Use of Inshore Benthic Cages for Storage and On-growing of Adult Lobsters *Homarus americanus*

by

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ABSTRACT

The goals of this research project were to determine if it is feasible to hold adult lobsters *Homarus americanus* in inshore benthic cages for extended periods. The underlying premise is that they would survive and grow. This larger product could then be sold outside of the regular season for a higher price. In the first experimental chapter, I investigated the use of the serum protein concentration (determined from the refractive index) as an indicator of the quality and physiological condition of adult lobsters. Serum protein concentration increased linearly with hepatopancreas mass, heart mass and edible meat content. There was a concomitant decrease in serum protein concentration with increasing moisture content of the hepatopancreas and muscle tissue. The serum protein concentration also changed over the molt cycle reaching its highest levels in the premolt stage followed by a sharp drop after the lobsters had molted. This rapid and non-invasive method is a valuable tool for determining quality and physiological status of commercially important decapod crustaceans. In the second series of experiments, I recorded the survival, molting, growth rates and serum protein concentrations in cage-held adult lobsters over a six month period. Laboratory experiments allowed parameters, such as water temperature, feed type, feeding frequency and cage size, to be manipulated to determine optimal conditions for survival and growth, while field experiments tested the feasibility of the storage and on-growing protocol. Temperature had a significant effect on molting rates, with a greater percentage of lobsters molting at higher temperatures. Feeding frequency influenced growth rates of both molted and non-molted animals. Animals with limited access to food had lower serum protein concentrations and

were of poorer quality. This project showed that benthic cages provide a viable method to store lobsters for up to six months allowing harvesters to sell them locally, outside the regular season.

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CO-AUTHORSHIP STATEMENT

The research described in this thesis was carried out by Guoqiang Wang, with guidance from Dr. Iain J. McGaw. Guoqiang Wang was responsible for conducting experiments, data collection and analysis, and thesis writing. Iain J. McGaw supervised the entire study and provided comments on the thesis.

Authorship for a publication arising from Chapter 2 is Guoqiang Wang and Iain J. McGaw. This manuscript has been published in December 2014 in the Journal of Shellfish Research. Authorship for a future publication arising from Chapter 3 is Guoqiang Wang and Iain J. McGaw. This manuscript is being prepared for submission to Aquaculture International or Canadian Journal of Fisheries and Aquatic Sciences.

CHAPTER 1: General Introduction

1.1 Introduction

The American lobster, *Homarus americanus*, ranges along the eastern seaboard of North America from Cape Hatteras, North Carolina to the Straits of Belle Isle, Newfoundland, Canada (Squires, 1990). In the USA, it is also called the Maine lobster or northern lobster, and in Canada it is known as the Atlantic lobster. Its normal coloration ranges from bluish green to greenish brown, although blue and yellow specimens can also be found (Van der Meeren et al., 2006). The American lobster's sex can be distinguished by its swimmerets (also referred to as pleopods) under the abdomen. Among its five pairs of swimmerets, the first pair differs between the male and female. For the male, the first pair of pleopods (named gonopods) are white in color, large in size and hard in texture; whereas in the female, they are smaller and softer in texture.

The American lobster is a very important fishery species in Atlantic Canada. It is also one of the most valuable seafood exports worldwide. Almost all marketed lobsters are wild captured. To maintain the fishery, landings are monitored and the number of licenses, fishing areas, type and size of fishing tool (traps), and fishing seasons are strictly regulated. The minimum legal landing sizes of lobsters in southern Gulf Lobster Fishing Areas vary between 71 and 80 mm in carapace length (DFO, 2011a). The smaller lobsters fetch a lower market price and are usually processed as meat products, rather than being sold as a live product. Lobsters larger than 81 mm in carapace length are most often sold as live animals. According to regulations, lobster smaller than 63.5 mm (sublegal size) have to be released unharmed. Many minimum legal sized lobsters are large enough to become valuable market products within one molt cycle.

1.1.1 Life stages

American lobster life stages can be categorized as embryonic, larval, juvenile, adolescent, and adult phase (Lawton and Lavalli, 1995). The life cycle of the lobster starts as fertilized eggs attached to the female's pleopods. Ten to eleven months are required for full embryonic development (from July or August of the first year to the next June or July), during which time the eggs are cared for and protected by the female (Holthuis, 1991). After several changes in embryonic morphology, including early cleavage, gastrulation, and organogenesis, the next developmental stage is the planktonic larvae; these larvae continue to molt through three stages, known as stage I, II, and III, which last around 10 - 20 days. These larval stages are strictly planktonic and are found high in the water column where they are transported by wind-driven currents (Templeman, 1936). When larvae go through their last molt, the animals become postlarvae, and at around 13 mm total length, they are known as stage IV. This postlarva now looks like a miniature adult. The postlarval lobsters begin to settle on the benthos as stage IV juveniles. The postlarval lobsters settle within a depth range of between 4 - 50 meters (Ennis, 1983). These inshore habitats can include mud, cobble, rock, peat reef, clay-silt and eelgrass beds; whereas in offshore areas, the habitat is commonly mud and clay-silt beds. In general, lobsters make use of these areas either sheltering in crevices or burrowing in soft substrate to avoid predators (Cooper and Uzmann, 1980).

The newly settled lobsters develop into juvenile stages. Juvenile lobsters are categorized according to the degree of dependency on shelters - shelter-restricted, emergent, and vagile juveniles (Lawton and Lavalli, 1995). The movement of shelter-

restricted juveniles is limited, and they tend to feed on prey that drift into the shelter. The emergent juvenile of 15 - 25 mm in carapace length is able to forage outside the shelter, although it still spends most of its time in a shelter. The vagile juvenile with a carapace length greater than 25 mm has a much wider range, moving outside the shelters to explore and feed. Once the lobster reaches around 50 mm in carapace length, it is classified as an adolescent, and is still not sexually mature but exhibits the typical nocturnal behavior of an adult. Lobsters reach sexually maturity at a size larger than 50 mm in carapace length in about 6 - 8 years.

1.1.2 Molting and growth

Molting, also known as ecdysis or shedding, is a process of casting off an old shell and producing a new exoskeleton (Kelly, 1993). In the wild, lobster molt frequency is size related and the frequency is reduced as the animal increases in size (Campbell, 1983). In general, adult lobsters molt once per year, or even every two years (Comeau and Savoie, 2001). Lobster molting is primarily elicited by a temperature threshold. Wild lobsters in the warmer waters of the southwestern Gulf of St. Lawrence molt between early July and early September (Comeau and Savoie, 2001), whereas in the colder waters of Bay of Fundy, the molt season is between August and October (Campbell, 1983). In addition to size and temperature, molt frequency of lobsters is also impacted by habitat, light rhythms, and diet quality (Aiken and Waddy, 1976; Waddy et al., 1995). In culture conditions, the molt can be artificially induced. One method to accelerate lobster molting is by hormone injection. However this has proved unfeasible, due to high mortality rates (Rao et al., 1973; Gilgan and Zinc, 1975). Bilateral eyestalk ablation is more successful in

inducing molting and could be a potential technique in commercial culture (Mauviot and Castell, 1976; Castell et al., 1977).

Following molting, an increase in size and mass occurs via uptake of seawater, which results in expansion of the new soft shell (Mykles, 1980; Hartnoll, 1982). However, growth increment per molt is quite different among the different lobster life stages. The average increment of carapace length in larval stages is around 31%, while adult size lobsters only have around 14% increase in carapace length (Wilder, 1953). In general, one molt can result in a 45 - 54% increase in body mass in adult lobster (Wilder, 1953; McKay, 2009). The actual growth at molt is influenced by temperature, feeding rate, diet, sex, sexual development, and stocking density (McLeese, 1972a, b; Aiken and Waddy, 1976, 1986; Bryars and Geddes, 2005; Mente, 2010). Nevertheless, in the wild, the lobster is a slow-growing animal. Generally, it needs 4 - 12 years to reach minimum market size of around one pound. Because of this slow growth, research has been carried out to try and reduce the time for the animals to reach a marketable size. Hughes et al. (1972) and Lim et al. (1997) have successfully carried out the culture of American lobster from larval stage to market size, using warm seawater to accelerate lobster growth rate and to reduce culture period to as low as two to three years, but these methods have not proven economically effective.

1.1.3 General nutritional requirements

In nature, larval lobsters feed on zooplankton (including copepods and smaller invertebrate larvae) and phytoplankton (filamentous algae). Juvenile and adult stages have a wide food preference range, including other crustaceans (crabs), bivalves

(mussels), benthic worms, echinoderms, small fish, sea grass, and carrion (Weiss, 1970; Ennis, 1973; Carter and Steele, 1982). However, the preferred food items of lobsters are crabs, sea urchins, and mussels. The rock crab is a preferred prey item of the American lobster and this prey item plays an important role in growth and ovary development (Gendron et al., 2001). The reason is not only rock crab's high protein and energy content (Brawn et al., 1968), but also the presence of particular amino acids, such as arginine, lysine and methionine (Vonk, 1960; Boghen et al., 1982). During the molting periods, sea urchins and sea stars are the preferred prey due to their high calcium content, which helps to accelerate the process of exoskeleton hardening (Evans and Mann, 1977; Vadas et al., 1986).

Other natural prey commonly consumed by lobsters are bivalve molluscs, such as the blue mussel. A number of studies have examined the feasibility of using mussels in the diet for cultured lobsters, including species of spiny and clawed lobster (Conklin et al., 1980; James, 1998; James and Tong, 1998; Jeffs and James, 2001; Bryars and Geddes, 2005). Mussels are potentially a good diet for farmed lobsters because they are a natural prey item, occur in large numbers, are easy to harvest and their high calcium content aids molting and exoskeleton development. However, despite these attributes, the essential amino acids asparagine, alanine and glutamic acid are deficient in mussels (Brawn et al., 1968; Mente, 2010). Carotenoids are another important supplement in diets. The coloration of tropical spiny lobster is a very important index for determining their quality in market. Insufficiency of carotenoids in the diet not only produces pale color but also results in an inferior growth rate and poorer survival (Smith et al., 2005; Barclay et al., 2006).

1.1.4 Responses to environmental changes

Temperature, salinity and dissolved oxygen are the three most critical environmental factors affecting growth, distribution and abundance of lobsters (Hartnoll, 1982). The growth rate of the lobster increases with ambient temperature, until it reaches a temperature of 26˚C, after which growth rate decreases and mortality rate increases (Chittleborough, 1975). The lower lethal temperature for larval stages of *Homarus americanus* is 5˚C, whereas for juvenile and adult lobsters, the range of survival is between -1 and 30.5˚C (Huntsman, 1924). However, when temperature drops below 5˚C, lobster molting and thus growth is restricted (Aiken, 1980). In the wild, the distribution and catchability of lobsters is impacted by temperature. In the winter season, when the weather becomes cold and the water is more turbulent, the lobsters move from shallow to deeper areas; they enter torpor, become less active and feed less. In the spring as temperature increases, lobsters become more active, and migrate inshore again (Ennis, 1983; MacKenize and Moring, 1985). Lobster molt timing, frequency and growth rate are impacted by water temperature (Kelly, 1993). Lobsters from cold waters in Canada (Nova Scotia, Quebec) (7 - 18˚C from May to September) molt later and less frequently than their counterparts of similar size from the warmer waters in United States (Maine) (10 - 21˚C from May to September) (Aiken and Waddy, 1986). Thus it is the temperature range that improves molting precision: in warmer water habitats, lobsters achieve a faster growth rate than those from colder areas.

Prelarval stages can tolerate salinities between 21 and 30 psu. When salinity decreases from 30 to 21 psu, the mortality of larvae increases, and they cannot survive in

salinities below 17 psu. The tolerance of juvenile and adult lobsters is wider, ranging from 6 to 30 psu (McLeese, 1956). Molting lobsters are much more vulnerable to low salinity, due to weaker osmoregulatory abilities during the molt (MacKenzie and Moring, 1985). Low salinity combined with higher temperatures is more stressful for juvenile and adult American lobsters (Jury, 1992). When the salinity drops below 15 psu, there is a significant increase in energy consumption; when the salinity falls below 10 psu, the lobsters tend to become moribund (Jury, 1992). Because of this, lobsters tend to avoid low salinity areas, and choose salinities between 25 - 30 psu (Jury, 1992). In lower temperature ranges (< 5˚C), lobster can survive in salinity as low as 6 psu for extended periods. Nevertheless once the salinity drops below 6 psu, up to 100% mortality can occur within 48 hours (McLeese, 1956).

The lethal oxygen concentration is significantly affected by temperature and salinity (McLeese, 1956). Juvenile and adult lobsters can tolerate low oxygen concentrations between 3.1% at 5˚C in 30 psu seawater and 59.3% at 25˚C in 20 psu seawater. Oxygen consumption increases during foraging and subsequent digestion and with increasing seawater temperature (McLeese, 1964). Lobsters can respond to hypoxia by increasing the amount and the affinity of haemocyanin and by increasing ventilation rate (McMahon and Wilkens, 1975). However, despite these physiological interventions they can only survive for a short period when oxygen concentration falls below 15% saturation at 15˚C in 25 psu seawater (McLeese, 1956).

1.1.5 Serum protein

The American lobster has pale bluish blood, due to the pigment haemocyanin. Haemocyanin is the predominant protein in the blood, accounting for between 60 - 90% of total protein in the blood (Uglow, 1969). Its main physiological function is to transport oxygen throughout the body (Palacios et al., 2000; Lin and Chen, 2001). There are two traditional methods to measure serum protein concentration, these are a Vet-Test blood chemistry analyser (Ozbay and Riley, 2002) or a modified biuret method (Layne, 1957). Both methods have shortcomings: they are relatively complex and time consuming, quite expensive to perform and often involve sacrificing the animal. More recently a rapid non-destructive method has been developed. A simple hand held refractometer has been used with small volumes of crustacean blood (Ozbay and Riley, 2002; Lorenzon et al., 2011). The refractive index has been used effectively to calculate the actual serum protein concentration (Sunderman, 1944; Smith and Dall, 1982).

Measurement of serum protein concentration has direct applications for the lobster industry. It can be used to determine the physiological conditions of live lobsters during transportation to market. Healthier individuals with a high serum protein concentration can tolerate longer periods out of water (Trutchot, 1983). The second application enables marketers to determine lobster quality. Serum protein concentrations have a high correlation with body muscle tissue content, thus animals with high levels will have a higher meat yield (Stewart et al., 1967). Finally it also enables one to determine lobster nutritional status. A number of articles have demonstrated that serum protein concentrations are directly related to food intake and quality (Stewart et al., 1967; Castell

and Budson, 1974; Hagerman, 1983; Pascual et al., 2006). Starved American lobsters exhibit a decrease in serum protein concentration (Stewart et al., 1967). European lobsters fed with bivalves did not have as high a serum protein concentration as those fed with shrimp, various invertebrates (a mixture of bivalves, shrimp and echinoderms) or pellets (Hagerman, 1983). Serum protein concentrations are also affected by both environmental and physiological conditions. In the wild, serum protein concentrations increase in spring and summer time (Stewart and Li, 1969). This might be due to the increasing water temperature and abundance of natural prey for the lobsters. In addition, serum protein concentrations also vary during the molt cycle (Barlow and Ridgway, 1969; Hepper,1977; Hagerman, 1983). In general, the highest serum protein concentration occurs before molting and is at its lowest level immediately after molting. Thus serum protein concentration is a useful indicator for determining health, molting and physiology of lobsters.

1.2 Lobster aquaculture

The American lobster fishery is very important in Canada and the United States (Boudreau and Worms, 2010). In the maritime provinces of Canada, 50,000 - 55,000 tons of lobsters are landed annually, and the value of lobster exported worldwide was around $800 million in 2009 (DFO, 2011b). Interest in American lobster aquaculture is growing, driven by increasing market demand with high market value in Asian countries, and the limited resources of wild stocks. Lobster aquaculture can potentially be divided into three areas: 1) stock enhancement, 2) on-growing of captured lobsters, 3) whole cycle culture.

The landing of American lobsters varies substantially among all 45 lobster fishing areas in Atlantic Canada, which makes lobster hatchery programs a potential method to improve and replenish natural stocks. Here the primary goal is to culture lobsters from eggs to larval stage IV or above, before releasing them into the wild (Waddy and Aiken, 1998; Nicosia and Lavalli, 1999; Castro et al., 2002). At present most of the cultured larvae are released at stage IV; these can be produced in large quantities, but the mortality rate is relatively high following release. The most likely reason for postlarval lobsters' high mortality rate is that they are not ready for immediate benthic settlement and as such this exposes them to natural predators (Castro and Cobb, 2005). There are two possible ways to improve the survival rate of these released lobsters. The first one is to enhance the health and quality of the larvae (Carlson, 1954; Beal and Chapman, 2001). Those fed with diets enriched in polyunsaturated fatty acids are able to accelerate their process of benthic settlement and thus evasion of predators (Gendron et al., 2013). The second way is employed in the European lobster stock enhancement program. Here the size at release is much larger (> 10 mm in carapace length), leading to relatively high survival rate (Bannister and Addison, 1998; Beal et al., 2002; Benavente et al., 2010). A preliminary study releasing larger American lobster also demonstrated a lower mortality rate (Wahle and Steneck, 1992). However one of the major obstacles faced by the American lobster enhancement program is the field research component. It is very difficult and expensive to conduct follow-up field research to identify the contribution that enhancement program efforts make to wild stocks (French McCay et al., 2003).

On-growing of captured lobster is where captured juvenile or smaller adult animals are fed with the aim of increasing their size and quality (Waddy, 1988). For

American lobsters, holding the sublegal sized individuals is a potential option for improving production because these lobsters are large enough to reach market size after one molt (Wilder, 1963). However, the study of on-growing of captured American lobsters is limited. A preliminary study of on-growth of the American lobster in captivity failed due to high mortality rates, because the lobsters were housed together and cannibalism occurred (McLeese, 1972b). While these mortalities could have been avoided by using a different cage design, there appear to have been no follow-up studies. Rather than on-growth, most adult lobsters are simply held in large indoor (such as those at Clearwater, NS) or outdoor storage compounds (such as those in the Bay of Fundy) prior to sale. At Clearwater Seafood Company located in Bedford, Nova Scotia, the claws are banded and the temperature reduced to 0 - 2^{o}C to induce a torpor. In the Bay of Fundy, lobster impoundments (McLeese and Wilder, 1964; Smolowitz et al., 1992) are used at ambient temperature during winter and spring when the temperature is low. The lobsters are not or minimally fed during this time and can be held for several months with minimal loss of product.

Capture-based aquaculture of some tropical and temperate lobster species has seen some interesting developments in last few decades. To date, all these commercial productions are spiny lobsters and based on wild caught lobster seeds. Land-based aquaculture is very expensive for both the facility and its maintenance, which means it is not yet a profitable venture (Booth and Kittaka, 1994; Jeffs and Hooker, 2000). However, sea-cage aquaculture is showing more promise and a variety of techniques, such as settlement of cages on sea bottoms (Lozano-Alvarez, 1996), suspension of cages from the water surface (Jeffs and James, 2001), and floating net cages (Mojjada et al., 2012) are

being used. Multitrophic aquaculture is another option for lobster aquaculture. A study of suspended culture cages of spiny lobsters in mussel farms was conducted by Jeffs and James (2001). The survival and growth of pueruli stages was as similar to, or better than those reared in laboratory conditions. The pueruli stage is the settling stage: it resembles the juvenile in shape with 9 - 13 mm in carapace length, but is transparent. However, the culture of one and two year old lobsters failed, because they were not separated and suffered high mortality due to cannibalism. Although studies of this kind on spiny lobster have increased in recent years, to date there are no comparable studies on clawed lobsters.

The whole cycle culture of lobster starts from the hatching of eggs, with animals reared all the way to market size. Although American lobsters have been successfully reared from eggs to market size in a culture period of two to three years, it is not considered to be economically feasible on a large scale (Wilder, 1971; Hughes et al., 1972; Lim et al., 1997). The European lobster however reaches market size more quickly. Norwegian Lobster Farms operated the first land-based sea water recirculating system of European lobster aquaculture in the world, rearing animals from egg to adult (Kristiansen et al., 2004). However, there are still many obstacles to overcome such as high capacity tanks that hold lobsters individually, self-cleaning tanks and automatic feeders (Aiken and Waddy, 1995; Drengstig and Bergheim, 2013). In addition, some inherent disadvantages include high costs of labor and investment. The most promising whole cycle culture is for tropical rock lobster (*Panulirus ornatus*) and slipper lobsters (*Thenus* spp.) in the tropics (Rogers et al., 2010). The slipper lobster (*Scyllaridae*) has several favorable attributes for aquaculture. It has a high reproductive capacity, producing around 0.5 - 0.7 million eggs (MacDiarmid and Saint-Marie, 2006), a relatively short larval development, around 4 - 8

months (Williams, 2007) and a rapid growth rate reaching over 1 kg in less than 1.5 years (Hambrey et al., 2001). Furthermore, cannibalism is not observed in this kind of lobster (*Scyllaridae*) , so they can be housed together. Nevertheless, culture of slipper lobster (*Scyllaridae*) is still an emerging industry and there are many areas, especially with regard to feeding regimes and diet that require further research.

1.3 Overall objectives

The primary goal of my research was to investigate methods of storage and on-growing of adult American lobsters *Homarus americanus* in inshore cages. To meet these goals, I carried out the following experiments. The first part of my project was to determine if American lobster could be used for on-growing. To accomplish this, I characterized the effect of various conditions (such as temperature, feed type, cage size, and feeding frequency) on survival rates, growth, and physiological conditions of cage-held lobsters in the laboratory. The second part of the project was to test the feasibility of inshore cage culture of lobsters. This could have two advantages – on-growing of animals to produce a larger, better quality product and a method whereby harvesters could store lobsters for prolonged periods and release them onto the market when prices increase. To accomplish this, two field experiments were set up. In one experiment, lobsters were fed by harvesters, and in the second the cage-held lobsters were “self-feeding” and held on blue mussel farms where they would get the potential input of mussels dropping off culture lines. In these experiments the animals' survival, growth and quality were assessed during a six month period. I anticipate that the knowledge generated by project would be

helpful in the future development of commercial lobster aquaculture and improve harvester income when selling lobsters during the winter/Christmas market.

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CHAPTER 2: Use of serum protein concentration as an indicator of quality and physiological condition in the lobster, *Homarus americanus* (Milne-Edwards, 1837)

2.1 Abstract

Decapod crustaceans are valuable seafood products, and although their price is typically gauged by their overall weight, the quality of the product (organ/muscle mass and moisture content) is also important. Here we report the use of the serum protein concentration (SPC) as a reliable indicator of the quality and physiological condition of adult lobsters, *Homarus americanus*. Lobsters were maintained in a variety of feeding regimes and environmental conditions in both the laboratory and in the field for six months. A hand-held refractometer was used to indirectly determine SPC at regular intervals. At the end of the experimental period, the lobsters were sacrificed and the hepatopancreas and heart mass, edible meat content, and moisture content of the hepatopancreas and muscle tissue were measured. In each case, a significant correlation existed with serum protein concentration. SPC increased linearly with hepatopancreas mass, heart mass and edible meat content. There was a concomitant decrease in SPC with increasing moisture content of the hepatopancreas and muscle tissue. Food intake had a significant effect, with higher SPC and thus larger organs, in animals that were maintained on a high feeding frequency regime (twice feeding per week). SPC also changed over the molt cycle reaching its highest levels in the premolt stage following by a sharp drop after the lobsters had molted. The serum protein concentration slowly increased thereafter, and 12 weeks after molting had reached levels that were similar to those measured at the start of the experiment. This rapid and non-invasive method has the potential to be a valuable tool for determining quality and physiological status of commercially important decapod crustaceans.

