CHARACTERIZING THERMOREGULATORY TRADE-OFF BEHAVIOR IN JUVENILE AMERICAN LOBSTER, *HOMARUS AMERICANUS*

By

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I. Abstract

The American lobster, *Homarus americanus*, forms an important fishery in Atlantic Canada and New England. As such, a plethora of information exists on the biology of the larval dispersal phases, as well as that of the adult lobster. However, comparatively less is known about the behaviour and physiology of the juvenile stages. The juvenile phase is a critical period of life, characterized by high levels of mortality. As a result, population parameters such as abundance and distribution can be significantly influenced by events occurring during the juvenile phase. Newfoundland is the northern most range limit for *H. americanus,* and associated low temperatures may affect foraging and sheltering behaviours. Laboratory experiments showed that juveniles preferred temperatures of ~18°C and were most active between 10-20°C. Heat stroke occurred above 30°C, while basal activity stopped at ~2.0°C and reactions to sensory stimuli ceased at ~-1°C. Although juvenile lobsters preferred water of 18°C, they would choose thermal regimes below their preference range if shelter or food was available. When shelter was present, the juveniles increased activity levels to maintain the shelter. Because juveniles are vulnerable to predation the acquisition of shelter appeared to override both thermal preferences and foraging behaviour. Although shelter may protect against predation, the use of sub-optimal thermal habitats will influence metabolism and reduce potential for growth in juvenile lobsters.

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VI. List of Abbreviations and Symbols

CL	=	Carapace Length
T_B	=	Body Temperature
CTmax	=	Critical Temperature Maxima
CTmin		Critical Temperature Minima
PT	=	Pejus Temperature
T_{pref}	=	Preferred Temperature
GLM	=	General Linear Model
ANOVA		Analysis of Variance
SEM	=	Standard Error of the Mean

VII. 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. Iain J. McGaw contributed significantly to the research proposal, experimental design, and provided extensive editorial reviews of all the chapters. Co-authors for future publications resulting from these chapters will include Dr. McGaw.

1. Introduction

1.1 Ecological and Economical Importance of Lobster

Homarus americanus is an ecologically important species in the North West Atlantic (reviewed in Boudreau and Worm 2012), *H. americanus* plays the role in the control of other invertebrate populations, such as decreasing urchin populations and preventing large urchin aggregations (Vadas et. al. 1986). *H. americanus* is also a commercially important crustacean that is a popular food worldwide. The fishery is one of the most profitable fisheries on the northeast coast of North America. In Canada, it is the single most valuable fisheries export, accounting for 41% ($664.2 million) of all fish species landed in Atlantic Canada (DFO 2012). In Atlantic Canada, the landings of lobster, have increased by 174% over the last 20 years (DFO 2012) In the United States in Maine alone, the landing of lobster over the past 40 years have increased by 170% (Maine Department of Marine Resource 2011). It is unclear what this increasing fishing pressure will have on the lobster population and the overall ecosystem (Steneck and Wahle 2013), and the worry is that the population will crash. As a result, conservation efforts have been looked at in order to maintain the populations.

To date the main conservation efforts have been in the restricting of gear, legal carapace length, fishing season and restricting number of fishing days per week by fishermen (DFO 2003). More recently hatchery reared juvenile lobster have been reintroduced into the wild (DFO 2003, Nicosia and Lavalli 1999). However, these hatchery releases have had limited success (Bannister and Addison 1998, Wahle 2003, Jørstad et. al. 2005), and a significant correlation between settling post-larval release and in increase in adult *H. americanus* populations has yet to be shown. From a fisheries point of view, it is prudent to attempt to increase survivorship of these early stages (Cobb and Wahle 1994). As a result, further investigation into increasing survivorship in juvenile cohorts of *H. americanus* may increase adult cohort size, making this an important area of research.

In juvenile *H. americanus*, there is a link between survivorship and behavior. Juvenile *H. americanus* are cryptic and spend most of their time within a shelter (Cobb, 1971; Lawton 1987, Rossong et. al. 2006). However it is not known how temperature interacts with or influences these shelter seeking and foraging behaviors. Such knowledge could be useful for the lobster fishery because release of hatchery raised settling postlarvae usually occurs between July and October, when water temperatures are optimal for settlement and growth (Nicosia and Lavalli 1999). However, in the northern geographical limits of *H. americanus,* this warm season is short lived

and temperatures can drop to <0 °C from January until April. This is important because preliminary work shows that newly settled juveniles may emerge from shelters if they experience prolonged bouts of low temperatures (Lillis 2009). By leaving their shelters, juveniles increase their chances of predation and mortality significantly (Whale 1992). This would reduce recruitment to the adult population (Hunt and Scheibling 1997). As a result, the sheltering behaviors used by juvenile *H. americanus* in cold environments require further study to fully assess how this would impact conservation and management of the northern fisheries.

1.2 Larval and Juvenile Lobster Life History

The geographic range of American lobster, *Homarus americanus,* extends from Southern Labrador, Newfoundland to Cape Hatteras, North Carolina (Squires 1990, Lawton and Lavalli 1995). Adult *H. americanus* produce many thousands of eggs, of which only a few survive to adulthood. The early larval stages of *H. americanus* are free-swimming zooplankton, with larval stages that consist of 5 instars (Stages I – V). These stages are particularly sensitive to abiotic variables such as temperature change (Templeman 1936, MacKenzie 1988) and biotic variables such as food availability and predation intensity (reviewed in Pechenik 1987, Olson and Olson 1989), both of which can decrease growth rates and increase mortality. Stage IV larval lobster change to stage V postlarvae between 15

and 60 days after hatching (Ennis 1995). The change from larvae to post-larvae marks a shift from a planktonic to benthic lifestyle (Ennis 1995). This shift results in behavioral, morphological, and physiological changes (Lawton and Lavalli 1995). In optimal conditions, postlarvae may settle within 36 hours (Botero and Atema 1992). Successful settlement for postlarvae usually requires a preferred substrate and temperature. Preferred settlement substrate consists of areas of bedrock or boulder/cobble with pre-formed crevices and macroalgal cover (Botero and Atema 1982).

As early juvenile benthic phase individuals mature and grow, their behavior changes. The smallest, shelter- restricted juveniles are cryptic and remain within a shelter until reaching approximately 15-25 mm carapace length (CL). After reaching this size, they are classified as an emergent juvenile (25-30 mm CL). Emergent juveniles still spend of their time hidden, emerging from their shelters only for short explorations and foraging trips at night (Lawton and Lavalli 1995). These shelter restricted and emergent juvenile lobsters are most commonly distributed among cobble rocks within the first 20 m of water in coastal areas (Wahle and Steneck 1991, Wahle and Steneck 1992, Lawton and Lavalli 1995). However, competition for suitable shelter may force juveniles to migrate to less optimal habitats, resulting in decreased overall survivorship (Wahle and Steneck 1991, Paille et. al. 2002)

Shelter restricted and emergent juveniles primarily gain nutrients by filter feeding, and opportunistic foraging on items entering their shelter (Emmel 1908, D'Agostino 1980, Barshaw 1989, Lavalli 1991, Sainte-Marine and Chabot 2002, Brown 2006). Nevertheless stomach content analysis suggests both these stages may spend considerable time foraging outside their shelters (Sainte-Marie and Chabot 2002). When juveniles reach 30-40 mm CL they enter a vagile stage, in which they expand their foraging area outside of the shelter (Hudon 1987, Lawton 1987). As juvenile lobster grow, they begin to expand their exploration and foraging area until approximately 55-65 mm CL, at which point sexual maturation begins prior to becoming a full adult (Lawton and Lavalli 1995).

1.3 Effects of temperature on *Homarus americanus*

Low water temperatures (<8 °C) increase lobster development time from hatching to the postlarval stage (Templeman 1936) and result in decreased survivorship at the later larval stages (stage III, IV and V) (MacKenzie 1988). Increased temperature increases growth rates and decreases time to sexual maturity in *H. americanus* (Aiken 1977, Factor 1995). Molting, and thus growth rate, increases in lobster at temperatures between 15-20 °C (Aiken and Waddy 1980). However water temperatures below 5 °C can reduce number of molts and time between molts, in some

cases, completely stopping molting for up to 2 years (Aiken and Waddy 1986).

The larval stages avoid cold temperatures when selecting a settlement site, and remain in the water column and extend their planktonic life in the absence of optimal settlement temperatures (Boudreau et. al. 1992, Annis 2005, Annis et. al. 2013). This selectivity results in larvae settling closer to shorelines which typically exhibit warmer surface water temperatures (Jossi and Benway 2003). Historically, research on lobster settlement has focused on stage V postlarval behaviors, such as substrate and habitat preference (Botero and Atema 1982, Cobb et. al. 1983, Barshaw et. al. 1988), timing of settlement (Cobb et. al. 1989), shelter preference at settlement (Boudreau et. al. 1990, Burdett-Coutts et. al. 2014) and availability of suitable habitat (Whale and Steneck 1991, Lillis and Snelgrove 2010). Fewer studies have investigated the behavior of juvenile lobster (post-settlement). These studies have investigated shelter preferences (Cobb 1971) and conspecific interaction (Sastry 1980) in a laboratory setting and substrate and habitat use in the field (Hudon 1987, Able et. al. 1988, Wahle and Steneck 1991, 1992). However, all of this research has focused on animals maintained at 16-25 °C temperatures, and thus did not evaluate behaviors within the context of the full range of seasonal fluctuations in temperatures that lobster naturally encounter.

Adult lobster can survive in temperatures between -2 °C and 30.5 °C and are capable of withstanding sudden temperature drops of 20 °C (Harding 1992,); however, they prefer water temperatures of approximately 16-20 °C (Crossin et. al. 1998). Behavioral thermoregulation in adult lobsters consists of increasing activity and directional changes in movement, reducing activity once warmer temperatures are found. (Reynolds and Casterlin 1979, Crossin et. al. 1998). Adult *H. americanus* migrate seasonally with changes in temperature, moving inshore in the spring when waters start to warm and to deeper waters in winter months where water temperatures are more stable and less influenced by storm conditions (Cooper et. al. 1975, Cooper and Uzmann 1977, Ennis 1984a, 1984b). Recent studies have shown that there is some discrepancies between sexes and thermoregulation (Jury and Watson 2013), which may influence the migration patterns and seasonal changes in sex ratios.

1.4 Objectives

The objective of this research project consists of two parts. Objective 1A) investigate the temperature preference in juvenile *H. americanus* and the effects of juvenile size, acclimation temperature and origin (hatchery or

wild) on temperature preference. 1B) investigate basal activity rates across a temperature gradient with different habitat variables.

Objective 2A) determine the interactive effects of shelter and food on the temperature preferences of juvenile lobster. 2B). develop a decision tree model of the trade-offs juveniles make in order to secure food or shelter in relation to preferred temperature.

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2. Temperature Preference and Activity Level in Juvenile Lobster, *Homarus Americanus,* Exposed to Cold Environments

2.1 – Introduction

Temperature is among the most important environmental stimuli that affect the performance of ectotherms. Temperature influences physiological functions such as metabolic rate (reviewed by Clarke and Fraser 2004), immune function (Wang et. al. 2008), neural function (Montgomery and MacDonald 1990), digestion (Whiteley et. al. 2001, Robertson et. al. 2002) and growth rate (Angilletta et. al. 2004). Temperature also influences behaviors such as navigation (Lagerspetz and Vainio 2006) and foraging (Taylor and Collie 2003). Given the limited physiological mechanisms for maintaining body temperature (T_B) in ectotherms, behavioral thermoregulation is important for regulating T_B, because it uses minimal energy (Stevenson 1985).

All aquatic crustaceans are ectotherms and their T_B rapidly equilibrates with that of the surrounding environment (Payette and McGaw 2003). Crustaceans are capable of sensing changes in temperature

between 0.2 – 2.0 °C, depending on species, and primarily use movement to adjust T_B (Lagerspetz and Vainio 2006). Thermoregulatory movement is thought to result from klinokinesis, which is increased activity outside of preferred temperature ranges (Lagerspetz and Vainio 2006). Klinokinetic movement has been reported in a variety of crustaceans, including the crayfish *Orconectes rusticus*, which increases activity during periods of rapid temperature change to seek out more stable microclimates (Mundahl 1989). Likewise, the purple shore crab *Hemigrapsus nudus* exhibits rapid activity in order to find preferred temperatures when cooled (McGaw 2003). Klinokinetic movement is also seen in the crayfish *Orconectes immunis* (Crawshaw 1974), the isopod *Asellus aquaticus* (Lagerspetz 2003), the crayfish *Astacus astacus,* (Kivivouri 1980) and the American lobster, *Homarus americanus* (Reynolds and Casterlin 1979a, Crossin et. al. 1998).

In *H. americanus,* temperature influences physiological functions such as growth and moulting (Aiken 1977, Aiken and Waddy 1980, Aiken and Waddy 1986, Factor 1995), acid-base balance (Qadri et. al. 2007) and sensory function (McLeese 1970). *H. americanus* exhibits a broad thermal tolerance, ranging from 5 to 26 °C in larvae and -1.0 to 30.5 °C in adults (McLeese 1956, Hudon 1987). Adult *H. americanus* can sense changes in temperature of 0.5 °C (Jury and Watson 2000), and can tolerate abrupt temperature increases as large as 20 °C (Lawlor and Lavalli 1995). For *H.*

americanus, temperatures >25 °C result in an increased growth rate and metabolic rate, but also in higher incidences of mortality (Hartnoll 2001). Recent research has established the critical thermal maximum (*CTmax*) for adult *H. americanus* between 28 – 30 °C (Jost et. al. 2012). *CTmax* is the point where temperature forces the organism to change from aerobic to anaerobic respiration, which can result in increased mortality (Zielinski and Pörtner 1996). Frederich and Pörtner (2000) suggested defining temperatures outside the optimum range as the 'pejus range' and the boundaries of the optimum range as the pejus temperatures (*PT*). The *PT* for *H. americanus* is 14 – 16 °C and the pejus range 14 – 30 °C (Jost et. al. 2012). However, these authors only determined the *CTmax* and upper *PT* for *H. americanus*, and did not establish the critical thermal minimum (*CTmin*) and lower *PT*. These lower values are important because temperatures lower than 8 °C can result in inhibition of molting, reduced growth rates, and increased mortality for *H. americanus*, especially for larval stages (Aiken and Waddy 1986, MacKenzie 1988).

Temperature also influences behaviors in *H. americanus,* such as larval settlement (Boudreau et. al. 1992, Annis 2005, Annis et. al. 2013), movement (Reynolds and Casterlin 1979a, Crossin et. al. 1998, Cowan et. al. 2007) and shelter acquisition (Lillis 2009). The various life history stages of *H. americanus* use different thermoregulatory behaviors to maintain T_B

within its preferred temperature (T_{pref}). Larval *H. americanus* can sense and actively avoid cold thermoclines (decreases in temperature ≥6 °C), which are usually seen in the upper 30 m of water (Harding et. al. 1987). Stage V postlarvae avoid settling in temperatures <8 °C, and these postlarvae may move back into the water column and postpone settlement until the temperature increases above 10 °C (Boudreau et. al. 1992, Annis 2005). Adult *H. americanus* have a T_{pref} between 15 – 20 °C with a mean preference of 16 °C (Reynolds and Casterlin 1979a, Crossin et. al. 1998). Similar preferences occur in *H. americanus* in the wild, which migrates annually between deep and shallow water to maintain stable body temperatures (Cooper and Uzmann 1971). During the winter when nearshore ocean temperatures can drop below 0 °C, berried female *H. americanus* migrate to warmer (4 – 5 °C), deep water offshore, returning to inshore water in spring to expose their developing eggs to temperatures above 10 °C in order to decrease development time and for larval release (Cowan et. al. 2007).

While the physiological and behavioral effects of temperature change on larval and adult *H. americanus* are comparatively well documented, less is known about the thermophysiology and thermoregulatory behavior of juvenile *H. americanus*. Understanding juvenile thermoregulatory behavior is important because the early juvenile

period is a critical period of life, during which mortality typically ranges from 80 – 100% in marine invertebrates (Gosselin and Qian 1997). These high levels of juvenile mortality, combined with post-settlement dispersal in motile species, can disconnect larval settlement patterns from patterns of abundance of adults within the same population (Hunt and Scheibling 1997). As a result, events occurring specifically during juvenile stages can significantly influence population parameters such as abundance and distribution. This is important as lobster hatcheries usually release settling postlarvae and juvenile lobster into the wild at these life stages (Nicosia an Lavalli 1999). Better understanding of these life stages could result in increased hatchery success.

Physiologically, juvenile *H. americanus* are considered to be adults; they share similar morphology, anatomy, ventilatory and circulatory processes (Factor 1995). Juvenile *H. americanus* differ from their larval and adult counterparts in that they exhibit markedly different behaviors. Juveniles strongly prefer shelter and structurally complex substrates (Botero and Atema 1982, Hudon 1987, Johns and Mann 1987, Barshaw and Rich 1988, Wahle and Steneck 1991). Juvenile lobsters rely on these substrates and a cryptic shelter-restricted lifestyle after settlement because of their small size and lack of predatory defenses (Barshaw and Rich 1988). Temperature can alter the behavior of shelter restricted juveniles. Settled

shelter-restricted juveniles (4-6 mm CL), may emerge from shelter in response to sudden drops in temperature (>7 °C) in order to search for more suitable temperature regimes (Lillis 2009). However, the behaviors seen in this study used a low temperature of 2°C, which does not reflect the temperature changes that have been seen the northernmost geographic ranges of *H. americanus,* such as Newfoundland (Colbourne et. al. 2011), where *H. americanus* may experience rapid changes in temperature >7°C during the fall and winter months and sub-zero ocean temperatures for extended periods, where they are readily found, as determined by field study (Cowan et. al. 2001).

The goals of the present study were: 1) determine the T_{pref}, and behavioral *CTmin* for juvenile *H. americanus;* 2) investigate the interactions of temperature change, shelter availability, and food on the activity levels juvenile *H. americanus; and* 3) examine the effects of low temperature (<0 °C) on the activity and reaction responses of juvenile *H. americanus.*

2.2 – Materials and Methods

2.2.1 – Study Animals

I used juvenile American lobster, *Homarus americanus* between 7-30 mm CL (Stage V – IX) (Lawton and Lavalli 1995), in the temperature preference experiments. A total of 257 Juveniles were collected for these experiments. No individual lobster was used more than once, and all unused juveniles were handled as per Memorial University protocols. Juveniles were raised from larvae at the Ocean Sciences Center in Logy Bay, Newfoundland and Labrador. Juveniles were also collected from Cape Breton Island, Halifax, and Church Point, Nova Scotia, as well as from Walpole and West Boothbay Harbor, Maine. Prior to experiments the lobster were held in cylindrical cages (10 cm diameter x 13 cm x 15 cm) in flowing seawater. Two groups of 30 animals each were acclimated to either 8 °C or 18 °C for at least 60 days before experiments commenced. The 8 °C temperature was representative of the average annual ocean temperature in the near shore waters of Newfoundland (Colbourne et. al. 2011), where local juveniles are most likely to be found (Wahle and Steneck 1991). *H. americanus* hatcheries use a temperature of 18 °C as the standard culture temperature for hatcheries (Cobb and Phillips 1980, Nicosia and Lavalli 1999). Juveniles were fed a diet of fish, scallop meat, brine shrimp,

and mussel flesh once per week. Each individual lobster was fed ~5 g of food during each feeding, and excess food was removed after 48 h. Lobster were allowed to digest for at least 48 h after feeding and prior to use in experiments. The juveniles were constantly maintained in, and experiments were carried out in dim red light, because *H. americanus* is unable to sense red light (Bruno et. al. 1977), simulating a 24 h dark photoperiod. These conditions allowed visual observation of the lobster, while maintaining the high activity seen during 24 h dark photoperiod (Jury et. al. 2005). Once experiments started, they were carried out consecutively 24 h a day in order to reduce possible changes in activity from endogenous circadian rhythms.

