LOBSTER POSTLARVAL AND EARLY JUVENILE ECOLOGY: INTERACTING EFFECTS OF PHYSICAL PROCESSES AND BEHAVIOUR

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by

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ABSTRACT

Physical and biological processes interact during early life stages to determine the distribution and abundance of larvae of many marine benthic species, including the commercially and ecologically important American lobster, *Homarus americanus*. Following planktonic development, lobster postlarvae seek benthic habitat to occupy during a juvenile phase. Past studies of lobster settlement and post-settlement behaviour have rarely considered the effects of physical factors. To test whether lobster settlement is affected by flow, I conducted flume experiments. Flow increased settlement by increasing substrate encounter through passive deposition and modification of searching behaviours. To examine shelter fidelity of recently settled juvenile lobster, the behavioural response of 3-month-old lobsters to shelter warming and cooling was tested. Lobsters behaved aversively in response to cooler temperatures, and abandoned their shelter to move to warmer water. These studies of the interactions between physical factors and early lobster behaviour enhance our understanding of spatial and temporal variability in populations and our ability to identify habitats for conservation.

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Statement of Co-authorship

The author of this thesis designed the experiments, collected and analysed all of the data, and wrote the manuscripts herein. Dr. Paul V.R. Snelgrove contributed significantly to the research proposal, experimental design, discussion of ideas, and reviewed all of the chapters. Dr. Snelgrove is the second author of the manuscripts included in this thesis.

CHAPTER 1

Introduction and Overview

1.1 Importance of Physical Processes in Marine Ecosystems

The biology of marine organisms can only be understood in the context of the physical processes that determine the conditions for biological processes (Mann & Lazier 1996). In marine systems, the physical environment defines the fundamental physiological and ecological processes, and marine communities are constantly responding to environmental variability (Denny & Wethey 2001). Patterns in marine communities are driven by complex interactions between environmental factors and biological phenomena. Of these environmental factors, water motion and temperature are key physical variables that determine the types of organisms that colonize a particular location (Ekman 1953, Mann & Lazier 1996, Denny & Wethey 2001).

Flows influence all organisms that live in moving fluids (Vogel 1994). In the oceans, hydrodynamic forces affect organisms directly and indirectly at various scales and, in turn, contribute to the structure of marine communities (Denny & Wethey 2001). Water motion alters boundary layers around individual organisms and their habitats, thereby significantly influencing nutrient uptake and waste transport (Mann & Lazier 1996). Large-scale nutrient supply is also dependent on physical processes such as upwelling. Hydrodynamics can exert considerable influence over organism movement, dispersal, and sinking, and also imposes forces (e.g. drag, turbulence) on sessile organisms. Water motion can influence encounter rates with predators, food, or habitat, as well as restrict or alter behaviours.

A major consequence of water temperature is to limit the distribution of organisms. The thermal environment restricts the geographical range of marine species (Ekman 1953, Kinne 1963) via several mechanisms. Marine organisms may not colonize areas with thermal conditions that reduce survival, or may not occur in some locations because settling individuals do not survive. Physiological rates are largely temperature dependent, particularly in ectothermic animals, and organisms are typically adapted to a particular temperature regime (Kinne 1963). Adaptations to temperature are varied. Behaviour is sometimes thermally regulated, and mobile marine animals will often follow temperature gradients. Organisms living in sedimentary bottoms can avoid extreme or variable temperatures by burrowing. These adaptations to temperature are important drivers of the demography of marine species.

1.2 Importance of Early Life Stage Processes in the Recruitment of Marine Benthic Invertebrates

One major focus of marine benthic ecology is to improve our understanding of the processes that determine spatial and temporal patterns of recruitment, and to assess their importance in regulating benthic populations and community structure. Most benthic marine invertebrates have complex life cycles with early life stages that differ considerably from adults in their morphology, habitat, and lifestyle (Thorson 1950). Consequently, knowledge of the dynamics of both planktonic and benthic stages must be integrated to understand the biotic and abiotic factors that regulate marine populations (Roughgarden et al. 1988, Underwood & Fairweather 1989, Gaines & Lafferty 1995, Eckman 1996).

It has long been recognized that variations in the supply of early life stages (larvae and juveniles) can be important drivers of adult population dynamics (Underwood & Fairweather 1989, Rodriguez et al. 1993, Underwood & Keough 2001). Recruitment, generally defined as the addition of new individuals to a population through survival to a specific size or life stage (Hunt & Schiebling 1997), integrates variation from multiple processes including larval production, planktonic dispersal, mortality in the plankton, settlement, and post-settlement growth and survival. Knowledge of the biological and physical factors that affect these early life history events, as well as determination of the relative contribution of each process to recruitment, is therefore vital to understanding the distribution and abundance of marine species. The relative importance of pre- and post-settlement events in regulating recruitment dynamics depends on multiple biotic and abiotic factors, and can differ among species (e.g. Keough & Downes 1982, Hurlbut 1991, Palma et al. 1998) and among habitats within a species range or sites within habitats (e.g. Connell 1985, Bingham 1992, Eggleston & Armstrong 1995).

Most marine benthic species produce planktonic larvae that can disperse over long distances (Thorson 1950, Scheltema 1974). The transition from a planktonic to a benthic existence (settlement) is a key event in the life cycle of marine animals with a pelagic dispersal phase, particularly for sessile or sedentary species. Settlement into favourable habitat can be of profound importance for the survival of benthic species with sessile or sedentary juvenile and adult stages. Active selection of settlement habitat has been documented for many soft-sediment and hard-substrate species (Doyle 1975, Keough & Downes 1982, Butman 1987, Morgan 2001, Underwood & Keough 2001, Keough & Swearer 2007). For many marine species, larval settlement is triggered by a combination of physical, chemical, and biological factors, and larvae can exercise control over their settlement location by delaying settlement in the absence of suitable stimuli (reviewed by Rodriguez et al. 1993). Physical cues for settlement can include hydrodynamic conditions (e.g. Mullineaux & Butman 1991, Pawlik et al. 1991, Fuchs et al. 2004), luminosity (e.g. Boudreau et al. 1993, Maldonado & Young 1996) and substrate contour and texture (e.g. Wethey 1986, Berntsson et al. 2000). A suite of chemical and biochemical agents have been identified that induce settlement of marine organisms (see reviews by Crisp 1974, Pawlik 1992, Rodriguez et al. 1993). Because many larvae can actively choose where to settle, settlement patterns are not determined solely by larval supply, but also by larval responses to substrate characteristics. The importance of larval response in settlement varies at different spatial scales: active selection of settlement sites may be important on small scales (centimeters to tens of meters) but large scale settlement patterns will likely be determined by the oceanographic patterns that affect larval delivery (reviewed by Butman 1987). Selection of settlement habitat can lead to a coupling between settlement and recruitment patterns, because larvae are thought to choose habitats that confer high post-settlement survival. Under these circumstances, where post-settlement mortality is density-independent and low compared to settlement stages, recruitment patterns correspond to settlement patterns (Connell 1985).

Settling marine organisms are small in size and particularly vulnerable to sources of mortality. Post-settlement mortality can result from abiotic factors that cause disturbance or physiological stress, and from biological factors such as predation, competition, and bioturbation (reviewed by Gosselin & Qian 1997, Hunt &

Scheibling 1997). Different causes of mortality, often at varying magnitude, may act in different areas of a species range, resulting in variation in the contribution of pre- and post-settlement components to recruitment (Connell 1985, Hunt & Scheibling 1997). Post-settlement movement and mortality can be as important as settlement in determining recruitment patterns of marine benthic organisms. Patterns in early post-settlement mortality and migration will therefore determine the contribution of settlement to recruitment (Gosselin & Qian 1997, Underwood & Keough 2001, Keough & Swearer 2007).

1.3 Biological-Physical Interactions in Early Life History Processes

The complexity of the relationship between physical conditions and recruitment processes requires an understanding of how spatial and temporal variations in the physical environment influence each component of recruitment. No single component of recruitment acts singularly, but rather patterns result from the combination and interaction of early life-history processes (Keough & Swearer 2007). Physical processes affect each stage of recruitment— fertilization, larval survival, dispersal, settlement, metamorphosis and post-settlement survival— directly and indirectly, with complex and often cascading consequences. For example, if the vertical migratory behaviour of a planktonic larva is affected by tidal flow patterns, this behaviour will affect transport and settlement location, and can influence both larval and post-settlement survival. Physical variables (e.g.

temperature, light, nutrient availability) that limit physiological rates influences the planktonic duration of larval stages, which in turn affects larval mortality, dispersal potential, larval supply, larval condition at settlement, and subsequent post-settlement survival. These examples highlight the need to understand the physical variables that influence early life-history stage processes, and to better assess the regulatory role of these biological-physical interactions.

1.3.1 Settlement in Flow

The process of settlement is the result of a combination of hydrodynamic processes and behavioural responses, and the relative contribution of each has generated debate in the past partly because early laboratory investigations were primarily done in still water (Butman 1987, Morgan 2001, Underwood & Keough 2001). Where water motion has been considered, it has been shown to significantly affect the settlement of marine organisms on both small and large scales (Abelson & Denny 1997).

Settlement experiments performed in flowing water in the laboratory and the field have shown that larvae can respond to complex environmental cues in hydrodynamically active environments (Underwood & Keough 2001). This ability is dependent on the size and swimming capability of the larva, and differs between species. For instance, a small larva may exercise selective behaviour at the substrate surface after passive contact, through a binary decision to attach or re-enter the water column. On the other hand, a larva that is a competent swimmer may, even in a high-energy environment, be able to respond to environmental cues and actively find a suitable habitat. Additionally, studies of larvae in different flow regimes have identified various ways in which hydrodynamics might interact with larval behaviour to influence settlement (Abelson & Denny 1997). It is clear that behavioural and physical processes interact to influence settlement outcomes, and that it is essential to consider the hydrodynamic environment in studies of larval settlement. To better understand variability in recruitment of marine species requires the integration of larval behavioural capabilities into physical models (Sale 1990).

1.3.2 Early Post-Settlement Dynamics

Knowledge of early post-settlement dynamics is critical to determining the relative contribution of settlement and post-settlement patterns to recruitment patterns under varying environmental conditions. Abiotic factors such as temperature and salinity can fluctuate greatly in coastal marine environments over tidal and seasonal cycles, and early juveniles are typically more sensitive to these extremes than adults (Gosselin & Qian 1997). Marine benthic invertebrates that settle at specific times during the settlement season, when environmental conditions are more favourable, have been found to have higher growth and survivorship (e.g. James-Pirri et al. 1998). On a local scale, the physical environment may determine survival of settled organisms and set the distribution limits of populations (Butman 1987, Gosselin & Qian 1997). In addition, environmental conditions may affect distributions by influencing post-settlement migration in mobile species (Hunt & Scheibling 1997). Mortality and migration may vary among habitats or among sites within a habitat and thereby decouple the settler-recruit relationship (e.g. Eggleston & Armstrong 1995).

Predation is typically considered the primary source of mortality for early juveniles (Gosselin & Qian 1997, Hunt & Scheibling 1997). Predation risk varies among habitats for many species, and it is well documented that structurally complex substrates often have lower predation intensity (e.g. Barshaw & Lavalli 1988, Wahle & Steneck 1992, Eggleston & Armstrong 1995, Lovrich & Sainte-Marie 1997, Butler et al. 2001). Though less well known, the influence of physical factors on predator/prey behaviour can also have important implications for juvenile mortality (Hunt & Scheibling 1997). Fluctuations in physical variables, such as temperature, turbidity, or salinity, can increase or decrease predation risk by affecting the activity of predators and/or prey. By modifying junvenile behaviour, physical processes could indirectly influence mortality by predation and therefore further influence the abundance and distribution of marine organisms.

1.4 Study Species: the American Lobster

The North American lobster, *Homarus americanus*, is an important Atlantic species and has long been a valuable commercial species in Canada and the United States. Dramatic increases in lobster landings occurred during the 1990s, and they are currently at near historic highs in many areas (Fogarty 1995, Fogarty & Gendron 2004). However, precipitous declines in some stocks underscore the fact that, despite the relatively large body of knowledge about lobster biology, our ability to predict and explain changes in lobster populations remains limited.

Beginning over 100 years ago, biological research on the American lobster has produced a vast literature on its physiology, population and fisheries biology, aquaculture, ecology, behaviour, and sensory biology (Factor 1995). Like many marine organisms, the adult stages of lobster have been most frequently examined, and the ecology of early life stages is less well known. A fuller understanding of the early life history of *Homarus* is emerging, through the use of new approaches and tools in both the field and laboratory (Cobb & Castro 2006).

Studies of clawed lobster populations (Family: Nephropidae) have concluded that successful settlement can be a key driver of lobster demography (Fogarty & Idoine 1986, Palma et al. 1999, Wahle et al. 2004). In contrast to other large benthic decapod species found in North Atlantic coastal areas, *H. americanus* has relatively low fecundity, low settlement densities, and low early post-settlement mortality (Palma et al. 1998). Settling lobsters show strong preference for structurally complex substrate (Botero & Atema 1982, Wahle & Steneck 1991, Palma et al. 1999). Predation rates are high in the absence of suitable shelter (Johns & Mann 1987, Wahle & Steneck 1992) and vary among habitats depending on the type of predator (Lavalli & Barshaw 1986, Barshaw & Lavalli 1988). These findings help explain why early life history processes are particularly influential to lobster demography. Nonetheless, local factors that increase post-settlement mortality or migration of juveniles and adults can obscure settlement-recruitment relationships (Wahle 2003, Wahle et al. 2009), and the investigation of the relative contribution of both pre- and post-settlement processes is necessary to increase our understanding of lobster demography (Wahle & Incze 1997, Wahle 2003).

Lobsters display a wide range of complex behavioural adaptations at each life stage, and are particularly sensitive to environmental conditions that trigger specific behavioural responses (Childress & Jury 2006). Lobsters possess an array of sensory receptors across their bodies, particularly concentrated on antennules, limbs, and mouthparts, making them capable of detecting chemical (e.g. conspecifics/food odours) and mechanical (e.g. water movements, vibrations) stimuli (Atema & Voigt 1995). Lobsters are demonstrably able to detect and respond to a suite of environmental variables such as salinity, temperature, osmolarity, and hydrodynamics (Atema & Voigt 1995, Childress & Jury 2006). The ability to integrate this sensory information into key behavioural decisions such as habitat selection and migration has clear adaptive value.

The remarkable sensitivity of lobsters to environmental stimuli means that abiotic factors play a significant role in their ecology, not only by affecting physiological rates or by defining the environment in which biological processes occur, but also through their influence on organism sensory biology and behaviour. For example, as a result of the importance of olfactory signals in many aspects of lobster life (e.g. courting, agonistic behaviour, food detection), there is a complex relationship between hydrodynamics, habitat and odour detection (Childress & Jury 2006). Because environmental cues significantly influence behaviours and affect processes such as movement, habitat selection, and mating, they play an important role in the life history of lobsters. In their review of lobster behaviour, Childress and Jury (2006) recommend that in the study of the relationship between habitat and recruitment, a better understanding of the behavioural responses of lobsters to environmental change is needed.

The use of closed areas to help sustain lobster fisheries has attracted significant interest in the last 20 years (Childress 1997), and efforts to enhance stocks and habitats through seeding programs and the creation of artificial reefs have met with varied success (reviewed by Cobb & Castro 2006). An understanding of the interaction between behaviour, habitat, and physical factors, particularly in early life-history stages, is essential in the development of marine protected areas, artificial habitats, and larval or juvenile seeding ventures.

1.5 General Objectives

The aim of this research is to investigate the interaction of physical and ecological processes in the early life history of the American lobster and to identify ways in which these interactions may contribute to spatial and temporal variation in recruitment patterns. The two following chapters use experimental approaches to examine behavioural responses of settlement and post-settlement stage lobster to physical factors.