Keywords: American lobster, hepatopancreas, molting, quality, serum protein concentration

2.2 Introduction

Worldwide, decapod crustaceans support a multi-billion dollar fishing industry. In the last fifty years, advances in live storage and transport means these crustaceans can be shipped around the globe. In addition to the traditional fishing industry, commercial aquaculture of valuable crustacean species is growing rapidly (Wickens and Lee, 2002), and now exceeds the production volume and value of wild-captured crustaceans globally. Clawed lobsters (e.g. European lobster *Homarus gammarus*, American lobster *Homarus americanus*) and crabs (e.g. snow crab *Chionoecetes*, red king crab *Paralithodes camtschaticus*) are important species in fisheries in western countries, while spiny (*Jasus edwardsii*) and slipper lobsters (*Scyllarides latus*) are favored species for aquaculture in south-east Asia and Oceania (Jones, 2010). The increasing market demand for lobster species has led to inflated market prices and has driven interest in large scale lobster aquaculture. Because of this, many aspects of lobster biology have received extensive research attention (Van Olst et al., 1980; Wickens and Lee, 2002; Cox and Johnston, 2003; Lavalli and Spanier, 2007; Phillips, 2013). These studies have included, but are not limited to, growth and reproduction under various culture conditions such as temperature (Bartley et al., 1980; Thomas et al., 2000), feeding type and feeding regime (Shleser, 1974; Bartley et al., 1980; Lim et al., 1997; Mente et al., 2001; Barclay et al., 2006; Bryars and Geddes, 2005) and cage and pen design (Shleser, 1974). Although the value of the product is typically gauged by size (body mass and carapace length), health and quality are also critical measurements for economic and ethical reasons to produce a premium product and to reduce mortality.

Historically, several methods have been used to assess the general health and physiological condition of crustaceans. Direct observations of external injuries and vitality are used as quick and simple indicators. More invasive procedures include assessment of size and moisture content of the hepatopancreas and heart. The hepatopancreas and heart size correlate directly with the quality of the feed (Stewart et al., 1972). Lobsters fed with high levels of dietary protein (60%) have a larger hepatopancreas and heart than those fed with lower dietary protein levels (20% or 40%) (Castell and Budson, 1974). This trend is accompanied by a concomitant decrease in moisture content of hepatopancreas and body muscle tissue (Castell and Budson, 1974). The edible meat content of an animal is typically measured directly by cooking the animal and calculating muscle percentage as a function of total live weight (Stewart et al., 1967b; Stewart et al., 1972). Although these methods are accurate health and quality indicators, they are time consuming to perform and involve sacrificing some of the product.

In addition to visual inspection of health and direct sampling of organs or meat content, tests of physiological condition may be used as more in-depth indicators of health and quality status (Lorenzon et al., 2008; Stoner, 2012). Hemolymph glucose levels have traditionally been used as a stress indicator. Hemolymph glucose increases during transport processes such as emersion and handling stress and also in lobsters and crabs suffering from septicemia and bacteremia (Paterson et al., 2005; Basti et al., 2010; Woll et al., 2010). Increased lactate levels also have been used as a stress indicator, especially during transport to market when animals undergo long periods of aerial exposure or poor handling occurs (Barrento et al., 2010). However, since lactate levels are influenced by temperature, season and even time of day, its use as a general health

indicator is somewhat limited. Although these methods are precise and reliable, all samples have to be analyzed in the laboratory which makes the operation time consuming and expensive. In addition, some of these physiological parameters are very sensitive and change quickly with time.

Changes in hemolymph protein levels have also been used to identify the physiological condition of crustaceans (Rosas et al., 2004). The major blood protein in crustacean hemolymph is hemocyanin, accounting for 60 - 90% of total protein in the blood (Uglow, 1969). Its main physiological function is to transport oxygen throughout the body (Palacios et al., 2000; Lin and Chen, 2001). Hemolymph proteins are also important for reproduction, adaptation to environmental stress and function in the immune response (Hall et al., 1999; Montaño-Pérez et al., 1999; Vargas-Albores and Yepiz-Plascencia, 2000; Oliver and MacDiarmid, 2001; Arcos et al., 2003). Although the analysis of SPC is not as sensitive as other physiological indicators, decreasing levels may be a useful indicator of trauma (Fotedar et al., 2002) and stress (Chang, 2005). Furthermore, serum protein has also been used as an indicator for nutritional condition. In the lobster, *Homarus americanus,* hemocyte numbers and SPC are related to diet quality (Stewart et al., 1967a; McLeese, 1972; Hagerman, 1983). Lobsters fed with a cod and liver diet have a higher hemocyte count and SPC than those fed a herring diet (McLeese, 1972). Likewise European lobsters fed with shrimp or various invertebrates and pellets (commonly used as fish food) have higher SPC than those fed only with bivalves (Hagerman, 1983). White leg shrimp*, Litopenaeus vannamei*, fed with a high protein diet exhibited slower rates of serum protein decline during a period of starvation compared to those maintained on a low protein diet (Pascual et al., 2006). Moreover, SPC is correlated

with meat content, with higher SPC observed in animals with a higher live wet weight (Stewart et al., 1967b). Although serum protein levels are a useful indicator, one must be careful when interpreting results since several other factors affect SPC. For example, serum protein concentrations increase during the intermolt period, reaching highest levels just prior to molting, but then decrease to their lowest levels immediately following the molt (Barlow and Ridgway, 1969).

Two methods have been commonly used to measure SPC. These include a modified biuret method (Layne, 1957) and a Vet-Test blood chemistry analyser (Ozbay and Riley, 2002). Both methods have their limitations in that these laboratory tests are somewhat complicated, time consuming, and costly. In the last decade, progress has been made whereby specific gravity and density-salinity refractometers have been used to directly or indirectly measure serum protein concentration (Ozbay and Riley, 2002; Lorenzon et al., 2011). These rapid and non-destructive methods allow serum protein concentration to be determined on site in a matter of minutes.

Although there are a number of studies verifying the use of SPC as a useful indicator of nutritional status (Stewart et al., 1967a, b; Stewart and Li, 1969; Stewart et al., 1972; Hagerman, 1983), none of them show a direct relationship between serum protein levels and some of the more traditional and invasive procedures for assessing health (organ size and moisture content, meat levels). Therefore the aim of current study was to investigate the relationship between SPC (calculated from refractive index - RI) and the more traditional health and nutritional indicators such as organ size and moisture content and edible meat content of adult lobsters. This will validate the use of the

refractive index method as a rapid non-destructive and economical method to determine quality and physiological condition in commercially important crustaceans.

2.3 Materials and Methods

2.3.1 Animals and experimental design

The animals used in the present study were part of a larger project investigating growth and survival of cage-held adult lobsters (see Chapter 3). Adult male and female intermolt lobsters used in laboratory experiments were purchased from Clearwater, NS, and held at the Department of Ocean Sciences, Memorial University in running aerated seawater (30 - 32 psu). Lobsters used in the field experiments were purchased from local harvesters in the Triton, NL, in May 2013. Lobsters (n = 120) purchased from Clearwater had a mass (mean ± SD) of 364 ± 59 g and carapace length of 77 ± 3 mm, significantly smaller in size than those from Triton, NL; those (n = 192) from Triton had a mass of 601 ± 92 g and carapace length of 88 ± 3 mm.

Two separate experiments were undertaken in the laboratory, and one at field sites from June to December 2013. During experiments, the lobsters were held in plastic coated wire mesh (2.5 cm) cages of 1.2 ×0.9 × 0.3 m in depth, with one lobster housed in a single compartment of 0.15 x 0.3 x 0.3 m. In the first laboratory experiment, all 96 animals were fed a mixed (snow crab body *Chionoecetes*, pink shrimp *Pandalus borealis*, squid *Illex illecebrosus*, scallop mantle *Placopecten magellanicus* and mackerel *Scomber scombrus*) diet; 48 animals were fed twice weekly, while the remaining 48 lobsters were fed once per month. They were maintained on an ambient seawater temperature cycle

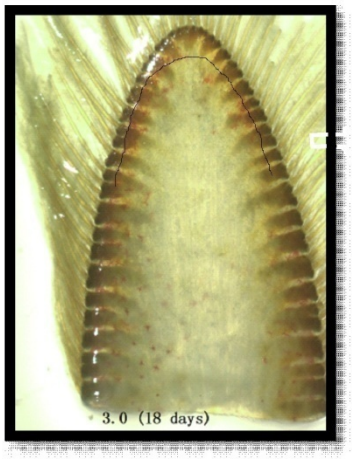
(June - December) with water pumped from Logy Bay, NL. In the second laboratory experiment, lobsters (n = 24) were maintained at a constant 15˚C and fed a mixed diet. Hemolymph samples were collected at one week intervals before and following molting. The process of hemolymph sampling was described below in **2.3.3. Parameter**. In the field experiment, 192 lobsters were held in 8 benthic cages near Triton, NL (N49^{o} 29' 03.03', W55^{o} 45' 03.58') between June and December 2013. The cages were set either directly under the mussel lines of Sunrise Fish Farms where lobsters would get the potential input of mussels falling off lines during harvesting, or they were set on the seabed where they would not get input of mussels, but could feed on benthic fauna that may have drifted into the cages.

Hemolymph samples were taken at monthly intervals in the laboratory and at three month intervals in the field. At the termination of the experiments (6 months), hemolymph samples were collected from 65 randomly selected lobsters from feeding frequency treatment and field. These individuals were then sacrificed and the hepatopancreas and heart were removed and the amount of muscle tissue in the second pair of walking legs calculated. Because the animals were maintained in a variety of conditions (feeding frequency, diet and temperature), this insured that there would potentially be a wide range of serum protein levels.

2.3.2 Molt stage identification

The identification of the different molt stages of adult lobster was carried out by staging the developmental morphology of the pleopods following the methods outlined in Aiken (1973) and Fig. 2-1. During intermolt and post-molt, there are no new setae formed

Fig. 2-1. Morphology of adult lobster pleopod in the proecdysis stage (3.0 - 5.5) using Infinity Capture Imaging Software at 40 x magnification. In 1 (stage 3.0), the new epidermis forms and becomes visible; in 2 (stage 3.5), new setae form but are not well developed; in 3 (stage 4.0), setae are well developed and the shafts become visible; in 4 (stage 4.5), shafts develop to a full length and the end of each shaft is bifurcate; in 5 (stage 5.0), shafts continue develop and the end of each shaft become blunt; in 6 (stage 5.5), shafts continue develop and become thicker and darker. The lines drawn in 2 represent the new setae appear; in 3, the lines represent the setae become visible; in 4, the lines represent the shafts fully developed and the end were bifurcate; in 5, the lines represent the end of shafts become blunt; in 6, the lines represent the shafts become thicker and darker (the definition of stages were referred to Aiken, 1973; the whole figures were from present study).



1

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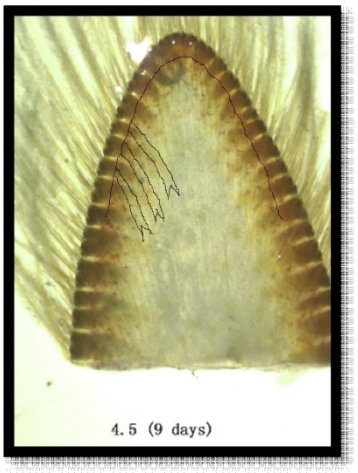

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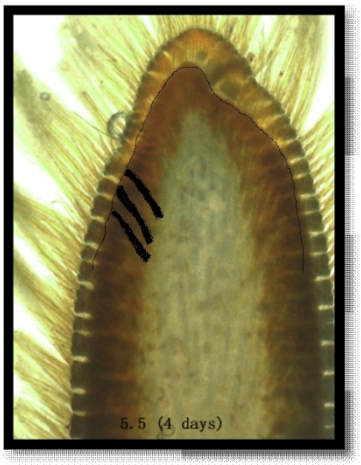


6

and no sign of the retraction of the epidermis. During proecdysis, newly forming setae are evident and retraction of the epidermis occurs. In the present study, lobster pleopods were sampled at one month intervals and each sample was immediately photographed using Infinity Capture Imaging Software at 40 x magnification.

2.3.3 Parameters

The refractive index (RI) was measured using a Brix/RI-Chek Digital Pocket Refractometer (Reichert Analytical Instruments, Depew, NY, USA). First the refractometer was calibrated with distilled water. A 500 µl sample of hemolymph was then withdrawn by inserting a 21 gauge needle and 1 ml syringe into the arthrodial membrane at the base of the fourth walking legs and the sample was injected onto the sample well. This method was shown to be innocuous and did not have any major impact on health and general behavior of lobster. Three components were automatically analyzed in sequence: temperature compensated percent solids (Brix-TC), temperature compensated refractive index (RI-TC), and refractive index (RI). The time between withdrawal of the hemolymph and processing of the sample did not exceed 90 secs. The actual serum protein concentration was calculated using the formula:

$$P = 510 \times (R.I.blood - R.I.water) - 1.81$$

Where P is in g/100 ml; R.I.blood is the refractive index of blood; R.I.water is the refractive index of water (Sunderman, 1944).

The wet mass of the hepatopancreas and heart were measured by sacrificing the animal by destroying the subesophageal ganglion with a pair of pliers. The organs were

then dissected out and patted dry with blotting paper for 3 to 5 min. After measurement of their wet mass, the organs were transferred to a Fisher Isotemp® drying oven and maintained at 80˚C for 72 hours to obtain a dry mass for each organ. The hepatopancreas and heart indices, and hepatopancreas and body muscle moisture content were calculated using the following formulas:

Hepatopancreas index = (hepatopancreas wet mass / total wet body mass) × 100.

Heart index = (heart wet mass / total wet body mass) × 100.

Hepatopancreas moisture index = (hepatopancreas wet mass – hepatopancreas dry mass) / hepatopancreas wet mass × 100.

Body muscle moisture index = (body muscle wet mass – body muscle dry mass) / body muscle wet mass × 100.

For meat content analysis, the second left and right walking legs were removed and boiled for 45 min. After being cooled, the walking legs were cut transversely, the cut leg was examined under a dissecting microscope and the cross section photographed using Infinity Capture Imaging Software. The horizontal and vertical diameter of both the leg muscle and its exoskeleton were measured using Infinity Analyze Software. The actual meat content of the lobster was then calculated following the methods outlined in James et al. (2013).

Meat index = $(S_1 / S_2) \times 100$

where S_1 is the cross sectional area of the lobster walking leg meat; S_2 is the cross sectional area of the lobster walking leg exoskeleton; S_1 and S_2 were calculated using the formula for an ellipse: $S = \pi \times a \times b$ (a is semi-major axis; b is semi-minor axis).

2.3.4 Statistical analysis

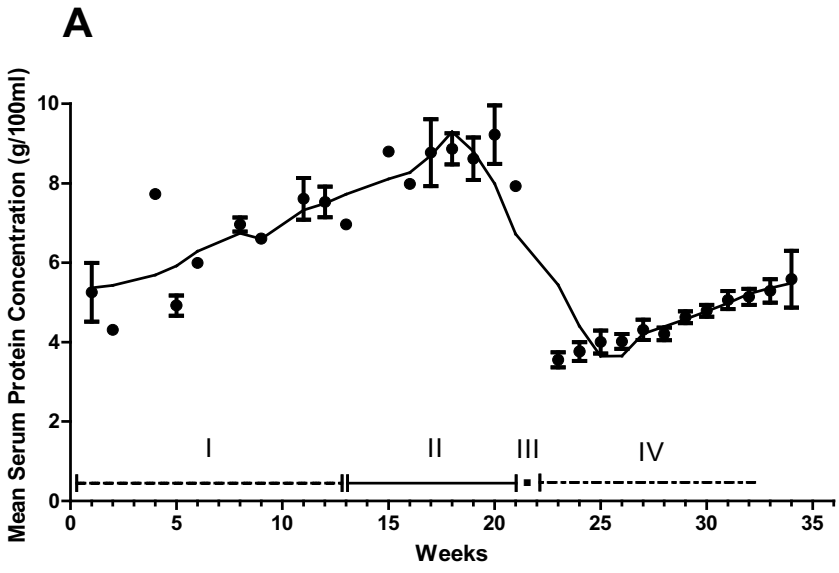
Statistical analyses were performed using Prism v5.0 and Minitab (GraphPad Software Inc., La Jolla. CA; v 16; State College, PA, USA). The relationship between serum protein concentration and health parameters, and changes in serum protein concentrations during the molt cycle were analyzed with linear regressions. The differences in serum protein levels in the two different feeding frequencies were determined using with Student t-tests with significance accepted at the $P < 0.05$ level.

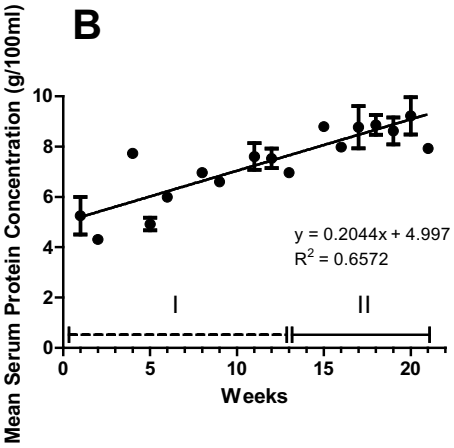
2.4 Results

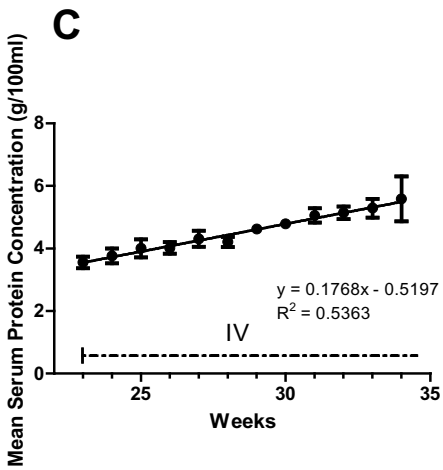
2.4.1 Serum protein concentration and molt stage

There was a significant change in serum protein concentration as a function of molt stage in lobsters maintained at 15˚C and fed a mixed diet (Fig. 2-2. A). Serum protein concentrations increased from an initial mean level of 5.25 ± 1.05 g/100 ml (intermolt stage I) reaching a peak level of 9.22 ± 1.28 g/100 ml after 21 weeks (intermolt stage II). The lobsters (n = 16) molted at the end of the 21st week and thereafter the mean serum protein concentration dropped to the lowest level of 3.55 ± 0.50 g/100 ml during the 22nd week. Serum protein concentrations slowly recovered during the post-molt stage

Fig. 2-2. A. Variation in serum protein concentration during the six-month period at different molting stages, where I is intermolt, II is proecdysis, III is ecdysis, and IV is post-molt. B. The linear regression line between serum protein concentration and time (weeks) for adult lobsters at stages I and II. C. The lobsters were bought from Clearwater seafood company (Bedford, Nova Scotia), held at 15˚C and fed mixed diet for a six-month period. The linear regression line for serum protein concentration and time (weeks) in adult lobsters at stage IV. The data in post-molt were collected in 13 weeks due to the time limit of experimental design. Linear regression was performed. Data are expressed as mean ± SD, n = 16.


A
Mean Serum Protein Concentration (g/100ml)
Weeks
I
II
III
IV


B
Mean Serum Protein Concentration (g/100ml)
Weeks
y = 0.2044x + 4.997
R2 = 0.6572
I
II

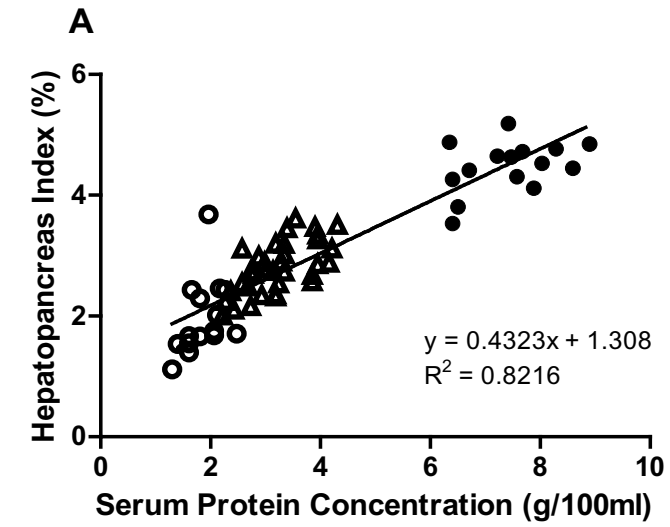

C
Mean Serum Protein Concentration (g/100ml)
Weeks
y = 0.1768x - 0.5197
R2 = 0.5363
IV

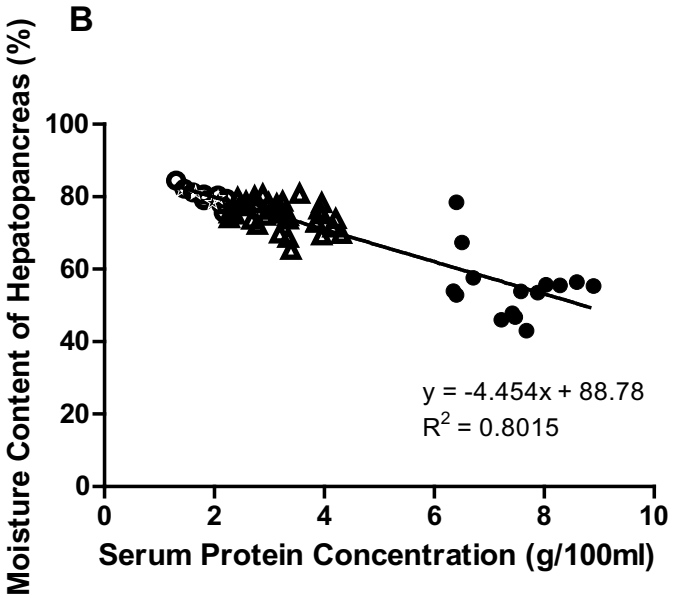
IV, reaching levels of 5.29 ± 0.67 g/100 ml at the end of the experimental period (33rd week), similar to those measured at the start of the experiment (week 1) (Student t test, t = 0.056, P = 0.957). Linear regression analysis was performed separately on the premolt and postmolt stages. In both cases, there was a significant increase in serum protein levels with time (Regression, t = 12.55, $P < 0.0001$, $R^2 = 0.657$; Regression, t = -2.53, $P < 0.0001$, $R^2 = 0.536$ for premolt and postmolt, respectively) (Fig. 2-2B, C), but the slopes of regression lines of changes in premolt and post-molt serum protein concentrations were not significantly different from one another (Linear regression, F = 0.86, P = 0.355). Based on the regression equation of serum protein concentrations during post-molt, it can be estimated that a newly molted lobster with a mean serum protein concentration of 3.55 ± 0.50 g/100 ml would require approximately 32 weeks after molting to regain maximal serum protein concentrations (9.22 ± 1.28 g/100 ml) similar to those measured during premolt, stage II.

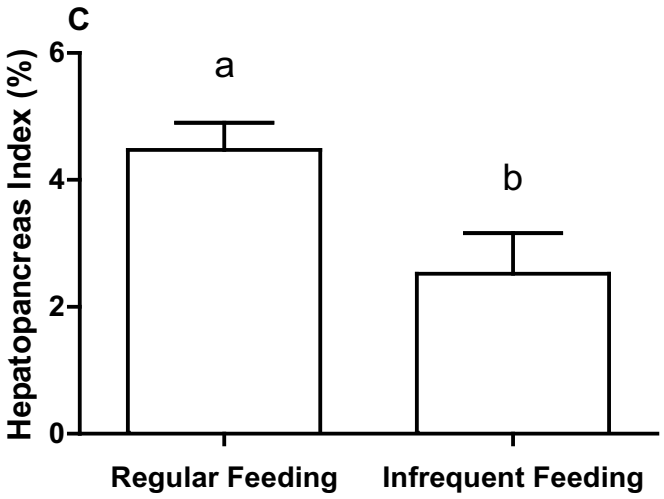
2.4.2 Serum protein concentration and organ size

Serum protein concentration was positively correlated with hepatopancreas wet mass (Regression, t = 11.72, $P < 0.0001$, $R^2 = 0.822$), while negatively correlated with the hepatopancreas moisture content (Regression, t = 72.29, $P < 0.0001$, $R^2 = 0.802$) (Fig. 2-3A, B). In concordance with the hepatopancreas wet mass, there was a positive relationship between serum protein concentration and heart wet mass (Regression, t = 18.37, $P < 0.0001$, $R^2 = 0.713$) (Fig. 2-4A). There was also a strong negative correlation between serum protein concentration and the moisture content of body muscle tissue (Regression, t = 464.75, $P < 0.0001$, $R^2 = 0.844$) (Fig. 2-5A).