2.2.2 – Temperature Preference Experiment

The experimental apparatus used to assess thermal preference consisted of a PVC pipe 330 cm in length and 10 cm in diameter, with 90° elbows affixed at each end. A 7 cm section was cut out along the top of the pipe, creating a long trough with sealed ends. The pipe was then insulated with neoprene foam along the outside of the wall to reduce fluctuations in temperature (Figure 2.1). An air diffusing tube or "curtain" in the bottom of the pipe maintained oxygenation of the water and also provided even mixing of the water throughout the apparatus, preventing

formation of a vertical temperature gradient. A heater with an adjustable controller [Finnex TH-500 plus] was placed at one end of the tube and a recirculating water bath attached to a stainless steel thermal transfer coil [VWR/polyscience 1180A] was used to cool the other end. Plastic grating placed in front of the cooling coil and heater prevented the juveniles from crawling too close to the heater or cooling coil. A low input (<1 l/h) of fresh seawater was added to the apparatus to maintain an ample supply of clean seawater in the apparatus. The experimental area of the tube was divided into 15 zones, each 15 cm in length, in order to record the relative position of the juvenile within the tube. The zones were labelled from 1 – 15 from left to right to span a temperature gradient of 5 – 25 °C. I used a temperature of 5 °C for the low temperature because in trial experiments, juveniles exposed to temperatures lower than 5 °C entered a moribund state similar to that seen in the spider crab, *Maja squinado* (Frederich and Pörtner 2000) and other subterranean crustaceans (reviewed in Issartel et. al. 2005). I set the temperature maximum at 25 °C because 100% mortality occurred in temperatures above 27 °C in trial experiments. Previous studies also show a 30 °C *CTmax* for most cold-water crustaceans and adult *H. americanus* (Frederich and Pörtner 2000, Jost et. al. 2012).

For experiments, I placed an individual lobster (n = 12 per acclimation temperature) at a randomly selected area along the length of the tube

and allowed it to choose its location. A random number generator was used for selecting the position. The position of the lobster was then recorded at 15 min intervals for a total time of 4 h. I chose a 4 h treatment period because preliminary trials showed that the animals chose their preferred temperature zone and moved little after 4 h. At each 15 min time period I recorded the temperature at the lobster's position using an electronic thermometer with a 0.5 mm diameter thermocouple [Sable Systems TC-1000 thermometer]. The thermocouple was sufficiently long (70 cm) to record the temperature next to the lobster without visually disturbing the lobster. Position data were used to calculate the distance travelled by the lobster during each 15 min period as well as total distance travelled in a 4 h period. The heating and cooling elements were switched to opposite ends of the tube for half of the replicates in order to correct for any possible end preferences by juvenile *H. americanus* unrelated to the temperature gradient (e.g. variation in ambient light etc.).

I included control experiments at 8 °C and 18 °C to test that lobster exhibited no preference for a specific area of the tube and also to provide baseline movement data for activity levels with no temperature gradient present. Juveniles acclimated to both 8 °C and (n=20) and 18 °C (n=20) were used in controls for a total of four combinations (ten 8 °C acclimated juveniles in 8 °C control, ten 8 °C acclimated juveniles in 18 °C control, ten

18 °C acclimated juveniles in 8 °C control, and ten 18 °C acclimated juveniles in 18 °C control). During the trials, I recorded lobster position and distance travelled as outlined for the temperature gradient experiments.

The preferred temperature range was determined by taking the mean temperature reading (±SEM) at the final position that each lobster chose within each temperature gradient (Crossin et. al. 1998, Haro 1991). I analyzed preferred temperature using student's t-tests to determine whether juveniles acclimated to different temperatures, if there were differences between juveniles from wild or hatchery origins, or differences between shelter-restricted (<25 mm CL; n = 20) and vagile (>25 mm CL; n = 20) size class of juveniles differed significantly in temperature preference. Juveniles from different geographic regions were acclimated to laboratory conditions for 60 days prior to experimentation to abolish previous acclimation temperatures. Juveniles of wild (n=20) or hatchery (n = 20) origin were tested to determine if there was a difference in temperature preference between the two groups. I chose juveniles of 25 mm CL because previous studies identify this as the approximate size when juveniles change from shelter restricted behaviors to more exploratory behaviors (Lawton and Lavalli 1995). A one-way ANOVA GLM with Tukey's post-hoc test was performed to test for significant differences in activity between the two controls, and the three temperature treatments.

2.2.3 –Activity Level Experiment

I investigated activity levels of juvenile lobster over a 5 – 20 °C temperature range. Lobsters ranging in size from 15 – 27 mm CL were acclimated to seawater of 8 – 10 °C for at least 60 days prior to experiments. This temperature was chosen because my temperature preference experiments showed no significant effect of acclimation on activity level or temperature preferences; however, lobster held at 16 – 18 °C exhibited 30 mortalities in 21 days. This high mortality is likely because the warmer temperatures induced moulting (Aiken 1977) that was beyond their physical capability to endure in a laboratory setting. Bacterial and fungal buildup in the tanks, which was noted as foul-smelling biofilms and was also more prevalent at these temperatures, and it is possible that pathological infection also attributed to some of the mortalities at these temperatures.

The experimental apparatus consisted of 5 individual circular chambers, 30 cm diameter x 25 cm high, with a 10 cm water depth. I drilled several 2 mm diameter holes in the sides and bottom of the chambers to allow free circulation of water. The chambers were placed in an insulated seawater table of 120 cm x 100 cm. A sump tank of aerated seawater was maintained at experimental temperatures using a recirculating water bath.

Water pumps provided a constant flow of each test temperature from the sump to the experimental chambers (Figure 2.2). I monitored velocity and distance travelled for each animal using Noldus Ethovision XT™ video recording and analysis software. The software recorded relative XY coordinates of each specimen, and calculated the distance travelled (cm) and velocity (cm/s). I then calculated velocity (cm/min) from these data. The entire tank apparatus and video monitoring system was enclosed in black vinyl sheeting and illuminated using red fluorescent lighting that minimized visual disturbance to the juveniles as the juveniles are incapable of sensing red light (Bruno et. al. 1977), this also had the added effect of stabilizing the lobster's circadian cycles, preventing maintaining a more stable level of activity (Jury et. al. 2005).

I placed an individual juvenile lobster in each chamber at 20 °C for 5 h immediately after transfer to determine the effects of handling and transfer on behavior. Following these initial observations I maintained and monitored the lobster at this starting temperature (20 °C) and monitored for further 5 h. I then cooled the temperature to 15 °C over a period of 5 h, a temperature change of 1°C per hour was used because it is known to illicit a physiological change in *H. americanus* (Jury and Watson 2000), and we wished to know there was a difference in activity between changing and stable temperatures. The temperature was maintained at 15 °C for a further

5 h. This process was repeated, dropping the temperature over 5 h to 10 °C and maintaining this temperature for a further 5 h, before the temperatures was dropped again to 5 °C. The experiment was then carried out in reverse (using a different individual for all experiments) starting at 5 °C and increasing the water temperature in 5 °C increments until it reached the highest temperature (20 °C).

The experiments consisted of four different treatments (n=80), with 20 replicates per treatment (10 lobsters in cooling and 10 in warming). The first treatment was the control, which contained only a single *H. americanus* in each chamber. This data established baseline activity level for a novel environment. In the second series of experiments, a shelter was added to each chamber. The shelter was similar to the shelters used by Cobb (1971) and consisted of an opaque acrylic tube (10 cm x 5 cm diameter) open at both ends. The shelter was placed on the left side of the chamber for half the replicates and on the right side for the other half, in order to prevent any side bias. In the third series of experiments, I added scallop meat (*Placopecten magellanicus*) to the chamber. Scallop meat was used because it is a readily available, known food source for lobster with similar coloration as the chamber that did not interfere with the video tracking system. I sealed the food inside a perforated Eppendorf tube and placed on the left side of the chamber for half the replicates and the right side for

the other half. This strategy eliminated any potential side biases. The final treatment combined the shelter and food treatments. In this case I placed the food and shelter on the left and right side of the apparatus, respectively, and switched to opposite sides for half the replicates, as was done in the food and shelter experiment.

I investigated the activity levels of animals (cm/min) separately for the changeover and stable temperature periods. Initial analysis showed no significant difference between warming and cooling treatments, so I combined these data for further analysis. A 2-way ANOVA with Tukey's post hoc test was used to analyse activity levels between the 5 hour changeover/stable temperature periods. I compared activity levels at each stable temperature and each treatment with a 2-way repeated measures ANOVA, followed by a Tukey post-hoc test where significant differences occurred.

I analyzed the activity levels of the animals during the first 5 h of data after the lobsters were placed in the apparatus to investigate the effects of handling and rapid thermal shift on behaviour. The data were averaged over 15 min intervals, removing outliers (±3 standard deviations from the mean) that represented tracking errors from the contrast tracking system picking up shadows (personal observation) These outliers were rare (<5% of total data), and are common with this type of contrast imaging system.

After transforming the data into a running average I performed a repeated measures ANOVA with Tukey's post-hoc test to determine significant differences in activity between the different 15-minute intervals following the initial transfer of the animals into the apparatus.

2.2.4 – Extreme Low Temperature Behavior Experiment

As a result of the difficulty in reducing and maintaining seawater temperatures below 5 °C and the torpor like state that juvenile *H. americanus* entered at temperatures of approximately 0 – 3 °C (personal observation), I performed a separate experiment to investigate the behavioral responses of juvenile lobster in temperatures below 5 °C. Ten cylindrical cages (25 x 15 cm diameter) were placed in a tank (70 x 50 x 15 cm) and an individual lobster was placed into each cage (n=20). The apparatus was maintained in a walk-in freezer (-12 °C) in total darkness, using a red light to inspect the tank during data collection. A titanium aquarium heater [Finnex TH-500 plus] and water pump [Eheim 1200 Universal] maintained the tank at an initial temperature of 5 °C. The juvenile *H. americanus* were transferred to the experimental apparatus and allowed to acclimate to the initial experimental conditions for 2 h, as it was found in the activity experiments that only 90min of acclimation time was needed

to see normal activity levels. Following this procedure I reduced the water temperature to -2 °C, over a 4.5 h period. This temperature drop represents a similar drop in temperature (>7°C) to those experiments done by Lillis (2009). During this time, each lobster was assessed every 15 min for three levels of reaction: 1) Basal activity, which included movement of the mouthparts, antennae, chelae and pereiopods. 2) Reaction to visual stimuli, observing reactions of lobster to 5 s of bright light, which was produced by using a underwater LED flashlight held over the tank at a distance of ~30 cm. 3) Reaction to physical stimuli, where I attempted to flip the lobster over with a glass probe and observed any reaction or righting response.

Once -2.5 °C was reached, I maintained the juvenile *H. americanus* at this temperature and assessed each for the three conditions once every 15 min until all individuals ceased responding to physical stimuli. I then raised the water temperature at a rate of1 °C/h to prevent heat shock. The status of the juveniles was monitored at 1, 2, 4, and 8 h, recording any mortalities and removing moribund individuals from the tank. The juveniles were then placed back in their 8 °C holding tanks and observed 12, 24 and 48 h later for the three conditions and any mortalities. I then grouped the data into 0.5 °C categories (5 °C to -2.5 °C) and analyzed the different variables separately using a chi-square test to test for significant differences in the

number of responding individuals at different temperatures, which allowed me to discern the temperature at which the lobsters' became non-responsive to the different reaction levels.

2.2.5 – Near-Shore Seasonal Ocean Temperature Assessment

Near shore ocean temperatures were monitored in coastal Newfoundland by placing temperature data loggers [Alpha Mach Inc. iBCod, Ste-Julie, QC, Canada] at 3, 6, and 9 m depths at Bay Bulls, NL (N 47°18.170', W 52°48.100'). This location was used because it gave a good profile of temperatures at a site that was actively used for lobster fishing, and was previously assessed as a potential hatchery release site for lobster. These loggers recorded the water temperature at 4 hour intervals for ~1 year before recovery. I then binned these data into average daily temperatures and analyzed the time series data with a one-way ANOVA to determine significant differences in temperature between the three depths.

2.3 – Results

2.3.1 – Temperature Preference Experiment

In the gradient experiments, I found no difference in temperature preference between hatchery raised or wild lobsters (t-test, df = 38, t = 2.14, P = 0.148); the T_{pref} of hatchery raised lobster was 15.1 ± 2.3 °C and the T_{pref} of wild caught lobster was 17.3 ± 2.0 °C. I also found no difference in T_{pref} between lobsters held at 8 °C or 18 °C acclimation temperature (t-test, df = 38, t = 0.47, P = 0.494); the T_{pref} of lobster acclimated to 8 °C was 17.4 ± 2.2 °C and the T_{pref} of lobster acclimated to 18 °C was 15.00 ± 2.0 °C. There was no difference in T_{pref} between lobsters of <25 mm CL and >25 mm CL (t-test, df = 38, t = 1.71., P = 0.195); lobsters <25 mm CL preferred 16.6 ± 2.5 °C and lobsters >25 mm CL preferred 16.2 ± 1.6 °C. Given the absence of significant differences in T_{pref} (Table 2.1) as a function of origin, acclimation temperature, or size, I pooled these data for subsequent analyses.

Distance travelled differed significantly between the control treatments and the temperature gradient treatments. Individuals in the two control treatments (8 °C and 18 °C) traveled significantly greater distances than individuals in the temperature gradient treatments (ANOVA, df = 4, F = 298.33, P < 0.0001). The average distance travelled in the stable control treatments was 1164.2 ± 36.7 cm over 4 hours compared to 325.1 ± 31.8 cm

in the temperature gradient treatments over the same time period. Individuals in the two control treatments did not differ significantly in distance travelled.

2.3.2 – Activity Level and Handling Time Experiment

2.3.2.1 – Activity level experiment.

I found no significant difference in overall distance travelled between warming and cooling periods (ANOVA, df = 1, F = 0.22, P =0.645); the mean activity in the warming and cooling treatments were 7.47 ± 0.16 cm/min and 8.1 ± 0.15 cm/min respectively. As a result, I combined warming and cooling data for further analysis.

The 5-hour changeover and stable temperature periods differed significantly (ANOVA, df = 1, F = 16.78, P < 0.0001), with a significant interactive effect between temperature and changeover / stable periods (ANOVA, df = 3, F = 8.82, P < 0.001; Figure 2.3). The lowest activity occurred in the 5 °C stable period (5.27 ± 0.17 cm/min), and the highest hourly activity occurred during the 15 – 20 °C changeover period (9.85 ± 0.42 cm/min). The activity in 5 °C water was significantly lower compared to all other temperature ranges (Tukey HSD, P < 0.001). I observed a pronounced increase in activity when raising the water temperature from 5 to 10 °C, but

activity remained unchanged once the water temperature reached the 10 °C stable treatment period (Tukey HSD, P = 0.9978). Activity remained unchanged when the water temperature was raised again from 10 – 15 °C (Tukey HSD, P = 0.1792), with no significant difference in activity between the 10 and 15 °C stable temperature periods (Tukey HSD, P = 1.000). Activity increased rapidly when the water temperature was raised from 15 to 20 °C (Tukey HSD, P = 0.001). Thereafter, activity declined significantly during 20 °C stable period (Tukey HSD, P = 0.003) to levels that were similar to those measured during the 15 °C stable temperature period.

In comparing activity levels between each stable temperature and experimental treatment I found a significant temperature effect (ANOVA, df = 3, F = 31.61, P < 0.001; Figure 2.4), with the lowest activity at 5 °C (5.27 ± 0.17 cm/min) and increasing activity with increasing temperature up to 7.96 ± 0.28 cm/min at 15 °C. Activity increased significantly from 5 °C to 10 °C (Tukey HSD, P = 0.05), and from 10 °C to 15 °C (Tukey HSD, P < 0.001), but not from15 °C to 20 °C (Tukey HSD, P = 0.95), or 10 °C to 20 °C (Tukey HSD, P = 0.143).

Activity also differed significantly between treatments (ANOVA, df = 3, F = 62.82, P < 0.001; Figure 2.5). The highest activity occurred in the combined food and shelter treatment (9.39 ± 0.3 cm/min), and this activity

was significantly higher than all other treatments (STAT). The lowest activity occurred in the control treatment (5.45 ± 0.19 cm/min). The distance travelled did not differ significantly between the control and shelter treatments (Tukey HSD, P = 0.148)1, but was significantly lower than the other 2 treatments (Tukey HSD, P < 0.001).

The significant interactive effect between treatment and temperature (ANOVA, df = 9, F = 2.92, P = 0.002; Figure 2.6) indicated a significant difference in how temperature influenced treatments. Despite similar patterns in temperature response in the control, food, and combined food and shelter treatments, there was a significant interaction between shelter and temperature. The shelter treatment did not follow the same pattern as other treatments (Figure 2.4). At 5 °C, the shelter treatment was not significantly different compared with the control treatment (Tukey HSD, P = 0.514) or food treatment (Tukey HSD, P = 0.937). At 10 °C activity rates in the shelter treatment were significantly higher than the control (Tukey HSD, P < 0.001), but similar to the food treatment at 10 °C (Tukey HSD, P = 0.208) and the combined food and shelter treatment (Tukey HSD, P = 0.208). At 15 °C activity declined significantly compared with the food treatment (Tukey HSD, P = 0.035), and the combined food and shelter treatment (Tukey HSD. P <0.001). At 15 °C and 20 °C the activity in the shelter treatment was similar to the control (Tukey HSD, P = 0.999), and the food (Tukey HSD, P = 1.000)

treatments, but significantly lower than the combined food and shelter treatment (Tukey HSD, $P < 0.001$).

2.3.2.2 – Handling time

In monitoring lobster activity levels for 5 h after they were first placed in the apparatus (Figure 2.7), I found a significant difference in distance travelled as a function of time (ANOVA, df = 20, $F = 3.02$, $P < 0.001$). The activity levels were significantly higher during the first 90 min (Tukey HSD, $P = 0.0292$), followed by a significant drop thereafter; activity levels were not significantly different from one another during the remaining 3.5 h experimental period (Tukey, $P>0.05$). As a result, I set the acclimation time to allow the animal to settle after handling at 90 minutes for all subsequent experiments.