Chapter 2 details experiments that investigated the effects of flow on the settlement of lobster postlarvae. Specifically, I explore the interaction of hydrodynamics and active postlarval behaviour on habitat searching and settlement. In this chapter, I identify and discuss ways in which near-bottom flow might contribute to spatial and temporal patterns of settlement.

Chapter 3 focuses on how environmental variability can affect early post-settlement lobster migration and mortality. This study describes the effect of temperature changes on early juvenile shelter-use behaviour. The influence of thermal environment on lobster post-settlement movement and the potential implications for demography are discussed.

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CHAPTER 2

Hydrodynamic Effects on Postlarval Settlement in the American Lobster, *Homarus americanus*

2.1 Abstract

Following planktonic larval development, American lobster (*Homarus americanus*) postlarvae seek out appropriate settling grounds in which to develop through a shelter-restricted juvenile phase. Previous studies have shown that lobster postlarvae exhibit strong directional swimming ability, distinct bottom-searching behaviours, and clear responses to physical and chemical cues. However, most experiments on larval and postlarval lobster have been conducted in still water, and little is known on the effect of hydrodynamics on settlement. Flume experiments were conducted to investigate the effect of a moderate flow on postlarval swimming behaviour and settlement. In 1-hour trials, postlarvae settled significantly more in flow than in still water. Postlarvae in flow were more likely to encounter substrate than postlarvae in still water. Behavioural observations indicated that postlarval behaviour was modified by the presence of flow. In contrast to postlarvae in still water, postlarvae in flow appeared to deliberately sink and were entrained to the substrate by turbulence, contributing to higher encounter and settlement rates. When subjected to flow, postlarvae performed significantly fewer dives and exhibited fewer bottom

searching behaviours than postlarvae in still water. The flow environment therefore has an effect on settlement frequency by influencing encounter rates through an interaction between passive and active processes. These results suggest that lobster settlement could be affected by spatial and temporal differences in flow and that the influence of hydrodynamics on behaviour needs to be considered when examining and predicting settlement patterns.

2.2 Introduction

Understanding the structure of marine populations requires knowledge of not only the processes that affect adult life histories, but also the processes that influence variation in the arrival and settlement of planktonic larvae (Roughgarden et al. 1988, Morgan 2001, Underwood & Keough 2001). Many marine benthic invertebrates have complex life cycles that include a planktonic larval phase, which is their primary mode of dispersal. For species with a planktonic larval phase, the settlement process is a critical transition to the seafloor environment and can be a major structuring factor for benthic populations (Underwood & Keough 2001). Because many of these animals are sessile or sedentary as juveniles and adults, larval delivery and settlement into favourable habitats may be particularly important in determining the structure of populations and recruitment levels into fisheries. Underlying the recruitment of larvae to adult populations are physical and behavioural processes that affect delivery to and settlement into suitable nursery grounds.

Studies on the settlement of marine invertebrate larvae of various groups such as molluscs, echinoderms, polychaetes, cnidarians, and crustaceans, have shown that these organisms can actively select habitats in response to physical, chemical and biological cues (Crisp 1955, Crisp & Stubbings 1957, Crisp 1974, Scheltema 1974, Pawlik 1992, Boudreau et al. 1993a, Rittschof et al. 1998, Forward et al. 2001). However, the scales at which active versus passive processes determine settlement distributions depend on the traits of a species (e.g. swimming ability, sensitivity to cues), as well as the hydrodynamic conditions in the settlement environment (Scheltema 1974, Hannan 1984, Butman 1986, Butman et al. 1988, Boudreau et al. 1990, Grassle et al. 1992). Unlike many smaller, weakly mobile planktonic larvae, decapod postlarvae are strong swimmers, and postlarvae of the American lobster, *Homarus americanus*, are particularly capable of oriented swimming (Ennis 1986, Cobb et al. 1989b). These characteristics have been hypothesized to allow relatively large-scale habitat selection by lobster postlarvae (Boudreau et al. 1990).

Lobster settlement occurs after metamorphosis from larval stage III to the postlarval stage. Postlarvae begin searching for suitable bottom habitat 2-6 days following metamorphosis (Cobb et al. 1989a). Timing of settlement is variable and, if suitable habitat is not encountered, postlarvae can delay their transition to the benthos until after a subsequent molt (Cobb et al. 1989a, Incze & Wahle 1991). Postlarval lobsters display a range of swimming and searching behaviours and respond to a suite of physical and chemical cues in controlled laboratory experiments (Hadley 1905, Botero & Atema 1982, Johns & Mann 1987, Cobb et al. 1989a, Boudreau et al. 1990, Boudreau 1991, Boudreau et al. 1992, Boudreau et al. 1993a,b). Postlarvae are known to exploit a variety of substrates, but laboratory experiments have demonstrated a preference for structurally-complex substrates such as cobble, rather than sand or mud (Botero & Atema 1982). Size of shelter substrate has been shown to correlate to body-size of juvenile and adult lobster (Cobb 1971, Wahle 1992) but a preference for a particular cobble size at settlement has not been examined. Johns and Mann (1987) found that lobster postlarvae choose habitats with seaweed or seaweed mimics more frequently than habitats without. Light penetration has also been identified as one of the most important substrate characteristics for lobster at settlement, and it has been suggested that a hierarchy of cues act to ultimately determine settlement location (Boudreau et al. 1990). While settling postlarval lobsters have been shown to respond to many physical and chemical factors, the difficulty of observing the settlement process in the field has resulted in settlement studies that have been done almost exclusively in the laboratory and, with few exceptions, in still water.

Water flow is a ubiquitous and highly variable factor in the coastal marine environment, and inevitably has a strong influence on ecological processes in the marine realm. Laboratory and field studies have clearly demonstrated that flow can have a range of effects on the settlement of benthic marine invertebrates on soft and hard bottoms (e.g. Eckman 1983, Butman et al. 1988, Mullineaux & Butman 1991, Pawlik et al. 1991, Snelgrove et al. 1993, Snelgrove et al. 1998). Abelson and Denny (1997) review three levels at which water motion may influence settlement of marine propagules: (1) Hydrodynamic forces may affect a settling organism's encounter rate with substrata, and/or subsequent behaviours, (2) Flow regime could serve as a settlement cue that induces active behaviour and determines site selection, and (3) Flow may mediate the detection of settlement cues in the environment. Because lobster settlement has been investigated primarily under static flow conditions, the effect of water motion is largely unknown. Without incorporating flow regime, it is unclear how the settlement behaviours and selectivity observed in laboratory experiments might translate to field conditions. Furthermore, the focus on still water experiments overlooks the possibility that habitat-associated flow can act as a physical settlement cue for lobster.

Investigation of the factors that influence the settlement process of marine species is fundamental to understanding and predicting patterns of recruitment, identifying critical habitat, and managing populations. Lobsters are effectively sedentary in the first years following settlement, and appear to experience little post-settlement mortality (Incze & Wahle 1991, Palma et al. 1998); lobster densities could therefore be largely determined by postlarval delivery and settlement patterns (Palma et al. 1998, Palma et al. 1999). Despite the ecological and economic importance of this species, and the significance of early life history stages in determining recruitment success, the behaviour of postlarval lobsters is not well-known (Lawton & Lavalli 1995, Phillips et al. 2006).

The purpose of this study was to investigate effects of water flow on postlarval lobster settlement. In a laboratory experiment to examine the effect of flow on settlement, I examined the settlement of individual lobster postlarvae of two age groups under either still-water conditions or in a moderate flow. Two sizes of cobble were offered as settlement substrate to test for postlarval selection of substrate size in the two flow regimes. In a separate experiment, lobster postlarval swimming and searching behaviours were measured in response to flow, to further examine the active component of substrate encounter.

2.3 Materials and Methods

2.3.1 Lobster Larval Culture

Homarus americanus postlarvae were raised at the Ocean Sciences Centre of Memorial University. Fifteen ovigerous female lobsters were collected from Notre Dame Bay, Newfoundland using standard lobster traps during July 2007. Females were transported to the Ocean Sciences Centre laboratory and maintained in separate tanks under ambient light with flowing seawater at ambient surface temperature (range: 8-15 °C). The lobsters were fed a ration of squid or mussels every 3 days. When hatching occurred, lobster larvae were collected from the adult tanks using a fine mesh net. During this period, tanks were checked frequently for larvae in order to ensure they were collected soon after hatch. Larvae of the same age (hatched within the previous 24 h period) were cultured together in static, 10-L culture containers filled with filtered seawater maintained at 17-19 °C. Larvae were kept under artificial light (12 h light : 12 h dark cycle). Initial culture densities ranged from 20-40 larvae•L^{-1}. Strong aeration decreased cannibalism in the culture containers. Larvae were fed ad libitum a mixed diet of enriched frozen adult artemia and live artemia nauplii. Culture water was changed and dead larvae removed from the cultures every 2 days. After molting to the fourth stage (postlarval stage), larvae were separated from their initial culture and reared, using the same methods, with individuals of the same molt date in order to reduce cannibalism and to document the number of days past molt (within 24 hours).

2.3.2 Settlement Experiments

Settlement experiments were conducted late August – mid-September 2007 during the daylight portion of the light cycle. A total of 62 mid-stage (7-9 days past molt) and 48 late-stage (12-16 days past molt) lobster postlarvae were used in individual trials. Within the mid-stage age group, 31 trials were conducted in flow and 31 in still water. Within the late-stage age group, 27 trials were performed in flow and 21 in still water.

Experiments were carried out in a 7-m long linear flume (Fig. 2.1a) with a 0.5-m wide channel and a 0.88-m long test section. Water depth was 10 cm during all experiments. Flow is generated in the flume by an impellor driven by a variable-speed motor. The flume was filled with unfiltered seawater maintained between 16-19°C by a heater/chiller unit that connected to a titanium heat exchange panel in the flume return pipe. To create two substrate choices, the test section substrate array of the flume was filled with a single layer of seawater-conditioned quarry cobble of two size ranges (small: 9.1 ± 0.2 cm, large: 17.2 ± 0.2 cm) on top of medium grain sand, divided on the longitudinal axis (Fig. 2.1b). The sand in the test section sloped down from the edge of the smooth upstream bottom so that the sand between cobble pieces was approximately 2 cm deeper and created a smooth transition from the acrylic flume bottom to the cobble test section. Cobble was arranged to abut so that crevices formed between adjacent stones. Small cobble protruded ~3 cm above the sand bottom, and large cobble pieces protruded ~5 cm (Fig. 2.1c). The two cobble sizes offered postlarvae contrasting habitat characteristics in 2 key ways: (1) different crevice sizes in which to shelter; (2) in flowing water, different hydrodynamic environments over the different-sized protruding structures. Cobble was chosen as the substrate for this experiment because it is a preferred settlement substrate for lobster postlarvae (Botero & Atema 1982, Hudon 1987, Wahle & Steneck 1991), but the preference for a particular grain size at settlement has not been fully assessed.

Every 2-3 days during the experimental period, the flume water was replaced, and the test section reconfigured to ensure that each side of the test section held each cobble treatment for half of the trials, to avoid any cross-stream location bias.

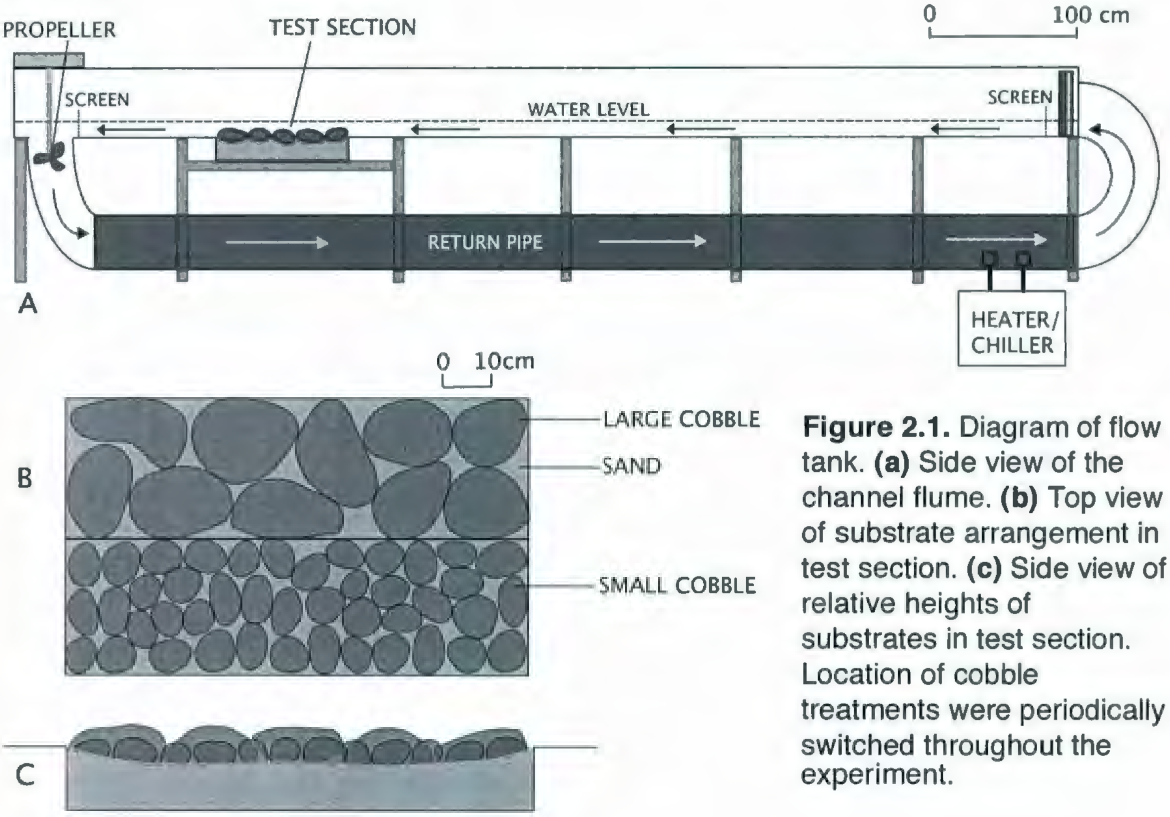

Figure 2.1. Diagram of flow tank. **(a)** Side view of the channel flume. **(b)** Top view of substrate arrangement in test section. **(c)** Side view of relative heights of substrates in test section. Location of cobble treatments were periodically switched throughout the experiment.

At the onset of each trial, an individual postlarva was gently introduced at the upstream edge of the flume test section using a dip net. Postlarvae exited the net themselves by crawling or swimming out. Constant observations of location and behaviour were made for 15 minutes following introduction; if settlement did not occur within this time period, the postlarva was left in the flume and observed for 5-minute periods every 15 minutes. Trials were discontinued when settlement occurred or at t = 1h. Postlarvae were considered settled when they became sheltered under or between rocks for longer than 5 minutes. Time to settlement and settlement substrate were recorded for settling individuals. If settlement occurred during unobserved periods, the time was recorded to the nearest 15-minute interval. Individuals not settling by the end of the 1-hour trial were scored as not settled.

Count data obtained from these experiments were analysed using the generalized linear model procedure (SAS statistical software). Response variables of (1) settlement (settled / not settled), (2) settlement substrate (small / large cobble), (3) substrate encounter during observation (encounter / no encounter, and (4) settlement following encounter (encountered and settled/ encountered and did not settle), were modeled in terms of the nominal predictor variables postlarval age (mid-stage / late-stage) and flow treatment (flow / still water). Models for these binary data used a binomial distribution and a logit link function. Effects of postlarval age and flow treatment on time to settlement were analyzed using a generalized linear model procedure with a poisson distribution and log link. For all analyses, plots of residuals versus fitted values were used to evaluate assumptions of homogenous errors and whether the structural models adequately represented the relationships. See Appendix A for details of each analysis and for sample diagnostic plots.