Fig. 2-3. A. The linear regression line for serum protein concentration and hepatopancreas size in adult lobsters from both laboratory and field studies. B. The linear regression line for serum protein concentration and hepatopancreas moisture content in adult lobsters from both laboratory and field studies. The open triangle values were from lobsters in laboratory at one feeding/month and open circle values from lobsters in the field (Triton area); the solid circle values were from lobsters in laboratory at two feedings/week. C. Comparison of hepatopancreas size between high and low feeding frequency during six month growth trial for adult lobsters from only laboratory study. D. Comparison of hepatopancreas moisture content between high and low feeding frequency during six month growth trial for adult lobsters from only laboratory study. Linear regression and t tests were performed. Data are expressed as mean ± SD, n = 65. Different letters indicate a significant difference between two feeding frequency levels ($P < 0.0001$).


A
Hepatopancreas Index (%)
6
4
2
0
0
2
4
6
8
10
Serum Protein Concentration (g/100ml)
y = 0.4323x + 1.308
R2 = 0.8216


B
Moisture Content of Hepatopancreas (%)
100
80
60
40
20
0
0
2
4
6
8
10
Serum Protein Concentration (g/100ml)
y = -4.454x + 88.78
R2 = 0.8015


C
Hepatopancreas Index (%)
6
4
2
0
a
b
Regular Feeding
Infrequent Feeding

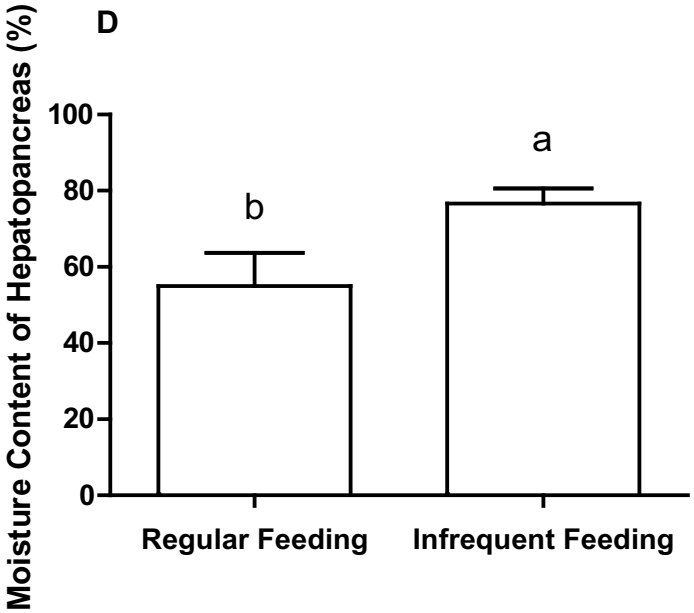
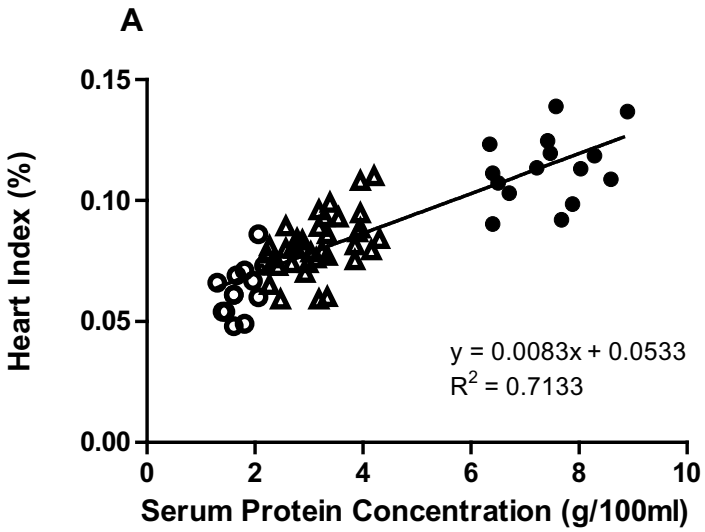

D
Moisture Content of Hepatopancreas (%)
100
80
60
40
20
0
b
a
Regular Feeding
Infrequent Feeding

Fig. 2-4. A. The linear regression line for serum protein concentration and heart index in adult lobsters from both laboratory and field studies. The open triangle values were from lobsters in laboratory at one feeding/month and open circle values from lobsters in the field (Triton area); the solid circle values were from lobsters in laboratory at two feedings/week. B. Comparison of heart size between high and low feeding frequency during six month growth trial for adult lobsters from only the laboratory study. Linear regression and t test were performed. Data are expressed as mean ± SD, n = 65. Different letters indicate significant difference between two feeding frequency levels ($P < 0.0001$).


A
0.15
0.10
0.05
0.00
Heart Index (%)
y = 0.0083x + 0.0533
R2 = 0.7133
0
2
4
6
8
10
Serum Protein Concentration (g/100ml)

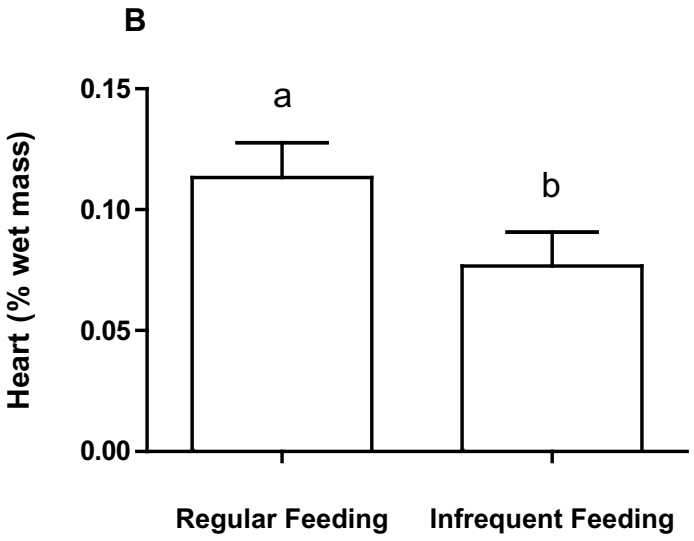
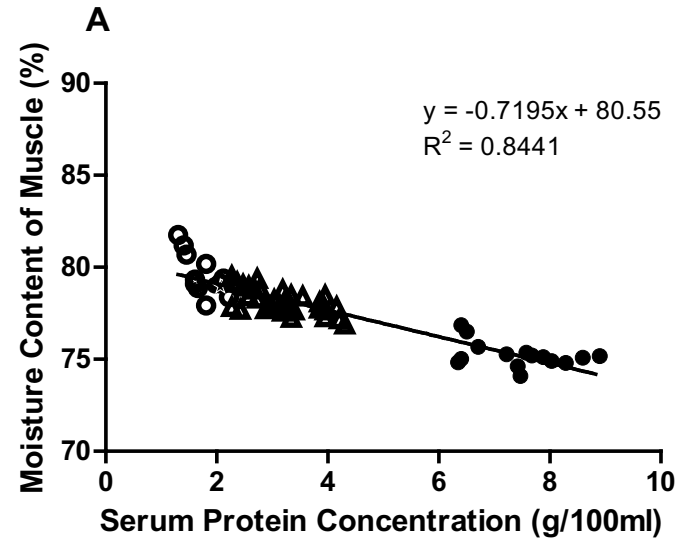
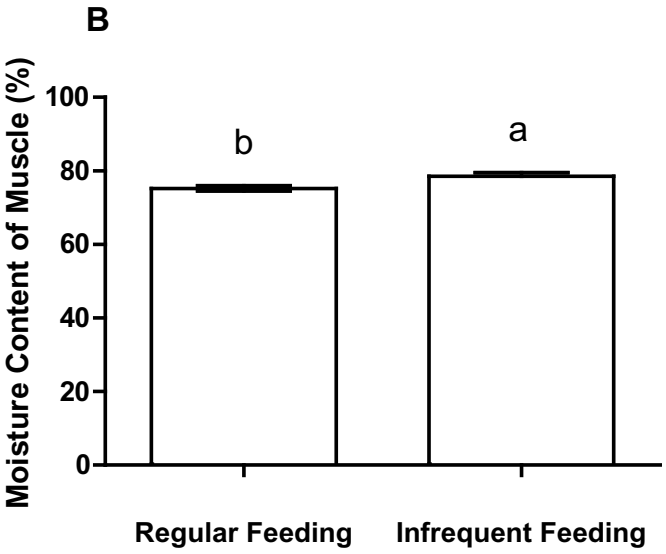

B
0.15
0.10
0.05
0.00
Heart (% wet mass)
a
b
Regular Feeding
Infrequent Feeding

Fig. 2-5. A. The linear regression line for serum protein concentration and body muscle moisture content in adult lobsters. The open triangle values were from lobsters in laboratory at one feeding/month and open circle values from lobsters in the field (Triton area); the solid circle values were from lobsters in laboratory at two feedings/week. B. Comparison of body muscle moisture content between high and low feeding frequency during six month growth trial for adult lobsters. Linear regression and t test were performed. Data are expressed as mean ± SD, n = 65. Different letters indicate significant difference between two feeding frequency levels ($p < 0.0001$).


A
y = -0.7195x + 80.55
R2 = 0.8441
Moisture Content of Muscle (%)
90
85
80
75
70
0
2
4
6
8
10
Serum Protein Concentration (g/100ml)

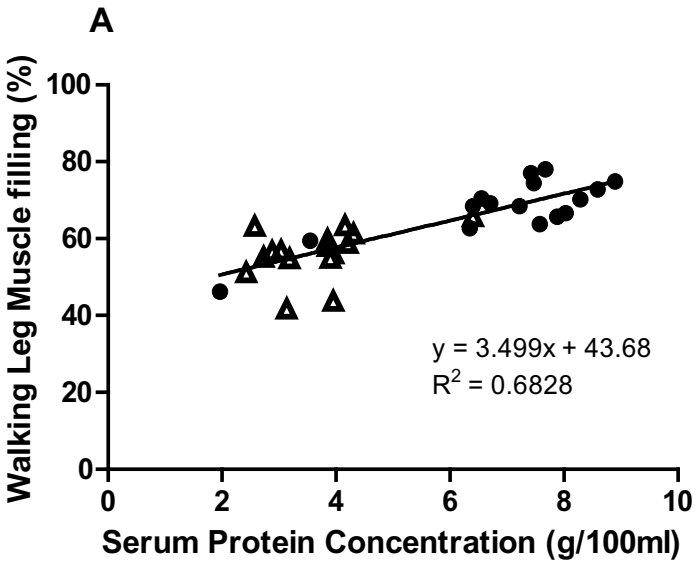

B
Moisture Content of Muscle (%)
100
80
60
40
20
0
b
a
Regular Feeding
Infrequent Feeding

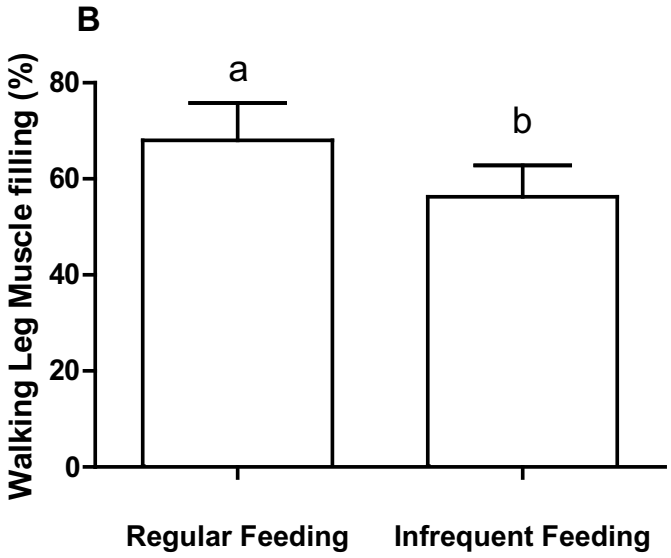
On each graph, it is interesting to note that the data were composed of two distinct groups. The data from each of these graphs were obtained from the laboratory experiments with animals fed either twice per week or once per month and from the field experiment at Triton where they had restricted access to food. Lobsters fed twice a week acquired a larger size of heart (Student t test, t = 8.75, $P < 0.0001$) (Fig. 2-4B) and hepatopancreas (Student t test, t = 13.66, $P < 0.0001$) (Fig. 2-3C), and had a lower moisture content in the hepatopancreas (Student t test, t = -9.35, $P < 0.0001$) (Fig. 2-3D) and body muscle tissue content (Student t test, t = -14.50, $P < 0.0001$) (Fig. 2-5B) and thus significantly different serum protein concentrations compared with lobsters fed once a month or lobsters maintained in benthic cages in the field.

2.4.3 Serum protein and walking leg muscle content

There was a clear linear relationship between serum protein concentration and muscle content of the walking leg (Fig. 2-6A) (Regression, t = 17.59, $P < 0.0001$, $R^2 = 0.687$). In addition, when the muscle content of the walking legs was analyzed as a function of feeding frequency (Fig. 2-6B), the lobsters fed twice per week had mean serum protein levels that were 12% higher than those fed once per month (Student t test, t = -7.11, $P < 0.05$). The mean edible meat content in the legs of the animals fed twice a week was also significant higher, 69.90 ± 4.71%, compared with 55.35 ± 6.30% for lobsters maintained on an infrequent feeding regime.

Fig. 2-6. A. The linear regression line for serum protein concentration and walking leg muscle content in adult lobsters. The open triangle values were from lobsters in laboratory at one feeding/month and open circle values from lobsters in the field (Triton area); the solid circle values were from lobsters in laboratory at two feedings/week. B. Comparison of walking leg muscle filling percent between high and low feeding frequency during six month growth trial for adult lobsters. Linear regression and t test were performed. Data are expressed as mean ± SD, n = 32. Different letters indicate significant difference between two feeding frequency levels ($P < 0.0001$).


A
Walking Leg Muscle filling (%)
100
80
60
40
20
0
0
2
4
6
8
10
Serum Protein Concentration (g/100ml)
y = 3.499x + 43.68
R2 = 0.6828


B
Walking Leg Muscle filling (%)
80
60
40
20
0
a
b
Regular Feeding
Infrequent Feeding

2.5 Discussion

Decapod crustaceans are one of the most valuable seafood commodities, and in particular live animals command a high price (Estrella, 1993; Holiday and O'Bonnon, 1994). Although the price is usually dictated by overall mass, consumers are becoming more aware of the quality of the product (Anderson and Anderson, 1991; Jaffry et al., 2004; Bremner, 2005). Quality control is not only important for the consumer; a healthy animal is more likely to survive transport to, and storage at market (Lorenzon et al, 2007). The actual meat content relative to the moisture content is important, and inferior products have a higher water content (Castell and Budson, 1974; Rosa and Nunes, 2004). In addition to the meat content, the hepatopancreas or digestive gland of decapod crustaceans is considered a delicacy, especially in Europe and the far East (Lawrence et al., 1994), with higher quality lobsters having a larger more fatty hepatopancreas (Barrento et al., 2009). Traditional methods to assess meat or hepatopancreas quality involve cooking the whole animal (Castell and Budson, 1974), or removing part of the animal, such as a leg, to calculate edible meat content (James et al., 2013).

The decapod crustacean hepatopancreas functions in the absorption, transport, and storage of nutrients (Yonge, 1924; Vogt et al., 1989; Johnston et al., 1998; Wen et al., 2006; Jiang et al., 2009). As such, a healthy animal with an optimal energy intake would be expected to have larger organs and a greater muscle mass with a low moisture content. Indeed, the wet mass and moisture content of the hepatopancreas and muscle tissue have been used extensively as important indicators of the adequacy and quality of experimental diets for crustaceans (e.g., Stewart et al., 1972; Castell and Budson, 1974; Lawrence et

al., 1979; Sánchez-Paz et al., 2007; Mente, 2010; Mente et al., 2011). A significant decrease in hepatopancreas size and an increase in its moisture content occur in starved lobsters *Homarus americanus* (Stewart et al., 1972; Castell and Budson, 1974), western rock lobster *Panulirus longipes* (Dall, 1974) and Pacific white shrimp *Litopenaeus vannamei* (Sánchez-Paz et al., 2007). In addition, both the hepatopancreas and heart size of *Homarus americanus* is positively correlated with levels of dietary protein (Castell and Budson, 1974). The results of our study confirmed these findings. The hepatopancreas and heart index were 1.8 and 1.5 fold larger respectively for lobsters fed twice per week compared with those fed monthly or maintained in the field with restricted access to food. In addition, the moisture content of hepatopancreas and body muscle tissues were 20.2% and 3.2% higher respectively for lobsters in the low feeding frequency compared with those in the high feeding frequency treatment. In line with the muscle tissue moisture content, the lobsters in the high frequency feeding treatment had a greater muscle mass (edible meat content) that was 12.3% higher than those fed in the low feeding regime. This was somewhat higher than the 7.5% difference between fed and starved lobsters reported by Stewart et al. (1967b).

Although direct observation of organ size and moisture content provides accurate and reliable information, such measurements are time consuming and either involve damage to the animal or sacrificing some of the product. Analysis of the serum protein concentration, while not as precise, has been used as a less invasive indicator of health and nutritional status of crustaceans (reviewed in Lorenzon et al., 2011). Historically serum protein concentration was measured using laboratory-based tests (Layne, 1957; Ozbay and Riley, 2002), while more recently hand-held refractometers have been shown

to provide an accurate means to determine serum protein concentration in a variety of crustacean species (Lorenzon et al., 2011).

Stewart et al. (1967a, b) were the first report of the use of the serum protein concentration as an indicator of lobster nutritive status, with higher serum protein concentrations in fed lobsters compared to lobsters that were starved for 3 - 8 months (Stewart et al., 1967a,b, 1972). This trend extends to the level of food intake: animals with a higher feeding frequency attain higher serum protein levels compared with those on a restricted diet (Stewart and Li, 1969; McLeese, 1972; Smith and Dall, 1982; Moore et al., 2000; Pascual et al., 2006). This was also observed in the present study: lobsters fed frequently in the laboratory had a 2.7 fold higher serum protein levels compared to their counterparts fed infrequently and those with limited access to natural food resources in the field. Stewart et al. (1967b) also report a significant correlation between serum protein concentration and edible meat content, but this was only found in starved lobsters, and there was no correlation between serum protein and meat content for fed lobsters. We were able to investigate a wider range of serum concentrations (1.1 to 9.3 g/100 ml, compared with 1.5 to 6.3 g/100 ml) and found strong positive correlations between serum protein concentration and hepatopancreas and heart size and edible meat content. As the size of the organs decreased, their water content increased and there was a strong negative relationship between serum protein concentration and moisture content of hepatopancreas and body muscle tissue (Fig. 2-2, 2-3, and 2-4). These relationships remained consistent when the lobsters were fed different diets, maintained at different temperatures and at different holding techniques in the laboratory and field (Chapter 3). These suggest that

serum protein concentration is a reliable indicator of animal quality over a wide range of conditions.

In addition to food intake, the reproductive status and infection from pathogens influence the variation of serum protein concentration (proportion and quantity) (Arcos et al., 2003). Environmental factors such as temperature (McLeese, 1972), feed type (McLeese, 1972; Hagerman, 1983), light intensity (Stewart et al., 1967b), salinity levels (Depledge and Bjerregaard, 1989; Perazzolo et al., 2002) and aquatic hypoxia (Depledge and Bjerregaard, 1989), also influence lobster serum protein concentration. However, these tend to influence feeding behaviours and appetite rather than having a direct effect on the serum protein concentration itself.

In the present study, serum protein concentration changed over the molt cycle; it increased during premolt, reached maximal levels just before molting and then dropped sharply immediately after the lobster molted. This pattern has also been reported before for both European lobsters (*Homarus gammarus*) and American lobsters (*Homarus americanus*) (Barlow and Ridgway, 1969; Hepper, 1977; Hagerman, 1983), brown shrimp (*Crangon vulgaris*) (Djangmah, 1970) and Norway lobster (*Nephrops norvegicus*) (Philp and Marteinsdottír, 2013). The sudden drop of serum protein concentration during lobsters' ecdysis is a result of a dilution effect associated with water uptake which is used for expansion of the body. In addition to the primary function of supplying oxygen, haemocyanin and other serum proteins might be involved with transport of hormones and phenols to be used as components in the newly formed exoskeleton (Terwilliger, 1999). To the best of my knowledge, there is no data showing changes over time in post-molt lobsters (Hepper, 1977; Hagerman, 1983; Philp and Marteinsdottír, 2013). The

subsequent observed increase in serum protein concentration in postmolt probably occurs as the lobster feeds and grows replacing the water in the body tissue with muscle (Smith and Dall, 1982; Oliver and MacDiarmid, 2001).

The fact that serum protein levels are influenced by the molt stage would appear to limit the use of this technique. However, recently molted lobsters (with low serum protein concentrations) are easy to distinguish by their crispy soft shell, and newly molted lobsters are seldom caught in the wild because of their reduced movement and food intake (Lipcius and Herrnkind, 1982). Once the animals start to harden the shell, the molt stage may not be readily apparent. However, although a low serum level might not necessarily equate with an under-nourished or unhealthy animal, it will directly correlate with important attributes of organ size and water content. Recently molted crustaceans, such as snow crabs *Chionoecetes opilio* have a low meat yield relative to their overall body mass and the flesh tends to be of poor quality (Moriyasu and Mallet, 1986). This is because the dilution effect associated with the uptake of water contributes to the sharp drop in serum protein concentration (Oliver and MacDiarmid, 2001). As a result, the newly molted lobster has a much higher percentage of water relative to meat content in shells. Thereafter, newly molted lobsters continue to feed and grow and in doing so increase muscle and organ mass with a concomitant increase in serum protein concentration (Smith and Dall, 1982; Oliver and MacDiarmid, 2001).

When the lobsters that were maintained in the field were transported to the Department of Ocean Sciences at the end of the six month experiment (10 h emersion), a high mortality rate occurred. In addition to an indicator of quality, the level of serum protein may also be important for live transport of crustaceans, where they often undergo

prolonged periods of exposure to air. This might not be unexpected since the majority of the serum protein is haemocyanin which is used for oxygen transport (Uglow, 1969; Palacios et al., 2000; Lin and Chen, 2001). Healthy lobsters are able to increase their total protein levels, especially haemocyanin, during air exposure (Lorenzon et al.,2007). Since haemocyanin predominantly functions for transport of oxygen throughout the whole body, such a mechanism would be important for survival (Taylor, 1981; Taylor and Whiteley, 1989). In contrast, lobsters with a low serum protein concentration would have a reduced ability to bind oxygen when exposure to air during transport.

2.6 Conclusions

Most of the recent studies on serum protein concentration have focused on its use as an indicator for crustacean nutritional status under various conditions (e.g., molting cycle, hypoxia, temperature, feeding, and type of diet). In the present study, we were able to show that serum protein concentration is correlated with the quality of the lobster destined for market. This relationship was consistent even when animals were maintained in a variety of conditions. Although this method is used to indirectly estimate lobster serum protein concentration, it is rapid and cost effective in large scale work. The important linear correlations included: 1) positive relationship between serum protein concentration and hepatopancreas index, heart index and body muscle tissue index; 2) negative relationship between serum protein concentration and moisture content of hepatopancreas and body muscle tissue.