2.3.3 – Extreme Low Temperature Behavior Experiment

The number of juveniles displaying basal activity (X^2, df = 14, $X^2 = 135.385$, $P < 0.001$; Figure 2.8) and the number of juveniles responding to visual stimuli (X^2, df = 14, $X^2 = 43.3367$, $P < 0.001$) changed significantly as temperature decreased. Basal activity ceased in all lobsters at ~1 °C, and

reaction to visual stimuli ceased at approximately -2 °C. However, I observed no significant difference in the number of lobsters responding to physical stimuli between 5 °C and -2 °C (X^2, df = 14, X^2 = 3.29412, P = 0.998). Thirteen of the 20 juveniles reacted to physical stimuli even at -2.5 °C. Of these 13 juveniles, all lost righting response after exposure to -2.5 °C for an additional 2 h. As a result, despite the absence of a significant difference in the number of lobsters responding to physical stimuli as temperature decreased, there was a significant difference in the number of lobsters responding to physical stimuli as a function of exposure duration (X^2, df = 14, X^2 = 50.4549, P < 0.001). After the experiment was completed, I noted only one mortality during the recovery period, which occurred 12 h after experiment completion, all lobsters were observed for a total of 48 hours and no other mortalities were recorded.

2.3.4 Near-Shore Seasonal Ocean Temperature Assessment

Near shore ocean temperatures for Bay Bulls, NL ranged from -1.5 °C to 14 °C over the course of the year. The lowest temperatures occurred in March through April and the highest temperatures in September through October (Figure 2.9). There was no significant difference between temperatures at 3, 6 and 9 m (ANOVA df =2, F = 1.15, P = 0.316), despite

some variation in pattern. At the beginning of the fall, there were periods when pronounced bouts of low temperature occurred. However, these drops only lasted 2-3 days before rising again to a stable temperature.

2.4 – Discussion

2.4.1 – Temperature Preference

Juvenile *Homarus americanus* exhibited a fairly wide temperature preference range from 13 °C to 19 °C. These findings parallel previous studies on adult lobster (Reynolds and Casterlin 1979a, Crossin et. al. 1998, Jury and Watson 2013) and support the inference that juvenile lobster are physiologically similar to adults (Factor 1995). Juvenile lobster avoided temperatures below 5 °C. Previous research showed that adults avoided temperatures below 5 °C, resulting in increased activity directed away from cooler temperatures (Reynolds and Casterlin 1979a, Crossin et. al. 1998, Jury and Watson 2013). Avoidance of these low temperatures probably occurs because it approaches the pejus range and lower critical thermal minimum (*CTmin*) for lobsters, where the low temperature reduces their aerobic capacity (Frederich and Pörtner 2000, Pörtner et. al. 2010). The fact that animals entered a moribund state and began to lose their righting response when exposed to temperatures below < 5°C supports this inference (personal observation). Other life stages of *H. americanus* also avoid low

temperature regimes; settling stage IV and V lobster avoid low temperatures below thermoclines (Boudreau et. al. 1992, Annis 2005, Annis et. al. 2013) and newly settled stage V and VI juveniles become more active and seek new environments when the temperature drops below 7 °C (Lillis 2009).

Juvenile lobsters did not show a pronounced avoidance behaviour in higher temperatures (>25 °C). In the experimental trials,10 juveniles entered temperatures above 27°C during these experiments, upon entering temperatures above 27 °C, four immediately exited the area (personal observation), and the remaining 6 individuals died within 30 min (personal observation). Although these juveniles may be seeking warmer temperatures to help increase growth (Templeman 1936, Aiken 1977, Aiken and Waddy 1986), it was likely that the rapid thermal equilibration led to heat shock and mortality (Jury and Watson 2000, Jost et. al. 2013). Adult *H. americanus* also suffer increased mortality when rapidly exposed to 30 °C without prior acclimation to temperatures >20 °C, however their larger body and increased thermal inertia means this occurs less rapidly than observed here for juveniles (McLeese 1956).

The T_{pref} for juvenile *H. americanu*s used in lab experiments appeared independent of acclimation temperature. In contrast, T_{pref} in adult lobster depends strongly on acclimation temperature, exhibiting a higher

preference range with increasing acclimation temperature (McLeese 1956, Reynolds and Casterlin 1979a, Crossin et. al. 1998, Jury and Watson 2013). Similar changes have been observed in preference as a function of acclimation temperature in the purple shore crab, *Hemigrapsus nudus* (McGaw 2003), the bluegill sunfish, *Lepomis macrochirus* (Reynolds and Casterlin 1979b), chinook salmon and sockeye salmon (Brett 1952). Because the T_{pref} was quite broad in juvenile lobster, temperature is probably not a primary defining factor in habitat choice. Juvenile *H. americanus* depend on a cryptic lifestyle to defend themselves against predation (Wahle and Steneck 1992, Spanier et. al. 1998, Oppenheim and Wahle 2013) and prefer rocky cobble or boulder patches where they can hide between the cracks (Cobb 1971, Botero and Atema 1982, Barshaw and rich 1988, Wahle and Steneck 1992). During our experiments juvenile lobster tended to remain near the edges of the apparatus or sit next to the air stone holders in the temperature gradient tube (personal observation). Juvenile lobsters actively seek out shelter when exposed to a variety of substrates and move against the edges of tanks if no shelter is available (Barshaw and Rich 1988). Potentially, this thigmotactic, shelter seeking behavior may have led to a broader preference range that obscured any effects of acclimation temperature.

The temperature preference of juvenile lobsters in the lab was also unrelated to their geographical origin. Adult lobsters collected from different locations in New Brunswick, Canada and New Hampshire, USA exhibit similar thermal preferences (McLeese and Wilder 1958, Crossin et. al. 1998, Jury and Watson 2013). Thermal histories may be abolished after approximately 30 days of exposure to new temperature regimes (McLeese 1956, Reynolds and Casterlin 1979a, Rastrick and Whiteley 2011) and the 60 day period over which lobsters in my study were acclimated to laboratory conditions was probably ample time to remove any effects of geographical origin. Stilman and Somero (2000) noted that for some populations of crustaceans (e.g. genus *Petrolisthes*) acclimation will not abolish their original T_{pref} despite broad temperature preferences. However, it should be noted that these populations live close to their *CTmax* and are therefore incapable of adapting well (Stilman and Somero 2000, Stilman 2003).

Lobsters were more active in the stable temperature control experiments (8 °C and 18 °C) compared with the temperature gradient experiment. Given that temperature influences metabolic rate in *H. americanus* (Worden et. al. 2006) one would expect greater activity in lobsters in the 18 °C control treatments. Although lobsters in the 18 °C control did exhibit a slightly higher (1265.6 ± 46.4 cm) total distance travelled

than those in the 8 °C controls (1162.7 ± 52.0cm) the difference was statistically insignificant. The experimental apparatus contained air curtain holders situated 15 cm apart along the gradient tube (Figure 2.1), the lobsters appeared to sit next to these curtain holders using thigmotactic feedback as a shelter response. When given a choice between multiple habitats in the lab, juvenile lobsters migrate between multiple habitats, increasing their overall activity (Cobb 1971), which likely occurred here as well. Adult lobsters do not actively avoid temperatures of 8 °C and there is no significant increase in activity within the 8 – 18 °C temperature range (McLeese and wilder 1958, Jury 1999). As a result, the similar activity seen at 8 °C and 18 °C for juvenile suggests a modest role for temperature. More likely, access to multiple shelters of similar value and a uniform temperature across the tube resulted in increased exploration (Cobb 1971).

2.4.2 – Handling Time and Activity Levels

2.4.2.1 – Handling Time

When H. americanus juveniles were transferred from the holding tanks into the apparatus their activity levels were high during the first 90 minutes. This high level of activity was followed by a constant and more gradual decline in activity over the next 4 h (Figure 2.7). These results parallel those

seen in experiments on adult lobster, where activity declined after 30 – 60 minutes (Reynolds and Casterlin 1979a, Crossin et. al. 1998). *H. americanus* is sensitive to rapid temperature changes (>0.5° C/min), and can sense these changes in as little as 4 seconds, resulting in increased in heart rate (Jury and Watson 2000). When I added a lobster to the apparatus, it immediately experienced a temperature change of either 3 °C (for lobsters in the warming treatments) or 11 °C (for lobsters in the cooling treatments). The emersion/immersion and temperature change was the only time lobsters were handled in this experiment. *Homarus gammarus* adults recover rapidly from disturbance and emersion (Whiteley et. al. 1990), and their stress indicators return to pre-handling rates within 30 min, even after 14 h of emersion (Whiteley and Taylor 1992). Eliciting a physiological response to emersion and handling stress in juvenile *H. americanus* requires at least 15 min of exposure to air or a temperature change of at least 13 °C (Chang et. al. 1999, Spees et. al. 2002). Given that my emersion periods were less than 5 min and initial temperature changes less than 13 °C, juveniles likely experienced negligible thermal, emersion and handling stress; the fact that activity rates decreased substantially within the first 90 minutes backs up this assumption. Thus, I omitted the first 90 minutes of observations from my analysis to remove handling effects from my experimental data.

2.4.2.2 – Activity level experiment

I observed a parabolic relationship between temperature and activity, with the highest activity at 15 °C, followed by a slight but insignificant decrease at 20 °C. Activity peaked near T_{pref} and then declined as temperatures moved towards the pejus ranges (McLeese 1956, McLeese and Wilder 1958, Reynolds and Casterlin 1979a, Jury and Watson 2013). Lyons et al. (2013) used accelerometers to show a direct link between adult lobster activity and metabolic rate. Therefore, the activity pattern as a function of temperature may be considered as the metabolic performance curve for juvenile *H. americanus, with* maximized activity within an optimal performance range (Huey and Kingsolver 1989).

The build-up of metabolites may drive the decline in activity levels at the highest temperature tested. Lactate concentration, heat shock protein and AMP-activated protein kinase, which are all indicators of stress and reduced oxygen uptake, begin to increase with increasing temperature in *H. americanus,* starting as low as 14 °C (Jost et. al. 2012). At temperatures ≥20 °C these stress indicators begin to affect oxygen uptake, resulting in reduced activity rates in *H. americanus* (Jost et al. 2012. The pejus range for adult lobster spans 12 – 16 °C (Jost et. al. 2012). However, this pejus range

lies within the preferred temperature range demonstrated in my study, and indeed falls within the T_{pref} range of *H. americanus* reported in other studies (McLeese 1956, Reynolds and Casterlin 1979a, Crossin et. al. 1998, Jost et. al. 2012). This means that the pejus temperature of individual *H. americanus* may vary, and could be potentially influenced by both biotic and abiotic factors. Studies of pejus ranges in *Cancer irroratus* (Frederich et. al. 2009), and *Maja squidano* (Frederich and Pörtner 2000) show a point where stress-related factors such as lactate, succinate, and HSP70 begin to accumulate, eventually influencing individual oxygen uptake ability. Previous studies define the point where these physiological changes begin to reduce oxygen uptake and activity as the *PT* for that species (Jost et. al. 2012). Some individuals tolerate these changes more than others (Frederich et. al. 2009); the basis for this variation in tolerance remains unclear, and requires further study.

Activity rates were higher during the changeover periods compared to the associated stable temperature period. *H. americanus* respond immediately to temperature changes as low as 0.5 °C/min with significant changes in heart rate (Jury and Watson 2000). These responses appear to be a temporary stress response, given the regaining of normal heart rates within 6 minutes of temperature acclimation (Jury and Watson 2000). The temperature change in my experiment was slower (0.017 °C/min), however,

increased juvenile activity during the changeover periods suggests a capacity to sense and respond to small changes in temperature; Jury and Watson (2000) noted that *H. americanus* could sense temperature changes as low as 0.15 °C per minute.

Activity rates were lowest in empty chambers and did not change significantly when a shelter was added. The addition of food significantly increased hourly activity rates, which increased further when shelter was added. In a bare environment, increased activity is associated with exploration (Cobb 1971); small lobsters are vulnerable to predation and constantly seek shelter (Wahle and Steneck 1992, Oppenheim and Wahle 2013). I found that adding shelter produced no overall change in activity. Given that juveniles retreat and remain in shelter for protection (Barshaw and Rich 1988), decreased overall activity might be expected relative to an empty chamber. However lobsters do not simply occupy a shelter, they actively maintain it (Cobb 1971, Lawton 1987, Karnofsky et. al. 1989, Rossong et. al. 2011). Maintaining shelter includes removing sand and debris from the inside of the shelter, and moving rocks to one end of the shelter to create a barrier (Cobb 1971, Lawton 1987, Karnofsky et. al. 1989, Rossong et. al. 2011). In my lab experiments, shelter maintenance included movement in and around the shelter, changing shelter orientation, and attempted movement of the shelter itself (personal observations). I also

observed an interactive effect between temperature and shelter; activity rates were similar for 15 and 20 °C control and shelter treatments, but higher when shelter was present in 10 °C and 5 °C. In these warmer temperatures, reduced juvenile activity suggests they are at T_{pref} and maintaining their shelter (Cobb 1971, Lawton 1987). Given that metabolism and activity relate directly to temperature in *H. americanus* (Lyons et. al. 2013) lower juvenile activity might be expected at cooler temperatures. Newly settling juveniles actually respond to sudden drops in temperatures below 7 °C by becoming more active and exiting their shelter (Lillis, 2009), possibly to re-enter the water column. Alternatively these older juveniles may have left their shelters in order to find more optimal temperature regimes (Lillis 2009). However, the results of this experiment are somewhat contrived because the lobsters were experiencing rapid changes temperature, which they would only experience rarely in the wild due to upwelling/downwelling events.

At all temperatures, addition of food to the chamber resulted in increased distance travelled at all temperatures. When a food odor is picked up by chemosensory setae on the lobster antennules, it often initiates a searching response (Derby and Atema 1982, Stein et. al. 1975). In my experiments this search response caused increased activity. The lobster handled and attempted to eat the food. Once crustaceans have fed they

tend to become inactive to allow digestion (McGaw, 2007, Bernatis et. al. 2007). However, I sealed the food sealed inside a tube, precluding consumption, and multiple attempts to access the food likely produced the continued high activity.

The addition of a shelter when food was present further increased the distance travelled compared to food alone. With shelter alone, juveniles spend their time maintaining their shelter (Cobb 1971, Lawton 1987, Karnofsky et. al. 1989), which increases activity. In my experiments food addition increased the frequency of excursions and duration of active foraging away from shelter. Lobsters could not access the food in my experiments, resulting in multiple attempts, including unsuccessful attempts by juveniles to move the food closer to their shelter (personal observation). These foraging and shelter-maintaining activities resulted in increased activity to secure food near their shelter.

2.4.3 – Effects of Extreme Cooling

Juvenile *H. americanus* cease basal activity (movement of mouthparts, pleopods, and antennae/antennules) at 1.0 °C. Loss of visual reaction follows at -2.0 °C, with loss of reaction to physical stimuli and righting response after three hours at -2.0 °C. Other decapods that inhabit

temperature ranges similar to *H. americanus,* (*Cambarus acuminatus* (Mirenda and Dimock 1985) *Astacus astacus* (Kivivouri 1980) and *Maja squinado* (Frederich and Pörtner 2000) also show reduced basal activity and reaction to physical stimuli at a similar temperatures range (<1 °C).

The cooling period of this experiment was 4 h (rate of 0.05°C/min), which is a rapid change in temperature than only occurs rarely in the field (Figure 2.9). Temperature logger data showed near-shore ocean temperatures reach lows of approximately -1.9 °C, and that the greatest temperature change occurred in mid-September, changing 9.5 °C over 24 h (rate of ~0.007 °C/min) (figure 2.9, an order of magnitude difference. My experiment demonstrated the loss of basal function and reaction to visual and physical stimuli to prolonged exposure to -2.0 °C. However, juveniles in the wild are active at these temperatures, and explore outside their shelters and react to stimuli, though their movement rates are compromised (Cowan et al. 2001). My experiment suggests that juveniles cannot deal with rapid temperature changes and lose reaction ability when seawater temperatures drop to near freezing. However, Juveniles can adjust to seasonal changes which take place slowly, maintaining sensory and motor function, and rarely experience such rapid temperature change in the wild (Figure 2.9, Hudon 1987, Jossi and Benway 2003, Colbourne et al. 2011), rarely increasing their risk of predation.

Chilling is used to transport adult lobsters because it reduces their metabolic rate and decreases oxygen requirements, allowing transport of live lobsters without inducing fatal systemic hypoxia or ion imbalances (Lorenzon et. al . 2007). Even chilling periods in excess of 24 h result in low mortality (Danford et. al. 1999). I observed only one instance of mortality within 48 h of the completion of the experiment, and found no evidence to link the mortality to temperature. Juvenile *H. americanus* can experience temperatures below 0 °C for several months in the wild, especially in the northern boundaries of their geographic range (Jossi and Benway 2003, Colbourne et. al. 2011)., Even in these low temperatures, temperature apparently has less effect on lobster than the need for shelter acquisition, as shown in the next chapter.

2.5 – Tables and Figures

Table 2.1 - Temperature preference (±SEM) for the different temperature gradient variables tested.

Variable	Temperature Preference (°C)
Acclimation Temperature	
8 °C	17.4 ± 2.2 °C
18 °C	15.0 ± 2.0 °C
Lobster Origin	
Hatchery	15.1 ± 2.3 °C
Wild	17.3 ± 2.0 °C
Size Class	
<25 mm CL	16.6 ± 2.5 °C
>25 mm CL	16.2 ± 1.6 °C

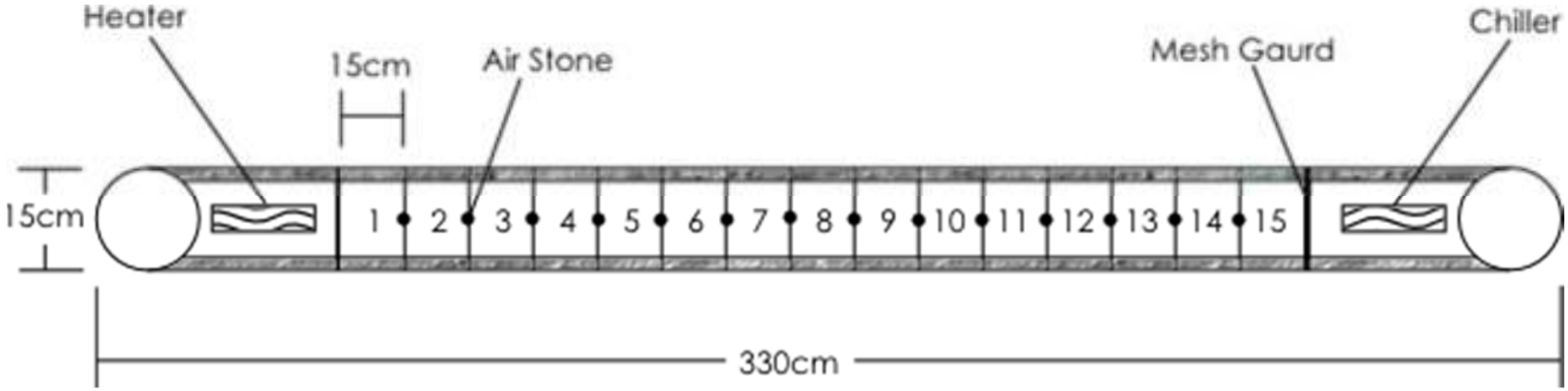

Figure 2.1 – Temperature gradient apparatus (top view). Direction of water flow into the system was randomly selected for each treatment, entering at either the left or right end of the system.