2.3.3 Flow Characterization

To characterize the hydrodynamic environment in the flume during the flow trials, vertical depth profiles of velocity were measured at different locations in the flume: 0.5 m upstream of the test section in the middle of the channel, and above the top of a rock and a crevice for each substrate treatment mid-way along the test section (Fig.2.2). The bounding surface at each location (i.e. rock surface, flume bottom) was the bottom from which height (z) measurements were made. All flow measurements were performed with a Sontek 16 Hz Acoustic Doppler Velocimeter (ADV) connected to a positioning system to allow measurements at defined coordinates (x,y,z) in the flume channel. A sampling volume of 0.75 mm^3 was used and each point was sampled for approximately 300 seconds (> 3000 samples per point).

All flow experiments were conducted using a moderate, smooth-turbulent flow, with a free-stream velocity of approximately 7.5 $cm \cdot s^{-1}$. Boundary shear velocity, u_*, is a measure of the shear stress acting on a boundary and is typically used as a convenient velocity parameter to characterize near-bottom flow (Nowell & Jumars 1984). The shear velocity (u_*) upstream of the test section was calculated to be 0.34 $cm \cdot s^{-1}$ using the log layer of the upstream velocity profile (Fig. 2.2a) and the formula:

$$\ln(z) = \frac{\kappa}{u_*} u + \ln(z_0)$$

where κ is von Karman's constant (=0.41), u is the velocity at height z, and z_0 is the bottom roughness parameter. This u_* is typical of mid-range near-bottom flow in a coastal embayment (Butman 1986, Grassle et al. 1992).

Horizontal flow accelerated as it moved over the roughness elements in the substrate test section, reaching approximately 8 $\mathrm{cm \cdot s^{-1}}$ and 8.5 $\mathrm{cm \cdot s^{-1}}$ above the small and large cobble, respectively (Fig. 2.2b). Flow weakened above the bottom within crevices, and crevices in the large cobble section, where rocks reached ~4.5 cm above the bottom, created flow eddies with increased vertical and transverse velocities, and a reverse horizontal flow (Fig. 2.2b). The temporal fluctuation in horizontal velocities (span of 95% confidence intervals) can be used as a measure of turbulence and was generally greater in the substrate test section compared to the upstream profile, and was more pronounced in areas of the vertical profile over the large cobble compared to the small cobble (Fig. 2.2c).

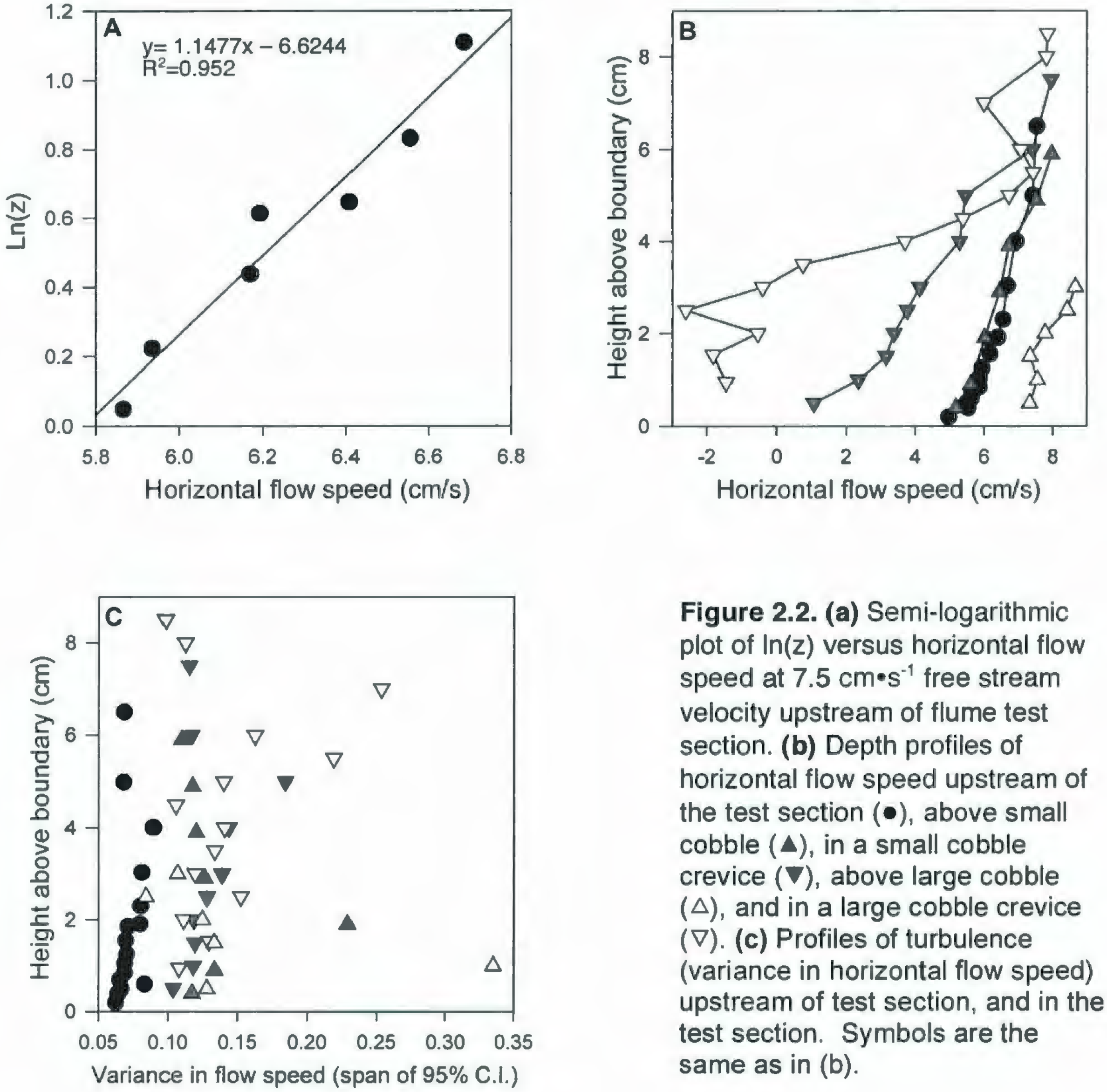

Figure 2.2. (a) Semi-logarithmic plot of ln(z) versus horizontal flow speed at 7.5 cm•s^{-1} free stream velocity upstream of flume test section. **(b)** Depth profiles of horizontal flow speed upstream of the test section (●), above small cobble (▲), in a small cobble crevice (▼), above large cobble (△), and in a large cobble crevice (▽). **(c)** Profiles of turbulence (variance in horizontal flow speed) upstream of test section, and in the test section. Symbols are the same as in (b).

2.3.4 Swimming Behaviour Experiments

The swimming behaviour responses of lobster postlarvae to flow were tested in the daylight period of the light cycle. The flume was filled to 10 cm depth with unfiltered 16-19 °C seawater. A total of 25 postlarvae (9-16 days past molt) were used in swimming behaviour trials. In the flume, with the cobble substrate made unavailable, each postlarva was observed for 15 minutes in a moderate flow (same speed as in settlement experiments) and for 15 minutes in still water. Time spent at the surface (top 1 cm), time spent at the bottom (within 1 cm of the flume bottom) and time spent in the water column was recorded. Three behaviours were characterized (adapted from Cobb et al. 1989a; Fig. 2.3) and enumerated during each trial: the number of full descents, the number of partial descents (departures from the surface to the mid-water, returning to the surface within 30 seconds), and the number of lift-offs (departures from the bottom to mid-water, returning to the bottom within 30 seconds). Between the two swimming trials, the postlarva was removed from the flume for 10 minutes before it was reintroduced for the alternative treatment. It is possible that experimental handling affected lobster behaviour, but every effort was made to minimize handling time and lobsters experienced consistent handling at each introduction; there was no systematic change observed in behaviour as the experiments progressed. To ensure that the sequence of treatments did not affect the individuals' responses, the order of exposure to the two experimental conditions was alternated among the test subjects, so that the order of trials was flow followed by still water for half of the postlarvae, and still water followed by flow for the remaining postlarvae.

Paired t-tests were utilized to analyze the results of the swimming behaviour trials. The mean number of descents (full and partial) and lift-offs performed, as well as the mean time spent by postlarvae in surface, mid, and bottom waters, was compared between still-water and flow trials.

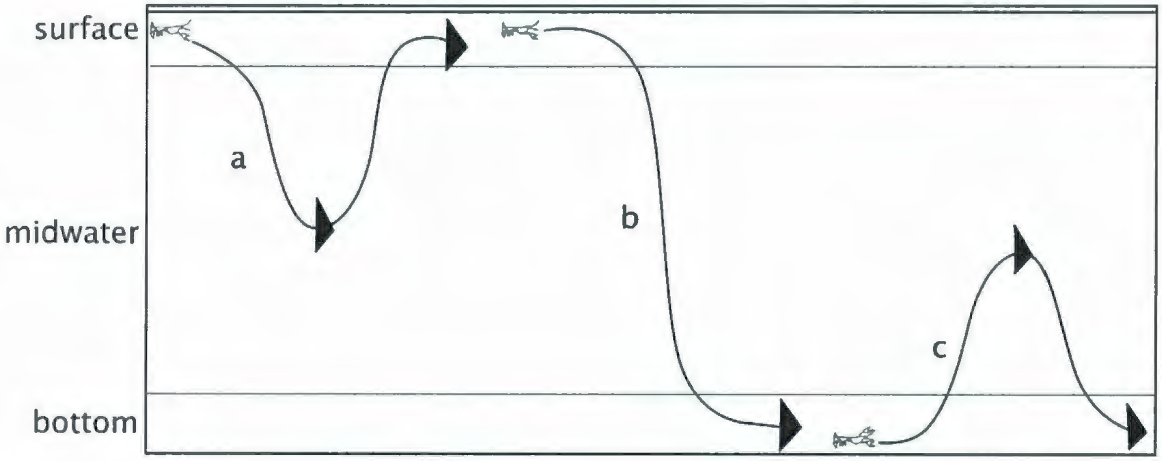

Figure 2.3. Diagram of postlarval searching behaviours: **(a)** Partial descent, **(b)** Full descent **(c)** Lift-off

2.4 Results

2.4.1 Settlement Experiments

Flowing seawater significantly affected postlarval lobster settlement. More postlarvae settled in flow treatment trials compared to still-water trials (Fig. 2.4; Table 2.1). The effect of flow was consistent across both age classes; however, a higher proportion of late-stage postlarvae settled compared to younger individuals. In flow trials, many postlarvae were observed to quickly descend following introduction, by deliberate sinking with claws and abdomen extended while being transported by the current. When the postlarvae entered the turbulent area above the substrate section, this behaviour typically resulted in advection of the postlarva to the substrate. In contrast, postlarvae in still-water trials most often swam actively at introduction and encountered substrate through diving and/or sinking.

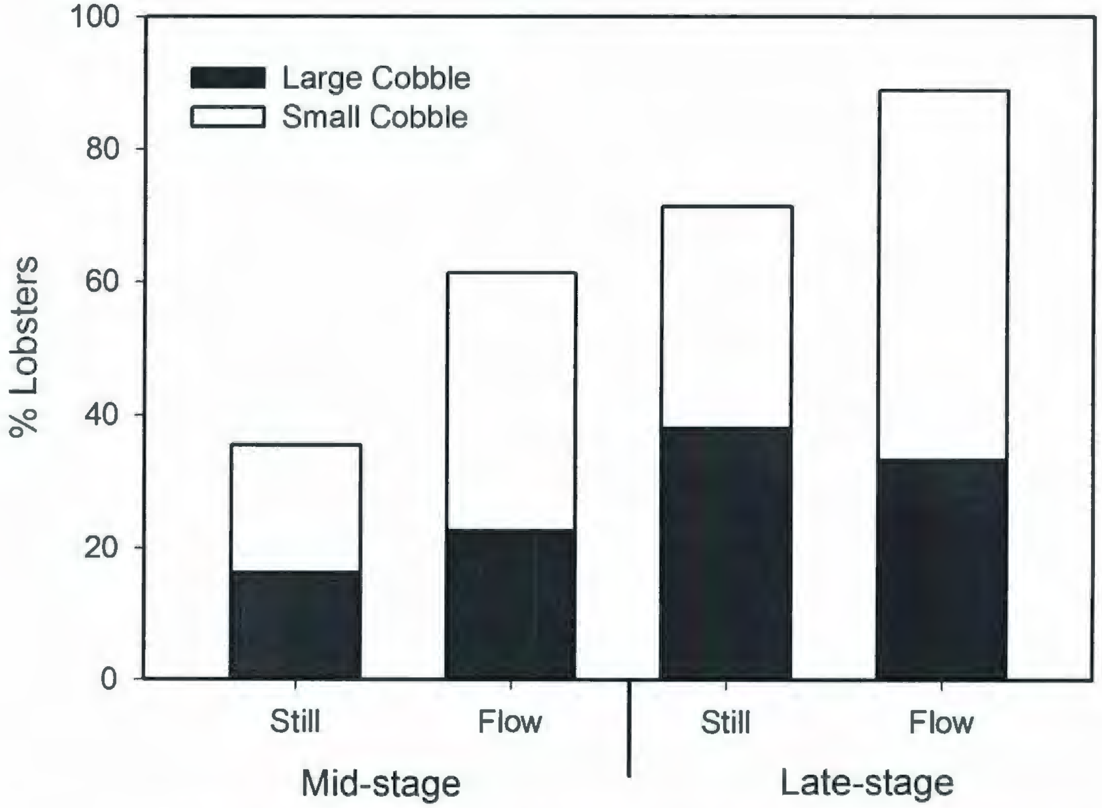

Figure 2.4. Percentage of lobsters in each age class settling under still-water and flow conditions. Bars are shaded to represent the percentage of lobsters that chose each substrate type.

Table 2.1. Summary statistics for generalized linear models of proportional settlement and encounter data. Asterices indicate statistical significance (*= p<0.05, **= p<0.01).

Dependent variable	Predictor	χ^2	p-value
Settlement (yes/no)	Age	12.68	0.0004 **
	Flow treatment	5.88	0.0153 *
	Interaction	1.869	0.9103
Settlement substrate (small/large)	Age	0.965	0.326
	Flow treatment	0.114	0.736
	Interaction	0.080	0.778
Substrate encounter (yes/no)	Age	4.702	0.030 *
	Flow treatment	7.569	0.006 **
	Interaction	1.869	0.172
Encountered substrate but did not settle	Age	6.696	0.010 *
	Flow treatment	0.019	0.889
	Interaction	0.397	0.529

There was no significant preference for small or large cobble as a settlement substrate in this experiment. Although a slightly larger percentage of postlarvae settled in small cobble in flow compared to still-water treatments (Fig. 2.4), this difference was not significant, and the proportion of postlarvae settling in each cobble type did not differ between the two age groups (Table 2.1).

During the first 15 minutes of trials, 73% of all lobsters tested encountered the substrate section. However, lobsters were significantly more likely to encounter substrate in the flow treatment than in the still-water treatment (Fig. 2.5; Table 2.1). The proportion of lobsters that encountered substrate was significantly different between the two age groups; in each flow treatment, late-stage postlarvae encountered substrate more frequently than mid-stage postlarvae (Table 2.1). The majority of lobsters that encountered substrate eventually settled during the trials: only 19 of 80 lobsters (24%) that encountered the substrate within the first 15 minutes did not settle during the trial period. Of these individuals that did not settle, significantly more were mid-stage lobsters (Fig. 2.5; Table 2.1). Flow condition did not have an effect on this relationship, in that lobsters of both age groups were as likely to settle after encountering substrate in flow or still water.