2.7 Acknowledgements

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CHAPTER 3: Storage and on-growing of adult American lobster, *Homarus americanus*, in inshore benthic cages

3.1 Abstract

The use of inshore benthic cages was investigated as an alternative method to hold adult lobsters *Homarus americanus*. The aims were to determine if lobsters can be maintained for prolonged periods and survive and grow: the larger product could then be sold outside of the regular fishing season for a higher price. The effects of temperature, diet type, feeding frequency and cage size on the survival, molting, growth rates and blood protein concentrations were monitored at regular intervals over a six month period, in both the laboratory and the field. In laboratory experiments, various environmental and biotic factors were manipulated to determine the optimal conditions for survival and growth. In the field, experiments tested the feasibility of the inshore cages. In the laboratory, molting was highest at 15˚C and survival lowest at 5˚C, and lobsters fed a mixed versus a mussel only diet were healthier. In a separate laboratory experiment, lobsters that were fed twice weekly grew larger than those fed once per month. However, feeding frequency did not affect survival or the number of animals that molted. Two separate experiments were conducted in the field. One group of lobsters were fed by local harvesters at regular intervals. Lobster survival rate was 88% and just over one third of the lobsters molted. Lobster cages were also set under mussel farms, with the idea that they would feed on mussels dropping off lines. Although survival rates were high (> 95%), the molting rate was low (13%) and analysis of blood protein concentration showed they were in a poorer condition than the fed lobsters. This project showed that these benthic cages provide a viable method to store lobsters for up to six months, allowing harvesters to sell them locally, outside the regular season.

Keywords: Aquaculture, growth, lobster, molting, serum protein, survival

3.2 Introduction

The American lobster *Homarus americanus* is found along the Atlantic coast of North America from Labrador to North Carolina (Squires, 1990). The commercial fishery is primarily based in Atlantic Canada and New England and can be traced back to the late 1800s. In Atlantic Canada, the lobster fishing season varies in duration among 45 lobster fishing areas (43 inshore, one offshore and one closed as a conservation area), ranging from two to six months (Department of Fisheries and Oceans Canada, 2011). On the island of Newfoundland, the lobster fishing season is comparatively short, starting between April and May and closing between June and July, depending on the lobster molting stage, assessment of wild stock, and the water temperature.

The life cycle of American lobster involves several developmental stages from egg through planktonic larvae, to a benthic lifestyle. Growth is achieved by molting (Campbell, 1983) and the increase in carapace length (CL) and body mass (BM) per molt, as well as molting frequency differ among the developmental stages and geographical areas (Wilder, 1953). For example, in the southwestern Gulf of St. Lawrence adult lobsters molt every year or every two years between early July to early September (Comeau and Savoie, 2001), which is much earlier and more frequent than lobsters from colder areas of the Bay of Fundy and Nova Scotia (Aiken and Waddy, 1986). Many factors impact the increments of CL and BM following molting, such as temperature, feeding rate, diet, sex, and stocking density (McLeese, 1972a, b; Aiken and Waddy, 1976, 1986; Comeau and Savoie, 2001; Bryars and Geddes, 2005; Mente, 2010), but it

generally takes between 4 to 12 years for a lobster to reach marketable size (Copper, 1977; Gulf of Marine Research Institute, 2012). Because of the long growth time and limited scientific knowledge on clawed lobster nutritional and environmental requirements, commercial culture of lobsters from egg to market size is not economically feasible. To date, the culture of clawed lobsters is mainly for wild stock enhancement. Larvae are hatched from ovigerous females and raised to the benthic settling stage (4 - 5th stage larva) before being released into the wild (Waddy and Aiken, 1998; Nicosia and Lavalli, 1999; Castro et al., 2002). However, because of their small size, these newly settling lobsters are vulnerable to predation and mortality rates are potentially very high (Castro and Cobb, 2005). In European lobster stock enhancement programs, the animals are released at a much larger size (> 10 mm in CL), leading to relatively high survival rate (Bannister and Addison, 1998; Beal et al., 2002; Benavente et al., 2010).

There has been some work on on-growing of juvenile clawed lobsters in the field. Container type was found to influence growth and survival of early cultured juvenile *Homarus americanus* (Beal, 2012). Lobsters maintained in soda bottles (350 ml) had a very low survival rate because the containers became blocked with muddy sediment, while survival and growth are improved when the animals are held in mesh covered Petri dishes (Beal, 2012). The survival rate and growth of the lobsters also increases as a function of water flow and container size (Beal and Protopopescu, 2012). Strong currents prevent deposition of sediment and the large container area provides more surface area for settling organisms, on which the lobster feed, as well as room for expansion of the new exoskeleton when the lobsters molt (Beal and Protopopescu, 2012). Juvenile

European lobsters (*Homarus gammarus*) also exhibit a high survival when held in suspended oyster cages, and it was assumed that these animals fed on larvae and zooplankton settling on the cages (Beal et al., 2002; Benevente et al., 2010).

Aquaculture of other lobster species such as tropical spiny and slipper lobsters is more advanced since these species mature more quickly (Rogers et al., 2010). To date, most of the spiny lobster aquaculture involves collecting wild juvenile seed stock and on-growing them in cages (Jones, 2010). These sea cages may be secured on the sea bottom (Lozano-Alvarez, 1996), or suspended from the water surface (Jeffs and James, 2002), and since these lobsters do not exhibit high levels of cannibalism, they may also be held together in floating net cages (Rogers et al., 2010; Mojjada et al., 2012). These practices still involve collecting wild seed stock; some Australian companies are now attempting to culture animals all the way from egg to adult in order to reduce the exploitation of wild seed lobsters (Jones, 2010).

Since the aquaculture of clawed lobsters is limited, research involving the commercial trade of American lobsters has largely focused on holding methods and transport of live product. For example, most of the lobster marketing companies in Nova Scotia and New Brunswick hold lobsters in seawater tanks at 1 - 3˚C. This low temperature reduces the lobster's metabolic rate and maintains them in the intermolt (hard shell) stage, allowing the animals to be held for several months with minimal loss of product. The lobsters are not fed during this time and in order to combat the effects of starvation only lobsters with high serum protein concentrations can be stored in this way (Stewart Lamont, Tangier Lobster Company Limited, pers. comm.). However, the

maintenance and logistics required for this holding method are expensive, and cannot be universally employed. Therefore, the goal of the present study was to investigate an alternative method for storage, and to determine if lobsters could survive and grow in inshore benthic cages when held for extended periods. The larger product could then be sold locally, outside the regular fishing season, for a higher price. Experiments were conducted in both the laboratory and the field. In the laboratory, various environmental and biotic factors were manipulated to determine the optimal conditions for growth and survival. In the field the experiments tested the feasibility of the inshore cages under different feeding frequencies and on different types of sea bottom. One experiment was carried out to determine if lobsters could be ranched in a multitrophic manner under blue mussel farms. Here mussels dropping off the culture lines could supply a food resource for lobster growth, while in turn the lobsters could help remove fallen mussels that would otherwise rot and stagnate on the bottom. This experiment also answered important questions as to whether mussel farms have a deleterious impact on lobster health. The second field experiment investigated the feasibility of regularly feeding the animals to try and encourage molting and growth, and thus produce a larger more valuable lobster for market.

3.3 Methods

3.3.1 Animals and cages

Four series of experiments were carried out, two were performed in the laboratory where factors could be manipulated and the other two were performed in the field to test

the validity of the out-growth protocol. The animals used in the laboratory experiments were purchased from Clearwater Seafoods®, Nova Scotia, and both male and female intermolt animals were used. The intermolt lobsters used in the field experiments were purchased from local harvesters and in line with DFO permitting requirements, only male lobsters were used. All the experimental animals were held in individual compartments in plastic coated 2.5 cm wire mesh cages during the six month experimental period. The cages measured 1.20 × 0.90 × 0.30 m in depth either with 24 compartments, individually measuring 0.30 × 0.15 × 0.30 m in depth, or with 12 larger compartments, individually measuring 0.45 × 0.20 × 0.30 m in depth. The individual compartments served as repetitions. Each lobster within the separate compartments was individually fed and could not touch or interact with each other.

4.3.2 Factors monitored

The lobsters in the laboratory experiments were checked every other day and any mortalities were recorded and removed, at the same time any lobsters undergoing molting were noted. The following parameters were measured once every two months in the laboratory and once every three months in the field.

Lobster growth was measured by recording body mass (BM) and carapace length (CL). For BM, the lobsters were removed from the cages and the water was allowed to drain from the branchial chambers for three to five minutes. The animals were then wiped dry and measured to the nearest 0.1 g. The CL was measured along the dorsal line between the eye socket and the posterior margin of the carapace. In molted lobsters, their BM was measured eight weeks after they first molted; and CL was measured between one

to seven days post-molt at which time both of these parameters (BM and CL) have stabilized (McLeese, 1972b).

The serum protein concentration was measured by withdrawing a 500 µl sample of hemolymph by inserting a 21 gauge needle connected to a one milliliter syringe into the arthrodial membrane at the base of the fourth walking legs. This sample was then injected onto the sample well of a pre-calibrated Brix/RI-Chek Digital Pocket Refractometer (Reichert Analytical Instruments, Depew, NY, USA). Three components were automatically analyzed in sequence: temperature compensated percent solids (Brix-TC), temperature compensated refractive index (RI-TC), and refractive index (RI). The time between withdrawal of the hemolymph and processing of the sample did not exceed 90 secs. The total serum protein concentration was then calculated from the RI using the following formula.

$$P = 510 \times (R.I._{blood} - R.I._{water}) - 1.81$$

Where P is in g/100 ml; $R.I._{blood}$ is the refractive index of blood; $R.I._{water}$ is the refractive index of water (Sunderman, 1944).

The wet mass of the hepatopancreas and heart were measured at the end of the experimental period in a subset of randomly selected animals. The lobsters were sacrificed by destroying the subesophageal ganglion with a pair of pliers. The organs were then dissected out and patted dry with blotting paper for three to five minutes. After measurement of their wet mass, the organs (hepatopancreas and body muscle tissue) were transferred to a Fisher Isotemp® drying oven and maintained at 80˚C for 72 hours in order

to obtain the dry mass for each organ. The following formulas were used to calculate organ size and moisture content.

Hepatopancreas size = (hepatopancreas wet mass / total body mass) × 100.

Heart size = (heart wet mass / total body mass) × 100.

Hepatopancreas moisture content = (hepatopancreas wet mass – hepatopancreas dry mass) / hepatopancreas wet mass × 100.

Body muscle tissue moisture content = (muscle tissue wet mass – muscle tissue dry mass) / muscle tissue wet mass.

For meat content analysis, the second left and right walking legs were removed and boiled in freshwater for 45 min. After being cooled, the walking legs were cut transversely, the cut leg was examined under a dissecting microscope and the cross section photographed using Infinity Capture Imaging Software. The horizontal and vertical diameter of both the leg muscle and its exoskeleton were measured using Infinity Analyze Software. The actual meat content of the lobster was then calculated following the methods outlined in James et al., (2013).

Meat content = $(S_1 / S_2) \times 100$

Where S_1 is the cross sectional area of the lobster walking leg meat; S_2 is the cross sectional area of the lobster walking leg exoskeleton; S_1 and S_2 were calculated

using the formula for an ellipse: $S = \pi \times a \times b$ (a is the semi-major axis; b is the semi-minor axis).

3.3.3 Experimental protocols

The first laboratory experiment was designed to test the effects of temperature and diet type on survival and growth of cage-held lobsters. The lobsters were held in flow-through seawater tanks maintained at either 5, 10 or 15˚C, representing typical temperature ranges experienced by lobsters in the wild (McLeese, 1956; Stewart et al., 1972; McLeese, 1972a, b). The temperature in each tank was checked daily; the temperature in the 10 and 15˚C tanks varied ± 1˚C, while the 5˚C tanks fluctuated ± 2˚C, during the six month experimental period. Each tank was equipped with air diffuser stones which maintained the water oxygen content between 92 - 98% saturation. The photoperiod was maintained on a 12L : 12D cycle with light level of 360 - 400 lux during the day time. Light intensity in the field was much weaker than that in the laboratory. In the field, lobster cages were located beneath mussel long line systems which reduce the light level to around 30 - 50 lux during the day time. Approximately equal number of lobsters (intermolt stage) of both sexes, with a BM (mean ± SD of 548.8 ± 53.3 g were purchased from Clearwater Seafoods®, NS. The lobsters were acclimated to laboratory conditions for one month before the experiments commenced. The experiment was carried out between January and June 2013, and forty eight lobsters were held at each temperature (5, 10, 15˚C) with 24 lobsters per cage. The animals were fed to satiation twice weekly; one group of lobsters (n = 24) in each temperature regime was fed a mixed diet (shrimp, squid, fish, scallop mantle and crab) while the other group (n = 24) was only

fed blue mussels. The tanks were cleaned at the end of each week, when any uneaten food and mortalities were removed.

The identification of the different molt stages of adult lobster was carried out by staging the developmental morphology of the pleopods following the methods outlined in Aiken (1973) and Fig. 1. During intermolt and post-molt, there are no new setae formed and no sign of retraction of the epidermis. During proecdysis, newly forming setae are evident and retraction of the epidermis occurs. In the present study, lobster pleopods were sampled at one month intervals and each sample was immediately photographed using Infinity Capture Imaging Software at 40 x magnification. Hemolymph samples were collected weekly for lobsters in premolt and those that underwent molting and the serum concentration calculated until the experiment was terminated.

In the second laboratory experiment the effect of feeding frequency and cage compartment size was investigated. The animals were maintained in the laboratory from June to December 2013 in a flow through tank, using ambient aerated sea water from Logy Bay, Newfoundland, Canada. Temperature data loggers (iBCod, type G, Ste-Juline, QC, Canada) were attached to cages and recorded water temperature every four hours. Ninety six intermolt male and female lobsters with a BM (mean ± SD) of 363.0 ± 59.3 g were purchased from Clearwater in May 2013. Half of the lobsters (n = 48) were held in cages in individual compartments of 0.20 × 0.30 × 0.30 m in depth, while the other half were held in larger compartments of 0.45 × 0.20 × 0.30 m in depth. The lobsters were fed a mixed diet of fish, squid, crab, blue mussel and scallop mantle. One group (n = 24 per both cage sizes) was fed to satiation twice weekly, while the remaining lobsters were fed

once per month. To avoid fouling of the water, any remaining feed was removed at the end of each week.

The objective of the first field experiment was to assess the potential input of blue mussels as a food source as well as the compartment size on lobster survival, growth, and health status. Lobsters had a BM (mean ± SD) of 601.0 ± 92.8 g. The lobsters (n = 196) were held in benthic cages (10 - 13 m depth) at Sunrise Mussel Farms near Triton, NL (N49^{o} 29' 03.03', W55^{o} 45' 03.58'). Half of the lobsters were maintained in cages with regular sized compartments, while the other half were kept in cages with the larger compartments. Temperature loggers (iBCod, type G, Ste-Juline, QC, Canada) were attached to the cages and recorded the temperature every four hours during the six months (June to December 2013) experimental period. Half of the experimental animals (n = 48 in regular compartments, 48 in large compartments) were placed directly under the mussel culture lines of Sunrise Farms, the idea being that they would be able to feed on mussels dropping off the culture lines. The remaining cages (n = 96) were set outside the mussel farm where the lobsters would not get the potential input of mussels.

The objective of the second field experiment was to determine the effect of water depth (temperature), feeding frequency and cage size on survival and growth of adult lobsters. Ninety six intermolt male lobsters with a BM (mean ± SD) of 502.2 ± 47.9 g were donated by Jerseyman Island Fisheries Ltd. The cages were set on the seabed near Rushoon, NL (N47º 20′ 42.21", W54º 54' 46.45"). Temperature loggers attached to the cages recorded the temperature change from June to December 2013. Lobsters were held in the two different compartment sizes at two water depths of 6 - 8 m and 12 - 14 m.

The original experimental design involved the staff of Jerseyman Island Fisheries, SCUBA diving on the cages and feeding the lobsters (herring, cod and scallop mantle) by hand. Half the lobsters were to be fed twice weekly and the other group once monthly. However, due to a strong underwater current, food was transported from the frequent feeding set of cages into the cages on the monthly feeding cycle. This made it difficult to accurately control the amount of food each group of lobsters received, therefore the feeding frequency component was abandoned and all the animals were fed twice weekly. Due to inclement weather and water conditions, twice weekly feeding was abandoned in October and once the water temperature dropped below 5°C, the feeding schedule was changed to monthly feeding for the remaining months.

3.3.4 Statistical analysis

Statistical analyses of lobsters mortality and molting rates in various conditions, were made using a Kaplan-Meier test (Prism v5.0, GraphPad Software Inc., La Jolla.CA) with statistical significance accepted at the 5% level. The Kaplan-Meier survival curves plot fractional survival/molting (Y) as a function of time (X). It can be used to analyze the time to any event (usually death/molt) that can only happen once.

Serum protein concentration as a function of time (weeks) was analyzed with Spearman rank correlation and linear regression analyses, using Prism (GraphPad Software Inc., La Jolla.CA). Growth (BM and CL increments) and physiological parameters (serum protein concentration, hepatopancreas/heart size, and moisture content) were analysed using ANOVAs. If significant differences were obtained they were further analysed with Tukey post-hoc tests. Statistical significance in all tests was

accepted at the 5% level. All the data is presented as the mean value ± the standard deviation.

3.4 Results

3.4.1 Laboratory Experiment 1

Temperature had a significant effect on lobster survival rate (Table 3-1) (Log-rank (Mantel-Cox) Test, Chi square = 53.49, $P < 0.0001$). The lowest mortality occurred at 10˚C (4.2 - 12.5%), and at this temperature the diet did not have a significant effect on survival rate (Log-rank (Mantel-Cox) Test, Chi square = 1.09, $P = 0.297$). In addition, these mortalities only occurred during the final two months of the experiment. The highest mortality rate (79.2%) was recorded at 5˚C for lobsters fed the mussel only diet; this mortality rate was significantly higher than the group fed a mixed diet at 5˚C (Log-rank (Mantel-Cox) Test, Chi square = 4.11, $P < 0.05$). For the group fed the mussel diet at 5˚C, the first animal died after just six weeks and the cumulative mortality rate increased steadily throughout the six month experimental period. In contrast, mortality rates only increased substantially during the last two months in lobsters fed a mixed diet at 5˚C. The mortality rate was also relatively high (50%) for lobsters in 15˚C fed on the mussel diet and this was significantly higher than its counterparts (12.5%) fed with a mixed diet (Log-rank (Mantel-Cox) Test, Chi square = 6.58, $P < 0.05$). Interestingly the mortalities in the 15˚C mussel diet nearly all occurred during the final month of the study and all these were animals that had recently molted. In contrast, no mortalities were observed in post-molt lobsters fed a mixed diet at 15˚C (Table 3-1).

Table 3-1. Mortality rates of adult lobsters maintained at three temperature and two diet types over a six month period.

Temperature (˚C)	Diet	Number	Cumulative Mortality (Number and Percent) in 6 Months					
			1	2	3	4	5	6
5	Mussel	24	1 (4.2%)	2 (8.3%)	6 (25.0%)	9 (37.5%)	13 (54.2%)	19 (79.2%) ^{aA}
5	Mixed	24	0 (0)	1 (4.2%)	2 (8.3%)	3 (12.5%)	8 (33.3%)	12 (50.0%) ^{bA}
10	Mussel	24	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.2%)	1 (4.2%) ^{aC}
10	Mixed	24	0 (0)	0 (0)	0 (0)	0 (0)	2 (8.3%)	3 (12.5%) ^{aB}
15	Mussel	24	0 (0)	1 (4.2%)	1 (4.2%)	1 (4.2%)	2 (8.3%)	12 (50.0%) ^{aB}
15	Mixed	24	0 (0)	0 (0)	3 (12.5%)	3 (12.5%)	3 (12.5%)	3 (12.5%) ^{bB}

Log-rank (Mantel-Cox) Test was performed on final mortality rate. Different lowercase letters indicate significant differences ($P < 0.05$) between two diets in the same temperature; different capital letters indicate significant differences ($P < 0.05$) among the three temperatures for the same diet.

The experimental temperature also influenced the incidence of molting (Table 3-2) (Log-rank (Mantel-Cox) Test, Chi square = 71.70, $P < 0.0001$. In 5˚C, only two lobsters molted during the six month experimental period, these occurred at beginning of the study and both of these animals were maintained on a mixed diet. There was a significant effect of diet on molting at 10˚C (Log-rank (Mantel-Cox) Test, Chi square = 7.65, $P < 0.01$). Molting rates were similar (and low) between the two groups during the first five months; there was a substantial increase in the molting rate for the mixed diet lobsters in the final month, but none of the animals fed the mussel only diet molted at this time. The highest molting rates occurred in lobsters maintained at 15˚C with most of the animals undergoing this process during the final three months of the study (66.7% for mixed diet and 83.3% for mussel diet). Although there was no significant effect of diet on actual numbers of animals molting (Log-rank (Mantel-Cox), Chi square = 1.93, $P = 0.165$) at 15˚C, the time to 50% molting occurred 30 days earlier for the lobsters fed the mussel diet.

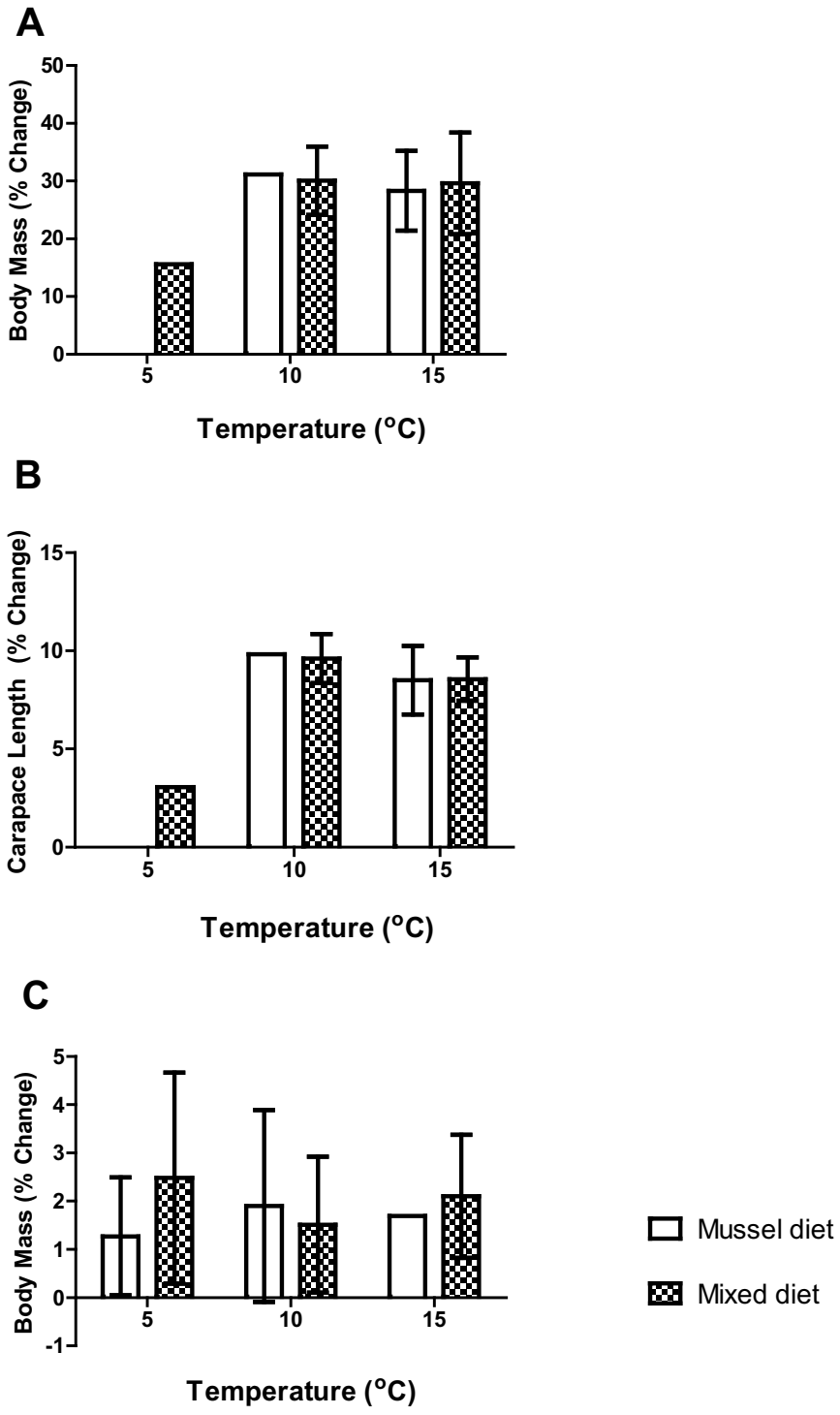
A significant increase in both BM and CL occurred following molting (Fig. 3-1). The limited amount of data for the 5˚C treatment and 10˚C mussel diet precluded statistical analysis on all combinations. Analysis of the remaining data showed no significant effect of temperature or diet on growth (One way ANOVA, F = 0.20, $P = 0.818$). For molted lobsters the increase in BM was 29.32 ± 7.21% while an increase of CL of 8.89 ± 1.36% was observed (Fig. 3-1A, B). There were only slight increases (1.3 - 2.5%) in BM for non-molted lobsters (Fig. 3-1C) and there was no consistent trend in change in BM as a function of temperature or diet in these animals.

