, and are caught more frequently in collectors on cobble, either because they are more likely to be displaced by dominant individuals on cobble bottom where densities are much higher (Paille *et al.* 2002, Steneck 2006), and/or because they are more likely to forage over larger areas on structurally complex bottom than over more homogeneous mud bottom (Hovel and Wahle 2010). This latter hypothesis may initially appear contradictory to one proposed earlier, which suggested that the relative abundance of smaller individuals 13-16 mm CL was greater inside collectors on mud than those on cobble due to an increased tendency to

move about on mud when shelters degrade. The 13-16 mm CL individuals are predominantly shelter-restricted and are believed to remain tightly-bound to their shelters, at least when living on structurally complex cobble bottom where shelters are sturdy and more permanent. On mud bottom, however, shelters may collapse and force these small individuals to move to find or construct another shelter, hence 13-16 mm CL lobsters may move more on mud than on cobble, thereby appearing more frequently in collectors on mud than in those on cobble. In contrast, the larger 17-36 mm CL individuals (emergent and vagile phases) are thought to need to leave their shelters to forage over larger areas (Lavalli and Lawton 1996), including on cobble bottom. When in a cobble habitat, these individuals may be able exploit the complexity of their environment to move more freely, farther, and safely, than those on a homogenous mud bottom, hence 17-36 mm CL lobsters may move more on cobble than on mud, thereby appearing more frequently in collectors on cobble than in those on mud. It is not possible to say from my data which of these hypotheses is most likely, and clearly empirical work is needed to quantify survival and movement of juvenile lobsters on cobble and mud bottom.

In summary, the differences in lobster abundance and size frequencies between collectors at the Pooled Rock, Pooled Edge Mud, and Centre Mud areas suggest a pattern of habitat use that includes settlement on mud and also juvenile range expansion from cobble into mud habitat as juveniles grow. In the lab, postlarvae settle from the water column onto both cobble and mud habitat although cobble habitat is settled preferentially (Botero and Atema 1982), and the same is probably true in nature. The high numbers of postlarvae on cobble beds give rise to high numbers of small shelter-restricted, emergent,

vagile, and adolescent size classes in this habitat. All these life phases of juveniles, with the exception of adolescents, appear to be markedly less abundant on mud seafloor, probably due to the lower number of initial YOY settlers there, but they are nonetheless present. As juveniles grow in size, they increasingly move over larger distances and, given their densities are much greater on cobble than on mud bottom, these movements result in a net movement of juveniles from cobble to mud bottom. The largest juveniles disperse the farthest and appear to be spread out more uniformly across different seafloor substrates in Maces Bay. This, in turn, allows the population to spread out from its initial settlement sites (Wahle and Incze 1997), until as adults, lobsters are found in a great variety of habitats (Cooper and Uzmann 1977). The results of my study indicate that we may have underestimated the use of mud substrate by juvenile lobsters (7-51 mm CL), which in Maces Bay seem to be using mud habitat as initial settlement grounds, as habitat during early development, and as expansion habitat as they grow.

Conclusion

The results of this study are consistent with earlier work demonstrating that lobster postlarvae prefer to settle upon structurally-complex cobble bottom rather than upon more homogenous substrates such as mud, bedrock or sand, and that they will delay settlement when swimming over these homogenous substrates (Botero and Atema 1982). These findings strongly suggest that modelling efforts to understand stock structure and connectivity need to incorporate seafloor substrate as a parameter when estimating dispersal and settlement. This study also shows, for the first time, that there is a significant development cost and potential growth cost associated with delayed

settlement. However, more experimental work is needed to determine the actual relationship between settlement delay and postlarval development and growth to stage V under natural conditions, including natural food availability, and to determine whether the effects of settlement delay have longer-term consequences. Irrespective of the precise shape of the relationship between settlement delay, development, and growth, it appears that the costs of delaying settlement limit the amount of time postlarvae will swim in search of cobble habitat, and this likely explains why they have been observed to settle on structurally simpler substrates in the lab when the preferred cobble substrate was not available (Botero and Atema 1982, this study). Somewhat surprisingly, there is very little empirical evidence of postlarvae settling on substrates of low structural complexity in nature (see Introduction), and my field study in Maces Bay provides some of the scant evidence to date of lobster settlement on bare mud (see also Cooper and Uzmann 1977, Normandeau Associates Inc. 1999). I believe the scarcity of data pointing to postlarval settlement on structurally simple bottom is due to a combination of low settlement densities on such substrates, few attempts to survey non-cobble habitats for early benthic phase lobsters, and large challenges involved with sampling for lobster settlement in these habitats.