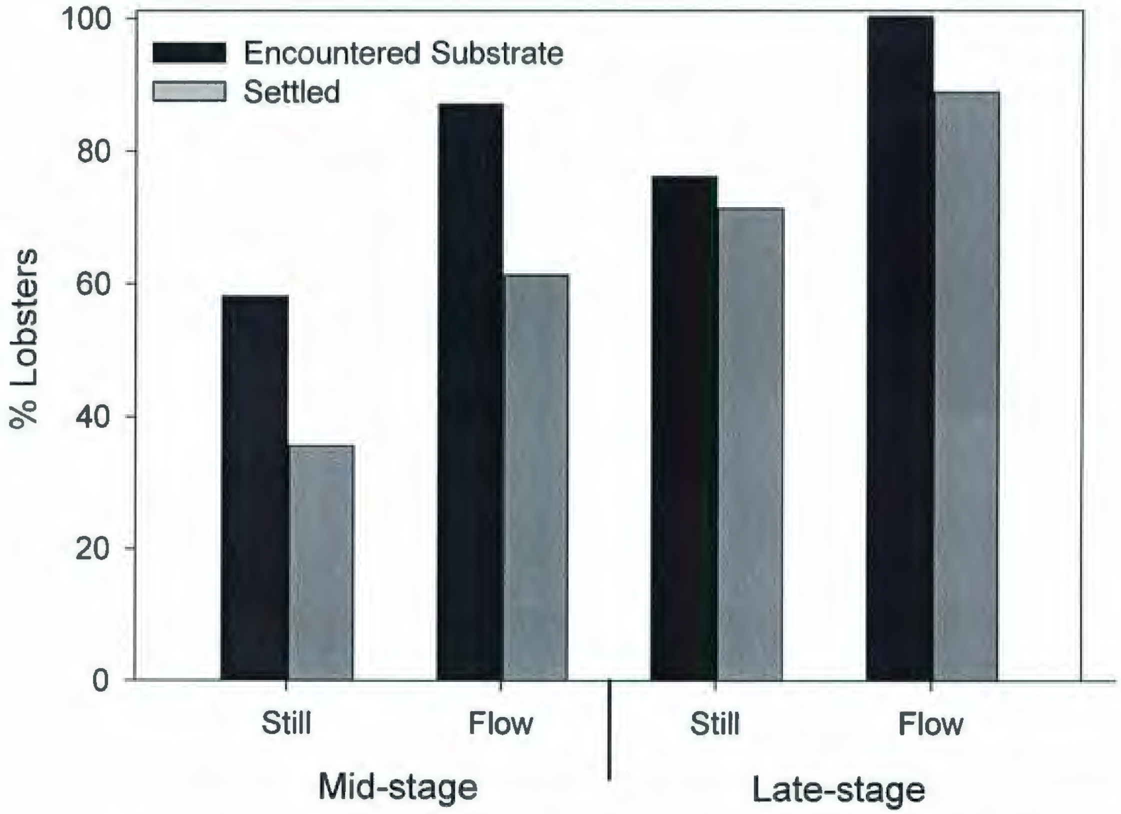

Figure 2.5. Percentages of mid-stage and late-stage postlarvae in still water and in flow that encountered substrate within 15 minutes of trial start and percentages that settled during 1h trials.

Flow and postlarval age had an interactive effect on the time to settlement (Fig. 2.6; χ^2=4.69, p=0.0136); therefore, each predictor was analysed separately (Table 2.2, Appendix A). No significant difference in time to settlement was found between the two flow treatments for either age group (Table 2.2). Late-stage postlarvae settled more quickly than mid-stage postlarvae in both flow and still water (Table 2.2; Fig. 2.6). In these experiments, time to settlement included two components: the time to encounter substrate and the time spent in the substrate choosing or creating a shelter. The younger postlarvae generally explored more within the substrate compared to older individuals, and took more time choosing a crevice or creating a burrow in which to remain. Younger postlarvae were also often observed to make multiple contacts with the substrate, re-entering the water column, whereas late-stage postlarvae typically remained in the substrate after the initial encounter. The interaction detected in the analysis is due to a difference in magnitude of the effect of age on time to settlement between flow and still water (Table 2.2). Flow increased the age effect, most likely by decreasing the time it took to encounter substrate, which led to quicker settlement by the late-stage postlarvae with a higher settlement drive than mid-stage postlarvae. Individual variation in searching times within the substrate possibily negated any direct effect of the decreased encounter time conferred by the flow on total time to settlement.

Table 2.2. Summary of statistical analysis of the interaction of postlarval age and flow treatment on time to settlement. Asterices indicate statistical significance (*= p<0.05, **= p<0.01).

Observations used	**Predictor**	χ^2	**p-value**
Mid-stage	Flow treatment	2.86	0.0909
Late-stage	Flow treatment	3.23	0.0721
Still	Postlarval age	4.39	0.0362 *
Flow	Postlarval age	45.32	<0.0001 **

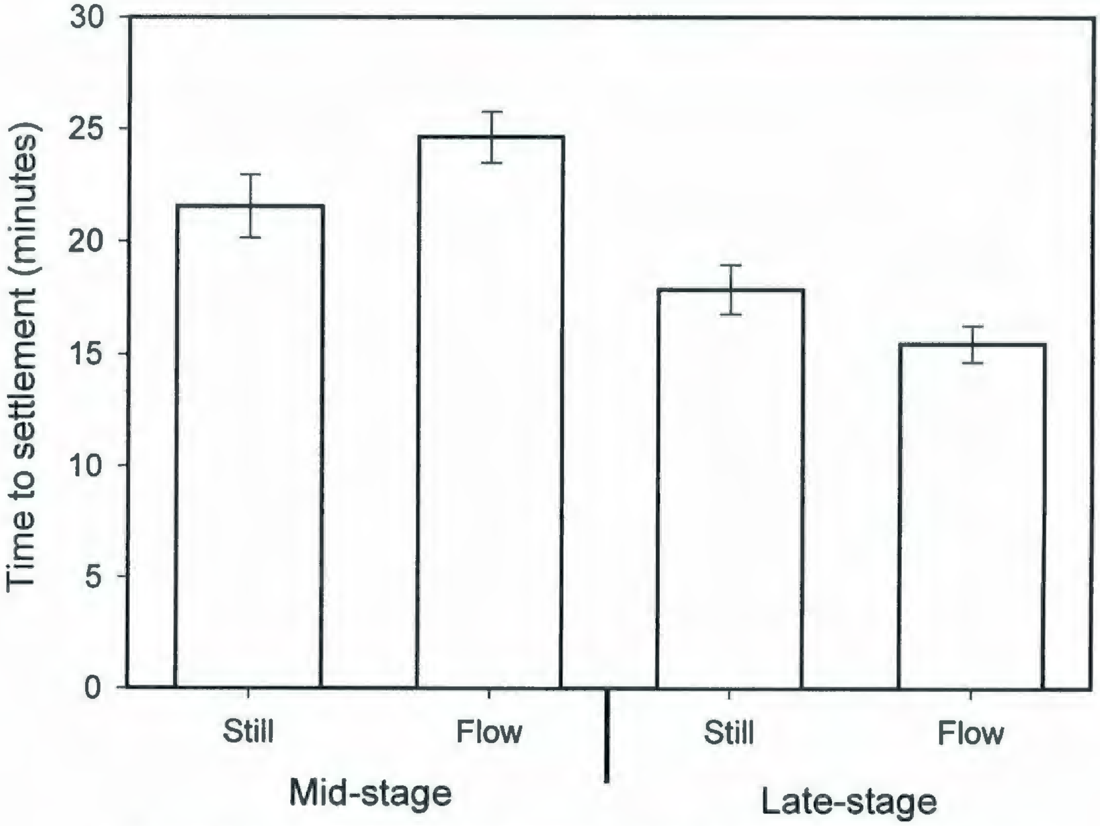

Figure 2.6. Mean time to settlement (± SE) for mid-stage and late-stage postlarvae that settled in 1 h still water and flow trials.

2.4.2 Swimming Behaviour Trials

Postlarvae were more active in their searching while in still water. In still-water trials, the mean number of full descents by postlarvae was significantly greater than in flow trials (Fig. 2.7 ; t-test: $t=3.05$, $p<0.01$). Postlarvae also performed significantly more partial descents ($t=3.32$, $p<0.01$) and lift-off behaviours ($t=2.94$, $p<0.01$) in still water compared to flow (Fig. 2.7). The postlarvae actively swam during flow trials, typically at speeds equal to or slightly less than the current speed. In flow, postlarvae bottom searched with deliberate sinking behaviour, using the current to transport them backwards and to the bottom, with their claws out and abdomen extended. This behaviour was in contrast to active diving as the main mode of descent in still-water trials. In flow, once in contact with the bottom, postlarvae often remained, and explored the surface by walking, whereas in still water, lift-offs were the preferred method of searching.

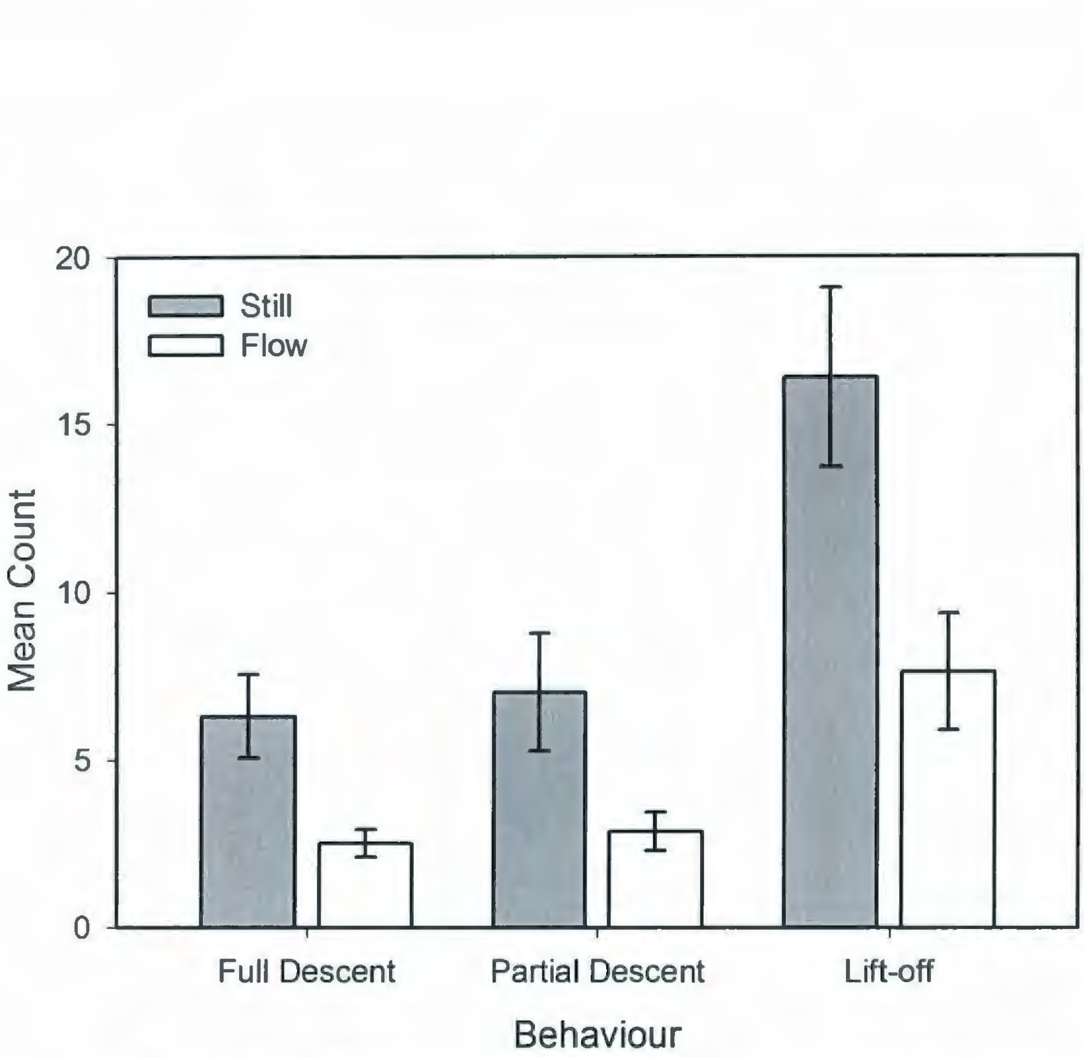

Figure 2.7. Mean number of full descents, shallow descents, and lift-offs performed by postlarvae in still- water and in flow trials. Error bars represent 1 S.E.

No significant difference was found between still and flow trials in the time spent by postlarvae at the surface (Fig. 2.8; t=0.672, p=0.508). On average postlarvae spent more time on the bottom in flow compared to still water, but this difference was not significant (t=1.907, p=0.101). However, the postlarvae spent significantly more time in mid-water during still-water trials (t=3.861, p<0.01), as a result of the increase in diving and lift-off behaviours that brought them into the mid-section of the water column.

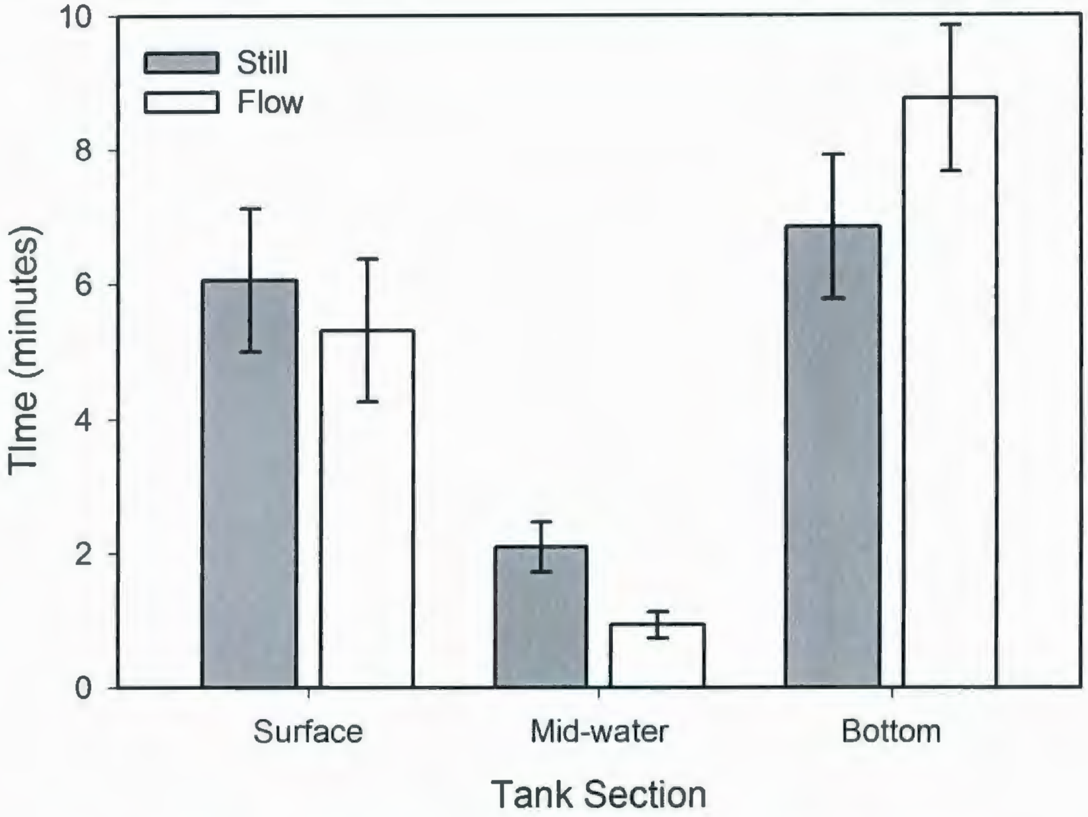

Figure 2.8. Mean time spent in the surface, mid-water, and bottom sections by postlarvae in still-water and flow trials. Error bars represent 1 S.E.