Table 3-2. Molting rates of adult lobsters at different temperatures and diets in a six month period.

Temperature (˚C)	Diet	Number	Cumulative Molting (Number and Percent) in 6 Months						Days to 50% Molting
			1	2	3	4	5	6	
5	Mussel	24	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0) ^{aB}	N/A
5	Mixed	24	1 (4.2%)	2 (8.3%)	2 (8.3%)	2 (8.3%)	2 (8.3%)	2 (8.3%) ^{aC}	N/A
10	Mussel	24	0 (0)	1 (4.2%)	1 (4.2%)	1 (4.2%)	1 (4.2%)	1 (4.2%) ^{bB}	N/A
10	Mixed	24	0 (0)	1 (4.2%)	1 (4.2%)	2 (8.3%)	2 (8.3%)	9 (37.5%) ^{aB}	N/A
15	Mussel	24	0 (0)	0 (0)	3 (12.5%)	11(45.8%)	15 (62.5%)	20 (83.3%) ^{aA}	131
15	Mixed	24	0 (0)	0 (0)	2 (8.3%)	7 (29.2%)	11 (45.8%)	16 (66.7%) ^{aA}	159

Log-rank (Mantel-Cox) Test was performed on final molting rate. Different lowercase letters indicate significant differences ($P < 0.05$) between two diets in the same temperature; different capital letters indicate significant differences ($P < 0.05$) among the three temperatures for the same diet.

Fig. 3-1. Effect of temperature and diet on growth of adult American lobsters, *Homarus americanus*. One way ANOVA was performed on final measurement. The data represents mean ± standard deviation of the changes observed at the end of a six month experimental period. (A) percent increase in body mass for molted lobsters (n = 9 - 18); (B) percent increase in carapace length for molted lobsters (n = 9 - 18); (C) percent increase in body mass for non-molted lobsters (n = 11 - 21).


A
Body Mass (% Change)
50
40
30
20
10
0
5
10
15
Temperature (°C)
B
Carapace Length (% Change)
15
10
5
0
5
10
15
Temperature (°C)
C
Body Mass (% Change)
5
4
3
2
1
0
-1
5
10
15
Temperature (°C)
Mussel diet
Mixed diet

Serum protein concentration was used as an indicator of the physiological and nutritional status of the animal (Wang and McGaw, 2014). Temperature and diet had a significant effect on the final serum protein concentration (Table 3-3). Serum protein concentration increased with increasing temperature in non-molted lobsters after four months (2 way repeated measures ANOVA, $F = 105.10$, $P < 0.0001$). The majority of the lobsters in 15˚C molted after four months, and significantly lower serum protein levels were measured in these animals at the termination of the six month study. Lobsters fed a mixed diet had higher serum protein levels than those fed the mussel diet at 5˚C and 15˚C, but there was no effect of diet at 10˚C (Two way ANOVA; diet, $F = 5.96$, $P < 0.05$; interaction, diet and temperature, $F = 4.25$, $P < 0.05$) in the final measurement of serum protein concentration of non-molted animals (5 and 10˚C in the 6th month; 15˚C in the 4th month). In addition to temperature and diet type, the lobster serum protein concentration changed over time for both diet types (Three way ANOVA, $F = 15.19$, $P < 0.0001$; interaction of temperature and time, $F = 16.63$, $P < 0.0001$). At 5˚C, the serum protein concentration was maintained at stable levels during the first four months, but increased significantly during the last two months of the study (Tukey post-hoc test, $P < 0.05$). At 10˚C, the serum protein concentration increased significantly at each two month sampling period, reaching its highest level at the end of the six month experimental period (Tukey post-hoc test, $P < 0.0001$). Serum protein concentrations at 15˚C also increased significantly during the first four months, thereafter a significant decrease occurred as a result of most of the lobsters molting during the final two months (Tukey post-hoc test, $F = 37.27$, $P < 0.0001$).

Table 3-3. Serum protein concentrations (g/100 ml) in different temperature and diet treatments.

Temperature (˚C)	Diet	Serum Protein (g/100 ml) Over 6 Months (Mean ± SD)			
		0	2	4	6
5	Mussel	4.622 ± 0.751	4.986 ± 0.876	4.852 ± 0.802 ^{aC}	4.979 ± 1.441 ^{bB}
5	Mixed	4.621 ± 1.756	4.959 ± 1.236	5.668 ± 1.102 ^{aC}	6.322 ± 0.853 ^{aB}
10	Mussel	3.972 ± 1.179	5.549 ± 1.029	6.770 ± 0.997 ^{aA}	7.924 ± 1.045 ^{aA}
10	Mixed	5.117 ± 0.985	5.891 ± 1.063	6.690 ± 0.751 ^{aB}	7.646 ± 0.779 ^{aA}
15	Mussel	5.199 ± 0.895	6.365 ± 1.249	7.727 ± 1.848 ^{bAB}	*3.472 ± 0.476 ^{aC}
15	Mixed	5.223 ± 1.259	6.911 ± 0.786	8.354 ± 0.854 ^{aA}	*4.157 ± 1.827 ^{aC}

2 way repeated measures ANOVA was performed. For the 4th and 6th month, different lowercase letters indicate significant differences ($P < 0.05$) between the two diets in the same temperature regime; different capital letters indicate significant differences ($P < 0.05$) among three temperatures in the same feed type condition. At 15˚C, data of 0, 2nd, and 4th month were from non-molt lobsters; at 6th month, all previously sampled lobsters had molted. * indicates serum protein concentrations of post-molted lobsters.

Hemolymph samples were collected at weekly intervals in both pre and post-molt animals in 15˚C (Fig. 3-2). Serum protein concentrations (both diet types) increased steadily during intermolt and early proecdysis, reaching a peak in late proecdysis. There was a trend for the lobsters fed a mixed diet to exhibit higher serum protein levels than those fed the mussel diet (7.94 ± 1.87 g/100 ml for the mixed diet and 8.62 ± 0.76 g/100 ml for the mussel diet), however, this difference proved to be statistically insignificant (Linear regression, F = 2.98, $P = 0.0882$). The majority of the lobsters (n = 33) molted during the 22nd week. Following molting, serum protein levels dropped to their lowest levels of between 3.55 and 3.91 g/100 ml. Thereafter there was a significant effect of diet type on serum protein levels. Serum protein concentration slowly increased over the following 12 weeks in lobsters fed a mixed diet, reaching levels that were higher than those measured at the start of the experiment at week 31 (One way ANOVA, F = 6.60, $P < 0.0001$). In contrast, serum protein levels of lobsters fed a mussel only diet declined steadily reaching levels that were significantly lower than those of the mixed diet animals after 30 weeks (Two way ANOVA, diet type, F = 55.99, $P < 0.0001$; time, F = 28.34, $P < 0.0001$; interaction, F = 2.14, $P < 0.01$). Eighty nine percent (n = 19) of post-molted lobsters fed on a mussel diet died, while only 7% of post-molted lobsters (n = 14) fed a mixed diet died during the same time period.

A subset of randomly selected lobsters (n = 7) were sacrificed at the end of the six month study. Due to the high molting rate, all lobsters sampled at 15˚C were in post-molt; while those sampled from 5 and 10˚C were non-molted lobsters. The hepatopancreas size and moisture content were compared between the different treatments (Fig. 3-3). Lobsters

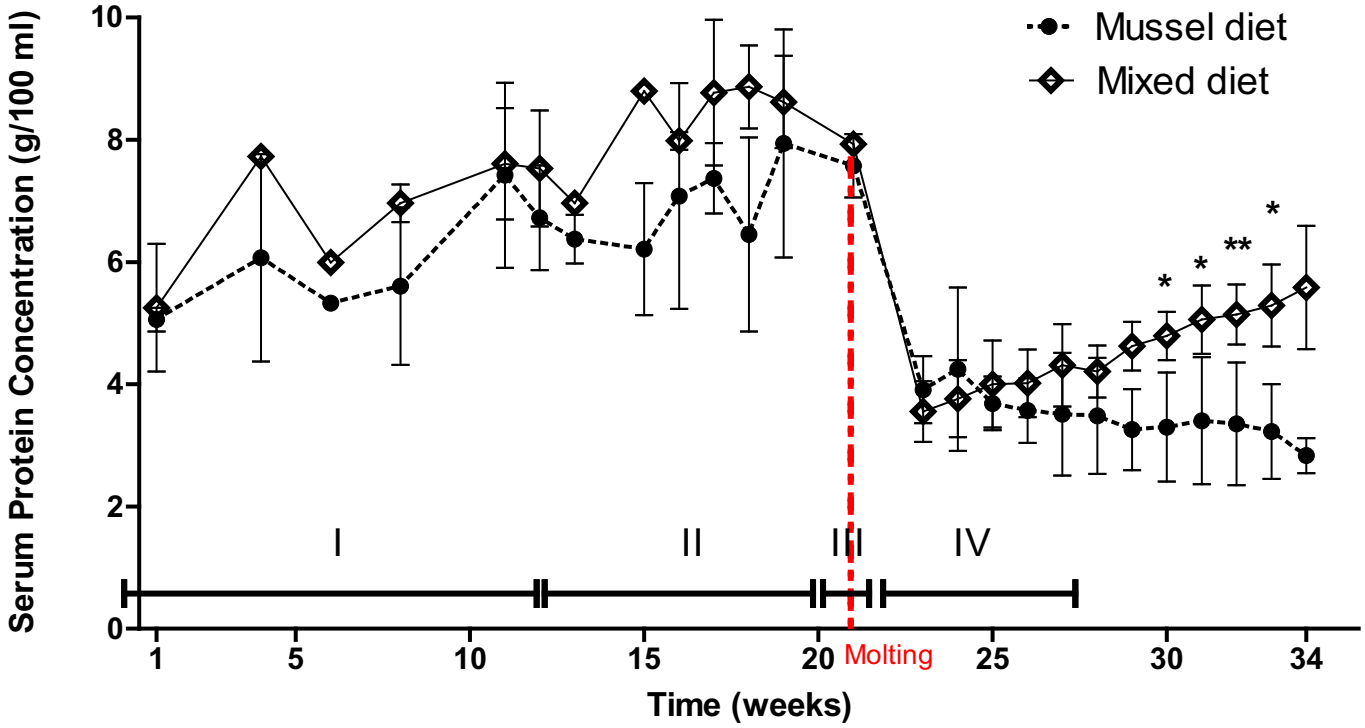
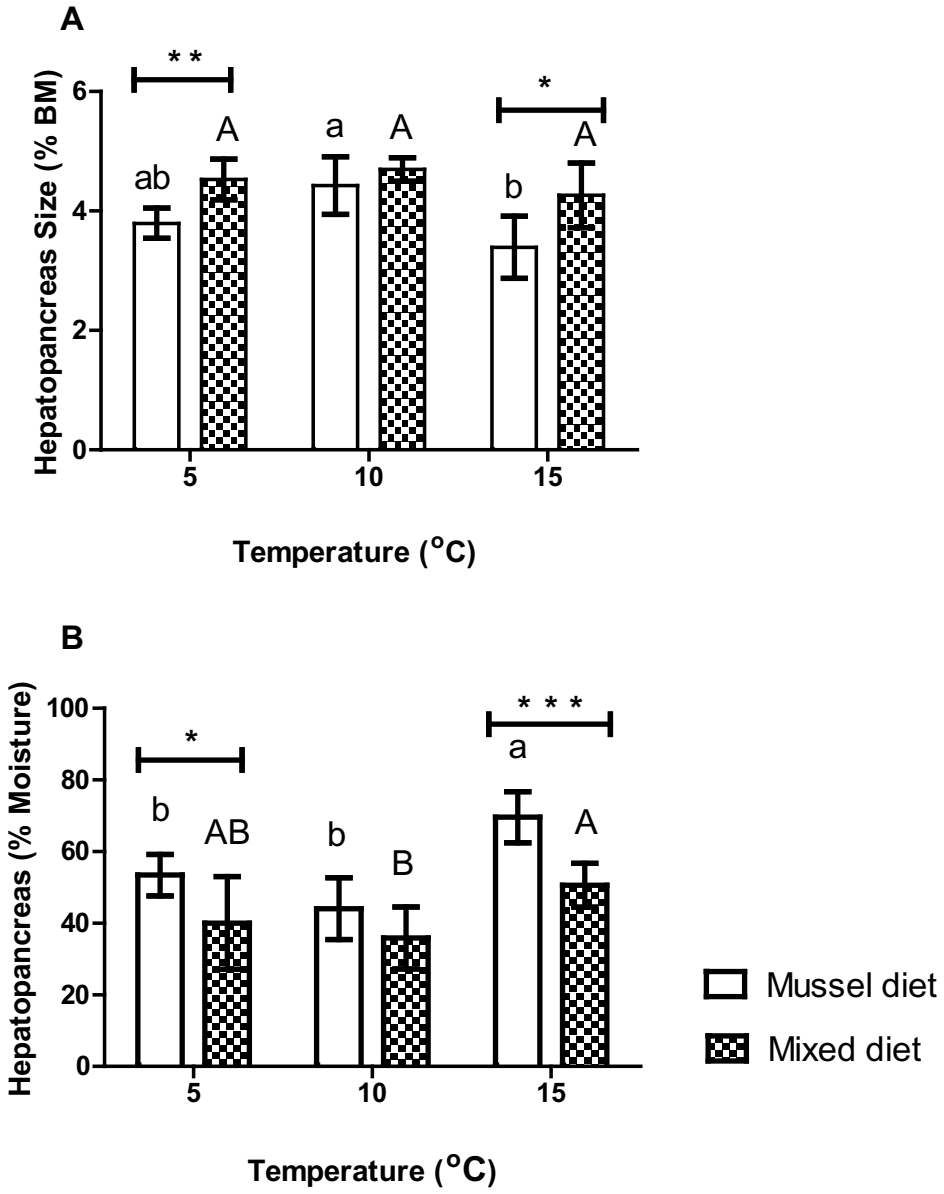


Fig. 3-2. Effect of two feed types on serum protein concentration of adult lobsters maintained for six months at 15˚C and fed either a mixed diet or a diet of blue mussels. Linear regression was performed. The different molt stages are indicated, where I is intermolt, II is proecdys, III is ecdysis, and IV is postmolt. The data represents the mean ± standard deviation of 14 lobsters fed mixed diet and 19 lobsters fed blue mussel diet. (* Significant 0.05 level; ** Significant 0.01 level).

Fig. 3-3. Effect of temperature and diet on (A) hepatopancreas size (% body mass); (B) hepatopancreas moisture content (%). Two way ANOVA was performed on final measurement. Data are expressed as the mean ± SD of n = 7. Different lowercase letters indicate significant differences ($P < 0.05$) among the three temperature levels for the mussel diet; different capital letters indicate significant differences ($P < 0.05$) among the three temperatures for mixed diet; an asterisk indicates significant difference as a function of diet at any one temperature (* Significant 0.05 level; ** Significant 0.01 level; *** Significant 0.001 level).


A
Hepatopancreas Size (% BM)
0
2
4
6
* *
*
ab
A
a
A
b
A
5
10
15
Temperature (°C)
B
Hepatopancreas (% Moisture)
0
20
40
60
80
100
*
* * *
b
AB
b
B
a
A
5
10
15
Temperature (°C)
Mussel diet
Mixed diet

fed a mixed diet had a significantly larger hepatopancreas than those fed the mussel diet at both 5˚C and 15˚C, although a similar trend was observed at 10˚C, this proved to be statistically insignificant (Two way ANOVA, diet, F = 20.88, $P < 0.0001$; temperature, F = 9.63, $P < 0.05$; interaction of diet and temperature, F = 1.76, $P = 0.188$). Molted lobsters at 15˚C fed a mussel only diet had a significantly smaller hepatopancreas than non-molted lobsters fed a mussel only diet at 10˚C (Tukey post-hoc test, $P < 0.01$). The moisture content of the hepatopancreas was inversely correlated with its size. In line with the hepatopancreas mass, lobsters fed the mussel diet had a significantly higher hepatopancreas moisture content than those fed mixed diet at both 5˚C and 15˚C, although a similar trend was observed at 10˚C, this proved to be statistically insignificant (Two way ANOVA, diet, F = 24.14, $P < 0.0001$; temperature, F = 18.45, $P < 0.0001$; interaction of diet and temperature, F = 1.29, $P = 0.289$). Molted lobsters at 15˚C fed the mussel diet had a significantly higher hepatopancreas moisture content compared with mussel fed non-molted lobster at 5 and 10˚C (Tukey post-hoc test, F = 20.94, $P < 0.0001$); similarly, the molted lobster at 15˚C fed a mixed diet had significantly higher hepatopancreas moisture content compared with non-molted lobster fed a mixed diet at 10˚C (One way ANOVA, F = 5.72, $P < 0.05$).

3.4.2 Laboratory Experiment 2

There was a pronounced variation in ambient water temperature during the six month experimental period (Fig. 3-4). Water temperature increased from around 5˚C at the start of the experiment in early June, reaching maximal levels of approximately 15.5˚C at the end of July. The temperature remained steady for around three months, after which

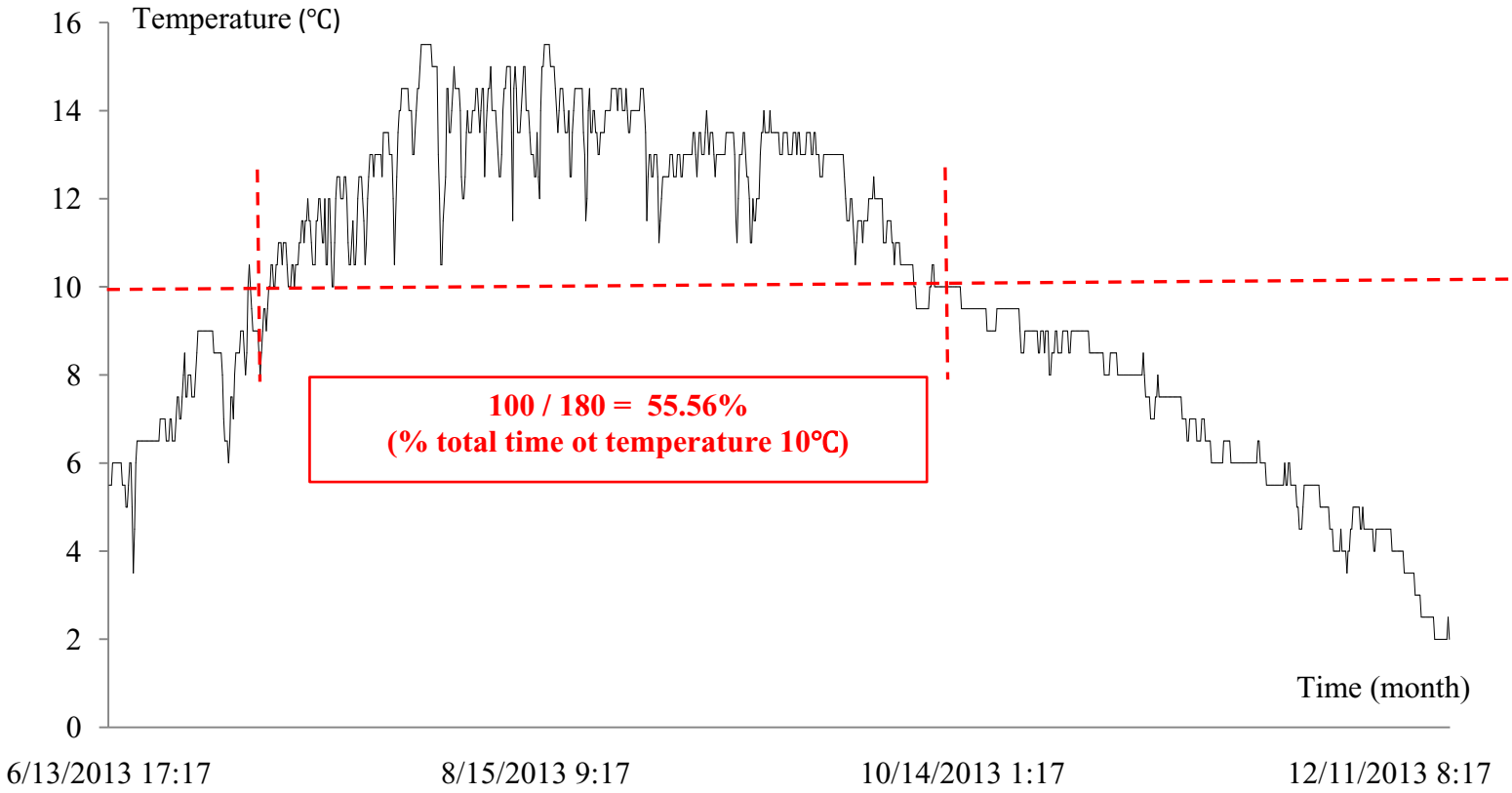


Fig. 3-4. Temperature range (˚C) from mid June to mid December (2013) in experimental tanks where the seawater was pumped directly from Logy Bay, St. John's, Newfoundland. Data was collected every 4h using iBCod data tags (type G, Ste-Juline, QC, Canada) affixed to the cages.

the water temperature decreased to 10˚C in early October. There was a further decrease from late October onwards, reaching the lowest measured temperature of 2˚C in mid-December.

The mortality rate ranged between 12.5 and 37.5% (Table 3-4) and a large proportion of these mortalities occurred in last 60 days in all treatments (Table 3-4). There was no significant effect of feeding frequency or compartment size on mortality rates (Log-rank (Mantel-Cox) Test, Chi square = 6.59, $P = 0.0862$). Feeding frequency and compartment size did not have any significant effect on molting rate, which ranged from 20.8 to 37.5% among the different treatments (Table 3-5) (Log-rank (Mantel-Cox) Test, Chi square = 0.093, $P = 0.761$). Molting started in mid-August, peaked September to October and declined substantially during November and December.