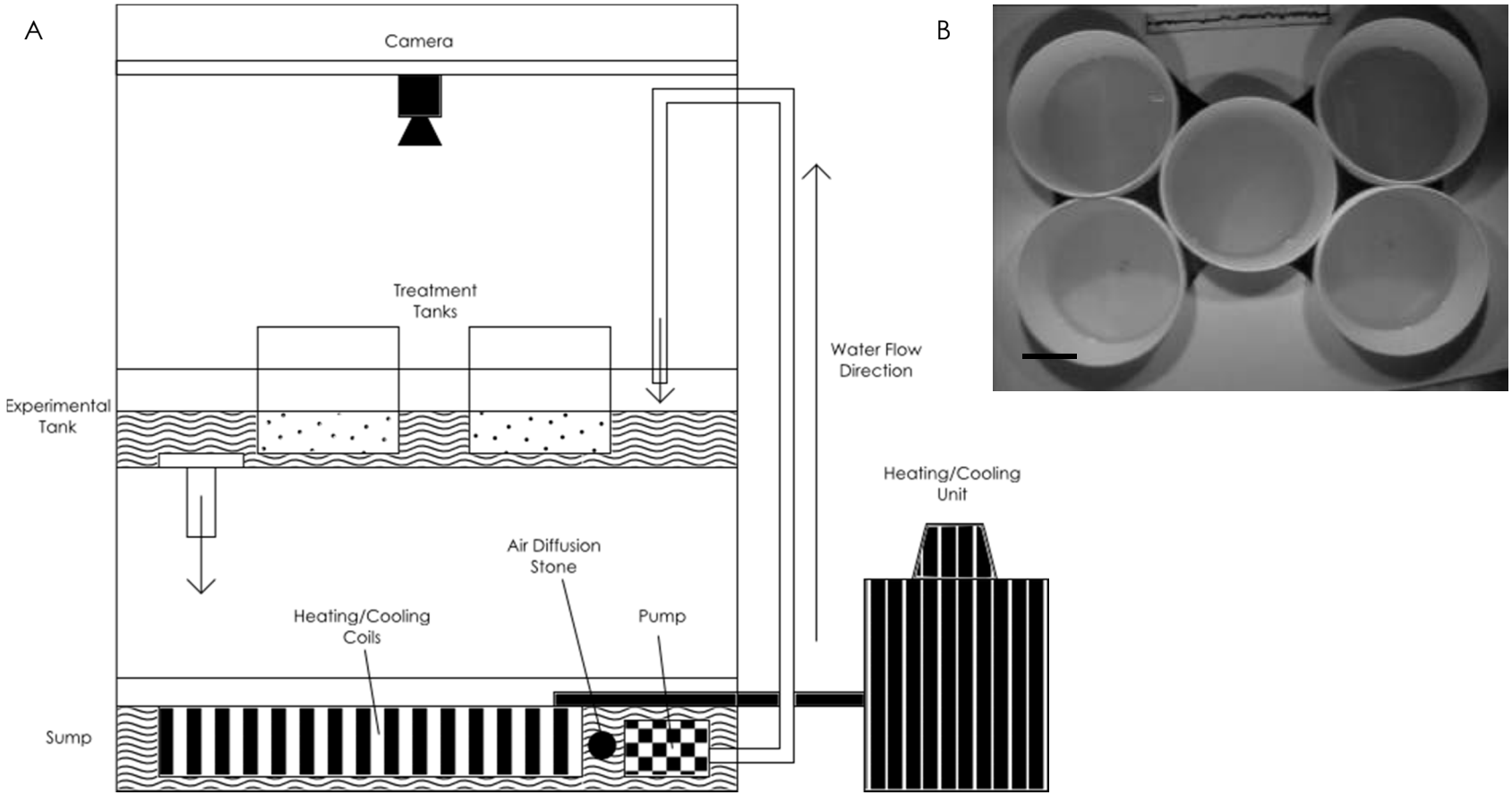

Figure 2.2 – A) Side view of the temperature control and activity analysis apparatus. Water was conditioned in the bottom tank and pumped to the top tank at a constant rate. Juvenile lobsters were viewed from above with a CCTV camera under red light. B) Treatment tanks viewed from above as seen by the CCTV camera using Noldus Ethovision™ software, scale bar represents 10 cm.

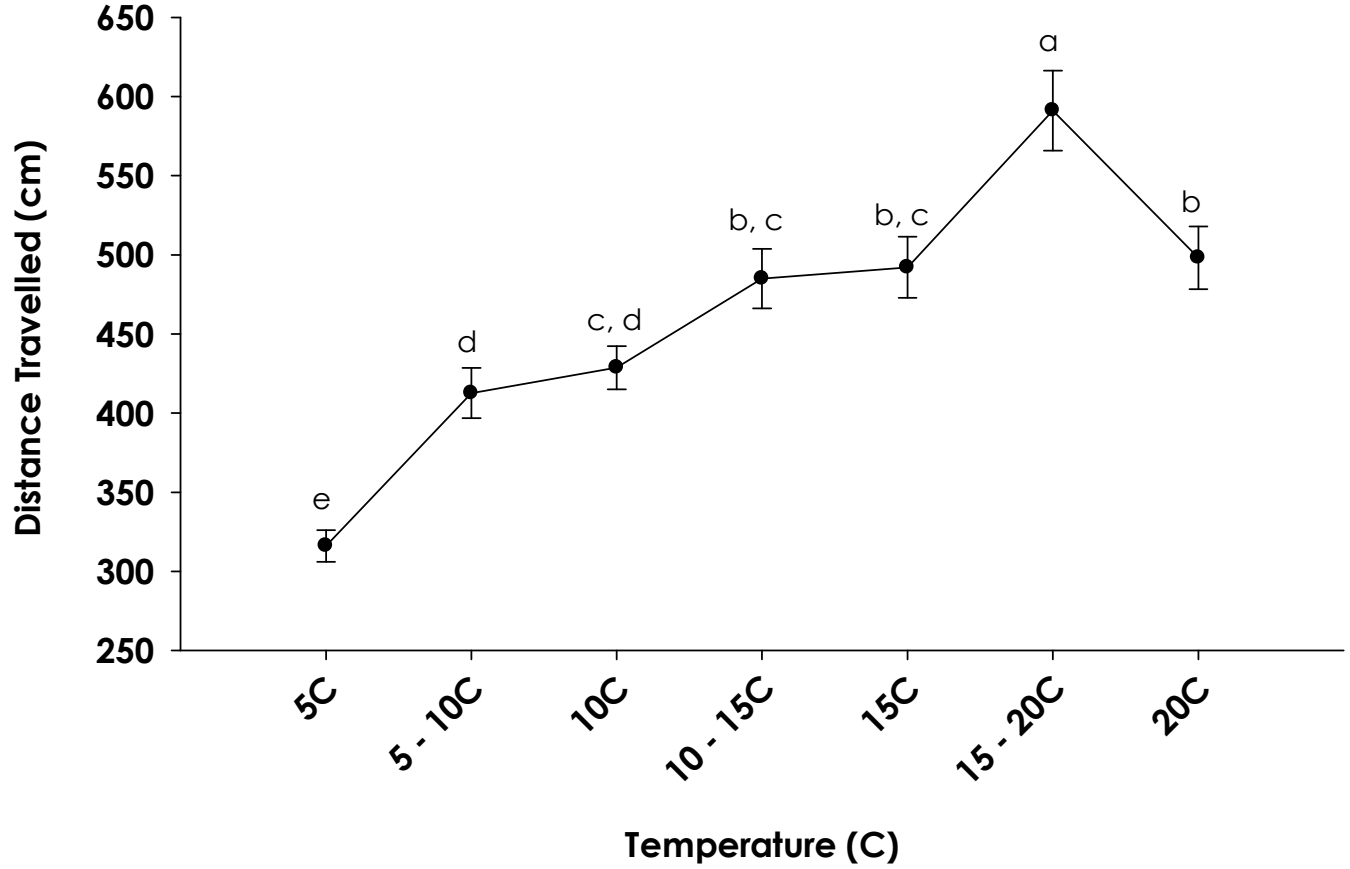

Figure 2.3 – The average level of activity (±SEM) for at each of the 5-h changeover and stable temperature periods. Each line represents pooled data from the four different treatments in this experiment (n = 80). Different letters denote significant differences among treatments (P<0.05).

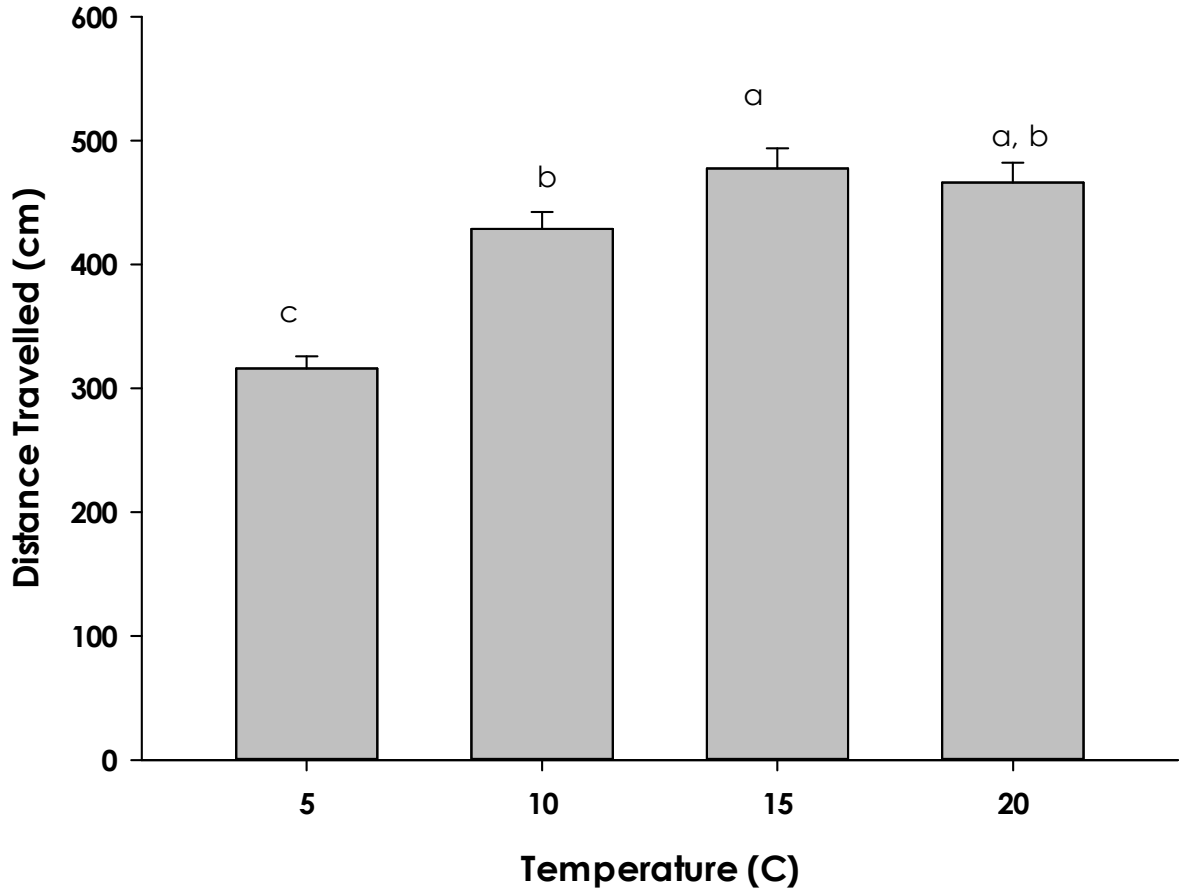

Figure 2.4 – Average activity (±SEM) organized by temperature. Each bar represents the pooled data from the four treatments (n = 80). Different letters denote significant differences among treatments (P<0.05).

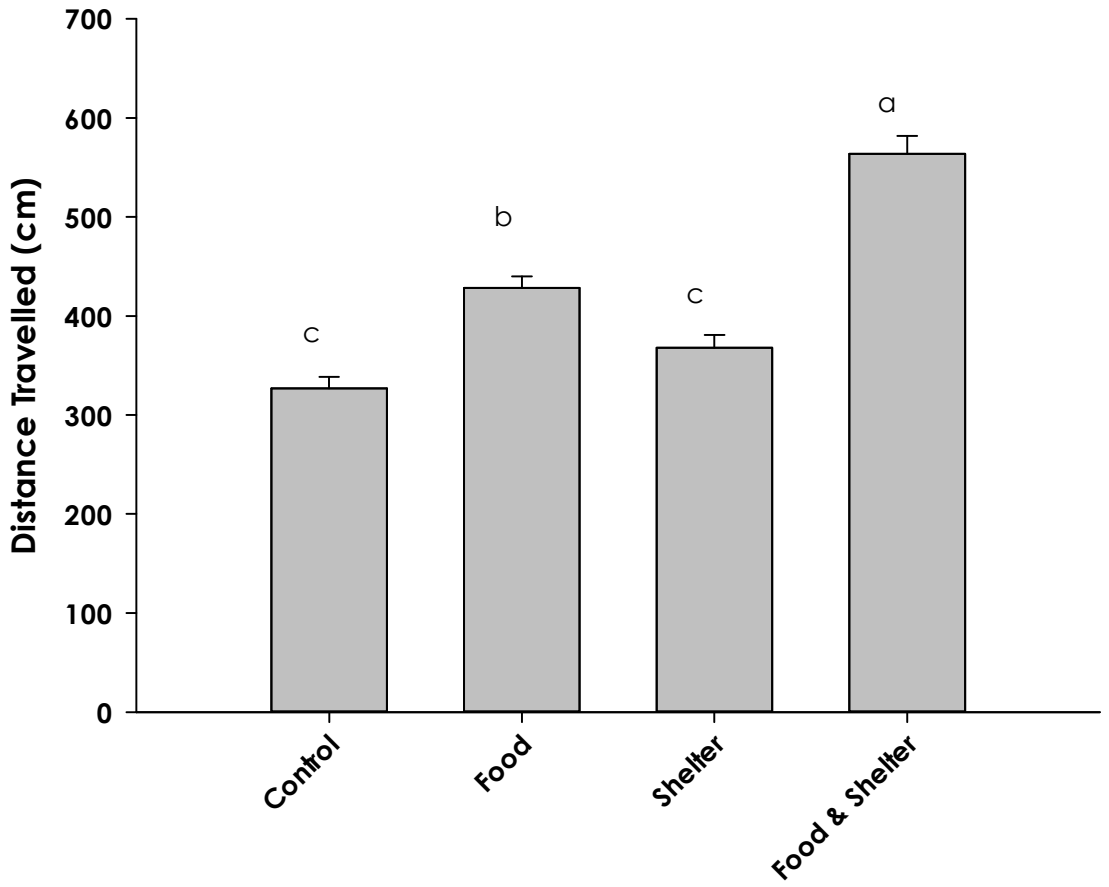

Figure 2.5 – Average activity (±SEM) organized by treatment. Each bar represents the pooled data from the four temperatures (n = 80). Different letters denote significant differences among treatments (P<0.05).

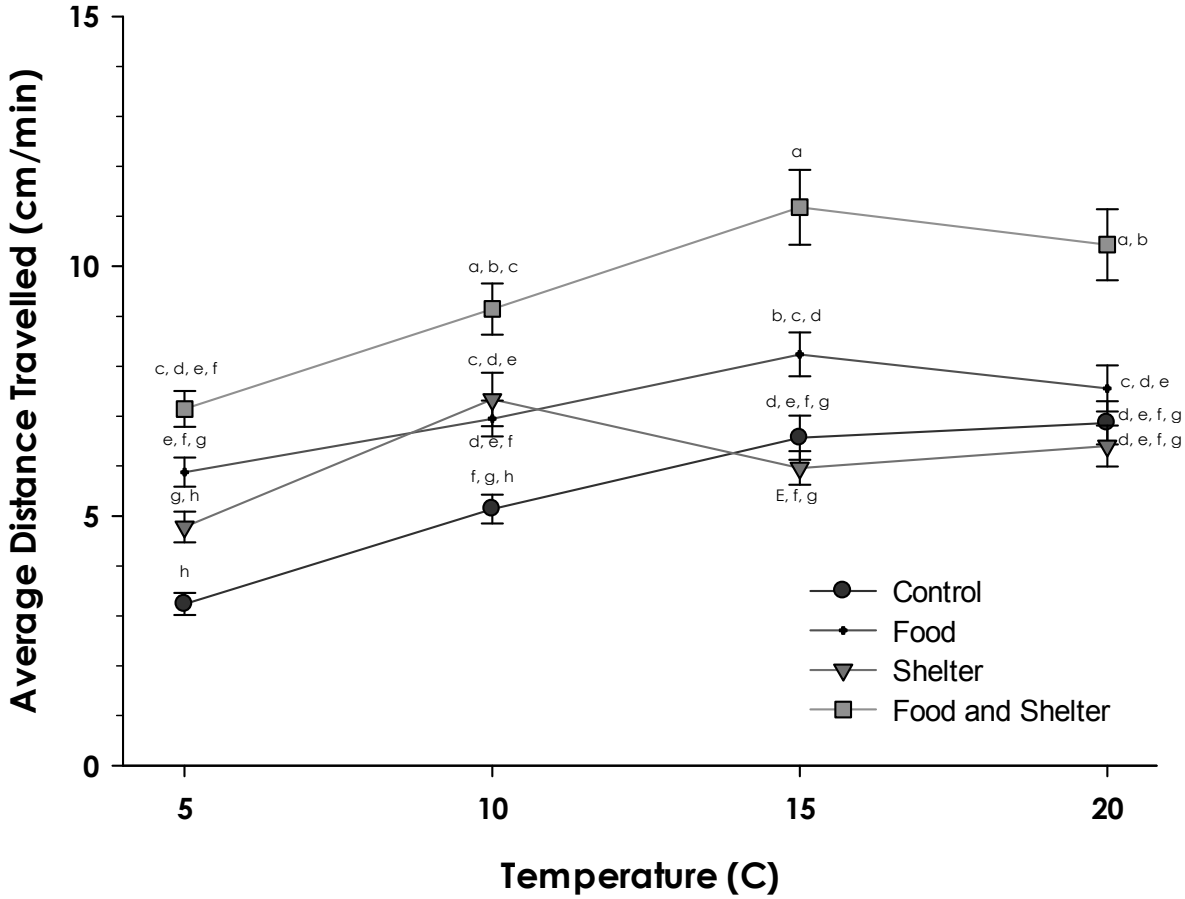

Figure 2.6 – Interactive effects plot of average activity (±SEM) organized by temperature and grouped into treatments. Each point represents averaged data from 5 h from each of 10 replicates (n = 80). Different letters denote significant differences among treatments (P<0.05).