2.5 Discussion

This is the first investigation to focus on the role of flow in American lobster postlarval settlement. Crustacean species such as *H. americanus* that exhibit strong swimming abilities, substrate preferences, and responsiveness to cues offer a compelling study subject to investigate the effects of hydrodynamics on larval settlement. The superior swimming capability of lobster postlarvae provides a contrast with the weaker swimming mollusc (e.g. Snelgrove et al. 1993, Snelgrove et al. 1998, Boxshall 2000, Crimaldi et al. 2002), barnacle (e.g. Crisp 1955, Mullineaux & Butman 1991, Berntsson et al. 2000) and polychaete (e.g. Butman et al. 1988, Butman & Grassle 1992, Grassle et al. 1992, Pawlik & Butman 1993, Snelgrove et al. 1993) larvae that have been the focus of previous studies of settlement in flow. The objective of the present study was to examine lobster settlement under realistic flow conditions and to evaluate the potential contribution of passive and active processes to lobster settlement.

The results presented here demonstrate that water flow can significantly influence lobster postlarval settlement. The observed differences in settlement and behaviour between flow and still water confirms that flow has potentially important implications for the settlement of *H. americanus.* The flow used in these experiments increased encounter rates of postlarvae with the substrate, and the probability of settlement was correspondingly higher and faster in flow compared to still-water treatments. The increased encounter rate and subsequent increase in settlement in flow was not simply a passive physical process, but rather one that was influenced by postlarval behavioural responses to flow. Lobster postlarvae are capable swimmers, and can sustain swimming at flow speeds well above the 7.5 cm•s^{-1} velocity used in this experiment (Ennis 1986, Cobb et al. 1989b). When exposed to the flow, however, postlarvae modifed their behaviour, deliberately sinking and subsequently becoming entrained to the bottom, particularly in the turbulent test section. These results indicate that the behavioural mechanisms by which postlarvae reach the substratum and ultimately settle can be influenced by hydrodynamic conditions.

Settlement requires two events to occur: (1) substrate encounter and (2) acceptance of the encountered substrate. The latter often requires an active behaviour on the part of the larva, whereas the initial component can occur passively. Two categories of conceptual behavioural models have been used to explain active settlement of marine larvae in flow (Mullineaux & Garland 1993, Abelson & Denny 1997, Dobretsov & Wahl 2008). Most commonly, larvae are thought to contact the substrate surface via advective flow, and subsequently accept or reject the surface (e.g. Butman & Grassle 1992, Snelgrove et al. 1993). If a larva rejects the surface, it returns to the water column. Alternatively, the "contact and explore" model suggests that certain larvae will contact a surface, and rather than make a binary accept/reject response, they will explore the substrate to locate a suitable settlement site. Neither of these models consider the influence larvae may have on substrate encounter, but rather, the "choice" comes when larvae accept or reject a substrate. Because larval decapods have a high swimming capacity, they may be able to select settlement sites over larger scales than smaller passive larvae (Luckenbach & Orth 1992, Fernandez 1994, Hedvall et al. 1998). Rather than contact the substrate solely by advective flow, they may actively contribute to probability of encounter with the substrate. The lobster postlarvae examined in this study demonstrated “contact and explore” behaviour but with a clear behavioural influence on initial contact. This behavioural influence was particularly important to settlement outcome; the increased settlement in flow resulted not from differential acceptance of substrate in flow and still water, but from differences in substrate encounter in flow and still water.

The variation seen between the two age classes of postlarvae in flow highlights the active behavioural contribution to substrate encounter — if the flow regime itself was the cause of the increased substrate encounter, a difference between mid- and late-stage postlarvae would not be expected. Instead, behaviour remains an important contributor to the first component of settlement in flow, and substrate encounter is higher for late-stage postlarvae. The behaviours used to encounter substrate may differ in flow versus still water, but the results presented here support and add to previous work that has demonstrated that lobster postlarvae increase bottom-related behaviours with age (Botero & Atema 1982, Cobb et al. 1989a, Boudreau et al. 1993b).

The behavioural differences quantified in the swimming experiment give further insight into the contrasting methods by which postlarvae find substratum in different flow environments. In still water, postlarvae actively dived and searched throughout the water column far more than in flow. This pattern suggests that in the absence of flow postlarvae must actively search significantly more than when flow is present and, in combination with the results of the settlement experiment, that this active searching does not equal the success of encounter attained through the use of near-bottom flow.

From an adaptive perspective, the postlarvae settling and searching behaviours in flow appear to maximize substrate encounter while minimizing energetic requirements. In the moderate flow used in these experiments, postlarvae actively sank or dove as they were carried across the more turbulent cobble-filled test section. Despite their capacity for swimming against the flow, the postlarvae rarely exhibited searching behaviour against the current, but instead alternated between drifting with the current while actively sinking, and swimming to hold position with the flow. In areas with even a moderate flow, such as that used here, postlarvae can utilize turbulence in order to contact substrate, and avoid the energy expenditure of repeated dives. This potential energy savings during settlement could have carry-over effects and confer an advantage to juveniles that settled through the use of flow. In field surveys, postlarvae are almost always caught in the surface layer (Hudon et al. 1986, Harding et al. 1987), a result that conflicts with the idea that postlarvae spend substantial time descending and ascending, testing bottom substrates, prior to settlement (Ennis 1995). Because postlarvae have been found to rarely cross thermal gradients, thermoclines have been suggested as a possible cue that confines them in surface waters and increases the likelihood of settlement in warmer shallow areas (Boudreau et al. 1991, Annis 2005). The behavioural responses to flow that have been documented here suggest an additional mechanism by which postlarvae avoid extensive active forays to the bottom in areas of innapropriate depths. Active movement that is limited to short vertical distances during transport in flow and the use of turbulent advection for substrate contact could increase the probability of substrate encounter primarily at shallow depths where appropriate settlement substrate is located.

Habitat-associated flows are potentially an important component of habitat selection (Crisp 1955, Eckman 1983, Mullineaux & Garland 1993, Abelson & Denny 1997). In the only other lobster settlement study that included flow as a variable, Johns and Mann (1987) found that lobster postlarvae that settled in a 10-30 $cm \cdot s^{-1}$ current preferred habitats with plastic seaweed mimics more than postlarvae in still water. They suggested that the lobsters seek shelter from water currents, because flow is reduced beneath the frond structures. In my study, the subtle differences in flow created by the two substrate sizes did not cause any significant difference in settlement. It is likely that in the moderate flow speed used, small and large cobble both provided sufficient refuge from the current. It is also possible that postlarvae do not seek shelter from moderate to low currents, but that these are ideal flow environments in which to live.

It is unclear if hydrodynamics could act as a settlement cue for lobster; although the flow promoted substrate contact it did not affect the acceptance or rejection of that substrate. It is possible, however, that the deliberate sinking and use of turbulence to contact substrate reflects a preference for a particular flow pattern. Only one flow speed was tested in this experiment so additional experiments are needed to determine whether there is a preference for settlement in a particular flow regime. Nonetheless, given that flow influenced behaviour and proportion of settled larvae, these results suggest that hydrodynamic cues are important for lobster settlement. Moreover, because early juvenile lobsters are generally shelter-restricted and rely on suspension feeding for the early part of their benthic life (Barshaw & Bryant-Rich 1988, Lavalli & Barshaw 1989, Lawton & Lavalli 1995), there would be an expected advantage to settling in an environment that confers a favourable, flow-mediated food supply. Because abiotic and biotic cues interact to determine settlement patterns, possible hydrodynamic cues should be investigated in conjunction with other known lobster settlement cues to evaluate their relative importance. An additional factor to be considered in future experiments is the possibility of gregarious effects of multiple settlers, or previously settled juveniles, on settlement behaviour in flow.

Examination of the interaction between active and passive processes during larval settlement is critical to determining the importance of the larval stage to the demography of a species (Butman & Grassle 1992). The results of this study demonstrate the importance of flow and its effect on active settlement in lobster. This laboratory study shows that hydrodynamics may have a considerable influence on active settlement with significant ramifications for settlement patterns in the field. If specific flow speeds promote lobster settlement, temporal (e.g. tides) and spatial variability in flow may be particularly influential in determing recruitment patterns. Although laboratory studies have implicated many exogenous factors in lobster habitat selection at settlement, little is known about the the ability and proclivity of postlarvae to respond to these physical, chemical and biological factors under different hydrodynamic conditions. The present experiment was not designed to fully examine the range of flow velocities and turbulence levels that postlarvae would be exposed to in the field, but rather, the results provide a starting point from which to examine the settlement of lobster under realistic flow conditions. Moreover, this study investigated the effects of water motion on initial settlement only, thus longer-term responses to flow remain to be evaluated. It is clear, however, that future investigations of lobster recruitment must consider the role of hydrodynamics in determining not only large-scale larval distribution patterns, but also smaller-scale temporal and spatial settlement patterns.

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CHAPTER 3

Behavioural Response to Temperature Change in Shelter-Restricted Early Juvenile American Lobster, *Homarus americanus*

3.1 Abstract

Early benthic juvenile American lobsters, *Homarus americanus,* rely on shelter-providing substrates for protection from predation, and are assumed to remain shelter-bound during their early post-settlement life. However, it is not known whether early juveniles will abandon the safety of their shelters under unfavourable environmental conditions. Larval and adult lobster are known to behaviourally thermoregulate in order to maintain metabolically favourable temperatures, but the behavioural response of early juvenile lobsters to temperature has not been investigated. The shelter fidelity of early post-settlement juvenile lobsters (4.2-6.2 mm carapace length) was examined in response to water temperature manipulation. In experiments to characterize the behavioural response to warming and cooling events, lobster shelters that were maintained in 9-10 °C water were gradually warmed or cooled by approximately 8 °C. During shelter cooling, 73.5% of lobsters demonstrated an aversive response and left the shelter to move into the warmer surrounding water, compared to only 2.9% movement in control trials. Warming did not produce a similar significant result, in that only 15% of lobsters abandoned the shelter. The

results indicate that, like larvae and adults, young juvenile lobsters can sense and respond to changing water temperatures, and will risk out-of-shelter movement to avoid unfavourable conditions. These results suggest that young lobsters, particularly those that inhabit thermally variable areas such as coastal areas subject to upwelling, may be less bound to a single shelter than has been previously thought, and this finding has important implications for lobster post-settlement migration and mortality.

3.2 Introduction

Thermal environment is of major importance in regulating marine community structure (Denny & Wethey 2001). Temperature, possibly more than any other single environmental factor, has a pervasive influence on the biological processes of marine organisms, and consequently plays a central role in the distribution and abundance of populations, and ultimately regulates the structure of marine communities. Indeed, the broad-scale biogeography of species in the marine environment is closely linked to seawater temperature (Ekman 1953, Kinne 1963, Crame 1993).

Temperature effects vary significantly among species and life history stages. The behaviour and movement of most crustaceans is strongly influenced by water temperature, and thermal preference behaviour can improve the fitness of species in different ways, most often by optimizing metabolic performance (Lagerspetz & Vainio 2006). The commercially and ecologically important American lobster (*Homarus americanus*) is one species for which temperature has complex effects on different life history stages. Planktonic larvae and postlarvae of American lobster have been found to regulate their vertical distribution in response to temperature, remaining in warmer surface waters above the seasonal thermocline (Boudreau et al. 1992, Annis 2005). This behaviour likely helps to prevent settlement in cooler, energetically unfavourable habitats. In adult lobsters, temperature is considered to be a major determinant of adult lobster distributions and movement (Lawton & Lavalli 1995); seasonal lobster migrations appear to follow thermal gradients (Ennis 1984, Watson et al. 1999) and short-term lobster movements in response to storms have also been linked to thermal variability (Jury et al. 1995). In general, studies on lobster movement suggest that individuals move to maintain residence in warm waters, a strategy that maximizes growth, reproduction, and survival (Aiken & Waddy 1986, Waddy et al. 1995). Although the thermal tolerance for this species is broad (from -1° to 30.5 °C in adults, 5° to 26°C in larvae), and they can survive abrupt temperature changes of up to 20 °C (Lawton & Lavalli 1995), adult lobster behaviour appears to maximize residence in metabolically optimal waters (Aiken & Waddy 1986, Crossin et al. 1998).

The post-settlement early benthic phase of the juvenile American lobster is behaviourally distinct from older juvenile and adult lobsters (Lawton & Lavalli 1995, Butler et al. 2006). Laboratory and field studies have demonstrated strong selection for structurally complex habitats by postlarval lobster at settlement (Botero & Atema 1982, Hudon 1987, Johns & Mann 1987, Barshaw & Bryant-Rich 1988, Wahle & Steneck 1991). The association of early benthic phase lobster with cobble, mussel shell, and peat reef habitats has been linked primarily to predator avoidance, and predation rates are significantly lower for juvenile lobsters in these structurally complex habitats compared to those living in habitats that do not provide shelter (Johns & Mann 1987, Barshaw & Bryant-Rich 1988, Wahle 1992, Barshaw et al. 1994, Ball et al. 2001). Following substrate selection, juveniles appear to be dependent on shelter-providing habitat, and adopt a highly cryptic, shelter-restricted lifestyle (Barshaw & Bryant-Rich 1988), a strategy that is likely made necessary by their small size and lack of structural defense mechanisms (Wahle 1992, Wahle & Steneck 1992, Atema & Voigt 1995, Lawton & Lavalli 1995). During the shelter-restricted phase, the energetic cost of reduced foraging is apparently overcome because young lobsters are able to meet their energetic needs with shelter-based food sources and suspension feeding (Barshaw & Bryant-Rich 1988, Lavalli & Barshaw 1989). Early benthic lobsters are strongly bound to their shelters during their first year or more, until they grow larger in size, become more mobile, and are better able to defend themselves (Barshaw & Lavalli 1988, Wahle & Steneck 1992, Incze et al. 1997).

The ecology and behaviour of lobsters during their early post-settlement months is largely unknown, other than the evidence of a shelter-restricted lifestyle. However, we do know that postlarval lobsters settle in dynamic shallow subtidal and lower intertidal habitats (Wahle & Steneck 1991, Cowan 1999), which are areas that are likely to be thermally unstable and at times subject to rapid temperature changes. Because lobsters respond to changing temperature during other life history phases, it is possible that shelter-restricted juveniles also exhibit a behavioural response to temperature. Although research suggests that early juveniles (<25 mm) are bound to their shelters (Lawton & Lavalli 1995), the possibility of shelter abandonment in response to a shift in environmental conditions has not been investigated. A recent study of year-round lobster abundance in intertidal areas showed seasonal differences in early juvenile abundance that was apparently unrelated to mortality, and pointed to seasonal movement of early stage juveniles in response to temperature (Cowan et al. 2001). In situations where temperature inhibits optimal metabolic function, behaviours may reflect a trade-off between the risks of out-of-shelter movement and the benefits of locating a metabolically favourable environment.

The behavioural responses of *H. americanus* to water temperature have been characterized for larval stages (Boudreau 1991, Boudreau et al. 1992, Ennis 1995, Lawton & Lavalli 1995) and adult lobsters (Crossin et al. 1998), but there is little data on juveniles. Given their distinct shelter-restricted behaviour, and habitation in thermally dynamic areas, it is of particular interest to investigate the response of early benthic juveniles to temperature change. Studies of the interactions between environmental factors and early juvenile lobster behaviour are needed to advance our understanding of their distributions and recruitment, and improving our ability to identify and protect key habitat.