My study presents evidence of postlarval and juvenile lobster use of mud bottom in Maces Bay, but does not clearly indicate how important mud bottom is to the demography of lobsters within the bay, let alone more broadly within the Bay of Fundy (although see Introduction for previous studies noting EBP juveniles on mud bottom in other locations) . Based upon densities of juveniles in the collectors, a conservative estimate suggests mud habitat could be important as lobster nursery grounds in these

areas. While the average density of juveniles in collectors was approximately 10 times higher on cobble (0.23 individuals m^{-2}) than on mud (0.021 individuals m^{-2}), at my study site in the north western portion of Maces Bay, mud bottom was roughly 4.5 times more common than cobble bottom. Furthermore, mud seafloor is prevalent in the Quoddy region in the Bay of Fundy (Fader *et al.* 1977, Shaw *et al.* 2012, Schumacher *et al.* in preparation) and across much of the lobsters' range (USGS 2005). Hence, even though mud may support a lower density of juveniles than cobble reefs do, mud's prevalence across the species' range could make this an important secondary habitat for juveniles.

These rough calculations must be taken with caution given that the collector is a passive sampling tool and hence, lobster densities in collectors do not necessarily correspond to *in situ* densities (see Methods). EBP lobster densities in collectors on cobble have been found to be similar to densities obtained by suction sampling quadrats (Wahle *et al.* 2009, Wahle *et al.* 2013). However, collectors on mud may artificially concentrate densities as the collectors offer a higher quality patch of structurally complex substrate than the surrounding homogenous habitat does. Now that my study provides strong evidence of use of sediment bottom by a large size range of juvenile lobsters, including for settlement, it will be worth exploring more logistically difficult and costly sampling approaches to attempt to obtain accurate estimates of densities of these lobsters on mud bottom. Based on preliminary attempts, I believe that quadrats sampled by SCUBA divers do not represent the best approach to obtain these estimates, given the low densities expected and the difficulty of sampling large areas of bottom where easily-disturbed sediments rapidly compromise visibility. Instead, I believe two approaches worth exploring are (i) a modified *Nephrops* trawl, which is designed to capture

crustaceans that reside in mud depressions and burrows (Conan *et al.* 1994), or (ii) a clam dredge, which uses jets of water to lift infauna buried in sediments for collection in the trawl (Smolowitz and Nulk 1982). These instruments sample a known area (instrument width x tow length), and would provide more reliable estimates of juvenile densities.

In addition to accurate estimates of juvenile lobster densities on mud bottom, estimating the real contribution of mud and cobble bottom to lobster productivity will require a better understanding and estimates of several vital processes. First, given the evidence that juvenile lobsters are moving between habitats, tracking studies should be done to quantify juvenile movements, and help determine the proportion of time individuals spend in different habitats at different phases of their life. Second, it will be important to compare the health, development, growth, and survival of juveniles on mud and on cobble bottom.

It is unclear if mud bottom has always served to some extent as lobster nursery, or whether this habitat has only recently become important as lobster populations in many areas have grown. Preferred cobble habitat could be undergoing crowding, thereby leading to increasing numbers of juvenile lobsters moving out of cobble reefs and onto mud. At the same time, survival on mud may be improving with declining predator populations in many areas (Acheson and Steneck 1997). Mud may be serving as expansion habitat, and may even help some lobster populations avoid a bottleneck caused by limited availability of cobble (Wahle and Steneck 1991). Interestingly, the movement of juveniles into mud habitat may even make this substrate a good place to detect signs of population expansion, or early signs of contraction, before they are visible in cobble habitats (Ross Claytor, pers. comm.). If the current upward trend in lobster populations

continues, the importance of mud seafloor as American lobster nursery habitat is likely to increase.

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