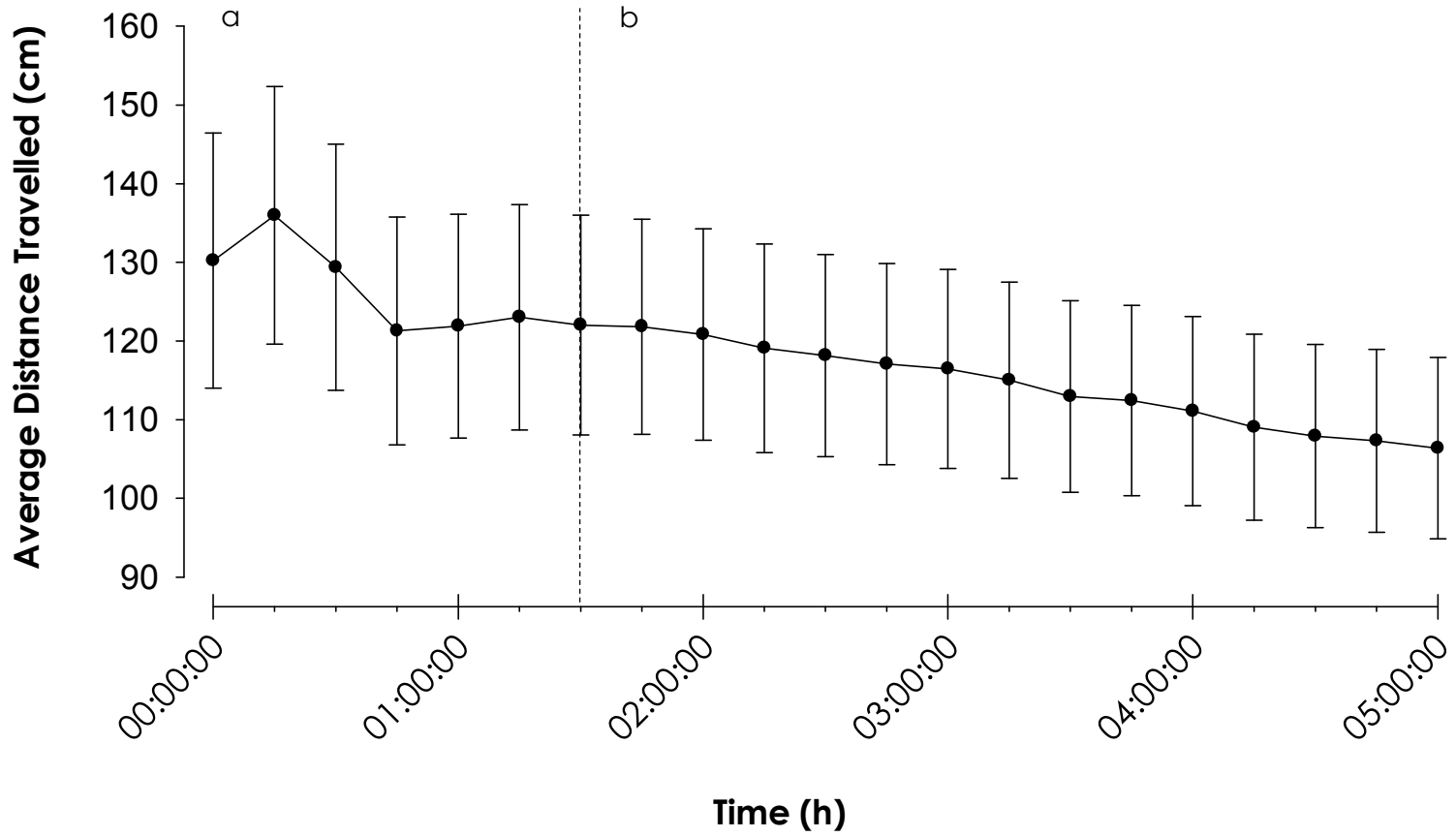

Figure 2.7 – Running average of distance travelled by juvenile *H. americanus* during the first 5 h of after being introduced into the apparatus. Data represents mean (±SEM) for all treatments. Sections that were significantly different from each other in post-hoc analysis are separated by vertical dashed lines and marked with letters.

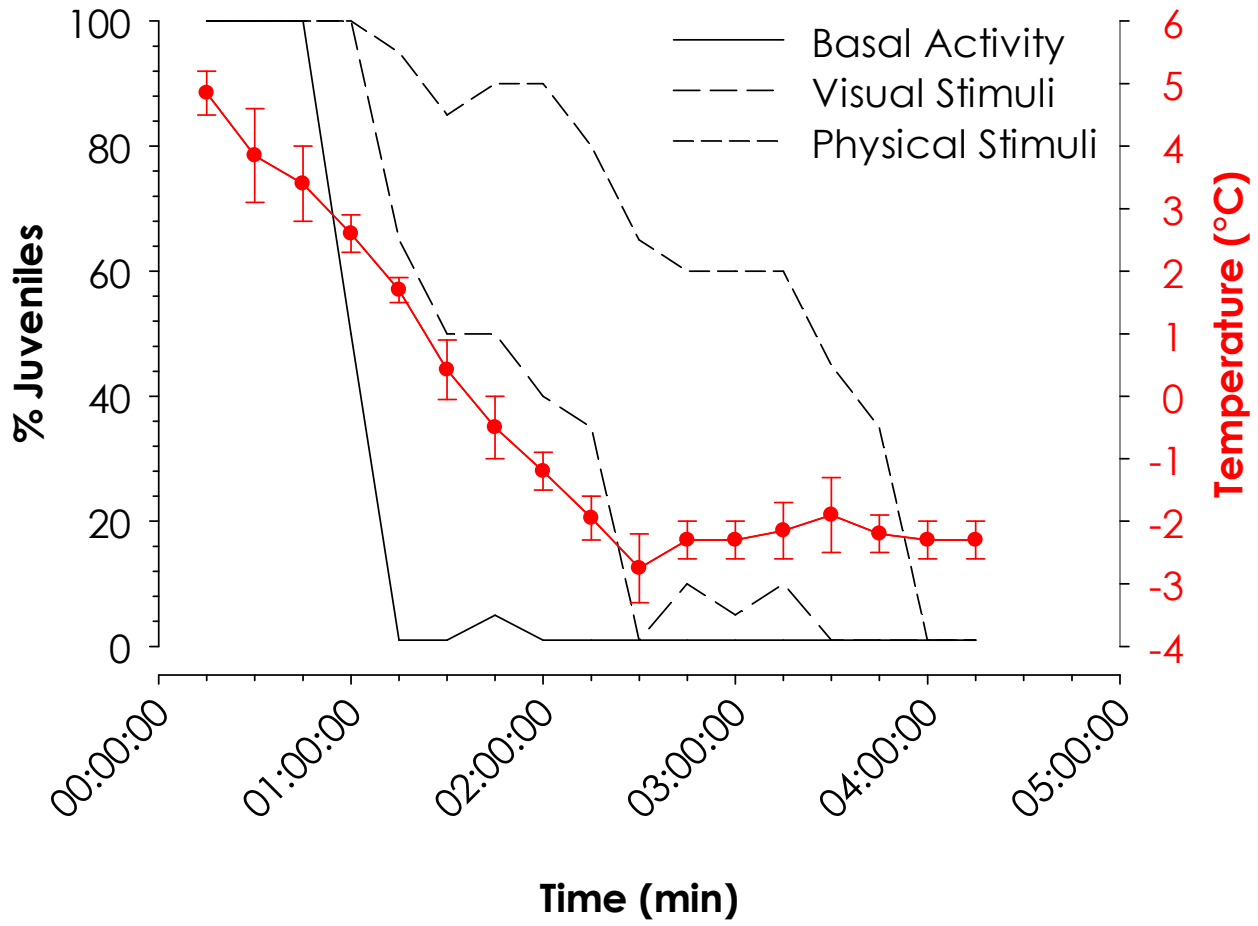

Figure 2.8 – The percentage of juvenile lobster showing basal activity and response to visual or physical stimuli over a 4 h temperature change. Each line represents pooled data from two separate experiments of 10 individuals (N=20). The red line represents the temperature of the apparatus at each time interval ± SEM.

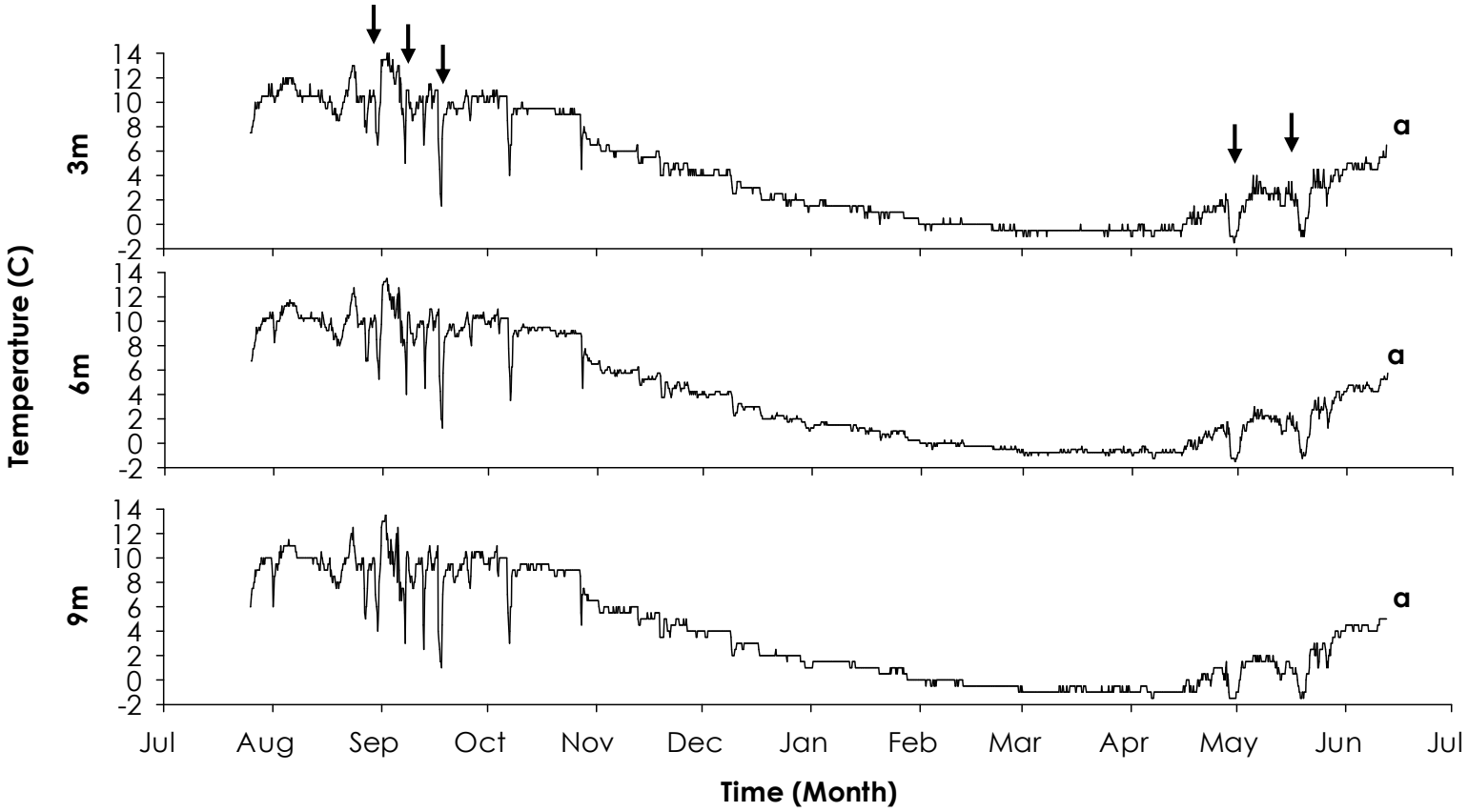

Figure 2.9 – Seasonal temperature change in Bay Bulls, NL. Temperature was recorded from July 24th, 2011 to June 15th, 2012. Temperature was recorded at 3, 6, and 9 m depths. Arrows represent the sudden drops in temperature associated with high wind events. These events occurred seasonally and were the same for all depths. Different letters denote significant differences in the data.

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3. Thermoregulatory Trade-Off Behavior when Exposed to Cold Environments.

3.1 – Introduction

All organisms must make choices based on the benefit of performing one action weighed against the risks to their survival of that action (Morrell 2004). These choices, or trade-offs in behavior, optimize organism fitness (Roff 1992). Predator-prey interactions epitomize the fundamental models of trade-off behavior, because successful predation attempts result in death for the prey – the greatest reduction in an organism's future fitness (reviewed by Lima and Dill 1990). However, predator-prey interactions are not the only time an organism makes trade-offs. Any decision that impedes another decision is a trade-off, and it does not have to be predation. Environmental variables can also induce a trade-off in behavior. Factors such as light causing salmon to change foraging and sheltering behavior (Fernö et. al. 1995), salinity influencing when and where foraging and digestion happen (Nielsen and Gosselin 2011, Curtis et. al. 2010), turbidity and hypoxic regions being used as a refuge for predation (Abrahams and Kattenfeld 1997, Hedges and Abrahams 2015), and

temperature being utilized to reduce the metabolic need to feed (Kessler and Lampert 2004) are all examples of environmental trade-offs.

The survival of environmental conformers, depends on the balance between maintaining internal homeostasis and access to resources. Cladocerans of the genus *Daphnia* will stay in unfavorable water temperatures to maintain access to food, because it decreases risk of predation (Kessler and Lampert 2004). Dungeness crabs, *Metacarcinus magister*, actively move into areas of low salinity in order to obtain food, but move out to higher salinity areas for digestion (Curtis and McGaw 2012). Juvenile Atlantic salmon, *Salmo salar*, switch from diurnal to nocturnal feeding when exposed to decreasing temperature in order to avoid seasonal predation (Fraser et. al. 1995). In many thermo-conformers, or ectotherms, trade-offs for resources can result in exposure to sub-optimal temperatures. Ants of the families Dolichonerinae, Myrmicinae and Formicinae forage in temperatures within 6 °C of their *CTmax*, risking heat-induced mortality for food (Cerdá et. al. 1998).

Marine crustaceans experience more rapid changes in temperature than their terrestrial counterparts because of the high thermal conductivity of water results in their body temperature mirroring their external environment. As a result, maintaining an optimal body temperature can require rapid trade-offs that reduce physiological

performance and therefore reduce fitness (Angilletta et. al. 2002). For example, the purple shore crab, *Hemigrapsus nudus,* and the crayfish *Procambarus clarkii* exit from warm water into air in order to reduce their body temperature through evaporative cooling (McGaw 2003, Payette and McGaw 2003). The crayfish, *Orconectes rusticus*, seeks out shelters with stable microclimates during periods of rapid temperature change (Mundahl 1989).

The American lobster, *Homarus americanus*, makes trade-offs in behavior that best suit each life stage. Larval *H. americanus* risk higher levels of predation by remaining within water temperatures above12 °C, typically above the 10-30 m thermoclines, where predation is more likely (Annis 2005, Annis et. al. 2013). Adult lobster migrate long distances to deeper offshore waters in order to maintain more stable temperatures and to provide their eggs with optimal temperatures for growth and development (Cowan et. al. 2007). To date, only one study has investigated such behaviors in juvenile *H. americanus* (Lillis 2009). This study showed that recently settled postlarvae and juveniles exit their shelters when exposed to rapid drops in temperature >7 °C. In the northern geographic limits of *H. americanus*, temperature drops > 7°C are not uncommon during seasonal changes, but usually occur over the course of days, and occasionally over the course of hours during extreme weather or upwelling / downwelling events (Chapter 2; Figure 2.9).

Patterns of movement and mortality of juveniles ultimately affect recruitment of adult *H. americanus* populations (Gosselin and Qian 1997, Hunt and Scheibling 1997). It is important to understand the thermoregulatory trade-off behavior of juvenile *H. americanus* because they rely on cryptic behavior and shelter acquisition to avoid predation (Barshaw and Rich 1988, 1997). If declining temperatures initiate exit behaviors from shelter (Lillis 2009) this may result in increased predation and mortality. Therefore, the objective of this study was to investigate choices and trade-offs by the juvenile *H. americanus* when given a choice between water temperature, access to food, and/or access to shelter,

3.2 – Materials and Methods

We used a Loligo Shuttlebox system [Loligo® systems #AB10203 Tjele, Denmark] to investigate the behaviour of juvenile *Homarus americanus* in response to temperature. The Shuttlebox system is a recirculating seawater system consisting of two circular arenas connected by a central walkway, through which the animals could freely move (Figure 3.1). The temperatures in each arena (5 °C and/or 18 °C) were independently maintained via a series of computer- controlled pumps connected to a cold or hot water bath. A USB camera [USB uEye® SE] was positioned 1.5 m above the

apparatus in order to fully visualize both arenas. A computer connected to the camera used the contrast between the organism and the arena to track the juvenile during experimental runs. These data consisted of XY coordinates gathered from the center of the contrasting image (i.e., the juvenile lobster) recorded to a text file every second. We used the recorded XY coordinates to determine which side of the apparatus the juvenile occupied, how frequently they switched sides, and the average time(s) spent in each side. The XY coordinates were further used to calculate velocity (cm/s), total distance travelled, and running average velocity (cm/s) of the juvenile lobster.

Lobsters were acclimated to 8 – 10 °C seawater for at least one month prior to beginning the experiment. This temperature regime was used rather than the preferred temperature of 18 °C because bacterial buildup in the higher temperature resulted in poor water quality that increased premature molting and mortality (personal observation). Moreover, experiments in Chapter 2 showed no effect of acclimation temperature on temperature preference (Chapter 2, Table 2.1). We used only juvenile lobsters between 15 – 27 mm CL in these experiments, excluding smaller individuals because they consistently sheltered in the inflow/outflow plumbing on the apparatus during trials, thus completely removing themselves from the recording area. Lobsters larger than 27 mm CL could

touch the carpus of their chelipeds on both sides of the central passage of the apparatus, using it as a shelter similar to that observed in other studies (Cobb 1971, Karnofsky et. al. 1989). The lobsters were fed weekly on a diet of scallop meat (*Placopecten magellanicus*), fasted animals used in experiments were not fed for at least 48 hours prior to use in experiments. Only animals that were within 48-72 hours fasting time were used in experiments, in order to standardize potential behavioral changes resulting from longer fasting periods.