The aim of this study was to investigate the possibility of behavioural thermoregulation in shelter-restricted early benthic lobster and to determine what temperature changes, if any, would prompt emergence from shelters. To examine the effect of temperature on shelter occupation in early juvenile lobster, I tested the behavioural response of young (approximately 3-month old) lobsters to short-term shelter warming and cooling. Lobster shelters were gradually warmed or cooled by approximately 8 °C from autumnal ambient water temperature to determine what temperatures elicited a response.

3.3 Materials and Methods

3.3.1 Lobster Rearing

Early juvenile lobsters (2-3 months old; carapace length 4.2-6.2 mm) were laboratory-reared from the broods of 15 females obtained from commercial fishermen in Notre Dame Bay, Newfoundland in August 2007. The early juveniles were held at the Ocean Sciences Centre in St. John's, Newfoundland in plastic cylinders (7 cm diameter x 15 cm high, 1 individual per cylinder) with mesh bottoms and mesh windows. The containers were positioned 5-cm off of the bottom in 1-m^2 tanks with flowing 8-10 ºC ambient seawater. Lobsters sheltered in small pieces of PVC pipe in their containers. Every two days, containers were cleaned and lobsters were fed *ad libitum* frozen enriched *Artemia* nauplii (San Francisco Bay Brand). Lobsters were maintained under an ambient light cycle from September until experiments were performed in November (range of light:dark was 13h:11h – 10h:14h). Lobster carapace lengths were measured prior to experiments using a dissecting microscope and all lobsters used were in an intermolt stage at the time of experiments.

3.3.2 Temperature Manipulation Experiments

34 early juvenile lobsters (stage VI-VII; 4.2-5.5 mm carapace length) were tested. Individual lobsters were placed in a 15 cm x 15 cm shelter box situated at one end of a 50 cm x 25 cm black-sided tank filled to 15 cm depth with unfiltered

ambient seawater (Fig. 3.1). The shelter enclosure was made of PVC with a removable door; the bottom of the enclosure was covered in sand. Cobble was placed at the rear of this enclosure so that lobsters would take cover in the location where the temperature change first occurred (Fig. 3.1). Shelter temperature was manipulated by circulating cold or warm propylene glycol through an exchange coil located behind the cobble side of the shelter. Submersible heaters and slow ambient water inflow were also used to maintain temperature gradients across the tanks. Digital thermometers were used to confirm the presence of a temperature gradient during manipulations. Gentle aeration was used to minimize vertical stratification or the development of convection currents. Experiments were performed in a temperature-controlled room set at 10 °C. To simulate the ambient light levels observed at ~10 m, the depths usually inhabited by early juvenile lobsters, dim red lighting was used, with only a small amount of white light entering the room. Red wavelengths have been shown to be poorly absorbed by lobster visual pigments (Bruno et al. 1977) and were used to mimic dark conditions while allowing for observation. Experimental tanks were black on all sides so that light entered from the top only.

Each individual was tested in separate warming, cooling, and control trials. Control runs were identical to experimental runs except that ambient water flowed through the cooling/heating coils and water inflows. Trials of a single individual were separated by at least 2 days. To avoid potential effects of the order of exposure to treatments, the sequence of treatments varied among lobsters, with equal numbers assigned to each of the 6 possible treatment sequences.

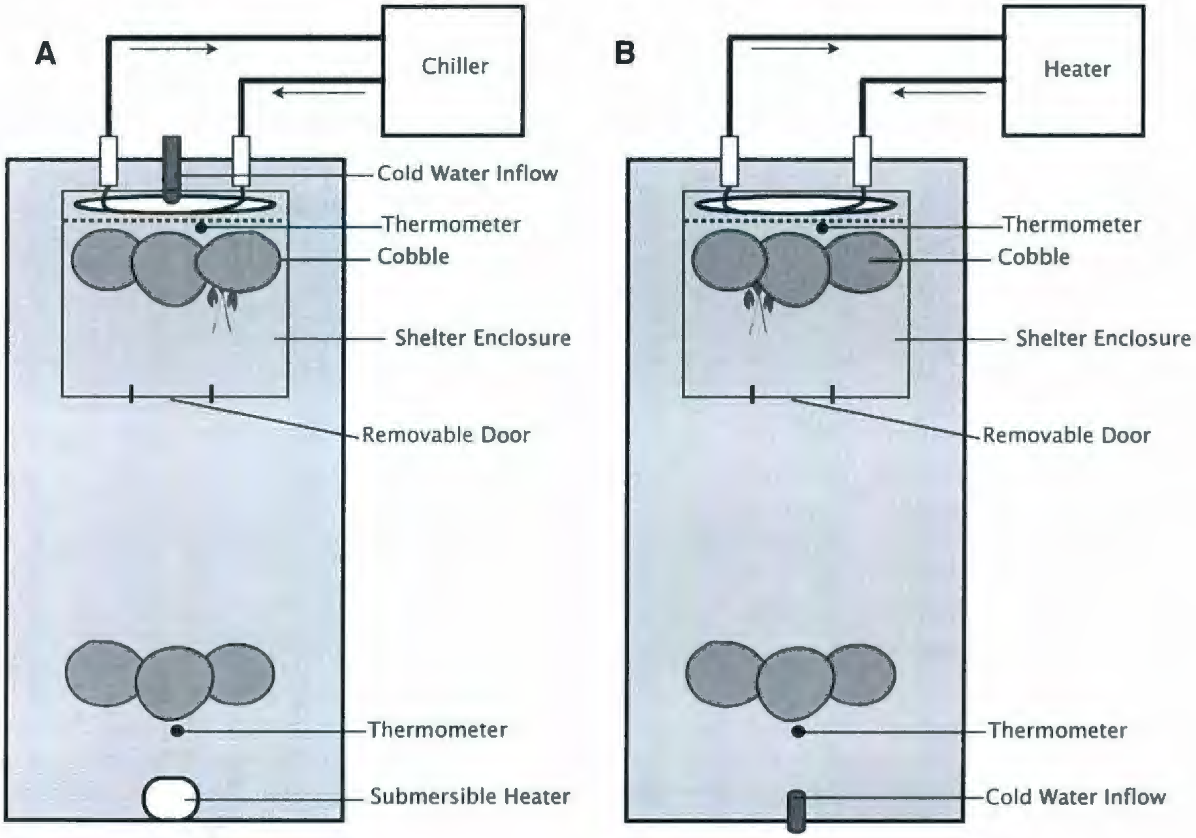

Figure 3.1. Thermal manipulation tanks (50 cm x 25 cm) viewed from above. **(A)** Shelter cooling was achieved by circulating coolant through an exchange coil, and by introducing cold water at the rear of the enclosure. A temperature gradient within the tank was maintained using a submersible heater at the end of the tank opposite the cooling apparatus. **(B)** Shelter warming was accomplished by circulating warm liquid through an exchange coil at the rear of the shelter enclosure. The temperature gradient was maintained in the tank during trials by introducing cold water at the end opposite the shelter enclosure.

Lobster juveniles were placed in the closed shelter box for a 30-minute acclimation period prior to the start of temperature manipulation. During this period, all lobsters disappeared from sight by sheltering in the cobble crevices or burrowing under the cobble. Following the acclimation period, the temperature manipulation began and the shelter door was opened to allow the lobster to move from the enclosure. In cooling treatments, the shelter was cooled from the initial ambient temperature of 9-10 °C to approximately 2 °C. In warming treatments, the shelter was heated from the ambient temperature to approximately 17 °C. This range of temperature change is within that observed in the shallow-water intake at the Ocean Sciences Centre during upwelling and downwelling events in late summer and early fall. On average, the temperature in the rear of the shelter changed at a rate of 0.4 °C/min during the first 20 minutes of the experiment, and remained at approximately that temperature for the remaining 10 minutes. Lobster activity was monitored during the trials and any visible movement or activity was recorded, as well as the temperature at which any activity occurred. Activity that occurred during a trial was classified as "normal" if no visible movement was observed or if the behaviour was identified as a burrow/shelter constructing behaviour (Cobb 1971). Any other behaviour exhibited by the lobsters were scored as "aversive"; these behaviours included movement from the shelter to open sand, movement to the top of the cobble, swimming, and departure from the shelter enclosure.

3.3.3 Statistical Analyses

Because the measures were repeated on animals for each of the three treatments, data was analysed using generalized estimating equations (GEE), an extension of the generalized linear model procedure that accounts for non-independent responses (Hardin & Hilbe 2003). This analysis used a binomial distribution and logit link function to determine whether treatment (cooling/warming/control) had an effect on juvenile lobster response (proportion of aversive versus normal responses). Pairwise comparisons were used to identify which of the treatments caused any significant differences in behaviour. Analyses were performed using SPSS Statistical Software. See Appendix B for detailed model and parameter information.

3.4 Results

Treatment had a significant effect on lobster behavioural response (x^2=43.0, p<0.001). Early juvenile lobsters demonstrated a significant aversion to shelter cooling. When the temperature in the experimental shelter was decreased from 9.9 ± 0.1 °C to 2.9 ± 0.4 °C (range 2.2-3.7 °C), most (73.5%) of the lobsters left their cobble shelter to move to warmer waters (Fig. 3.2). The high rate of shelter abandonment was clearly in response to the temperature manipulation, given that during the control trials, only 1 of the 34 lobsters (3%) left the shelter (pair-wise comparison: p<0.001).

Shelter warming did not elicit the same strong aversive response as cooling, however, given that only 5 of 34 lobster (15%) abandoned the experimental shelter during warming to 16.0 ± 0.5 °C (range 14.7-17.6 °C). The proportion of aversive responses in warming trials was not significantly different than in control trials (Fig. 3.2; p=0.13).

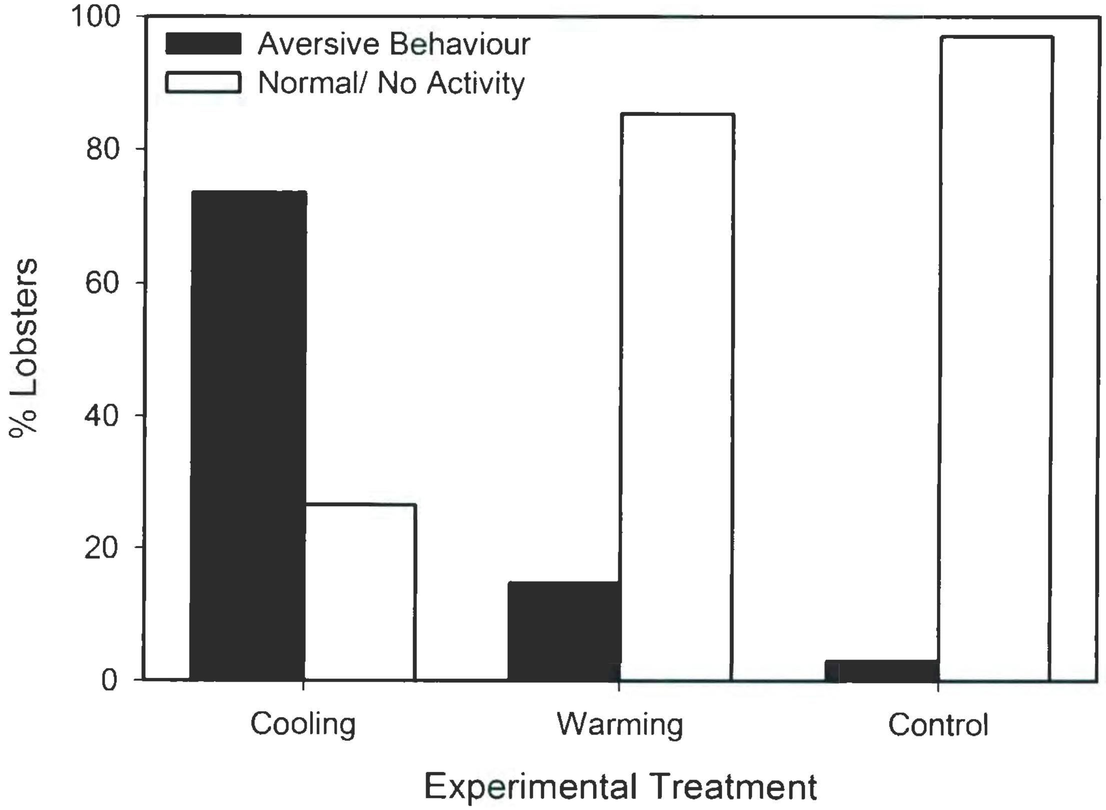

Figure 3.2. Comparison of the percent of juvenile lobsters that exhibited an aversive response during cooling, warming and control trials.

The most common aversive response observed was movement to open sand outside of shelters, followed by movement to the top of the cobble, upward swimming, and movement out of the shelter enclosure (Fig. 3.3). In the cooling experiment, of the 25 lobsters that exhibited aversive behaviour, 16 (64%) moved to exposed sand, 11 (44%) moved to the top of cobble, 5 (17%) swam, and 4 (14%) moved completely out of the shelter enclosure area. In the warming experiment, in which 5 lobsters responded to the treatment, all moved onto the open sand in front of the cobble and 2 moved further out of the shelter enclosure. The one individual that exhibited an atypical behaviour during the control trial moved around the front of the cobble shelter, walked to another rock, and resumed sheltering.

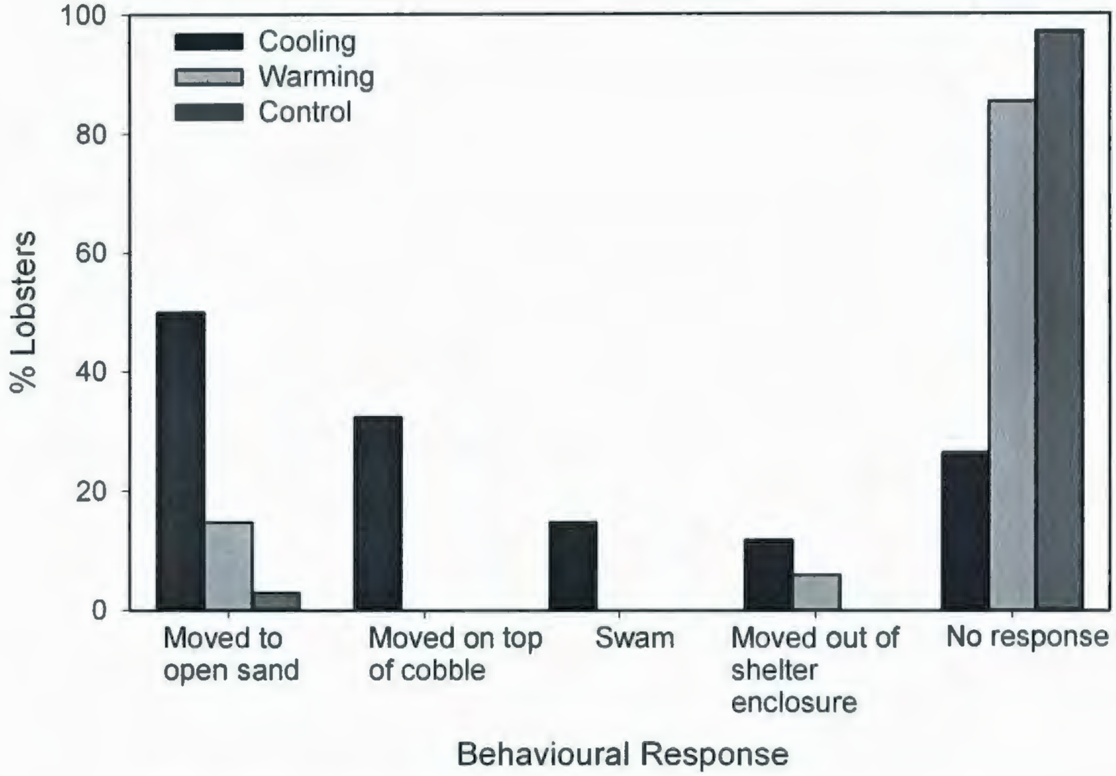

Figure 3.3. Percent of juvenile lobsters that exhibited specific aversive responses during cooling and warming manipulations.