Feeding frequency and to a lesser degree compartment size did have a significant effect on growth of molted lobsters (Fig. 3-5 A, B). Lobsters fed in the high feeding frequency treatment had a significantly higher increment of both BM and CL (37.07 ± 10.94% and 10.03 ± 2.07%, respectively) compared with lobsters in the low feeding frequency treatment (20.49 ± 7.39% and 6.85 ± 1.84%, respectively) in both compartment sizes (Two way ANOVA, F = 22.11, $P < 0.0001$; F = 13.03, $P < 0.01$ for BM and CL respectively). Lobsters maintained in large compartments exhibited a trend of a larger increment in BM and CL but the effect of compartment size on lobster growth was only statistically significant for BM in the low feeding frequency treatment (Two way ANOVA, F = 7.61, $P < 0.05$). There was also a change in BM for non-molted lobsters. Lobsters in the low feeding frequency could not maintain their BM and it decreased on

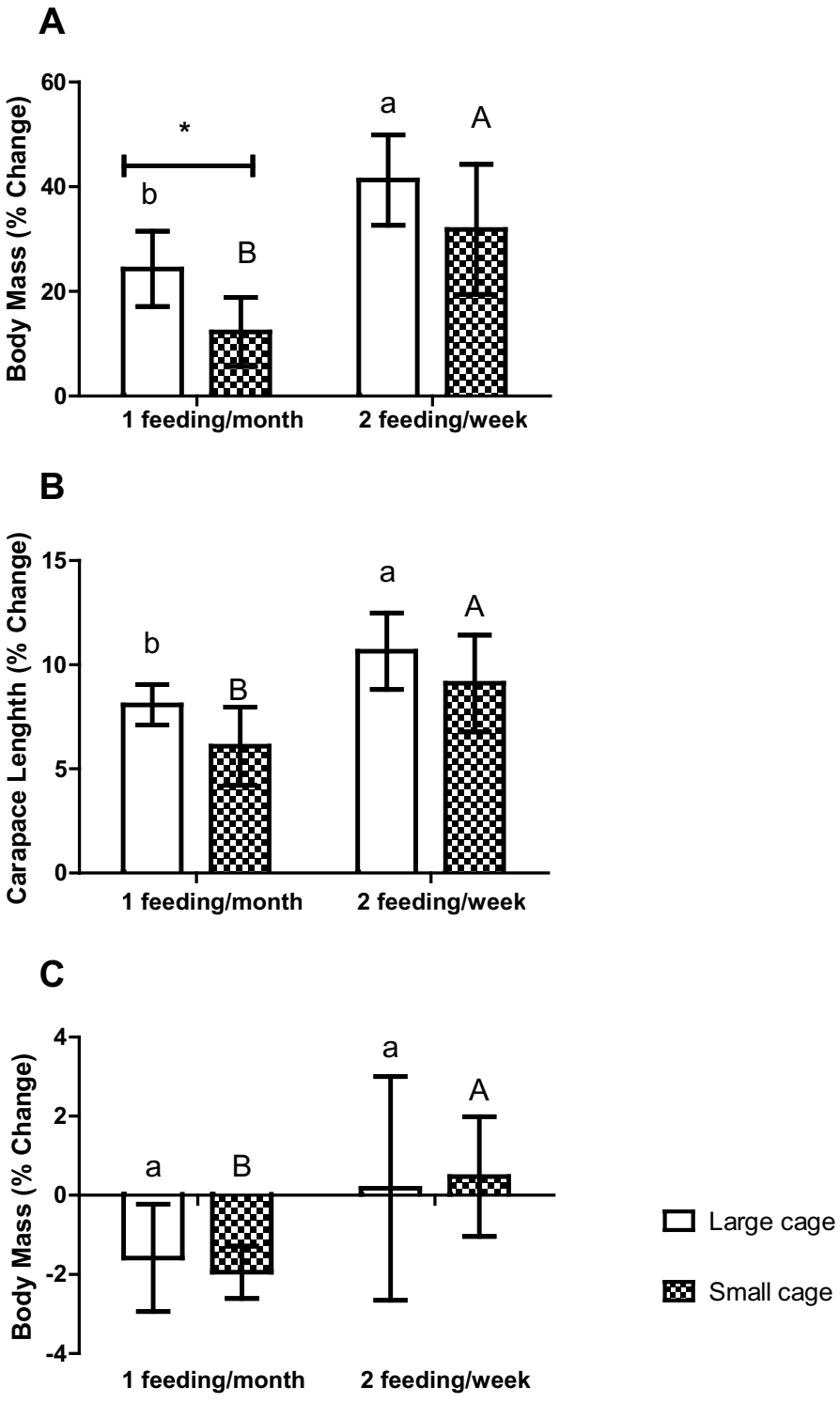
Table 3-4. Mortality rates of adult lobsters held in two compartment sizes and subjected to two feeding frequencies.

Feeding Frequency	Compartment Size	Number	Cumulative Mortality (Number and Percent) Over 6 Months					
			July	August	September	October	November	December
1 Feeding/Month	Large	24	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.2%)	3 (12.5%)
1 Feeding/Month	Small	24	0 (0)	1 (4.2%)	1 (4.2%)	1 (4.2%)	2 (8.3%)	3 (12.5%)
2 Feedings/Week	Large	24	1 (4.2%)	3 (12.5%)	3 (12.5%)	3 (12.5%)	5 (20.8%)	9 (37.5%)
2 Feedings/Week	Small	24	0 (0)	1 (4.2%)	1 (4.2%)	1 (4.2%)	3 (12.5%)	5 (20.8%)

Table 3-5. Molting rates of adult lobsters, effect of compartment size and feeding frequency.

Feeding Frequency	Compartment Size	Number	Cumulative Molting (Number and Percent) Over 6 Months					
			July	August	September	October	November	December
1 Feeding/Month	Large	24	0 (0)	2 (8.3%)	4 (16.7%)	4 (16.7%)	5 (20.8%)	5 (20.8%)
1 Feeding/Month	Small	24	0 (0)	1 (4.2%)	4 (16.7%)	5 (20.8%)	8 (33.3%)	9 (37.5%)
2 Feedings/Week	Large	24	0 (0)	2 (8.3%)	3 (12.5%)	5 (20.8%)	6 (25.0%)	7 (29.2%)
2 Feedings/Week	Small	24	0 (0)	1 (4.2%)	2 (8.3%)	2 (8.3%)	4 (16.7%)	6 (25.0%)

Fig. 3-5. Effect of feeding frequency and compartment size on growth of adult lobsters maintained in the laboratory for a six month period. (A) percent change in body mass for molted lobsters (n = 6 - 8); (B) percent increase in carapace length for molted lobsters (n = 6 - 8); (C) percent increase in body mass for non-molted lobsters (n = 13 - 16). A two way ANOVA was performed on the final measurement. Data are expressed as mean ± SD. Different lowercase letters indicate significant differences ($P < 0.05$) between the two feeding frequencies in the large compartment size; different capital letters indicate significant differences ($P < 0.05$) between the two feeding frequencies in small compartment size. An asterisk indicates significant difference as a function of compartment size at any feeding frequency treatment.


A
Body Mass (% Change)
60
40
20
0
*
b
B
a
A
1 feeding/month
2 feeding/week
B
Carapace Lenghth (% Change)
15
10
5
0
b
B
a
A
1 feeding/month
2 feeding/week
C
Body Mass (% Change)
4
2
0
-2
-4
a
B
a
A
1 feeding/month
2 feeding/week
Large cage
Small cage

average by 1.9% during the six month trial. Lobsters in high feeding frequency treatment maintained their BM with a mean increase of 0.5%. However, the effect of feeding frequency on non-molted lobster growth was only statistically significant in the small compartment sizes (Two way ANOVA, feeding frequency, $F = 20.91$, $P < 0.0001$).

The serum protein concentration of the lobsters was significantly impacted by feeding frequency, but was not affected by the compartment size (Table 3-6) (Two way ANOVA, feeding frequency, $F = 54.81$, $P < 0.0001$; compartment size, $F = 1.89$, $P = 0.175$). Non-molted lobsters with a high feeding frequency exhibited an increase in serum protein levels during the six month period. The highest levels were measured in mid-October and although serum protein levels decreased slightly in the last two months, they were still significantly higher (7.08 ± 2.06 g/100 ml) than levels measured at the start of the experiment (5.26 ± 0.92 g/100 ml) (Student t test, $T = 3.53$, $P < 0.01$). In contrast, the lobsters fed once per month were unable to maintain serum protein levels and they declined significantly from initial levels of 5.58 ± 0.94 g/100 ml, reaching their lowest levels of 3.79 ± 1.09 g/100 ml at the end of the experimental period (Student t test, $T = 9.54$, $P < 0.0001$).

Because of a large difference in the timing of the molt for individual lobsters and the close relationship between time after molting and serum protein levels (Fig. 3-2; Wang and McGaw, 2014), there were not enough replicates to perform reliable statistical analysis. Nevertheless, the trend for molted lobsters was consistent with the non-molted lobsters. In the low feeding frequency group, the serum protein concentration of molted lobsters decreased from 3.70 ± 1.15 g/100 ml down to 1.84 ± 0.25 g/100 ml (n = 2) at the

Table 3-6. Serum protein concentration of adult lobsters effect of compartment size and feeding frequency.

Feeding Frequency	Compartment Size	Serum Protein (g/100 ml) Over 6 Months (Mean ± SD)			
		June	August	October	December
1 Feeding/Month	Large	5.633 ± 0.966	4.856 ± 1.100	4.127 ± 1.051	3.812 ± 1.105 ^{aB}
1 Feeding/Month	Small	5.523 ± 0.938	4.740 ± 1.063	4.081 ± 1.137	3.756 ± 1.115 ^{aB}
		(8.747 ± 1.370, n=2)	(3.698 ± 1.154, n=2)	(2.066 ± 0.577, n=2)	(1.835 ± 0.250, n=2) ^{B}
2 Feedings/Week	Large	5.400 ± 0.362	5.247 ± 0.669	7.462 ± 2.197	6.242 ± 2.332 ^{aA}
2 Feedings/Week	Small	5.173 ± 1.126	5.895 ± 1.041	7.651 ± 1.824	7.556 ± 1.798 ^{aA}
		(8.679 ± 0.347, n=3)	(3.630 ± 0.212, n=3)	(6.026 ± 1.027, n=3)	(5.840 ± 0.487, n=3) ^{A}

Two way ANOVA was performed on final measurement. Different lowercase letters indicate significant differences ($P < 0.05$) between the two compartment sizes in the same feeding frequency conditions; different capital letters indicate significant differences ($P < 0.05$) between two feeding frequencies for the same compartment size. Data in parentheses are from molted lobsters.

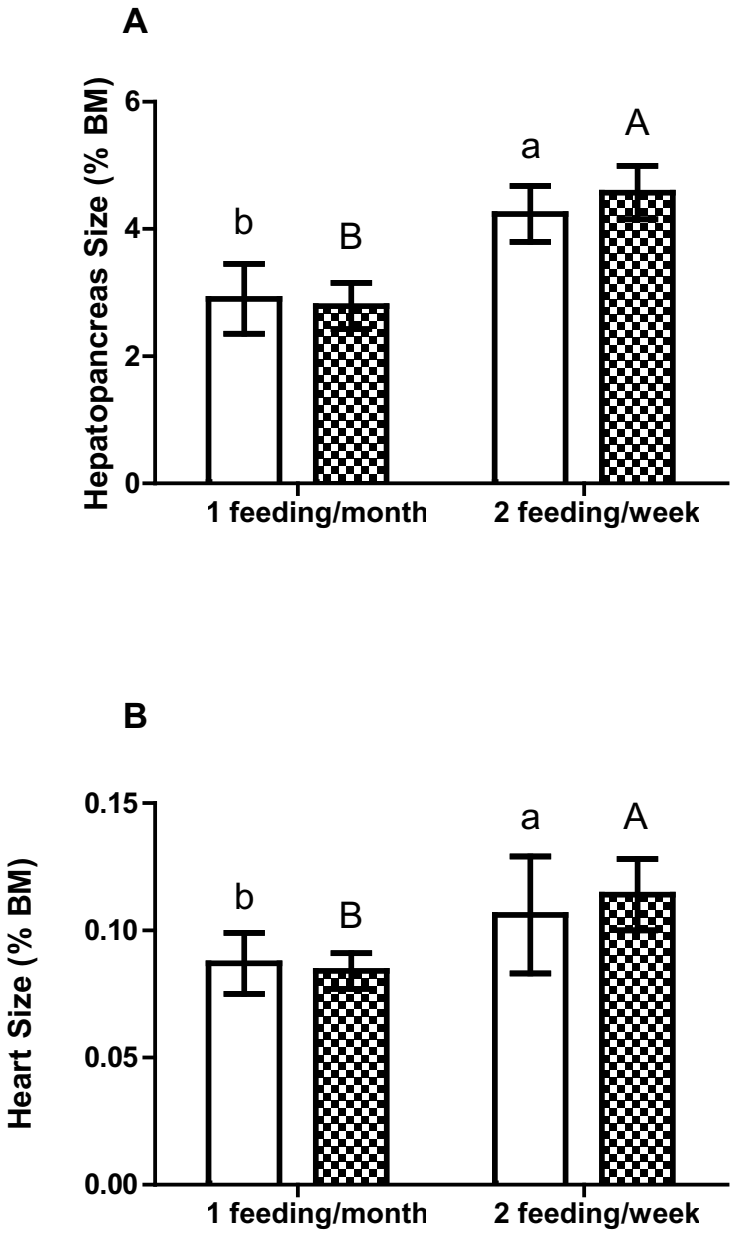
end of the experimental period. In the high feeding frequency group, the molted lobster's serum protein level increased from 3.63 ± 0.21 g/100 ml, reaching levels as high as 6.03 ± 1.03 g/100 ml (n = 3) after two months.

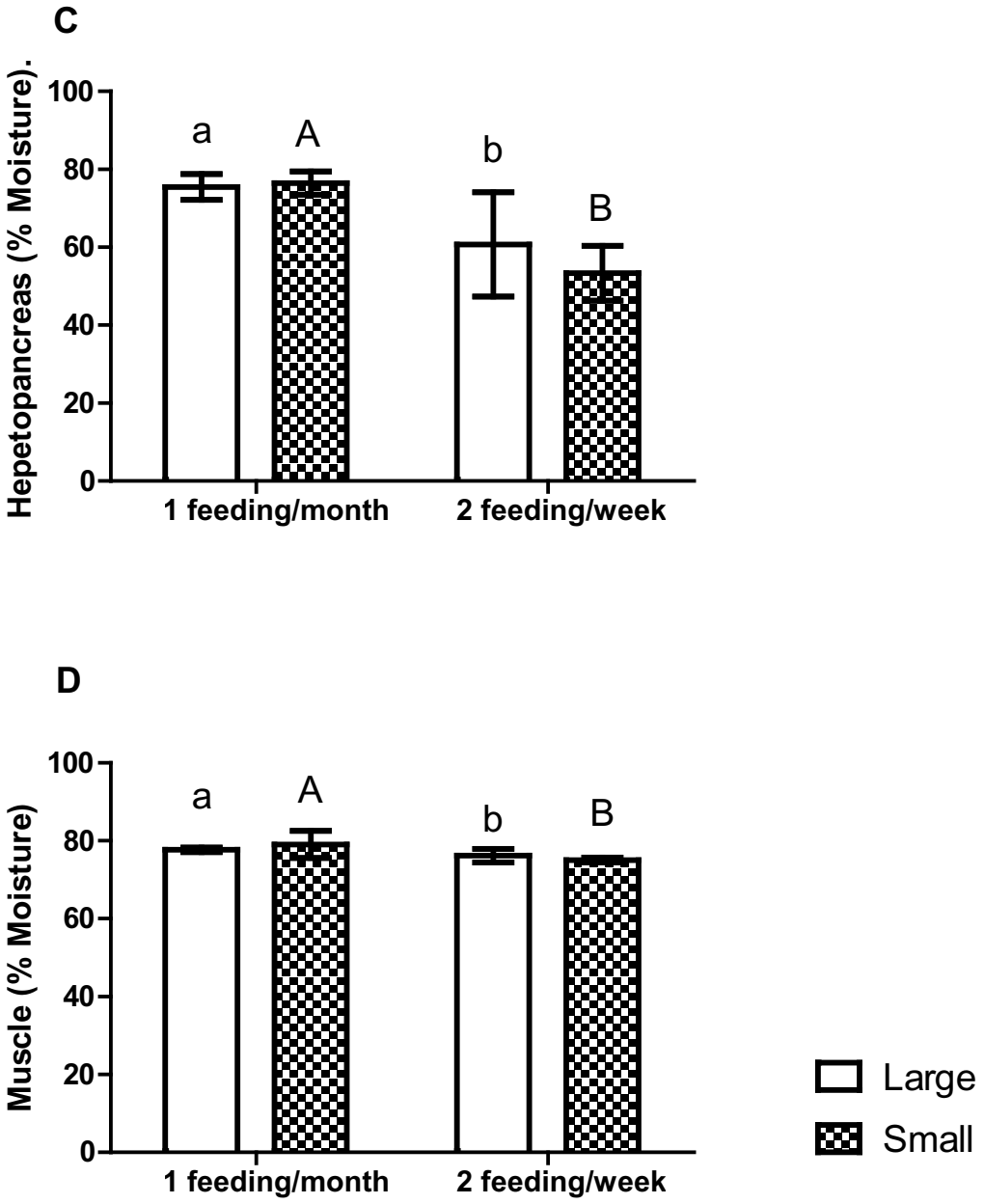
Feeding frequency, but not cage compartment size, also had a significant effect on the size of the hepatopancreas and heart and the moisture content of the hepatopancreas and body muscle tissue (Fig. 3-6). Lobsters fed twice per week had a larger hepatopancreas (4.43 ± 0.45% vs 2.85 ± 0.45%) (Two way ANOVA, F = 96.62, $P < 0.0001$) and heart (0.11 ± 0.02% vs 0.09 ± 0.01%) (Two way ANOVA, F = 21.89, $P < 0.0001$) than those fed once per month. In concordance with the smaller hepatopancreas, the hepatopancreas of lobsters fed once per month had a significantly higher moisture content of 75.99 ± 3.07% compared with 56.58 ± 10.59% for the twice weekly fed lobsters (Two way ANOVA, F = 49.72, $P < 0.0001$). Likewise the moisture content of the body muscle tissue was higher for the infrequent feeding treatment (78.39 ± 2.49%) compared to the frequent feeding trials (75.57 ± 1.33%) (Two way ANOVA, F = 15.74, $P < 0.0001$).

3.4.3 Field Experiment 1

The benthic water temperature (10 to 13 m) at Triton increased from 3.5˚C in late June reaching between 5.0 and 7.0˚C, during the period between mid-July to early November. Thereafter the water temperature dropped to around 2˚C by mid-December (Fig. 3-7). The temperatures recorded on cages under the mussel lines and those situated outside the mussel farm were almost identical to one another.

Fig. 3-6. Effect of feeding frequency and compartment size on internal organ characteristics: (A) hepatopancreas size (% BM); (B) heart size (% BM); (C) hepatopancreas moisture content (%); (D) muscle moisture content (%). Data are expressed as Mean ± SD (n = 7 - 9). Two way ANOVA was performed on final measurement. Different lowercase letters indicate significant differences ($P < 0.05$) between the two feeding frequencies for the large compartment size; different capital letters indicate significant differences ($P < 0.05$) between two feeding frequencies when animals were held in small compartments.


A
Hepatopancreas Size (% BM)
6
4
2
0
b
B
a
A
1 feeding/month
2 feeding/week
B
Heart Size (% BM)
0.15
0.10
0.05
0.00
b
B
a
A
1 feeding/month
2 feeding/week


C
Hepetopancreas (% Moisture).
100
80
60
40
20
0
a
A
b
B
1 feeding/month
2 feeding/week
D
Muscle (% Moisture)
100
80
60
40
20
0
a
A
b
B
1 feeding/month
2 feeding/week
Large
Small

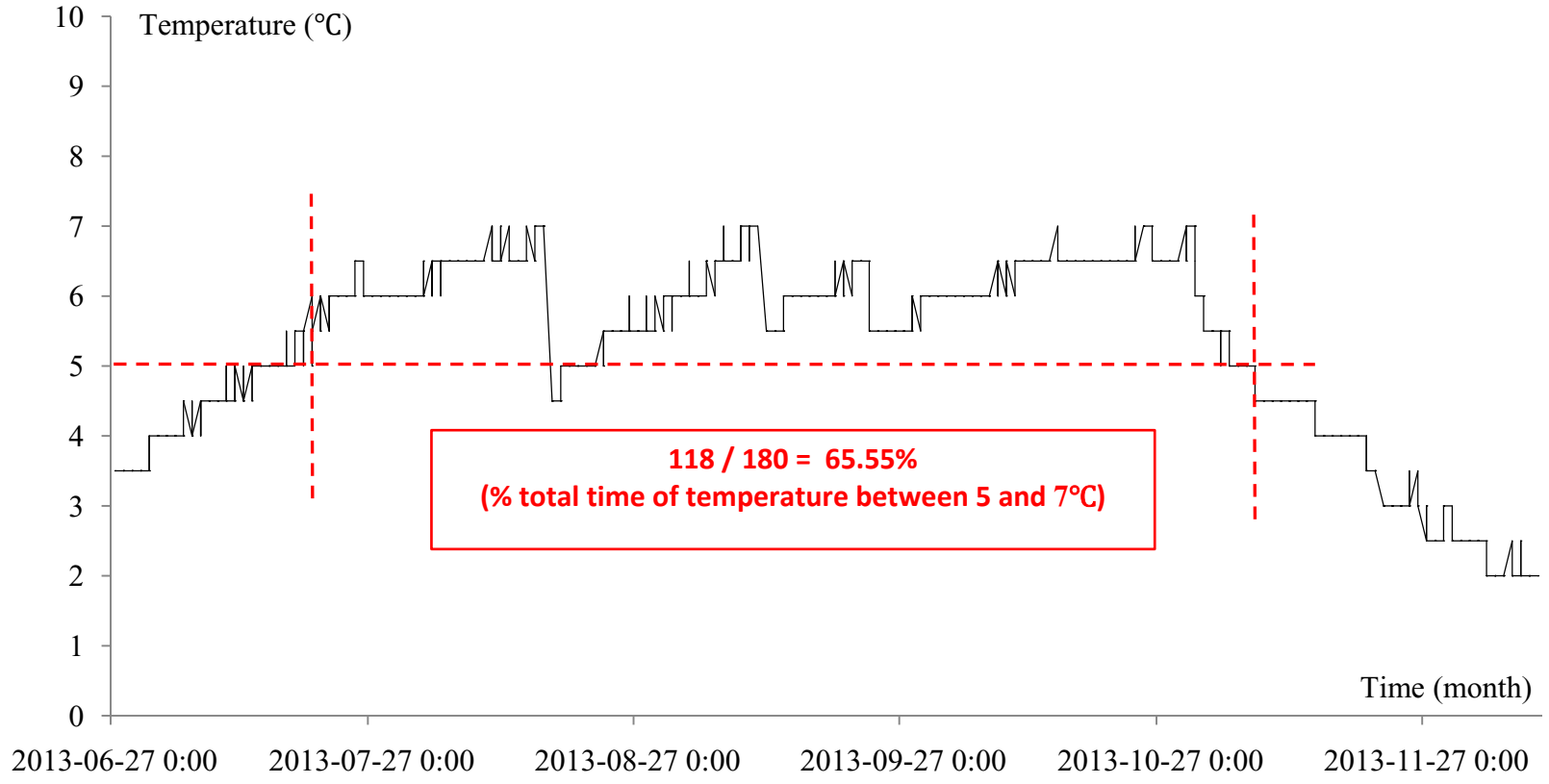


Fig. 3-7. Natural temperature range (˚C) from late June to mid December (2013) in the Triton area, northwestern coast of Newfoundland. Data loggers were attached to the cages which were set in 10 - 13m of water and recorded the temperature every four hours.

The lobsters held in cages in the field exhibited a very low mortality rate, ranging from 2.1 to 4.2% (Table 3-7) and the majority of these mortalities occurred in the last three months (mid-September to mid-December). In contrast to the high survival rate, lobsters in this trial exhibited a very low molting rate, ranging between 10.4 to 18.8% in all treatment groups (Table 3-8). Statistical analysis could not be performed on this data, because the limited inspection at the field sites (once per three months) did not allow accurate assessment of the exact time of each individual mortality/molting event.

The molted lobsters in cages on the open sea bottom exhibited an increase in BM and CL of 22.32 ± 7.74% and 6.45 ± 1.53%, respectively, while for those situated under mussel lines BM and CL increased by mean levels of 20.33 ± 4.70% and 7.04 ± 2.26%, respectively. There was no significant effect of compartment size or cage location on these values (Fig. 3-8) (Two way ANOVA, compartment size, $F = 0.12$, $P = 0.738$; cage location, $F = 0.58$, $P = 0.459$). Non-molted lobsters were able to maintain or even increase their overall BM during the six month period, nevertheless this increase was low, ranging between 0.39 and 1.92%, for both cage locations. Interestingly the non-molted lobsters in cages on the open seabed had a significantly higher increase in BM (1.85 ± 1.15%), than those maintained under mussel lines (0.67 ± 1.29%) (Two way ANOVA, $F = 32.23$, $P < 0.0001$). The compartment size did not have a significant impact on change in BM of non-molted lobsters (Two way ANOVA, $F = 0.92$, $P = 0.340$).