For each experiment, I placed a randomly chosen individual (n = 20) into one side of the apparatus and recording began immediately. Each replicate ran for 8 hours, and it was replicated 20 times with a new juvenile for each replicate. We performed 10 experiments, adding differing combinations of shelter and food to the temperature choices (Figure 3.2). To avoid side bias in the apparatus, I varied the starting position of the lobster and the experimental variables (temperature, shelter, and/or food) into 5 replicates each of the 4 different possible left or right side combinations.

For the control experiment the temperature in both arenas was maintained at 18°C. This uniformity allowed us to determine any side bias in the system, and established a baseline movement rate for the lobsters. In the second series of experiments, I manipulated a single variable

(temperature, shelter, or food, Figure 3.2). For temperature, I chose to set one side of the apparatus to 5 °C, which is outside preferred temperature range for adults and juveniles (McLeese 1956, Reynolds and Casterlin 1979, Crossin et. al. 1998, Jury and Watson 2013, Chapter 2), while maintaining a level that did not induce loss of righting response. I constructed shelters from white PVC pipe (10 x 5cm) cut longitudinally 1 cm from its edge to create a long c-shaped trough similar to those used by Cobb (1971). Painting the interior of the shelter black reduced light reflection inside the shelter. A series of 7 mm holes drilled in the top of the shelter allowed the video recording system to track the juvenile while in the shelter. For the food treatments, I glued a small (<5 g) piece of scallop meat to a microscope slide, and placed it in one of the arenas. These slides were soaked in filtered seawater for 4 hours before use in experiments and weighed before and after the experiments to determine if any food was consumed during the experiment. I chose scallop meat because it is part of lobsters' diet (Factor 1995), and it did not contrast with the apparatus, making it invisible to the video tracking system.

In the second set of experiments, I offered two of the variables in combination in order to examine possible trade-off behaviors (Figure 3.2). Three experiments investigated: 1) Whether juveniles utilise shelter in sub-optimal temperature; 2) Whether juveniles forage and consume food in

sub-optimal temperature; and 3) Whether lobsters prefer shelter or food when forced to decide between the two. A final series of experiments offered all three variables in combination: 1) Shelter in sub-optimal temperature versus food in a preferred temperature; 2) Shelter in a preferred temperature versus food in sub-optimal temperature; and 3) Food and shelter in sub-optimal temperature versus a preferred temperature.

I removed the first 90 minutes of data from the experimental analysis because of concerns over handling stress and temperature change when introduced to the apparatus. The data for velocity (cm/s) and total distance travelled (cumulative per second) was grouped into 1 minute intervals for analysis. From these data we also derived the time spent in each side of the apparatus, time spent foraging around the food source, and the time spent in or around a shelter. If a dataset contained more than 2 hours of unusable data (~35% of total collected data), such as contrast problems with the image capture, or the lobster crawled into the plumbing of the apparatus, it was removed from analysis.

I compared the time spent in each arena, the average time spent in each side between switches and the average velocity on each side with Student's two-sample t-tests. A switch occurred any time that the juvenile crossed form one side of the apparatus to the other. If I detected a significant difference in the time on each side, I then compared lobster

movements with the position of the shelter or food to determine the amount of time spent maintaining a shelter or foraging. In food experiments, I determined the number of lobsters that consumed food. We considered food consumed if any food had been removed from the microscope slide, this was determined by direct observation of the slide, or by comparing weight of the slide before and after the experiment if it was not possible to determine visually. I then compared the average velocity in each treatment to the each other using a Kruskal-Wallis test with a Dunn's post-hoc test in order to determine whether activity levels differed significantly between experiments. A one-way ANOVA with Tukey's post-hoc test determined if average number of switches differed significantly between experiments.

3.3 – Results

3.3.1 – Control Experiment

In the control experiment, we found no significant difference in the time spent on each side of the apparatus (see Table 3.1 for summarized statistics). The lobsters explored the entire apparatus before finally settling along one of the round walls or in the center of the apparatus (Figure 3.4). The number of switches between sides averaged 605.6 ± 90.3 over the 7 h

experimental period and the average time spent in each side between switches was not significantly different (See Table 3.2 for summary statistics). We found no significant difference between lobster velocities in each side (See Table 3.3 for summarized statistics).

3.3.2 – Single Variable Trade-offs

The amount of time spent on each side differed significantly for all the single variable experiments (Table 3.1). In the 18 °C vs. 5 °C experiment, lobsters spent 74% of the time in the 18 °C side (281.8 ± 19.5 min). Lobsters switched between sides an average of 340.3 ± 58.31 times, and spent significantly more time in the 18 °C arena between switches (Table 3.2). Average lobster velocity did not differ significantly between arenas of the apparatus (Table 3.3).

In shelter experiment, lobsters spent 85% of the duration (346.6 ± 24.1 min) in the arena with the shelter (Figure 3.4). Of this time, the lobsters spent almost 80% of their time in or around the shelter. The number of switches between the two arenas averaged 189.4 ± 45.0, and the lobsters spent significantly more time in the shelter arena between switches (Table 3.2). Lobster average velocity did not differ significantly with arena (Table 3.3).

In the food only experiment, lobsters spent 89% of their time (369.0 ± 18.5 min) in the side with the food, mainly around the edges of the arena (Figure 3.4). The lobsters only made short forays to the food area before moving the food back to the edge of the arena (personal observation). Of the 12 lobsters analysed, 11 consumed all of the food available, and only one lobster consumed no food. Lobsters spent significantly more time between switches in the arena with food (Table 3.2) The average switching frequency was low (180.3 ± 36.8 switches). The lobsters had significantly lower velocities in the arena with food than the arena without food (Table 3.3).

3.3.3 – Double Variable Trade-offs

When two variables were manipulated, the time spent in each arena differed significantly for all of the experiments (Table 3.1). In the shelter or food experiment (both arenas 18 °C), the lobster spent 84% of the duration (350.4 ± 27.5 min) in the arena with the shelter (Figure 3.5). Of this time, lobsters spent ~26% of their time inside or around the shelter. The lobsters spent an average of 9.2 ± 0.03min foraging in the food area. Eight out of ten lobsters consumed all available food. Switching frequency averaged 250.8 ± 96.0. The lobsters spent significantly more time in the shelter arena

between switches (Table 3.2). Average lobster velocity in each arena did not differ significantly in this set of experiments (Table 3.3).

In the 5 °C shelter experiment, lobsters spent 80% of their time (337.6 ± 21.2 min) in the 5 °C shelter arena, undertaking only brief explorations of the 18 °C arena (Figure 3.5). Individuals in this arena spent ~52% of their time in or around the shelter. Switching frequency averaged 240.5 ± 47.9. Lobsters spent significantly more time between switches in the 5 °C shelter arena (Table 3.2), with significantly lower average velocity in the 5 °C shelter side (Table 3.3).

In the 5 °C food experiment, lobsters spent 93% (392.0 ± 6.6 min) of their time in the 5 °C food area with only short excursions into the 18 °C arena (Figure 3.5). Of the time spent in the 5 °C food arena, lobsters spent ~11% of their time foraging in the food area. However, only 3 of 12 lobsters in this set of experiments consumed food. Switching frequency averaged 96.8 ± 20.9. Lobsters spent significantly more time between switches in the 5 °C food arena (Table 3.2). The average velocity was significantly lower in the 5 °C food arena (Table 3.3).

3.3.4 – Triple Variable Trade-offs

The amount of time spent in each arena differed significantly in all three variable experiments (Table 3.1). In the 5 °C shelter/18 °C food experiment, lobsters spent 88% of the duration (367.9 ± 20.9 min) in the 5 °C shelter arena, with only short excursions into the 18 °C food arena (Figure 3.5). Of the time spent in the 5 °C shelter arena, lobsters spent ~86% of their time in or around the shelter, and ~6% if their time foraging in the food area. Only 2 of 9 juveniles consumed food during this set of experiments. Switching frequency averaged 103.0 ± 31.8. Lobsters spent significantly more time between switches in the 5°C shelter arena (Table 3.2). Average velocity was significantly lower in the 5 °C shelter arena for this set of experiments (Table 3.3).

In the 5 °C food/18 °C shelter experiment, the lobsters spent 86% of their time (359.1 ± 17.5 min) in the 18 °C shelter arena, with only short explorations into the 5 °C food arena (Figure 3.5). Of the time spent in the 18 °C shelter arena, lobsters spent ~48% of their time inside and around the shelter. Of the time the lobsters spent in the 5 °C food arena, lobster spent ~7% of their time foraging in the food area. Six of the twelve lobster consumed food. Switching frequency averaged 169.3 ± 48.0 switches. Lobsters spent significantly more of their time between switches in the 18 °C

shelter arena (Table 3.2). Lobster average velocity did not differ between arenas in this set of experiments (Table 3.3).

In the 5 °C food and shelter versus 18 °C experiments, the lobsters spent 92% of their time (380.8 ± 13.5 min) in the 5 °C food and shelter arena, with only brief forays into the 18 °C arena (Figure 3.5). The number of switches averaged 56.6 ± 15.3. The lobsters spent significantly more of their time in the 5 °C food and shelter arena (Table 3.2). While in the 5 °C food and shelter arena, the lobsters spent ~86% of their time in or around the shelter and ~13% of their time foraging in the food area. However, of the 12 lobsters used in these experiments, only one consumed food. Average velocity was significantly lower in the 5 °C food and shelter arena.

3.3.5 – Trade off Experiment Comparisons

Average velocity differed significantly between experiments (Kruskal-Wallis, df = 9, X^2 = 22.357, P = 0.008; Figure 3.7). The lobsters in the 5 °C shelter and food experiments were most active (107.2 ± 43.5 cm/min), while the lobsters in the 5 °C food experiment were least active (18.0 ± 2.1 cm/min). Post-hoc analysis showed that the 5 °C food and shelter experiment had significantly higher activity than the 5 °C Food experiment (Dunn's post-hoc, P = 0.043). The velocities in all other experiments did not differ significantly from each other.

Though the number of switches in each experiment was high, the number of switches over time differed significantly (Chi-Square, df = 12, X^2 = 312.864, P < 0.0001). The number of switches was highest during the first 30 minutes, and declined to stable levels by the end of the 4th hour (Figure 3.8). The average number of switches differed significantly between experiments (ANOVA, df = 9, F = 7.48, P < 0.0001; Figure 3.9). Lobsters in the control experiment switched most often (606.6 ± 90.3) with the fewest switches in the 5 °C food and shelter vs. 18 °C experiments (69.3 ± 20.8). Post-hoc analysis showed significantly more switches in the control experiments than all of the other experiments (Tukey's HSD, P < 0.01). The 5 °C vs. 18 °C had significantly more (321.07 ± 58.31) switches than the 5 °C food and shelter vs. 18 °C experiments (Tukey's HSD, P = 0.049), but the number of switches differed significantly when compared to all other experiments. The lobsters in the 5 °C food and shelter vs. 18 °C experiment switched significantly less than both the control (Tukey HSD, P < 0.0001) and the 5 °C vs. 18 °C experiments (Tukey's HSD, P < 0.0001), but no other experiments differed significantly.

3.4 – Discussion

3.4.1 – Effects of Shelter

Temperature strongly influences the physiology, behavior, and distribution of aquatic ectotherms. Our temperature preference experiments showed that juvenile lobsters are no exception. Juvenile *H. americanus* prefer temperatures similar to those in adults, and like adults they avoid lower temperature (Reynolds and Casterlin 1979, Crossin et. al. 1998). In the Shuttlebox arenas, when given a choice between 18 °C (near their preferred temperature) and 5 °C (a sub-optimal temperature), juvenile lobsters spent the majority of their time in 18 °C. This and other experiments on thermal preference show that aquatic animals gravitate towards a preferred temperature (reviewed in Lagerspetz and Vainio 2006). These preferences may then be used to forecast the distribution of, and movement of, animals in their natural habitat (Chang et. al. 2010). However, the natural complexity of natural environments complicates such prediction, particularly given that animals seek food and shelter whilst avoiding predators.

When we added food or shelter to their environment, juvenile lobsters shifted their thermal preferences and spent significantly longer periods of time in 5 °C. Shelter provided the strongest stimulus, overriding preference for both food and a thermally optimal environment. Shelter is important to

juvenile *H. americanus* because cryptic behavior during this life stage facilitates predator avoidance (Wahle and Steneck 1991, 1992, Lawton and Lavalli 1995).

When shelter was available, juvenile *H. americanus* spent the majority of their time in and/or around shelter. This shelter seeking behavior is well documented for juvenile lobsters (Cobb 1971, Lawton 1987, Barshaw and Rich 1988, Karnofsky et. al. 1989, Wahle and Steneck 1991, Barshaw and Rich 1997). Juvenile *H. americanus* prefer shelters narrow enough that they can maintain contact with both sides of the shelter with their chelipeds (Cobb 1971, Lawton 1987, Barshaw and Rich 1988, Karnofsky et. al. 1989, Wahle and Steneck 1991, Barshaw and Rich 1997). Indeed, some of the larger individuals in our study attempted to use the walkway between the arenas in such a manner.

In the control experiment, juvenile *H. americanus* moved around the arena edges and moved frequently between the arenas in a figure-eight pattern (personal observation). When given access to a shelter, juveniles utilized this shelter exclusively, reducing their exploration and arena switching. Although activity tended to decrease when shelter was available when compared to the control, the trend was not statistically significant.

The juvenile lobsters also spent extended periods in a lower temperature when shelter was available. Our findings are supported by field surveys reporting that juveniles occur in shelters at/or below the sub-optimal temperatures (<5 °C) used in this experiment (Wahle and Steneck 1991, Cowan et. al. 2001). In contrast Lillis (2009), showed that juveniles leave shelter and increase activity if they experience a rapid drop in temperature. Kerkut and Taylor (1958) observed similar behavior in the crayfish, *Astacus astacus*, which increased activity when temperature decreased. However, the stimuli provided by the rapid change in temperature, rather than the actual temperature, may have driven the increase in activity (Kerkut and Taylor 1958). The only time a lobster experienced a rapid temperature change in our study was when they moved from 18 °C to 5 °C water. The lobsters initially increased activity in low temperature, like that reported in other crustaceans (Lagerspetz and Vainio 2006). This behavior may represent a mechanism to avoid sub-optimal temperatures. However, when shelter was available the lobsters remained in the sheltered area and thus tolerated to the lower temperature.

3.4.2 – Effects of Food

The lobsters spent significantly more time in an arena and reduced movements between arenas when food was present, even when the food was placed in the colder arena. We expected lobsters would move to the warmer arena after feeding because other crustaceans, such as the Dungeness crab, feed in stressful environments, but move to more favourable environments to digest food (Bernatis et. al. 2007, Curtis and McGaw 2012). Nevertheless, other animals such as Atlantic salmon *Salmo salar,* (Fraser et. al. 1993), and the crustacean *Daphnia pulex*, (Kessler and Lampert 2004) remain in sub-optimal temperatures in order to access food, trading off decreased growth rate for better food access and decreased predation. These experiments suggest that access to food offers a greater fitness benefit to juvenile lobster than the cost of staying in decreased temperatures (Kessler and Lampert 2004). In our food experiments juveniles moved the food (and slide to which it was attached) towards the edges or interface of the Shuttlebox, and at the end of each experiment the juveniles were found between the edge of the Shuttlebox and the food slide (personal observation). As a result, juveniles likely stayed with the food in 5 °C because they were using the thigmotactic stimulus of the food and arena edge as a shelter (Barshaw and Rich 1988, McGaw 2001).

When the lobsters actually consumed the food their general activity decreased; digestion reduces movement in ectotherms (Angilletta et. al. 2002, Bernatis et. al. 2007, McGaw 2007), because they direct energy towards digestion. These findings contradict our previous study (Chapter 2), in which juveniles' activity increased when exposed to food. However, our previous study only exposed juveniles to food odor, and they could not consume food. Their multiple handling attempts therefore increased activity..

Temperature apparently influenced both the proportion of animals consuming food and the total amount of food consumed by each individual. In all experiments, we scored removal of any food from the slide during a replicate as the lobster feeding. However, in the 18 °C experiments, not only did more juveniles consume food, but all individuals that consumed food consumed all available food on the slide (personal observation). In the 5 °C food experiments, even those few juveniles that consumed food left large proportions of uneaten food in the Shuttlebox and attached to the slide. Long-term exposure to sub-optimal temperature results in lower metabolism (Bullock 1955, Gillooly et. al. 2001, Worden et. al. 2006). In our experiments, we used activity as a qualitative estimate of metabolism, given the known correlation between metabolism and activity in adult *H. americanus* (Lyons et. al. 2013). Because of the reduced activity and

resulting decrease in metabolic rate, a reduction in numbers of individuals feeding and the proportion of food consumed may have reflected reduced metabolic activity and need for food.

3.5 – Tables and Figures

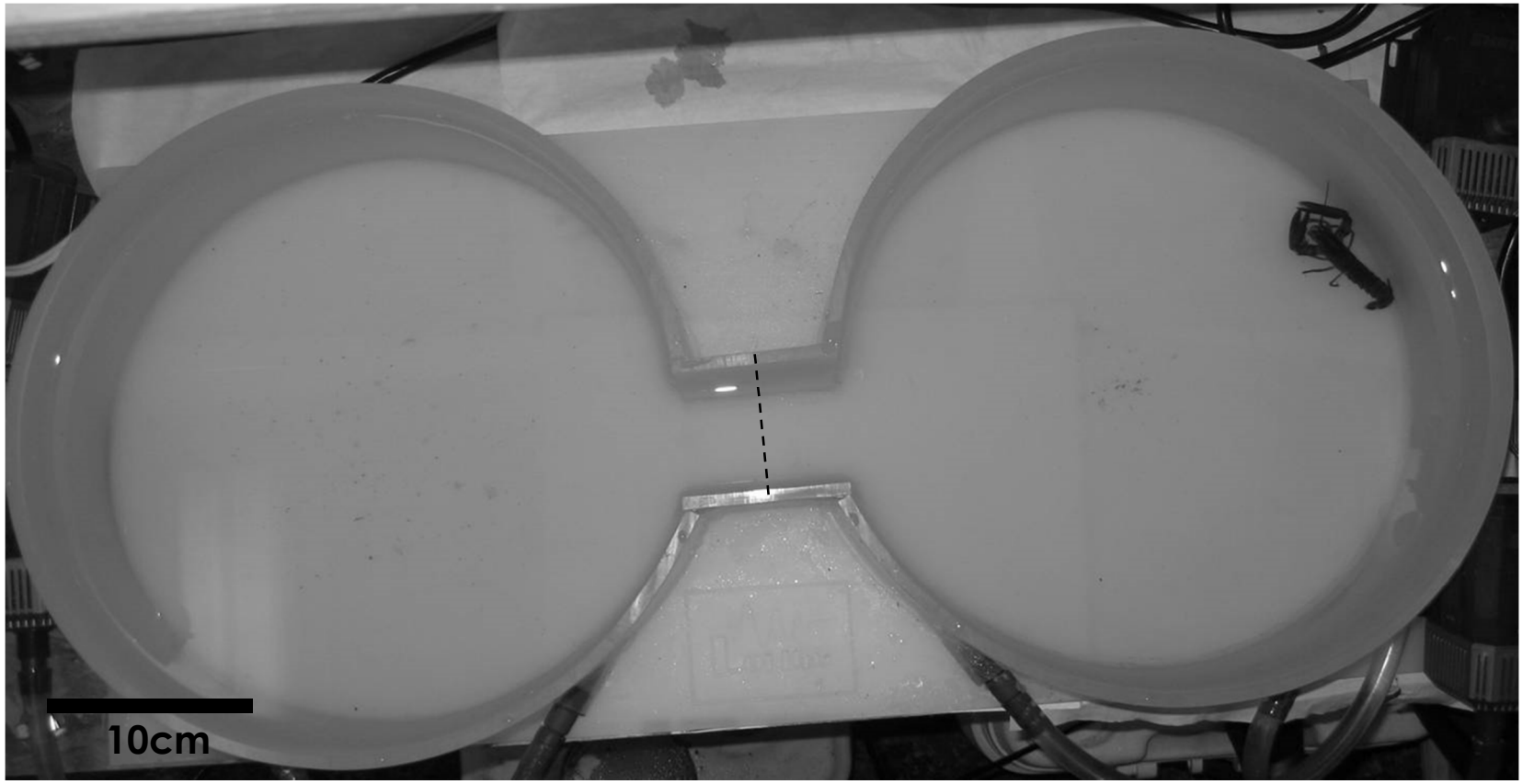

Figure 3.1 – Loligo Shuttlebox system in use with juvenile *Homarus americanus* during experimental setup before start of experiment start. The dashed line represents the boundary between the two chambers as defined by the video tracking software.