In the cooling experiments, the temperature at which lobsters exhibited responses was varied. No responses were observed during approximately the first two degrees of decrease, but subsequently, various responses occurred throughout the temperature range (Fig. 3.4). There were, however, patterns in the types of responses exhibited during the decrease in temperature. Horizontal movements onto open sand generally occurred earlier in the cooling period (at higher temperatures) than vertical movements onto the top of the cobble (Fig.

3.4; Fig. 3.5). There was no obvious trend in the less common responses (swimming and moving out of the shelter enclosure) in terms of the temperatures at which they occurred.

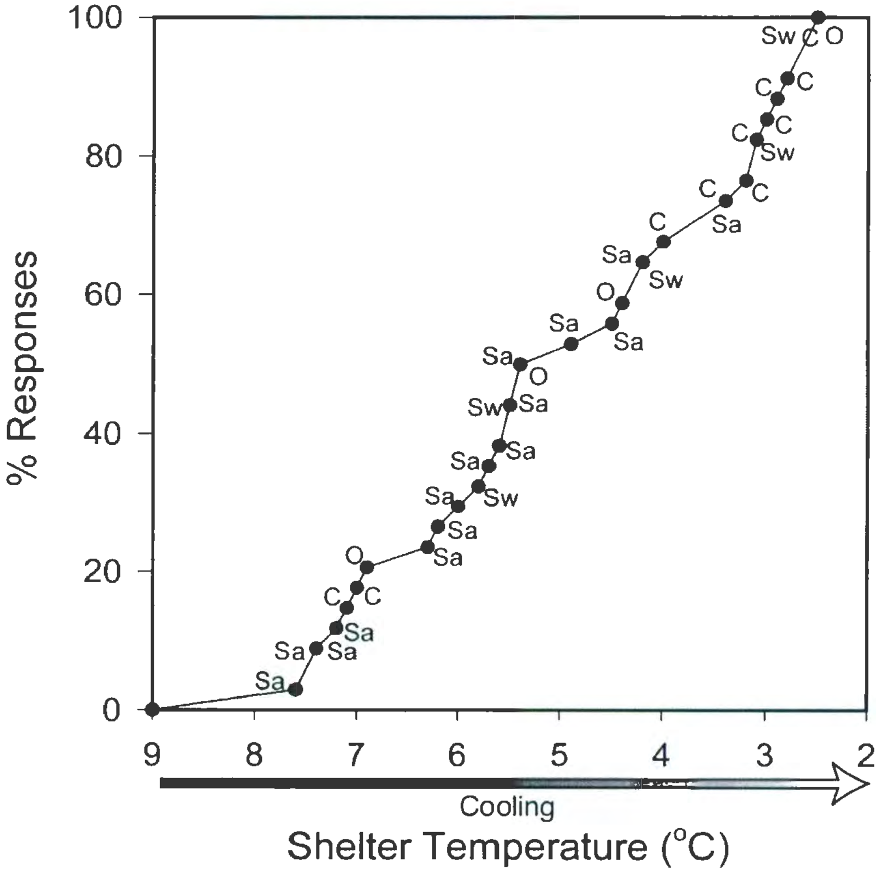

Figure 3.4. Cumulative percent of aversive responses occurring as temperature was decreased during shelter cooling. Letters indicate behaviours occurring at each temperature: Sa= moved to open sand, C= moved on top of cobble, Sw= swam, O= left shelter enclosure.

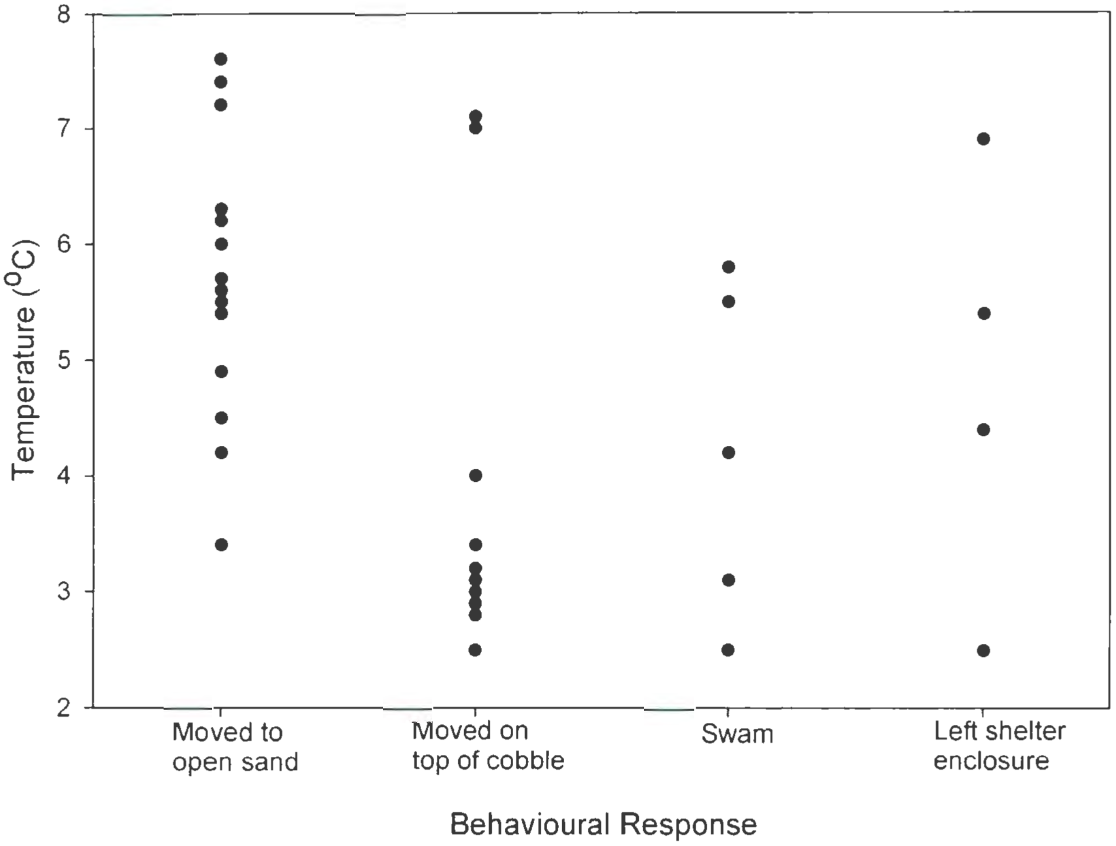

Figure 3.5. Temperatures at which specific behavioural responses occurred during shelter cooling experiments.

3.5 Discussion

Early juvenile lobster can detect and respond behaviourally to changes in environmental temperature. In cooling experiments, lobsters began to respond aversively when water temperatures decreased, and a majority chose to move out of their burrows. It is not surprising that shelter cooling elicited a stronger response from juvenile lobsters than warming, given that cooling has a potentially damaging effect on lobster metabolism and growth, whereas limited warming is likely to be physiologically beneficial within the range of temperatures tested in these experiments. This interpretation is consistent with evidence that lobsters generally move into warmer water that enhances their growth rate (Waddy et al. 1995). Metabolic implications of thermal manipulations would be worthwhile to investigate in future experiments by measuring physiological as well as behavioural responses of early juvenile lobster.

In their study of adult lobster thermal preferences, Crossin et al. (1998) found that animals prefer shelters 1.5 °C warmer than their acclimation temperature and demonstrate an avoidance response above 23.5 °C; responses to cooling were not examined. They determined that it was not the magnitude of the temperature increase that the lobsters found aversive, but rather temperatures above 23 °C . This finding is consistent with results of my heating experiment, in which juvenile lobsters were exposed to temperatures up to approximately 18 °C, and did not exhibit a significant negative response to the higher temperature. Within the context of climate change, modest warming may have little effect on juvenile behaviour, however, other temperature-related effects may be important to investigate since excessive warming has previously been found to contribute to massive mortality events in adult lobster populations (Pearce & Balcom 2005).

No threshold temperature was found at which responses to cooling occurred, but rather aversive behaviours were observed throughout the range of decrease in temperature. This experiment was not designed to detect specific temperatures at which responses occurred. Because temperatures were recorded at the rear of the shelter, it was not possible to assess the precise temperature to which each lobster was exposed when they displayed aversive behaviour. Thus, because individual lobster would burrow/shelter in different areas of the cobble, it is quite possible that cool water reached them at different rates. Not unlike wild conditions, heat transport processes might have brought sudden pulses of cold water into contact with postlarvae. In future experiments, studying several subjects simultaneously, as well as attaching temperature loggers to individual lobsters, may prove useful in detecting potential temperature thresholds. Even though information on specific temperature responses of juvenile lobsters is lacking, the data suggest that there may be different behavioural responses as water cools. Horizontal movements to sand outside of the shelters typically occurred at higher temperatures than vertical movements to the top of the cobble. Further studies to characterize the specific behaviours in terms of their induction temperatures as well as their associated predation risk would allow a more thorough evaluation of the implications of cooling events.

The data suggest that early juvenile lobsters (<8 mm CL) may move more than previously thought, despite being considered "shelter-restricted". It is possible that thermally-induced movements occur in early juvenile lobster that inhabit dynamic low intertidal and shallow coastal areas. Juvenile lobsters in Atlantic Canada are exposed to highly variable temperatures, particularly in the early post-settlement autumn months. Sudden cooling events, which are probable in the intertidal zone, may induce out-of-shelter movement in early juvenile stage lobster. Ellis and Cowan (2001) found a positive correlation between juvenile (<40 mm CL) abundances and substrate temperature, with the highest intertidal abundances in the warm summer months. Seasonal variation in abundance of young-of-the-year lobsters (<10 mm CL) in Maine revealed a peak in spring (Cowan et al. 2001), which is probably not indicative of a settlement event because hatching and settlement usually do not occur until summer. Instead, the seasonal variation they observed in small lobster abundance in the intertidal zone is possibly a result of behavioural responses to environmental conditions (Cowan et al. 2001).

This laboratory study helps to explain some distribution patterns observed in juvenile lobster, and suggests that temperature may be of significance in early juvenile lobster behaviour. Nonetheless, this work does not address the potential interaction between responses to temperature and other abiotic and biotic variables in the wild, such as light level, presence of predators and conspecifics, salinity, food supply, and hydrodynamics. Juvenile lobster are known to restrict activity outside their burrows in full light (Zeitlin-Hale & Sastry 1978, Spanier et al. 1998), and there is evidence that early juvenile lobster can detect and respond to predators (Johns & Mann 1987, Spanier et al. 1998) by increasing their use of shelters (Wahle 1992). It is likely that these and other stimuli interact with thermal conditions to determine juvenile sheltering behaviour. Circumstances that promote sheltering, such as high light or predator odour, may increase risk enough to offset the response to decreasing temperature, and deter a juvenile from abandoning its shelter during a mild cooling event. Further exploration of the conditions under which temperature change affects behavioural decisions of early juvenile lobster is needed to evaluate the potential for complex behavioural processes with a hierarchy of decision-making cues.

The demography of the American lobster is thought to be driven largely by settlement success, and less influenced by post-settlement mortality or the movements of juveniles (Lawton & Lavalli 1995, Butler et al. 2006). Recently, however, Wahle et al. (2009) illustrated how local factors that increase post-settlement mortality (such as incidence of disease) can weaken the settlement-recruitment relationship in a lobster population. Their research emphasizes the importance of examining the processes that influence both pre- and post-settlement stages in order to explain recruitment. My results highlight the need to consider the complex relationship between environment and behaviour by assessing the early post-settlement mortality risks and migration associated with different types of habitats or regions. Incze et al. (1997) and Palma et al. (1999) have suggested that juvenile lobsters remain within 1-2 m of where they settled during their first year, but findings here suggest that this fidelity could depend on whether they settle in thermally dynamic areas. Habitats with frequent temperature fluctuations may represent a greater mortality risk for juvenile lobster because of their behavioural response to temperature, above and beyond any direct physiological impacts. The thermal environment, in terms of average temperature and temperature variability, are likely to influence early juvenile lobster relocation and therefore predation mortality. These post-settlement processes have a significant effect on local population size and need to be considered in predictions of recruitment and models of habitat quality.

To assess more clearly the role that temperature variation may play in juvenile lobster movement, mortality, and recruitment in various habitats, the effect of substrate type also needs to be considered. Postlarval lobsters are known to preferentially settle in several types of substrate, including cobble on sand, cobble on bedrock, peat reef, and shell hash (reviewed by Lawton & Lavalli 1995). The present study examined juvenile lobster behaviour in cobble on sand, but it is possible that behavioural responses to changing temperature will vary among shelter substrates. For instance, during unfavourable temperature fluctuations, a juvenile lobster in cobble on soft sediment may be able to remain protected and burrow deeper into the substrate, whereas an individual that resides on hard substrate may be forced to abandon the protection of their shelter. If substrate-specific behavioural responses to temperature can be identified, then prediction of recruitment success may be improved for different types of habitat.

The use of laboratory-reared lobsters in this study could not be compared to field-caught test subjects because early benthic juvenile lobsters are rarely encountered in Newfoundland waters (Burdett-Coutts & Wilke, pers. comm.). Although any laboratory experiment raises the potential for artifacts, one study that examined sheltering differences between field-caught and hatchery-reared early juvenile lobsters found that hatchery-reared individuals spent more time in their shelter and were less likely to explore than individuals brought in from the wild (Castro & Cobb 2005). If this finding holds true for my laboratory-reared lobsters, the shelter abandonment response I found should be strengthened, and suggests that wild juvenile lobster may be even more likely to leave their shelter under changing temperatures. Nonetheless, experiments using wild-caught juveniles, particularly wild lobster from different thermal environments, would provide further insight into the significance of temperature avoidance behaviour of juvenile lobster.

Identification of the role of environmental variables such as temperature in the migration and mortality of early benthic stages is essential to evaluating the relative contribution of pre- and post-settlement events to spatial and temporal patterns in the recruitment of marine animals. In this study, I have demonstrated that early juvenile lobsters have the capacity to respond to temperature changes, and that they show distinct avoidance responses to cooling. These results shed light on the behaviour of lobsters during the poorly known, early post-settlement phase, and suggest that environmental conditions, through their influence on behaviours, could impact early juvenile movement and survival.

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CHAPTER 4

Summary

Processes that occur during the early life stages are critical to the recruitment and success of populations of benthic marine species. In marine systems, physical processes such as water flow and temperature can interact with biological processes during critical early life history stages to determine the distribution and abundance of a species. Through advances in the ability to simulate natural ocean environments using laboratory flumes (flow channels that attempt to create realistic sea-bottom conditions) and other controlled tank systems, there is the potential to gain a better understanding of the key processes that regulate benthic community structure. The investigation of early life history processes can improve our ability to predict population demography and identify important nursery habitats. This study of the interaction between physical processes (hydrodynamics and temperature) and lobster behaviour at early life-history stages serves to enhance our understanding of the processes that drive spatial and temporal variability in recruitment.

Previous laboratory experiments have provided information about lobster postlarval substrate preferences and responses to chemical and physical substrate characteristics. However, nearly all of these experiments have been conducted in still water. Chapter 2 showed that flow has an effect on settlement frequency by increasing encounter rates through both passive deposition and the modification of postlarval settlement behaviour. These results suggest that the flows associated with different benthic environments are important to consider as potential cues for habitat selection by lobsters at settlement, and that spatial and temporal hydrodynamic characteristics of habitat are important to incorporate into settlement and recruitment models. Further studies of lobster settlement behaviour in response to the range of flow speeds and turbulence levels seen in the field will make the implications of hydrodynamics on settlement more clear. This type of information could be applied in the development of marine reserves, specifically to identify habitats with flow regimes that may enhance settlement and recruitment.