The serum protein levels decreased significantly over the six month study for both lobsters under mussel lines and on the open seabed (Table 3-9) (Two way repeated measures ANOVA, $F = 279.30$, $P < 0.0001$). The decrease in final serum protein levels

Table 3-7. Mortality rates of adult lobsters at Triton, effect of cage location and compartment size.

Sea Cage Location	Number	Compartment Size	Cumulative Mortality Over 6 Months (Number and Percent)		
			June	September	December
Under Mussel Line	48	Large	0 (0)	1 (2.1%)	1 (2.1%)
Under Mussel Line	48	Small	0 (0)	1 (2.1%)	2 (4.2%)
Outside Mussel Farm	48	Large	0 (0)	0 (0)	2 (4.2%)
Outside Mussel Farm	48	Small	0 (0)	0 (0)	2 (4.2%)

Table 3-8. Molting rates of adult lobsters under different sea cage location and compartment size condition, measured at three month intervals from June to December 2013 in the Triton area, northwestern coast of Newfoundland.

Sea Cage Location	Number	Compartment Size	Cumulative Molting Over 6 Months (Number and Percent)		
			June	September	December
Under Mussel Line	48	Large	0 (0)	1 (2.1%)	6 (12.5%)
Under Mussel Line	48	Small	0 (0)	3 (6.3%)	5 (10.4%)
Outside Mussel Farm	48	Large	0 (0)	9 (18.8%)	9 (18.8%)
Outside Mussel Farm	48	Small	0 (0)	4 (8.3%)	5 (10.4%)

Fig. 3-8. Effect of cage location and compartment size on growth of adult lobsters maintained for six months in benthic cages near Triton, NL. (A) percent increase in body mass for molted lobsters (n = 6 - 8); (B) percent increase in carapace length for molted lobsters (n = 6 - 8); (C) percent increase in body mass for non-molted lobsters (n = 32 - 38). Two way ANOVA was performed on final measurement. Data are expressed as mean ± SD. Different lowercase letters indicate significant differences ($P < 0.05$) between the two cage locations for large compartments; different capital letters indicate significant differences ($P < 0.05$) between two locations for animals maintained in small compartments.

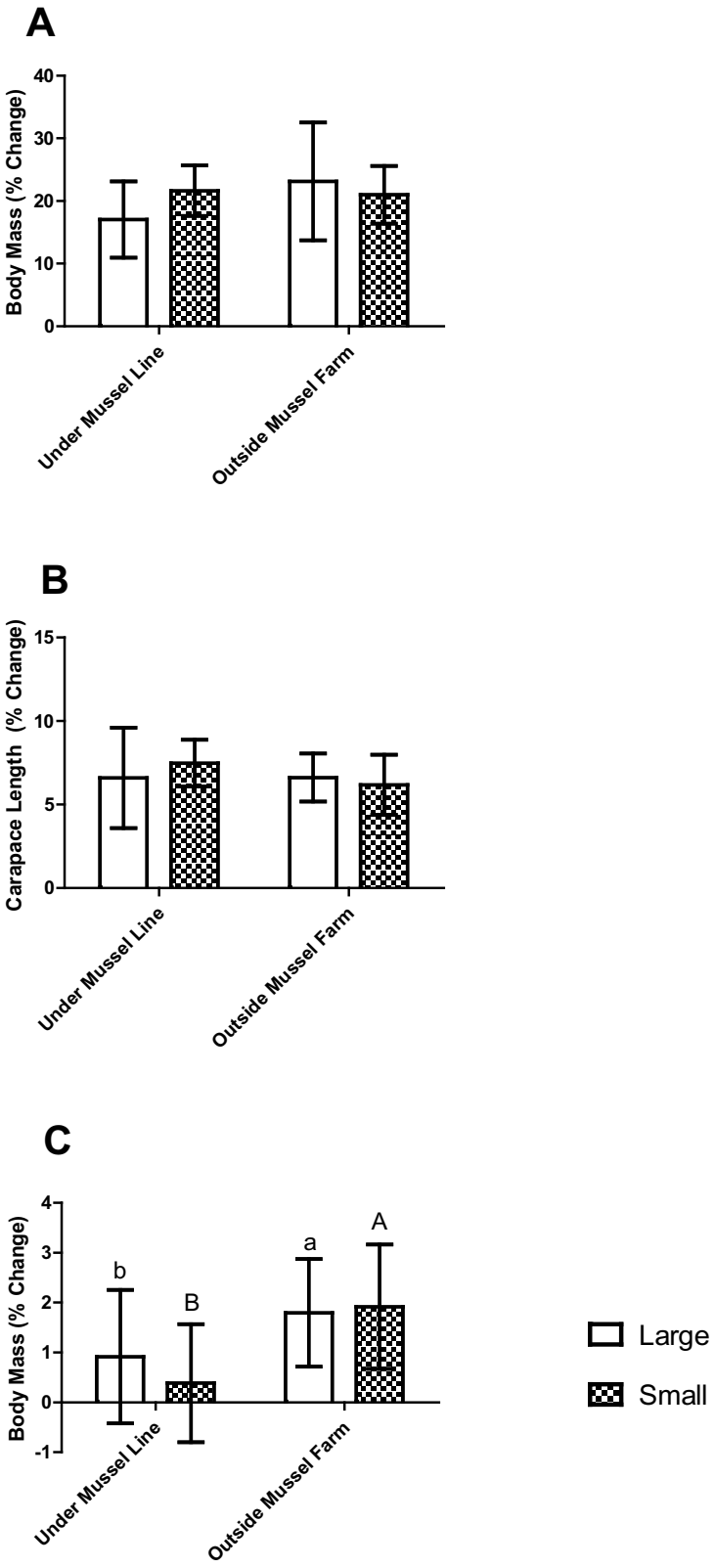

A
Body Mass (% Change)
40
30
20
10
0
Under Mussel Line
Outside Mussel Farm
B
Carapace Length (% Change)
15
10
5
0
Under Mussel Line
Outside Mussel Farm
C
Body Mass (% Change)
4
3
2
1
0
-1
b
B
a
A
Under Mussel Line
Outside Mussel Farm
Large
Small

Table 3-9. Serum protein concentration of adult lobsters in the Triton area, northwestern coast of Newfoundland, effect of cage location and compartment size.

Sea Cage Location	Compartment Size	Serum Protein (g/100 ml) Over 6 Months (Mean ± SD)		
		June	September	December
Under Mussel Line	Large	5.857 ± 1.111	3.857 ± 0.917	3.501 ± 0.916 ^{aA}
Under Mussel Line	Small	5.726 ± 0.901	3.600 ± 0.936	2.982 ± 0.859 ^{bA}
		(7.115 ± 1.678, n=3)	(2.202 ± 0.511, n=3)	(1.760 ± 0.568, n=3) ^{A}
Outside Mussel Farm	Large	6.045 ± 1.386	2.653 ± 1.009	2.218 ± 0.881 ^{aB}
Outside Mussel Farm	Small	5.961 ± 1.405	2.949 ± 1.182	2.469 ± 1.129 ^{aB}
		(8.325 ± 1.079, n=11)	(1.987 ± 0.515, n=11)	(1.463 ± 0.173, n=11) ^{A}

Two way ANOVA was performed on final measurement. Different lowercase letters indicate significant differences ($P < 0.05$) between the two compartment sizes in the same location; different capital letters indicate significant differences ($P < 0.05$) between two sea cage locations for the same compartment size. Data in parentheses are from molted lobsters.

was more pronounced in lobsters maintained on the open seabed (6.00 ± 1.39 to 2.35 ± 1.02 g/100 ml) compared with those held under mussel lines (5.83 ± 1.02 to 3.25 ± 0.91 g/100 ml) (Tukey post-hoc test, $P < 0.0001$). The compartment size also had a significant effect on the final serum protein concentration, but only for the lobsters ranched underneath mussel lines (Tukey post-hoc test, $P < 0.01$). The serum protein concentration of lobsters held in small compartments decreased from 5.73 ± 0.90 to 2.98 ± 0.86 g/100 ml, compared with a change of 5.86 ± 1.11 to 3.50 ± 0.92 g/100 ml for animals maintained in large compartments (Table 3-9). The surface area of the compartment also had an effect on serum protein levels (Fig. 3-9). For example in the 24-compartment cages, the 4 corner compartments had a large surface area that could potentially come into direct contact with organic material, while the lobsters in the middle 6 compartments were surrounded by animals in other compartments therefore had a much lower surface area (one upper and one lower) directly in contact with the environment. On the open seabed, the lobsters held in corner compartments with a larger surface area had significantly higher serum protein levels (2.70 ± 0.74 g/100 ml) than those held in the middle compartments with a smaller surface area (1.94 ± 0.56 g/100 ml) (Two way ANOVA, $F = 6.10$, $P < 0.05$). Although a similar trend was observed for lobsters held under the mussel lines (large surface area - 3.45 ± 0.73 g/100 ml, small surface area - 2.93 ± 0.90 g/100 ml), this proved to be statistically insignificant (Tukey post-hoc test, $P = 0.230$).

Only a few lobsters molted and because the cages were only checked once every three months, the data for molted lobsters was limited (shown in brackets in Table 3-9).

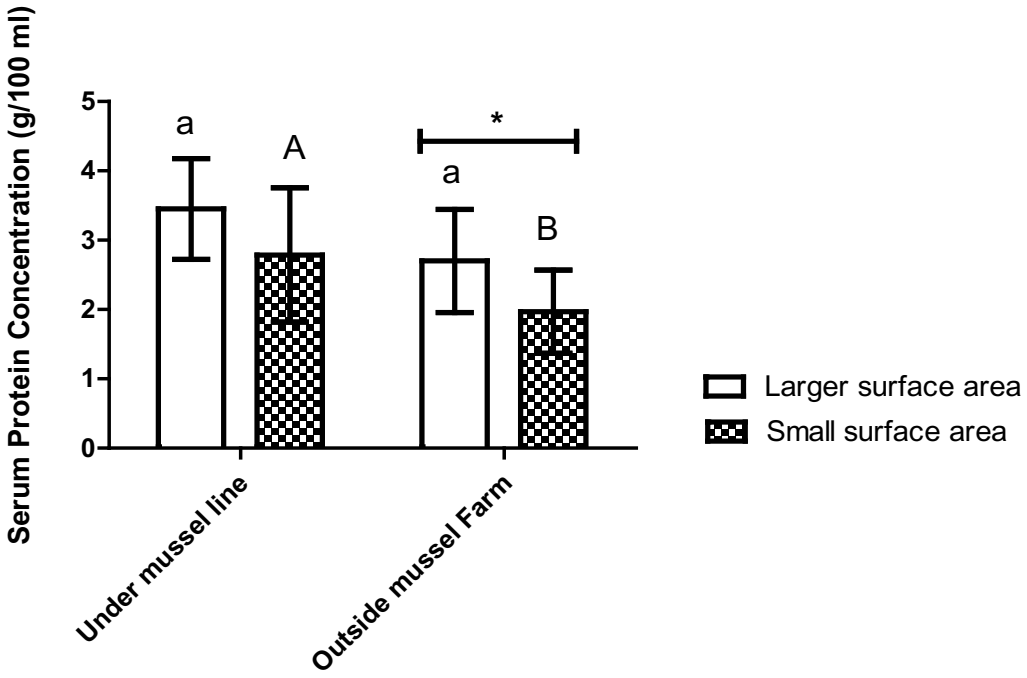
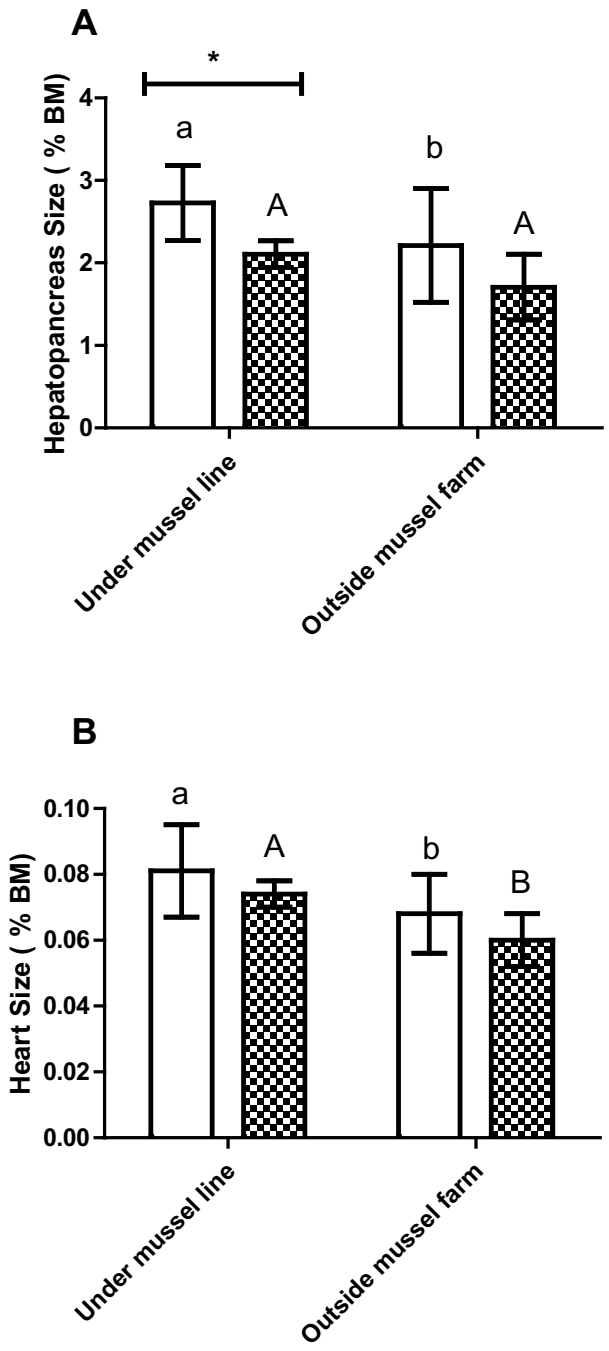


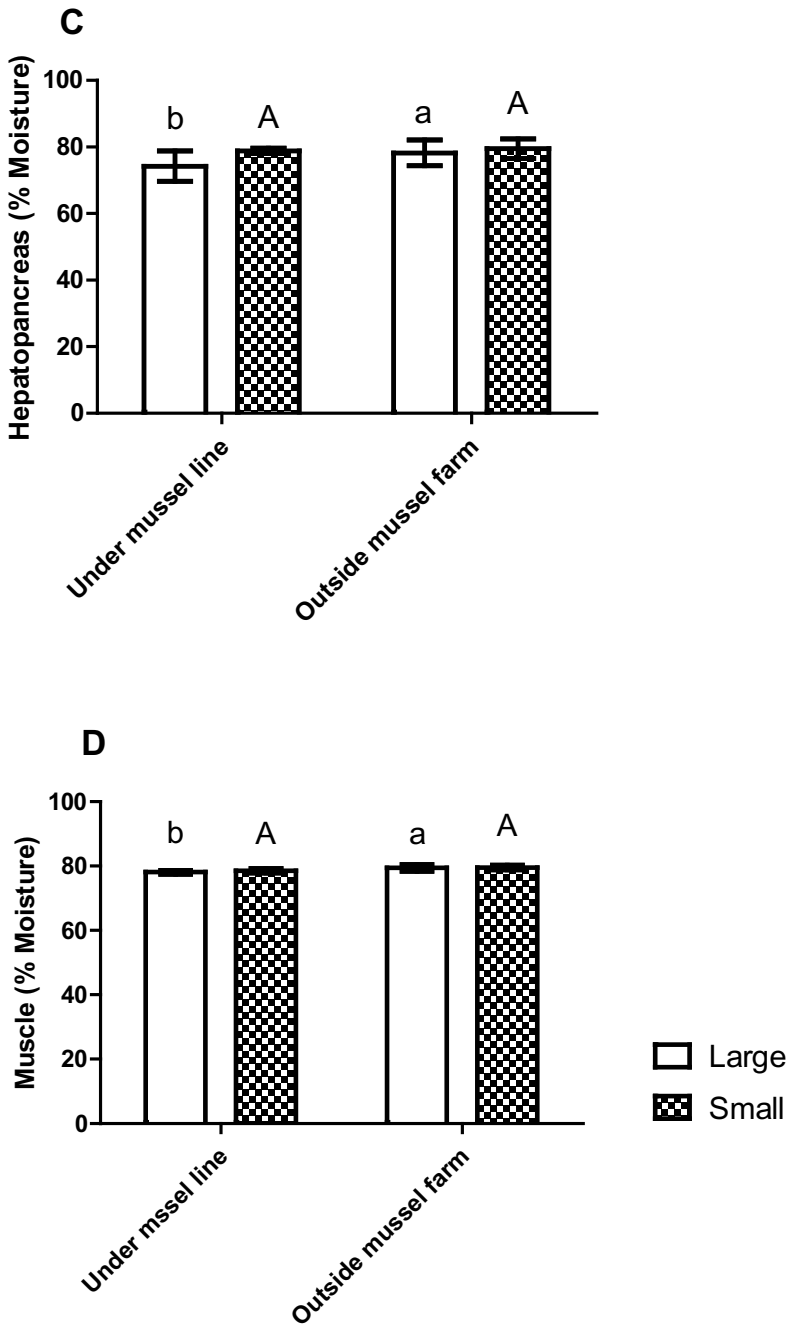
Fig. 3-9. Effect of compartment surface area (corner or middle) and sea cage location on lobster serum protein concentrations (g/100 ml). Data are expressed as mean ± SD (n = 7 - 9). Two way ANOVA was performed on final measurement. Different lowercases indicate significant differences ($P < 0.05$) between the two locations in large compartment surface area; different capital letters indicate significant differences ($P < 0.05$) between the two locations in small compartment surface area; asterisk indicate significant difference within group (* Significant 0.05 level).

The general trend was that serum protein levels dropped after molting, and serum protein levels (at both locations) continued to decrease thereafter. This decrease appeared to be more pronounced for lobsters settled on open seabed (8.84 ± 1.08 to 1.46 ± 0.17 g/100 ml), than for lobsters settled under mussel lines, (7.12 ± 1.68 to 1.76 ± 0.57 g/100 ml), however, this difference proved to be statistically insignificant (Student t test, T = 1.62, $P = 0.131$).

The cage location did have a significant effect on organ size and moisture content. Lobsters underneath the mussel lines were in a better condition than those held on the open seabed (Fig. 3-10). The percentage wet mass of the hepatopancreas of lobsters under mussel lines was significantly higher (2.62 ± 0.48%) than those on the open seabed (2.02 ± 0.64%) (Two way ANOVA, F = 10.05, $P < 0.01$). A similar trend was seen in the wet mass of the heart of lobsters from under mussel lines (0.080 ± 0.013%), and the open seabed (0.065 ± 0.011%) (Two way ANOVA, F = 7.50, $P < 0.05$). In concordance with organ mass, the moisture content of hepatopancreas of lobsters from under the mussel lines was significantly lower compared with those from the open seabed (75.04 ± 4.48% vs 78.70 ± 3.52%) (Two way ANOVA, F = 4.67, $P< 0.05$). A similar pattern was observed with respect to moisture content of the muscle tissue, with levels of 78.19 ± 0.66% under mussel lines versus 79.51 ± 0.95% for the open seabed (Two way ANOVA, F = 12.14, $P < 0.01$). The only effect of compartment size was for the lobsters settled under mussel lines. Here lobsters held in large compartments had a significantly larger hepatopancreas (2.73 ± 0.46%) than those held in small compartments (2.11 ± 0.16%) (Two way ANOVA, F = 15.25, $P < 0.0001$).

Fig. 3-10. Effect of cage location and compartment size on lobster organ indices: (A) hepatopancreas size (% BM) (n = 10 - 14); (B) heart size (% BM) (n = 10 - 14); (C) hepatopancreas moisture content (%) (n = 10 - 14); (D) muscle moisture content (%) (n = 10 - 14). Data are expressed as mean ± SD. Two way ANOVA was performed on final measurement. Different lowercase letters indicate significant differences ($P < 0.05$) between the two cage locations for animals held in large compartments; different capital letters indicate significant differences ($P < 0.05$) between the two locations for animals maintained for two months in small compartments.


A
*
Hepatopancreas Size (% BM)
4
3
2
1
0
a
A
b
A
Under mussel line
Outside mussel farm
B
Heart Size (% BM)
0.10
0.08
0.06
0.04
0.02
0.00
a
A
b
B
Under mussel line
Outside mussel farm


C
Hepatopancreas (% Moisture)
100
80
60
40
20
0
b
A
a
A
Under mussel line
Outside mussel farm
D
Muscle (% Moisture)
100
80
60
40
20
0
b
A
a
A
Under mssel line
Outside mussel farm
Large
Small

3.4.4. Field Experiment 2

The water temperature at Rushoon area increased from 6˚C during mid-June to around 15˚C in late July and was maintained at this level until early October. The temperature then decreased sharply in early October to 10˚C and declined thereafter reaching 2.5˚C in late December (Fig. 3-11). There was a trend in the variation of water temperature in different depth: the mean temperature range at the deeper water site (10 - 12 m) was approximately 1 - 4˚C lower than the shallow water site (6 - 8 m) from mid-June to early September. This trend reversed from early October to late December, at which time the temperature range in the deeper water site was approximately 0.5 - 1˚C higher than that measured at the shallow water site (Fig. 3-11).

The mortality rate ranged between 4.2 and 20.8% and nearly all of these deaths occurred during the first three months (Table 3-10). Lobster molting rate in this trial was higher than that in the Triton area, ranging between 33.3 and 41.7% (Table 3-11). Most of the molting occurred in the first three months between mid-June to mid-September. As with the field experiment at Triton, because we were unable to record the precise time of mortality and molting, statistical analysis could not be performed on this data.

There was no effect of compartment size or water depth on growth rates of molted lobsters, for BM (Fig. 3-12A) (Two way ANOVA, F = 1.21, P = 0.280), or for CL (Fig. 3-12B) (Two way ANOVA, F = 0.067, P = 0.080). The increase in BM and CL were 34.64 ± 10.52% and 8.50 ± 2.17%, respectively. Non-molted lobsters maintained their initial BM or showed a slight increase in BM, ranging between 0.28 and 3.49% (Fig. 3-12C). Although the lobsters held in larger compartments exhibited a greater increase in

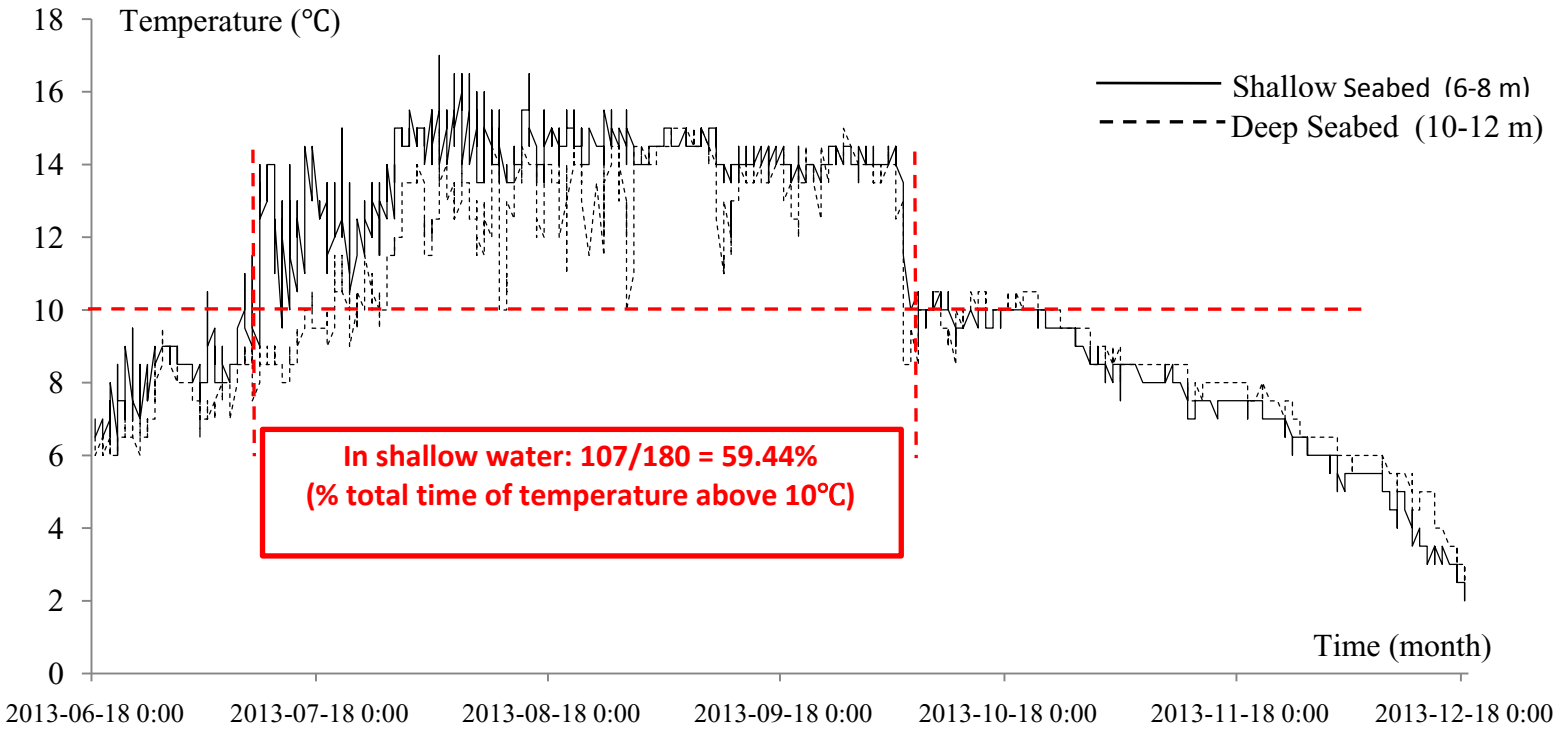


Fig. 3-11. Natural temperature range from mid June to mid December 2013 in the Rushoon area, Newfoundland. Data was collected every four hours using iBCod data tags (type G, Ste-Juline, QC, Canada) affixed to the cages. The solid line represents the temperature range from shallow seabed (around 6 - 8 m below water surface); the dash line represents the temperature range from deep seabed (around 10 - 12 m below water surface).