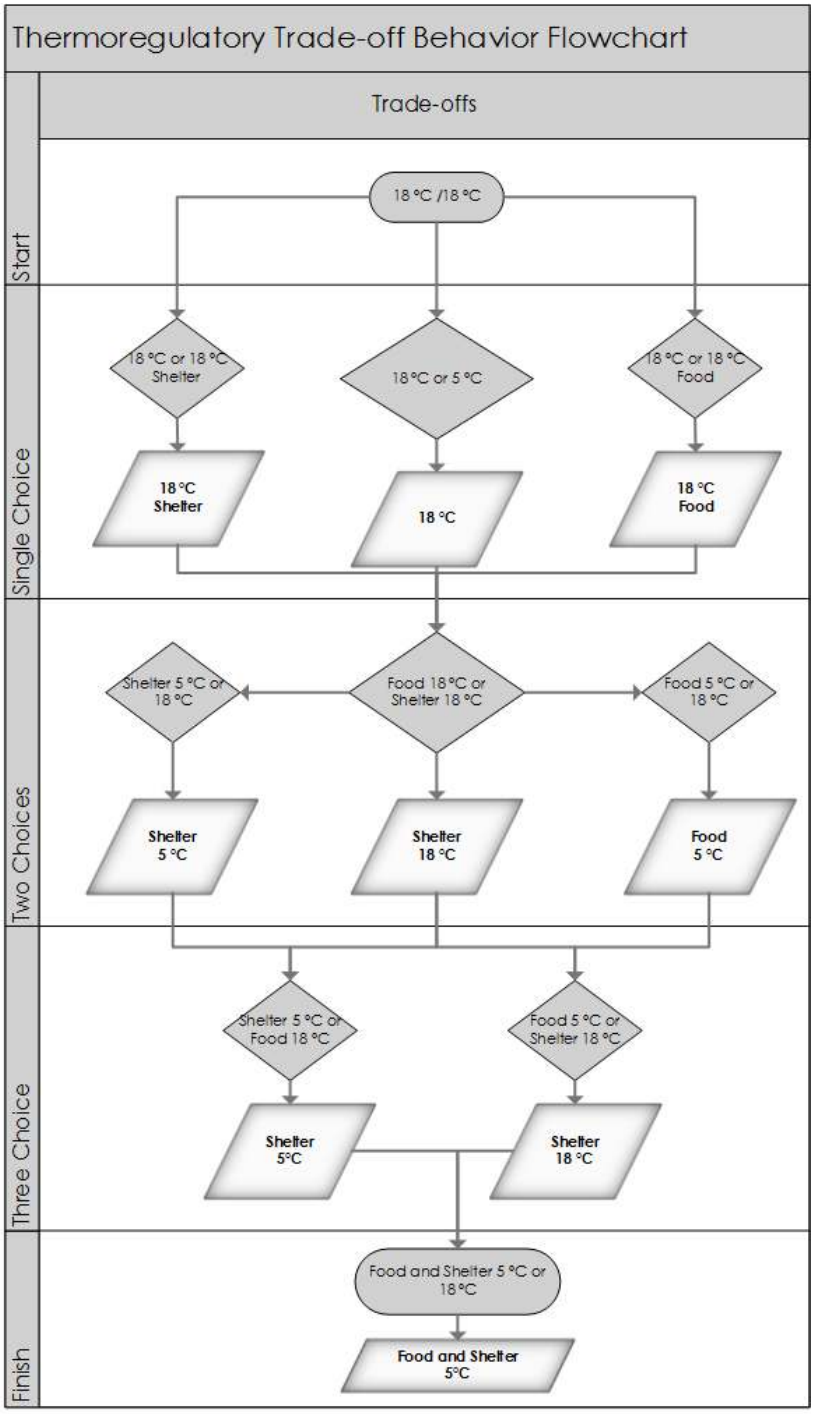

Figure 3.2 – Flowchart of the ten experimental experiments in this study. Each row represents the number of manipulated variables in the experiments (one, two, or three variables) and the diamonds represent an experiment where we tested a combination of those variables. The white Rhombus' show where juveniles spent significantly more time. The oval shaped boxes represent the first and last experiments.

Table 3.1 – Shuttlebox trade-off time in each side for all ten experiments (±SEM) and the Student's t-test values for each experiment. Significant differences are bolded. Significant arenas are marked with a *.

Variables	**Experiment**		**Mean time in each arena (min ± SEM)**		**df**	**t-stat**	**P-value**
	Left	***Right***	***Left***	***Right***			
0	18 °C	18 °C	185.91 ± 24.53	221.73 ± 24.65	9	-0.73	0.483
1	**5 °C**	**18 °C***	**104.53 ± 2.27**	**281.78 ± 19.52**	**11**	**-5.30**	**<0.0001**
	18 °C	**Shelter***	**62.78 ± 24.02**	**346.57 ± 24.06**	**12**	**-4.19**	**0.001**
	Food*	**18 °C**	**369.03 ± 18.45**	**46.74 ± 18.39**	**12**	**8.81**	**<0.0001**
2	**Shelter***	**Food**	**350.42 ± 27.46**	**64.96 ± 26.26**	**10**	**8.81**	**<0.0001**
	5 °C Shelter*	**18 °C**	**337.55 ± 21.22**	**82.45 ± 21.22**	**15**	**6.01**	**<0.0001**
	5 °C Food*	**18 °C**	**392.00 ± 6.61**	**28.00 ± 6.61**	**12**	**27.53**	**<0.0001**
3	**5 °C Shelter***	**18 °C Food**	**350.42 ± 27.46**	**64.96 ± 26.26**	**9**	**8.27**	**<0.0001**
	5 °C Food	**18 °C Shelter***	**52.15 ± 20.85**	**367.85 ± 20.85**	**12**	**-8.83**	**<0.0001**
	5 °C Food & Shelter*	**18 °C**	**376.84 ± 13.66**	**31.20 ± 10.65**	**12**	**14.47**	**<0.0001**

Table 3.2 –Number of switches between arenas and mean time in each arena between switches for all ten experiments (±SEM) and the Student's t-test values for each experiment. Significant differences are bolded. Significantly different arenas are marked with a *.

Variables	**Experiment**		**Number of Switches (±SEM)**	**Mean time in each side between switches (min ± SEM)**		**df**	**t-stat**	**P-value**
	Left	***Right***		***Left***	***Right***			
0	18 °C	18 °C	605.6 ± 90.3	0.8 ± 0.1	1.1 ± 0.7	9	-0.82	0.427
1	**5 °C***	**18 °C**	**340.3 ± 58.3**	**0.7 ± 0.1**	**2.9 ± 0.9**	**11**	**2.94**	**0.015**
	18 °C	**Shelter***	**189.4 ± 47.0**	**0.7 ± 0.2**	**7.8 ± 2.0**	**12**	**-3.19**	**0.009**
	Food*	**18 °C**	**180.3 ± 36.8**	**7.2 ± 2.2**	**0.5 ± 0.1**	**12**	**3.14**	**0.009**
2	**Shelter***	**Food**	**250.8 ± 96.0**	**7.0 ± 1.8**	**0.7 ± 0.2**	**10**	**3.57**	**0.006**
	5 °C Shelter*	**18 °C**	**240.5 ± 47.9**	**7.5 ± 2.3**	**0.6 ± 0.1**	**15**	**3.05**	**0.009**
	5 °C Food*	**18 °C**	**96.8 ± 20.9**	**13.5 ± 3.6**	**0.6 ± 0.1**	**12**	**3.55**	**0.005**
3	**5 °C Shelter***	**18 °C Food**	**103.0 ± 31.8**	**13.2 ± 3.0**	**1.7 ± 0.8**	**9**	**3.41**	**0.008**
	5 °C Food	**18 °C Shelter***	**169.3 ± 48.0**	**7.9 ± 1.6**	**0.8 ± 0.1**	**12**	**-4.30**	**0.001**
	5 °C Food & Shelter*	**18 °C**	**56.6 ± 15.3**	**36.1 ± 8.8**	**0.9 ± 0.1**	**12**	**3.71**	**0.003**

Table 3.3 – Mean velocity in each side for all ten experiments (±SEM) and the Student's t-test values for each experiment. Significant differences are bolded. Significant arenas are marked with a *.

Variables	**Experiment**		**Mean velocity in each side (cm/min±SEM)**		**df**	**t-stat**	**P-value**
	Left	***Right***	***Left***	***Right***			
0	18 °C	18 °C	77.9 ± 15.0	71.3 ± 13.0	9	0.33	0.746
1	5 °C	18 °C	70.4 ± 11.0	67.0 ± 13.0	**11**	0.20	0.845
	18 °C	Shelter	100.2 ± 21.0	61.0 ± 25.0	**12**	1.18	0.250
	Food	**18 °C***	**41.5 ± 9.4**	**122.6 ± 15.0**	**12**	**-4.55**	**<0.0001**
2	Shelter	Food	77.6 ± 25.0	102.0 ± 23.0	**10**	-0.72	0.482
	5 °C Shelter*	**18 °C**	**54.3 ± 12.0**	**133.7 ± 10.0**	**15**	**-5.17**	**<0.0001**
	5 °C Food*	**18 °C**	**14.3 ± 1.6**	**84.9 ± 8.7**	**12**	**-7.96**	**<0.0001**
3	**5 °C Shelter***	**18 °C Food**	**25.2 ± 7.1**	**65.2 ± 15.0**	**9**	**-2.39**	**0.036**
	5 °C Food	18 °C Shelter	56.3 ± 15.0	62.7 ± 14.0	**12**	0.31	0.760
	5 °C Food & Shelter*	**18 °C**	**25.5 ± 6.9**	**85.0 ± 7.8**	**12**	**-5.73**	**<0.0001**

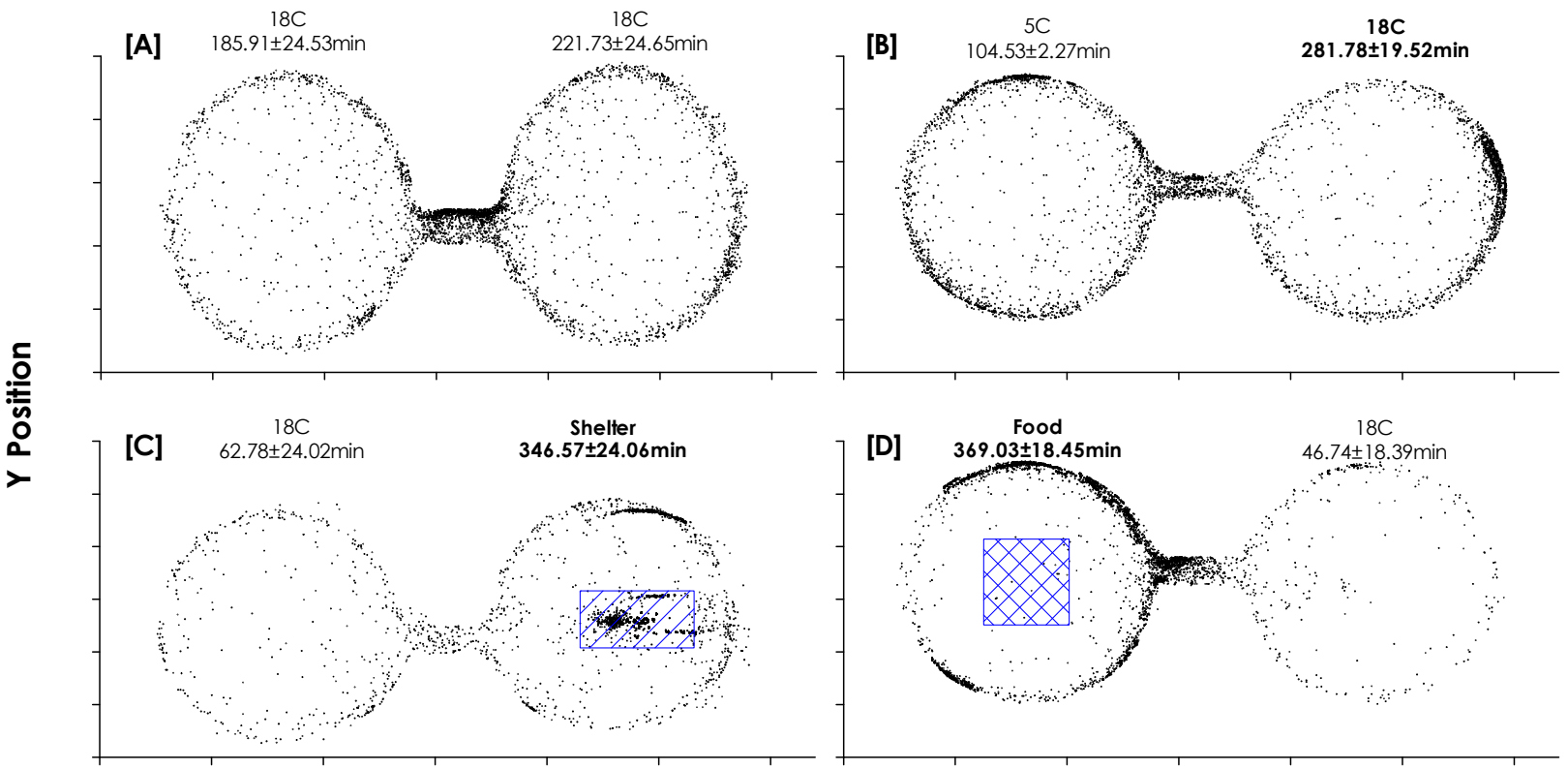

Figure 3.3 – XY coordinate scatter plots of juvenile *Homarus americanus* in A) the 18°C/18 °C control, B) the 18°C/5°C experiment, C) the 18 °C/shelter experiment, D) the food/18 °C experiment. Each point represents the XY position of a juvenile lobster at 1 min intervals. The variable(s) in each side and the time spent in that side is noted above the respective side. Significant differences are bolded. The overlaid lined box represents shelter placement in the apparatus, and the crosshatched box represents food placement. Graphs represent pooled data from all replicates used in data analysis.

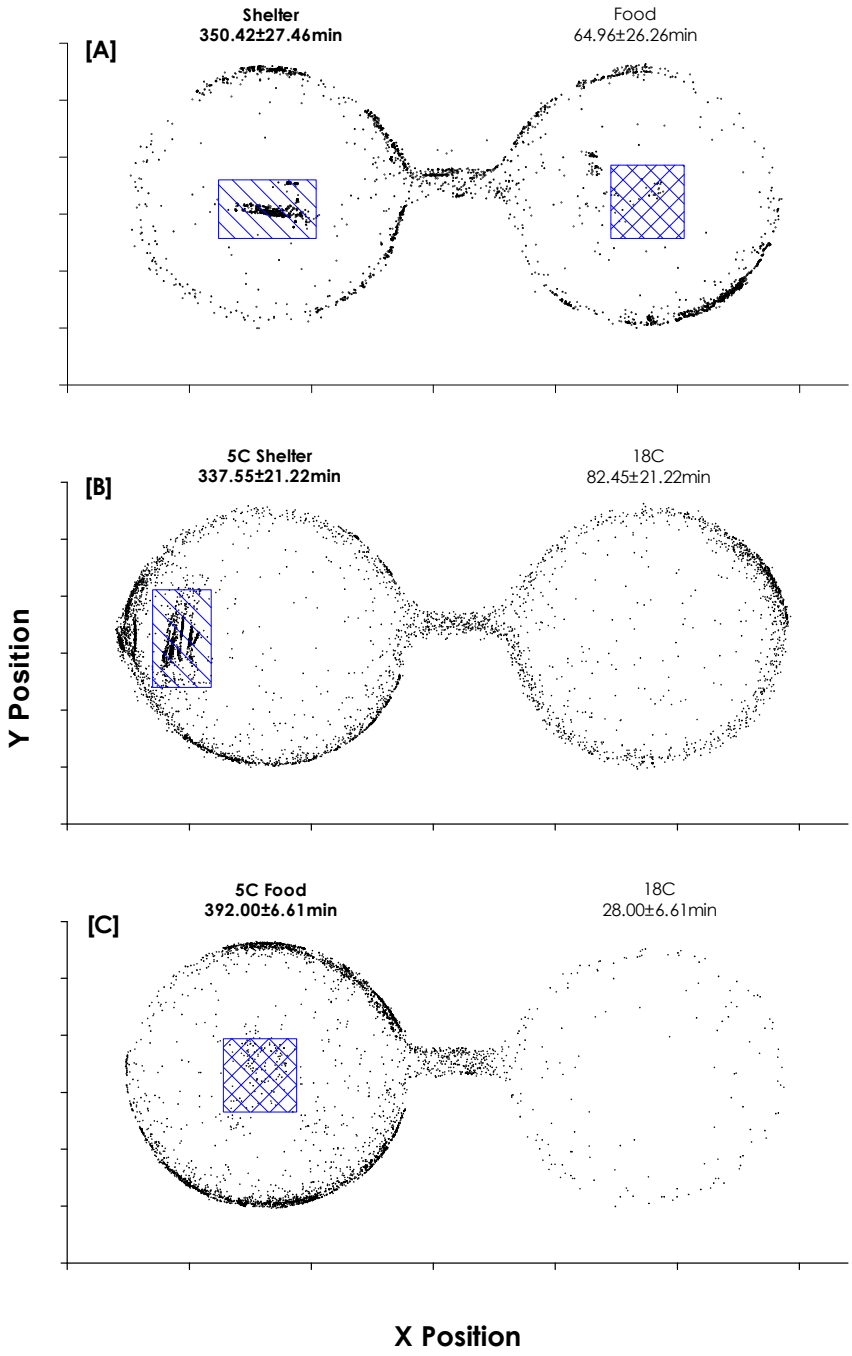

Figure 3.4 – XY coordinate scatter plots of juvenile *Homarus americanus* in the A) food/shelter experiment, B) 5 °C shelter/18 °C experiment, and C) the 5 °C food/18 °C experiment. Each point represents the XY position of a juvenile lobster at 1min intervals. The variable(s) in each side and the time spent in that side is noted above the respective side. Significant differences are bolded. The overlaid lined boxes represent shelter position and the crosshatched boxes represent food position. Graphs represent pooled data from all replicates used in analysis.