Very little is known about the ecology and behaviour of lobsters post-settlement (1-12 months old), although they are widely considered to be "shelter-restricted". In the study presented in Chapter 3, a significant number of lobsters demonstrated sensitivity to cooler temperatures, abandoning their shelters for warmer water during cooling. This pattern suggests that shelter-restricted juvenile lobsters may move from the protection of their shelter under unfavorable temperature conditions, and that early post-settlement mortality and migration may vary among habitats and therefore be more important than previously thought in determining recruitment patterns. In this case, post-settlement migration and mortality may be especially significant in regions that experience rapid changes in temperature, such as coastal Newfoundland. This also suggests that future environmental changes that lead to more unstable or unpredictable temperature regimes could negatively impact lobster populations through their effects on early juvenile behaviour.

These results emphasize the need for an integrated approach in studying lobster recruitment, where interactions between environmental fluctuations and behavioural plasticity are considered. Knowledge of the causes and outcomes of different behavioural strategies, and integration of these variables into population and fishery models, represents an important step towards increasing our ability to manage fishing activities on marine populations more sustainably than in the past. As climate change continues to alter the environmental conditions in marine systems, studies of biological-physical interactions are needed to evaluate the impacts of changing thermal and hydrodynamic regimes on marine communities. Effective implementation of marine protected areas and habitat enhancement projects also requires an understanding of how physical variables affect recruitment. The new insights provided here regarding the influence of physical variables on settlement and post-settlement behaviour could assist in the designation of marine reserves for lobster; the results highlight the need to consider the hydrodynamic conditions and thermal regime of areas when examining their suitability as lobster nursery habitat.

In summary, a more comprehensive understanding of the physical factors that influence survival, growth, and distributions of early life-history stages is of fundamental importance in determining the relationships between physical and ecological processes that regulate recruitment. Such information has the potential to be applied to fisheries management, conservation and mitigation of human impacts on marine communities.

Appendix A

Chapter 2 Statistical Analysis

1. Settlement Outcome

$$S= \beta_o + \beta_A \cdot A + \beta_F \cdot F + \beta_{A \times F} \cdot A \cdot F + \varepsilon$$

where
S= settlement outcome
A= age (mid- or late-stage)
F= flow treatment (flow or still)

Model Information

Dependent Variable	Settlement
Probability Distribution	Binomial
Link Function	Logit
No. Observations Used	110

Tests of Model Effects

Source	Type III		
	Chi-Square	df	Sig.
Age	12.68	1	.0004
Flowtrt	5.88	1	.0153
Age * Flowtrt	1.869	1	.9103

Dependent Variable: Settlement
Model: (Intercept), Age, Flowtrt, Age * Flowtrt

Analysis of Parameter Estimates

Parameter	Estimate	Std. Error	95% Confidence Interval		Hypothesis Test		
			Lower	Upper	Chi-Square	df	Sig.
(Intercept)	-.9163	.4830	-1.8630	.0305	3.60	1	.0578
Age=mid	1.5141	.6118	.3151	2.7131	6.13	1	.0133
Flowtrt=flow	-1.1632	.7800	-2.6918	.3655	2.22	1	.1359
Age=mid * Flowtrt=flow	.1058	.9409	-1.7383	1.9498	0.01	1	.9105

2. Settlement Substrate

$$\text{Sub} = \beta_o + \beta_A \cdot A + \beta_F \cdot F + \beta_{AxF} \cdot A \cdot F + \varepsilon$$

where:
Sub= substrate (small or large cobble)
A= age (mid- or late-stage)
F= flow treatment (flow or still)

Model Information

Dependent Variable	Settlement Substrate
Probability Distribution	Binomial
Link Function	Logit
No. Observations Used	69

Tests of Model Effects

Source	Type III		
	Chi-Square	df	Sig.
Flowtrt	.965	1	.326
Age	.114	1	.736
Flowtrt * Age	.080	1	.778

Dependent Variable: Substrate
Model: (Intercept), Flowtrt, Age, Flowtrt * Age

Parameter Estimates

Parameter	B	Std. Error	95%Confidence Interval		Hypothesis Test		
			Lower	Upper	Chi-Square	df	Sig.
(Intercept)	-.134	.5175	-1.148	.881	.067	1	.796
Flowtrt=flow	.316	.7966	-1.245	1.877	.157	1	.692
Age=mid	.644	.6676	-.664	1.953	.932	1	.334
Flowtrt=flow * Age=mid	-.288	1.0191	-2.285	1.710	.080	1	.778

3. Substrate Encounter

$$E= \beta_o + \beta_A \cdot A + \beta_F \cdot F + \beta_{AxF} \cdot A \cdot F + \varepsilon$$

E= encounter
A= age (mid- or late-stage)
F= flow treatment (flow or still)

Model Information

Dependent Variable	Encounter in 15
Probability Distribution	Binomial
Link Function	Logit
No. Observations Used	110

Tests of Model Effects

Source	Type III		
	Chi-Square	df	Sig.
Age	4.702	1	.030
Flowtrt	7.569	1	.006
Age * Flowtrt	1.869	1	.172

Parameter Estimates							
			95%ConfidenceInterval		Hypothesis Test		
Parameter	Estimate	Std. Error	Lower	Upper	Chi-Square	df	Sig.
(Intercept)	-.693	.4629	-1.600	.214	2.242	1	.134
Age	.499	.5870	-.651	1.649	.723	1	.395
Flowtrt	-2.565	1.1193	-4.759	-.371	5.252	1	.022
Age* Flowtrt	1.703	1.2456	-.738	4.144	1.869	1	.172

4. Encountered substrate but did not settle

$$\text{Ens}= \beta_o + \beta_A \cdot A + \beta_F \cdot F + \beta_{AxF} \cdot A \cdot F + \varepsilon$$

Ens= encounter without settlement
A= age (mid- or late-stage)
F= flow treatment (flow or still)

Model Information

Dependent Variable	Encounter_didnotsettle
Probability Distribution	Binomial
Link Function	Logit
No. Observations Used	81

Tests of Model Effects

Source	Type III		
	Chi-Square	df	Sig.
Age	6.696	1	.010
Flowtrt	.019	1	.889
Age * Flowtrt	.397	1	.529

Parameter Estimates

Parameter	B	Std. Error	95% Confidence Interval		Hypothesis Test		
			Lower	Upper	Chi-Square	df	Sig.
(Intercept)	2.565	1.0377	.531	4.599	6.109	1	.013
Age	-2.208	1.1488	-4.460	.043	3.695	1	.055
Flowtrt	-.528	1.2057	-2.891	1.835	.192	1	.661
Age* Flowtrt	.865	1.3726	-1.826	3.555	.397	1	.529

5. Time to settlement

$$TTS = \beta_o + \beta_A \cdot A + \beta_F \cdot F + \beta_{AxF} \cdot A \cdot F + \varepsilon$$

TTS= time to settlement
A= age (mid- or late-stage)
F= flow treatment (flow or still)

Model Information

Dependent Variable	TimetoSettlement
Probability Distribution	Poisson
Link Function	Log
No. Observations Used	69

Tests of Model Effects

Source	Type III		
	Chi-Square	df	Sig.
Age	39.81	1	<.0001
Flowtrt	.54	1	.9672
Age * Flowtrt	4.69	1	.0136

Parameter Estimates

Parameter	B	Std. Error	95% Confidence Interval		Hypothesis Test		
			Lower	Upper	Chi-Square	df	Sig.
(Intercept)	2.8829	0.0611	2.7632	3.0027	2227.43	1	<.0001
Age=mid	0.1872	0.0892	0.0125	.3260	4.41	1	.0358
Flowtrt=flow	-.1448	0.0802	-0.3019	0.0123	3.26	1	0.0709
Age=mid * Flowtrt=flow	.2787	0.1131	0.0571	0.5002	6.07	1	0.0137

```
Stem Leaf                      #             Boxplot
   7 14                        2                |
   6 2222                      4                |
   5 3468                      4                |
   4                                            |
   3 3388888                   7                |
   2 7                         1             +-----+
   1 11144688                  8             |     |
   0 1                         1             |     |
  -0 77761                     5             |  +  |
  -1 988855                    6             *-----*
  -2 99984211                  8             |     |
  -3 220                       3             |     |
  -4 99996541000              11             +-----+
  -5 777550                    6                |
  -6 611                       3                |
     ----+----+----+----+
```

Figure A-1. Boxplot diagnostic to determine appropriate distribution for generalized linear model.

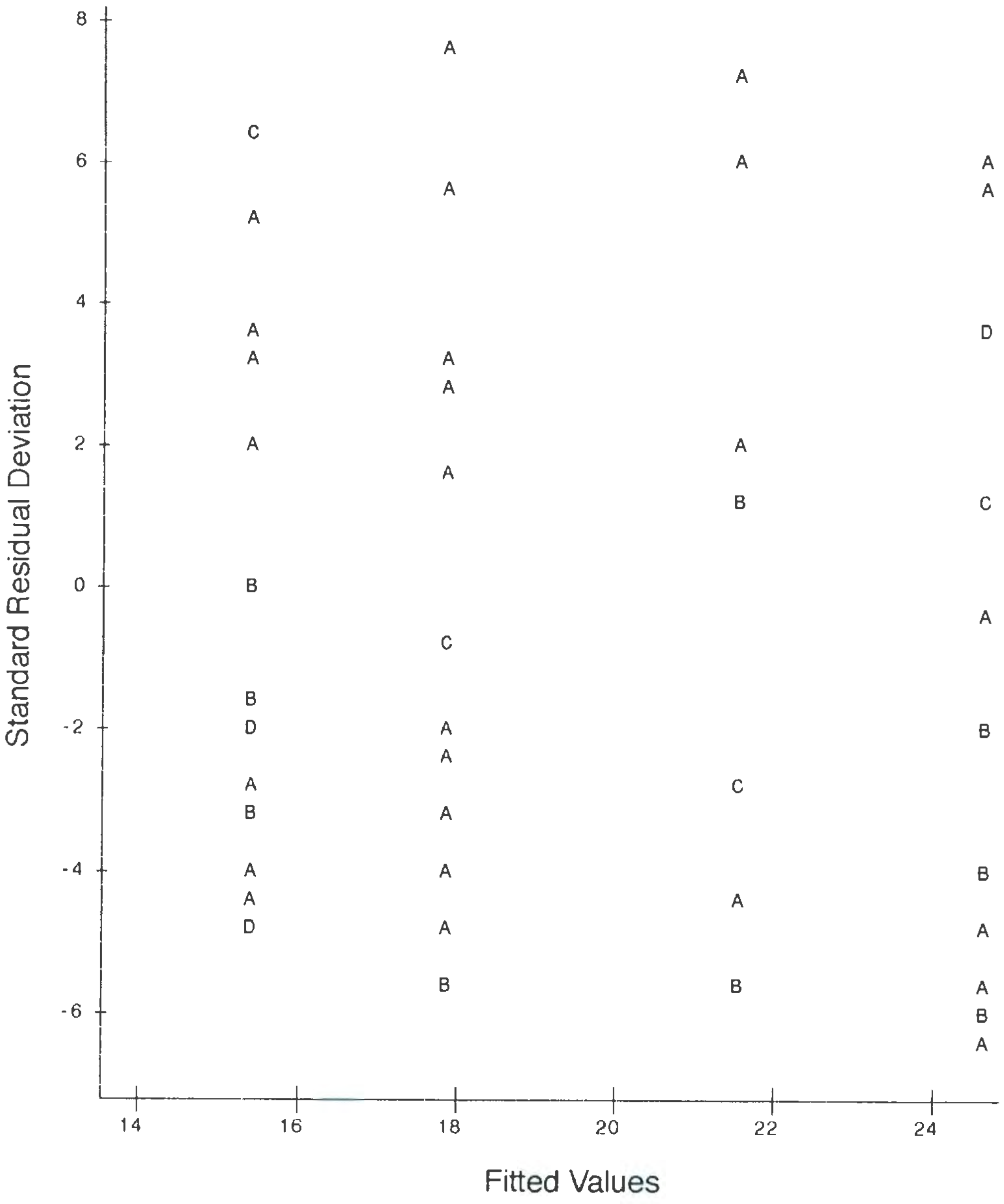

Figure A-2. Diagnostic plot of fitted values versus residuals to check for homogeneity of errors and fit of structural model.

Analysis of interaction for time to settlement:

Flow treatment

Mid-stage: flow vs. still

Model Information

Dependent Variable	Time to Settlement
Probability Distribution	Poisson
Link Function	Log
No. Observations Used	30

Tests of Model Effects

Source	Type III		
	Chi-Square	df	Sig.
Flow treatment	2.86	1	.0909

Late-stage: flow vs. still

Model Information

Dependent Variable	Time to Settlement
Probability Distribution	Poisson
Link Function	Log
No. Observations Used	39

Tests of Model Effects

Source	Type III		
	Chi-Square	df	Sig.
Flow treatment	3.23	1	.0721

Age

Still: Mid-stage vs. Late-stage

Model Information

Dependent Variable	Time to Settlement
Probability Distribution	Poisson
Link Function	Log
No. Observations Used	26

Tests of Model Effects

Source	Type III		
	Chi-Square	df	Sig.
Flow treatment	4.39	1	.0362

Flow: Mid-stage vs. Late-stage

Model Information

Dependent Variable	Time to Settlement
Probability Distribution	Poisson
Link Function	Log
No. Observations Used	43

Tests of Model Effects

Source	Type III		
	Chi-Square	df	Sig.
Flow treatment	45.32	1	<.0001

6. Swimming Behaviour Trials

Paired Samples Test

		Paired Differences							
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval		t	df	Sig. (2-tailed)
					Lower	Upper			
Pair 1	T Midwater flow-still	-1.16E0	1.508E0	.30163	-1.787E0	-5.42E-1	-3.861	24	.001
Pair 2	T Surface flow-still	-7.42E-1	5.5246E0	1.104E0	-3.023E0	1.538E0	-.672	24	.508
Pair 3	T Bottom flow-still	1.90733	5.59693	1.11939	-.40296	4.21763	1.704	24	.101
Pair 4	Lift-offs flow-still	-4.160	7.087	1.417	-7.085	-1.235	-2.935	24	.007
Pair 5	Partial Dives flow-still	8.760	13.182	2.636	3.319	14.201	3.323	24	.003
Pair 6	Full Dives flow-still	-3.800	6.238	1.248	-6.375	-1.225	-3.046	24	.006

Appendix B

Chapter 3 Statistical Analysis

$$Ab = \beta_o + \beta_T \cdot T + \varepsilon$$

where:
Ab= aversive behaviour (yes/no)
T= temperature treatment (warming/cooling/control)

Generalized Linear Model

Model Information

Dependent Variable		Behaviour
Probability Distribution		Binomial
Link Function		Logit
Subject Effect	1	Individual
Within-Subject Effect	1	Treatment
Working Correlation Matrix Structure		Independent

Tests of Model Effects

Source	Type III		
	Wald Chi-Square	df	Sig.
(Intercept)	11.779	1	.001
Treatment	43.000	2	.000

Dependent Variable: Behaviour
Model: (Intercept), Treatment

Pairwise Comparisons

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	df	Sig.	95% Wald Confidence Interval for Difference	
						Lower	Upper
Control	Cooling	-4.518[a]	1.048	1	.000	-6.573	-2.463
	Heating	-1.739	1.156	1	.133	-4.004	.527
Cooling	Control	4.518[a]	1.048	1	.000	2.463	6.573
	Heating	2.780[a]	.540	1	.000	1.721	3.838
Heating	Control	1.739	1.156	1	.133	-.527	4.004
	Cooling	-2.780[a]	.540	1	.000	-3.838	-1.721

Pairwise comparisons of estimated marginal means based on the linear predictor of dependent variable Behaviour

a. The mean difference is significant at the .05 level.