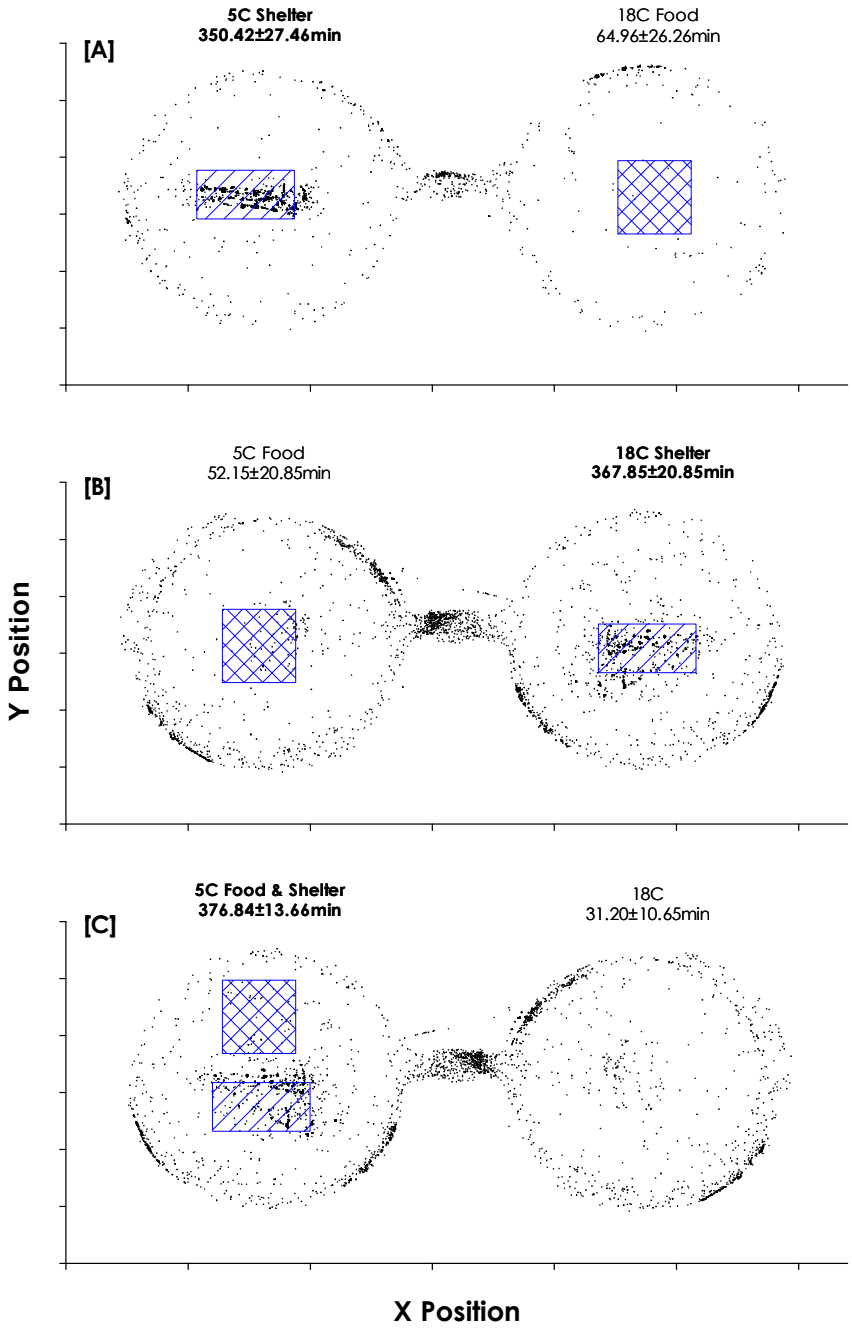

Figure 3.5 – XY coordinate scatter plots juvenile Homarus americanus in the A) 5 °C shelter/18 °C food experiment, B) 5 °C food/18 °C shelter experiment, and C) the 5 °C shelter and food/18 °C experiment. Each point represents the XY position of a juvenile lobster at 1min intervals. The variable(s) in each side and the time spent in that side is noted above the respective side. Significant differences are bolded. The overlaid lined boxes represent the position of shelter and the crosshatched boxes represent position of food. Graphs represent pooled data from all replicates used in analysis.

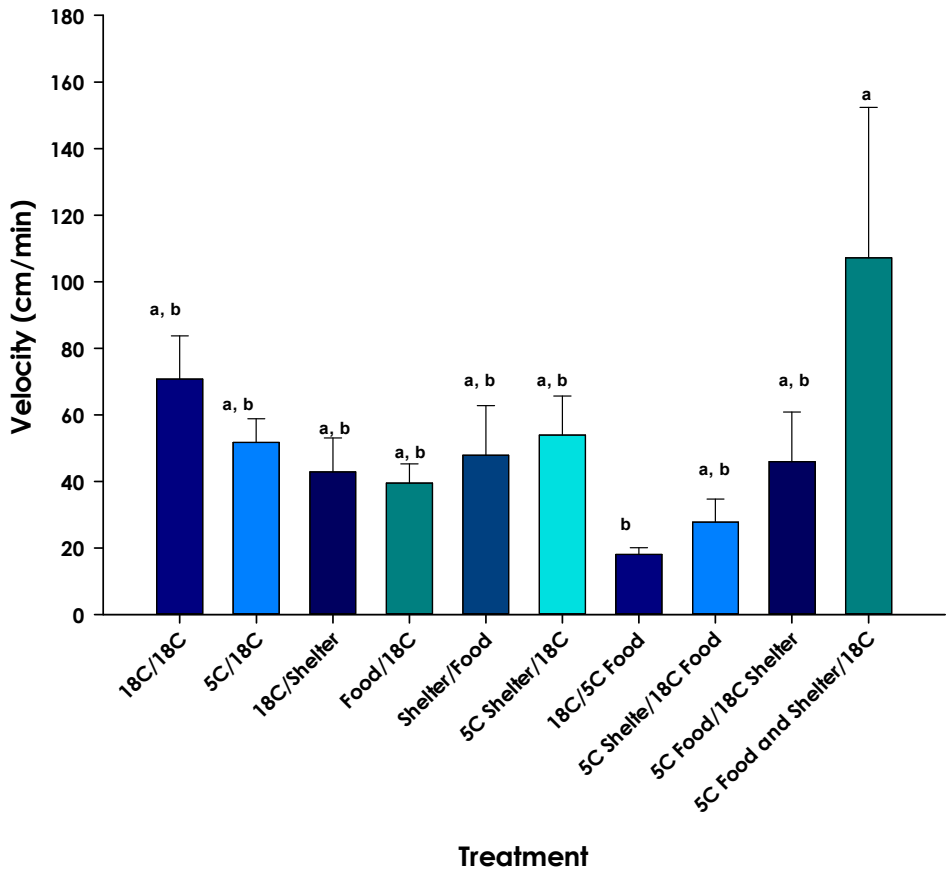

Figure 3.6 – The overall average velocity of juvenile *Homarus americanus* in each trade-off experiment. Each bar represents the pooled data from all replicates used in the analysis (±SEM). Different letters above mean velocities denote significant differences.

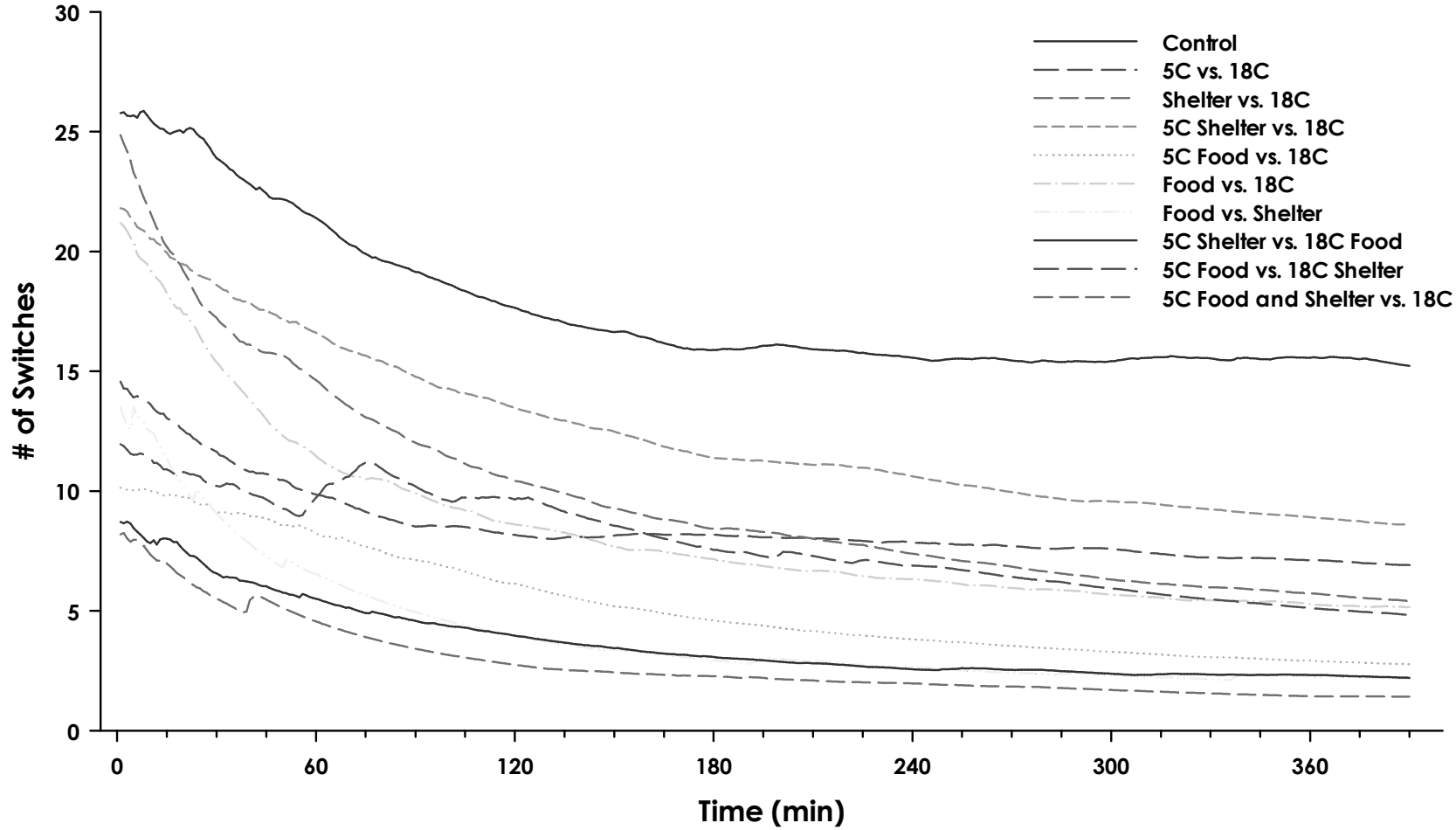

Figure 3.7 – Running average number of switches between sides of lobsters over the 6.5 hour experimental period, as per methods. We removed the first 90 minutes of data to reduce effects of handling and transfer. Colored lines denote the pooled data for each experimental experiment.

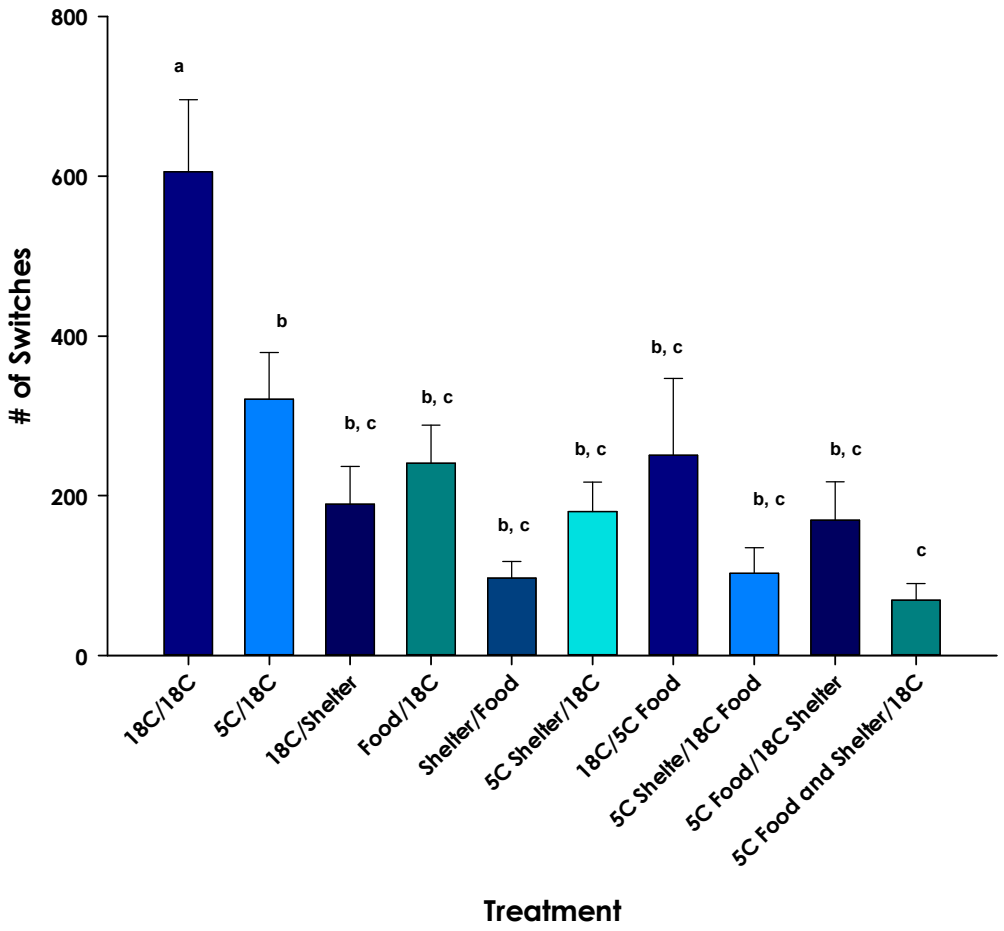

Figure 3.8 – The average number of switches between arenas made by juvenile *Homarus americanus* during each of the trade-off experiment experiments. Each bar represents the pooled data from all replicates used in the analysis (±SEM). Different letters denote significant differences.

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4. Summary and Conclusions

Temperature can influence the physiology, behavior, growth and reproductive status of an organism, and thus helps define its environment. Understanding the thermal biology of commercially important fish and crustacean species can influence regulation of fisheries, design of hatcheries and implementation of conservation measures where necessary. Chapter 1 reviews the life cycle of the American lobster, *Homarus americanus*, including its distribution, thermal biology, and past fishery conservation efforts. Chapter 2 investigated the thermal preferences of juvenile *H. americanus*, as well as their activity levels in a contrasting temperature range when exposed to both food and/or shelter. In addition, I examined the effects of handling on lobster behavior. Finally, Chapter 3 determined trade-offs in juvenile *H. americanus* when given choices between shelter, food, or thermally optimal temperature regimes.

Like adults, juvenile lobsters exhibit a broad survival range and can recover from exposure to -2 °C water. My data showed that such low temperatures only resulted in increased predation risk (by leaving the shelter or losing motor function) when juveniles were rapidly (<4 h) cooled. Such rapid temperature change rarely occurs in *H. americanus* habitats, as my temperature data showed. Although the lobsters died in water above 25 °C they rarely encounter these temperatures in their natural

environment. I observed temperature preferences in juvenile *H. americanus* similar to those reported in adults, which suggests many physiological similarities between juvenile and adult life stages. The activity experiments showed that the combined presence of food and shelter resulted in increased activity compared to food or shelter alone. Regardless of the direction of temperature change, activity was highest when temperatures were near or slightly lower than their preferred temperatures. Handling stress only appeared to affect juvenile *H. americanus* for short periods of time, before they adjusted to their new surroundings. The juvenile lobsters showed a pronounced decrease in activity after 90 min in the apparatus. The Shuttlebox experiments suggested that juveniles prefer access to shelter most, followed by access to food, and then by optimal temperature. This is the first study that examines the trade-offs of juvenile lobster between temperature, food and/or shelter.

Although temperature influences both the behavior and physiology of crustaceans, extrapolating results of thermal preferences obtained in the lab to distributions in the field requires caution. Juveniles differ from larval and adult life stages in their highly thigmotactic and shelter seeking behavior. Shelter is vital for avoiding predation during the vulnerable juvenile phase. As such, the acquisition of a shelter outweighs preference for a thermally optimal environment and acquisition of food. In the lab, the

acquisition of shelter overrode foraging responses, especially in cold conditions. However, in the wild, juveniles could potentially access particulate food that may drift into their shelters and sustain them during cold periods. Determining the levels of food deprivation required to initiate foraging behavior outside of the shelter requires further study.

Juvenile lobster exhibited markedly different activity levels (7-10 fold) in the activity and Shuttlebox experiments. This difference in activity should be interpreted with caution, however, because the activity experiment used a smaller chamber than the Shuttlebox, and juveniles were held in these chambers for prolonged periods. The changes in size of chamber and duration of experiment most likely resulted in lowered activity rates. Indeed, other studies using chambers with a similar area to our Shuttlebox experiment reported similar activity rates (Lawton 1987).

This study emphasizes the importance of evaluating animals in lab, or even in field settings, that create natural conditions. Simple laboratory experiments, although useful, may not accurately predict behaviors extrapolated into structurally complex natural habitats. Looking forward, anticipated temperature increases in the North Atlantic associated with climate changes may be particularly important in Newfoundland, where lobsters are on the northern most boundary of their geographic range. How these temperature changes will influence juvenile *H. americanus'*

development, growth, and behavior is unknown. However, the combination of these changes in concert with increased fishing pressure in the United States and Canada will undoubtedly lead to changes in the fishery (Wahle et. al. 2013). The information gained from this study provides insight into how juvenile *H. americanus* may behave in natural conditions, which will be important to any management and stock enhancement efforts, particularly near *H. americanus'* northern geographic limit.

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