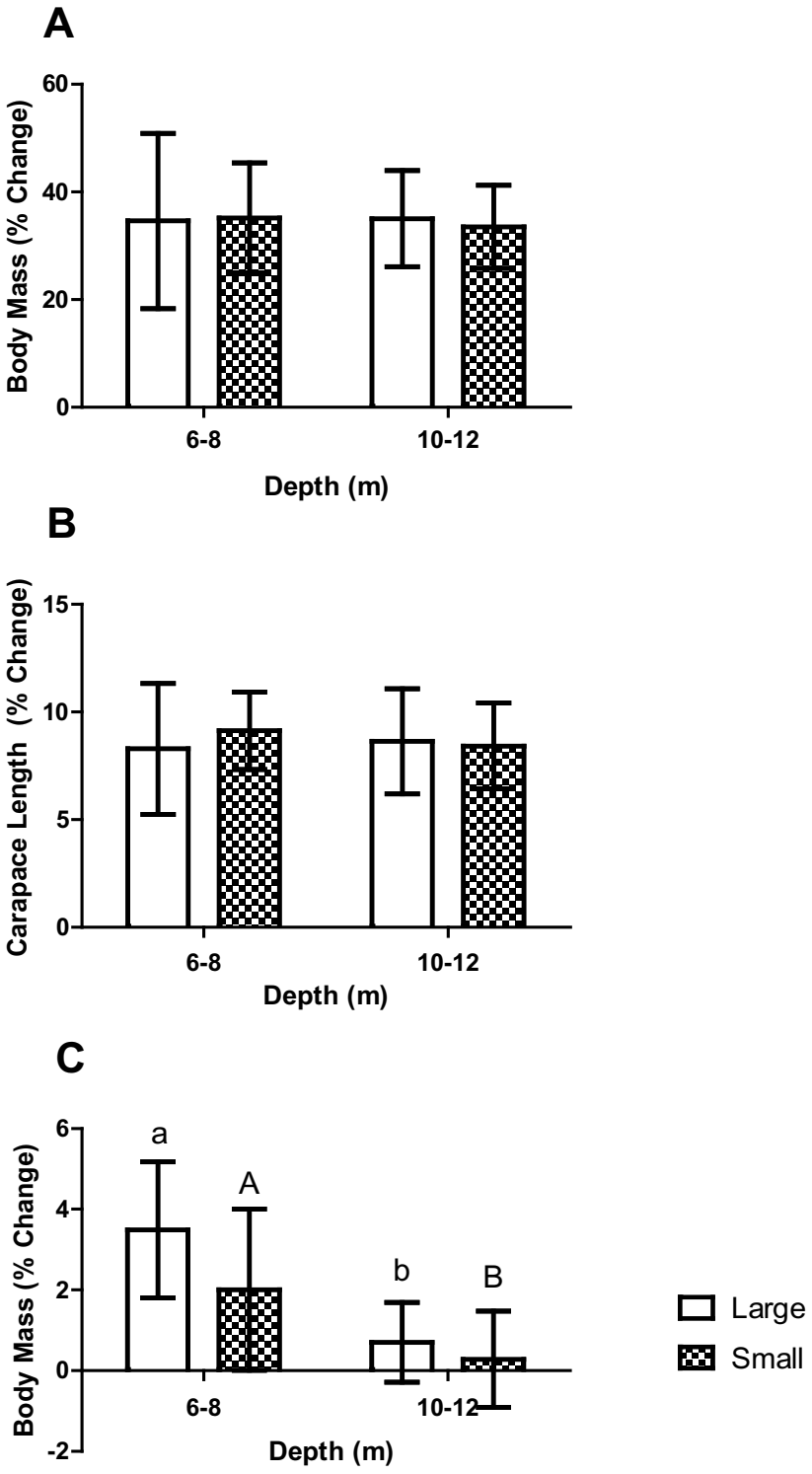
Table 3-10. Mortality rates of adult lobsters in the Rushoon area, effect of water depth and compartment size.

Depth (m)	Compartment Size	Number	Cumulative Mortality Over 6 Months (Number and Percent)		
			June	September	December
6 - 8	Large	24	0 (0)	5 (20.8%)	5 (20.8%)
6 - 8	Small	24	0 (0)	2 (8.3%)	2 (8.3%)
10 - 12	Large	24	0 (0)	1 (4.2%)	1 (4.2%)
10 - 12	Small	24	0 (0)	3 (12.5%)	4 (16.7%)

Table 3-11. Molting rates of adult lobsters maintained at different depth and two compartment sizes at Rushoon, NL.

Depth (m)	Compartment Size	Number	Cumulative Molting Over 6 Months (Number and Percent)		
			June	September	December
6 - 8	Large	24	0 (0)	4 (16.7%)	8 (33.3%)
6 - 8	Small	24	0 (0)	7 (29.2%)	9 (37.5%)
10 - 12	Large	24	0 (0)	9 (37.5%)	10 (41.7%)
10 - 12	Small	24	0 (0)	7 (29.3%)	8 (33.3%)

Fig. 3-12. Effect of water depth (m) and compartment size on growth of adult lobsters. (A) percent increase in body mass for molted lobsters (n = 7 - 10); (B) percent increase in carapace length for molted lobsters (n = 7 - 10); (C) percent increase in body mass for non-molted lobsters (n = 8 - 11). Two way ANOVA was performed on final measurement. Data are expressed as mean ± SD. Different lowercase letters indicate significant difference ($P < 0.05$) between the two water depths for lobsters held for six months in large compartments; different capital letters indicate significant differences ($P < 0.05$) between the two waters depths in small compartments.


A
Body Mass (% Change)
60
40
20
0
6-8
10-12
Depth (m)
B
Carapace Length (% Change)
15
10
5
0
6-8
10-12
Depth (m)
C
Body Mass (% Change)
6
4
2
0
-2
a
A
b
B
6-8
10-12
Depth (m)
Large
Small

BM (1.41 ± 1.70%) than those held in small cages (1.02 ± 1.80%), this proved to fall just shy of significance at $P < 0.05$ (Two way ANOVA, F = 3.85, $P = 0.058$). The water depth did have a significant effect on BM for non-molted lobsters; those in the shallow area added more mass than those in from the deeper location (2.50 ± 1.96% vs 0.45 ± 1.05%) (Two way ANOVA, F = 21.57, $P < 0.0001$).

The experimental design involved two feedings/week for the first three months and one feeding/month in last three months. The serum protein concentration of non-molted lobsters increased in the first three months, and then declined slightly during last three months. Despite the decline during the second half of the experiment, these final serum protein levels were significantly higher than the levels measured at the start of the experiment (Table 3-12). There was no significant effect of compartment size or water depth on serum protein concentrations. The trend of serum protein levels in molted lobsters was partially consistent with the other laboratory and field studies, with high levels in premolt and lower levels in newly molted lobsters. However, in the last three months, the serum protein concentration of the molted lobsters did not show a recovery to original levels (Table 3-12). Since the experimental lobsters were the property of Jerseyman Island Fisheries Ltd and were destined for market, the hepatopancreas and heart size, and hepatopancreas and body muscle moisture content were not sampled and tested for in this experiment.

Table 3-12. Serum protein concentrations of adult lobsters maintained at two different depths and two compartment sizes from June to December 2013 near Rushoon.

Depth (m)	Compartment Size	Serum Protein (g/100 ml) (Mean ± SD)		
		June	September	December
6 - 8	Large	5.400 ± 1.937	6.452 ± 1.276	5.775 ± 1.035 ^{aA}
6 - 8	Small	5.095 ± 1.518	5.876 ± 0.955	5.259 ± 0.801 ^{aB}
		(8.075 ± 1.193, n=10)	(3.922 ± 0.556, n=10)	(2.698 ± 0.461, n=10) ^{B}
10 - 12	Large	5.595 ± 1.492	5.848 ± 1.181	5.275 ± 1.125 ^{aA}
10 - 12	Small	5.295 ± 0.834	6.087 ± 0.630	5.883 ± 0.630 ^{aA}
		(7.966 ± 1.330, n=16)	(3.688 ± 0.630, n=16)	(3.302 ± 0.735, n=16) ^{A}

Two way ANOVA was performed on final measurement. Different lowercase letters indicate significant differences ($P < 0.05$) between two compartment sizes at the same depth; different capital letter superscripts indicate significant difference ($P < 0.05$) between two compartment sizes in the same deep seabed. Data inside parentheses are from molted lobsters.

3.5 Discussion

3.5.1 Survival rates

Temperature had a significant effect on survival rates, with noticeable differences between the laboratory and field experiments. Lobsters held in the laboratory at 5˚C exhibited a high mortality rate (> 50%). The water temperature range in the Triton area was also low (between 2 - 7˚C), and yet nearly all the lobsters survived the six month period, so temperature alone could not account for the high mortality. The metabolic rates of aquatic crustaceans are directly influenced by temperature (Nelson et al., 1977; Childress et al., 1990) and as the lobster's metabolic rate increased, it resulted in a greater food intake (Wang and McGaw, in prep.). The hemolymph protein concentration is directly related to food intake (Wang and McGaw, 2014), and it increased with increasing water temperature (Table 4-1). The hemolymph protein is primarily composed of hemocyanin; however, there are other proteins which are involved in the immune response (Hose et al., 1990; Sung et al., 1996; Le Moullac et al., 1997; Vargas-Albores et al., 1997). The total hemocyte count increases in the American lobster *Homarus americanus*, shore crab *Carcinus maenas*, and the shrimp *Penaeus stylitrostris* as temperature increases (McLeese, 1972a; Truscott and White, 1990; Le Moullac et al., 1998) and in lobsters, the rate of phagocytosis is positively related to temperature (Paterson and Stewart, 1974). Although the experimental tanks were supplied with a constant flow of seawater and were cleaned weekly, there is the potential “wall effect” where bacterial build-up occurs on flat surfaces in these semi-enclosed laboratory systems

(Zobell, 1943; Eilers et al., 2000; Baltar et al., 2012). Since serum protein concentrations were low in the animals maintained in 5˚C, it would suggest that they had compromised defense mechanisms, leaving them more vulnerable to infection from pathogens.

There was also a high mortality rate at 15˚C, but this was primarily for post-molted lobsters. Although mortality rates increase during molting, and recently molted lobsters are more physiologically sensitive and vulnerable (McLeese, 1956; Mykles, 1980; Bowser and Rosemark, 1981), this was not the case here. None of the lobsters in the present study died during the molting process, and only one lobster died within one day of molting. The mortalities occurred between 31 and 80 days after molting and nearly all of them were lobsters fed the mussel diet at 15˚C. Essential amino acids such as asparagine, alanine and glutamic acid are deficient in mussels (Brawn et al., 1968; Mente, 2010). Astaxanthin is also lacking in the mussel diet (Barclay et al., 2006) and this plays an important role in immunocompetence and stress tolerance in crustaceans (Chien et al., 2003). This suggests the mussel diet was not sufficient to provide nutrients for post-molt processes such as laying down muscle and hardening of the carapace and for dealing with increased pathogen loads in the experimental tanks.

In contrast to the high mortality rate in the laboratory at 5˚C, lobsters maintained in cold water at Triton (between 5 - 7˚C for 66% of time) exhibited a very high survival rate (> 95%). The lower temperature suppressed metabolism (Nelson et al., 1977; Childress et al., 1990) and reduced their activity and feeding rates (McLeese and Wilder, 1958; Wang and McGaw, in prep.). Such chill coma methods are used in commercial facilities to enhance live storage times, and for transport of crustaceans to market

(Lorenzon et al., 2007, 2008). Nevertheless, adult lobsters can only tolerate starvation for a finite period of time. There is an inverse relationship between temperature and tolerance of starvation (McLeese, 1965), and at temperatures between 1 and 5˚C lobsters can survive without food for three to four months (Stewart et al., 1967; Stewart et al., 1972). Since the lobsters held at Triton survived for six months this suggested that they were able to procure enough food to meet their basic survival needs. The lobsters held under culture lines were likely feeding on mussels that dropped off the lines during harvesting. Although we were unable to directly quantify this assumption, large numbers of empty shells were found around the cages. The lobsters under mussel lines were generally healthier (higher serum protein concentration) than those held outside the mussel farm, suggesting extra food input in the form of mussels. However, the lobsters on the open seafloor also had a high survival rate, albeit with a slightly lower serum protein concentration. Gastropod shells, seaweed, sea urchins and sea stars were found inside the cages when they were retrieved. As lobsters are omnivorous, it was likely they were feeding opportunistically on animals that entered the cages.

In addition to temperature, the diet influenced survival, with higher survival rates for animals fed the mixed diet at 5˚C and 15˚C. In the wild, American lobsters' food changes seasonally and geographically (Ennis, 1973). Crabs are a preferred prey of the lobster and play an important role in growth and ovary development (Gendron et al., 2001) because of their high protein and energy content (Brawn et al., 1968), and the presence of specific amino acids, such as arginine, lysine and methionine (Vonk, 1960; Boghen et al., 1982). Sea urchins, molluscs, sea stars, fish and seaweeds are also part of

the diet. The sea urchin, molluscs, and sea stars have high calcium content, which helps to accelerate the process of exoskeleton hardening (Evans and Mann, 1977). The high survival of lobsters fed mixed diet confirmed the correct balance of nutrients.

Although there were variations in the rate of mortality as a function of feeding frequency (2nd laboratory experiment), there were no statistically significant differences. In line with the experiments at a constant temperature of 15˚C, most of the mortalities occurred in the final months, however, these mortalities were not molting or recently molted lobsters, so it was unclear why rates were higher towards the end of the experiment, especially since serum protein concentrations were similar to the lobsters that survived.

The temperature profile and feeding regime at Rushoon was similar to that of the 2nd laboratory experiment, but at Rushoon, the mortalities predominately occurred in the first three months. These mortalities were probably related to the lobsters' initial condition. By referencing notes on the lobsters that succumb, they were found to have lower serum protein concentrations and were less active when handled. Therefore, the lobsters' original physiological condition and vitality likely has long lasting effects on survival (S. Lamont, pers. comm.).

3.5.2 Molting

The molting rate was temperature dependent; in the laboratory, molting rate was very low in 5˚C (4%) and most animals remained in the intermolt stage throughout the six month experimental period. The low molting rate in the Triton area (13%) could also be contributed to the cooler water temperatures (2 to 7˚C). In the laboratory at 10˚C, over

60% lobsters entered premolt in the 5th/6th month, and started to molt during the 6th month. At 15˚C, 75% lobsters were in premolt stage by the 3rd and 4th month, and these lobsters subsequently molted in the 5th and 6th months. This result was not consistent to a similar study on lobster growth in captivity where molting was not affected by temperature: the culture period was eight months, which is long enough for most lobsters to molt; but the temperatures were warmer 12, 17, and 22˚C (McLeese, 1972a), and all are within the range suitable for growth (Aiken and Waddy, 1980; Crossin et al., 1998). Rather than needing to be exposed to a certain temperature to induce molting, the lobsters might need to be exposed for a number of degree days. The growing degree day (thermal integral) is used as a reliable predictor of growth and development in fish species (Neuheimer and Taggart, 2007) and this likely also applies to crustacean molting and growth (Simon Webster, Bangor University, pers. comm.).

Using the regression equation of serum protein concentration during post-molt (Fig. 3-2), it can be estimated that a newly molted lobster would require approximately seven and half months to molt again if maintained in 15˚C and fed a mixed diet. This period is considerably shorter compared with animals living in the wild. Since lobsters spend a prolonged period in cold water during the winter months, this undoubtedly extends the postmolt/intermolt stage to at least 12 months (Campbell, 1983; Comeau and Savoie, 2001). Indeed, there is an increase in intermolt duration when comparing lobsters from their southern limits in North Carolina to those in northern most range in Newfoundland, as animals are exposed to longer periods of cold water.

When lobsters were held in the laboratory on an ambient temperature cycle, the majority of molting occurred in the warmer months from August to October 2013. This molt timing was the same as those cultured in the Rushoon and one month later than those in the warmer waters of the southwestern Gulf of St. Lawrence (Campbell, 1983; Comeau and Savoie, 2001). During these molting periods, the water temperature was above 10˚C for 100 days (55.6% of time) in Logy bay, in the Rushoon, temperature increased above 10˚C for 107 days (59.4% of time), while in the southwestern Gulf of St. Lawrence, temperature is above 10˚C for 133 days (66.5% of time) (Comeau and Savoie, 2001). The molting rate in Logy bay was 28.1% which was a little lower than those in the Rushoon (36.5%). The seasonal temperature range in these two locations likely explains the molting rate difference. Although the lobsters held in laboratory were smaller than those at Rushoon, size was unlikely to play a role since molt-related differences as a function of size are limited to sub-adult lobsters (Campbell, 1983).

Changes in food abundance impact molting frequency in larval and juvenile stages of clawed, rock and spiny lobsters (Templeman, 1936; Chittleborough, 1975; Vijayakumaran and Radhakrishnan, 1986). Crustaceans can refrain from molting during starvation in order to save energy to maintain basal metabolic functions (Comoglio et al., 2004). This was not the case here for adult lobsters. Lobsters fed once per month had similar molting rates to those fed twice weekly. In addition, lobsters maintained in the laboratory at 5˚C and fed had similar low molting rates to animals at Triton where food was limited. Crustaceans expend energy at molt and the hepatopancreas functions as a major source of energy during molting (Travis, 1955; Read and Caulton, 1980). Even

though the hepatopancreas was smaller in infrequently fed animals, it suggests that the lipid and glyceride stores were sufficient to facilitate molting.

The diets (mussel and mixed) used in present study had no significant effect on the molting rate of adult lobsters. This result is opposite to the previous studies where molt incidence decreases in adult *Homarus americanus* fed artificial meals with a low protein content (Castell and Budson, 1974). Artificial diets with cod liver oil also induce a higher molt incidence compared with diets containing corn oil or hydrogenated coconut oil (Castell and Covey, 1976). In the natural environment, the main prey items of lobster *Homarus americanus* include crabs, molluscs and echinoderms (Weiss, 1970; Ennis, 1973), which are relatively high in protein. The diet types used in this study were similar to the lobsters natural prey items and probably supplied a more balanced nutrition for lobster molting compared with the artificial diets used by the other researchers.

3.5. 3 Growth

Growth was quantified by changes in BM in non-molted lobsters, and increases in CL and BM for molted lobsters. Feeding increases with temperature, and lobsters consume 3, 4 and 5% of total body mass per sitting at 5, 10 and 15˚C, respectively (Wang and McGaw, in prep.). The cooler temperatures (5˚C) suppressed the lobsters metabolism (Nelson et al., 1977; Childress et al., 1990), and subsequently the lobsters consumed less food (Wang and McGaw, in prep.). At higher temperatures (10 and 15˚C), lobsters had a higher metabolic rate and fed more, as a result a more muscle was added (Wang and McGaw, 2014). Although lobster hepatopancreas and heart at 5˚C were smaller with a higher moisture content compared with those in 10 and 15˚C, they were still in a similar

range to wild caught lobsters. This suggests that even though they ate less at 5˚C, this was enough to sustain an optimal physiological condition. This likely occurs because the lower temperature slows the passage of food, which allows more time for absorption of nutrients from the meal (Wang and McGaw, in prep.).

In the Rushoon the molted lobster BM and CL was not impacted by water depth, even though the shallow water warmed up at a faster rate, it also cooled more rapidly and so the lobsters were essentially exposed to a similar range of degree days. Molted lobsters in the Rushoon had higher increment in BM (34.64 ± 10.52%) compared with those held at Triton (21.62 ± 6.77%) and the lobsters maintained on a similar temperature cycle in the laboratory (26.43 ± 13.82%), but were not different from those held at 10 and 15˚C (29.16 ± 7.36%). There are a couple of possible reasons for the higher increment of BM at Rushoon: the lobsters could potentially receive additional food that drifted into the cages and at the time of measurement they had been in the postmolt stage for longer than the animals in the laboratory and hence added more muscle mass.

The changes in CL of molted lobsters were similar among the laboratory and field treatments, except for lobsters at Triton, which were slightly lower (8.54 ± 1.98% vs. 6.71 ± 1.86% increment in CL). The lower growth rate at Triton was due to the combined effects of low temperature and a very low food input. Thus in addition to temperature, the growth increment of molted lobsters was impacted by feeding frequency. Lobsters fed frequently (in both the laboratory and the field) were significantly larger (CL and BM) than infrequently fed lobsters. Lobsters with access to enough food would lay down more muscle tissue and have energy adequate energy reserves to produce larger organs.

The effect of feeding frequency on growth of non-molted lobsters was somewhat different. Although one feeding per month was adequate to keep the lobsters alive, the non-molted lobsters in the laboratory experiment tended to lose some BM. In contrast, the lobsters at Triton(without artificial feeding) were able to maintain or even increase BM slightly. The lower temperatures at Triton probably slowed the lobsters metabolic rate and use of stored nutrients (Stewart et al., 1967; Stewart et al., 1972). Nevertheless, the lobsters at Triton had a more pronounced decrease in hepatopancreas size and edible meat content than those fed once per month in the laboratory. During starvation, crustaceans metabolize their tissues, resulting in a decrease in organ mass (Dall, 1974; Barclay et al., 1983; Dall and Smith, 1987; Depledge and Bjerregaard, 1989). One possible explanation for the starved lobsters at Triton maintaining or even slightly increasing (1 - 2%) their body mass is that an increased water uptake would compensate for the decrease in organ mass. The body mass of white shrimp *Litopenaeus vannamei,* and king crabs *Lithodes santolla*, also remains constant during short-term starvation and is likely due to an increased water content in the body (Comoglio et al., 2004; Comoglio et al., 2008).

Although there was no change in body mass for molted lobsters at Rushoon as a function of depth, there were different growth rates in non-molted lobsters, the mean body mass of lobsters in the shallow water was 2% higher than those at the deeper sites. The water current was stronger in the shallow water area, and this might carry more food into the cages, resulting in non-molted lobsters put on more muscle mass than those held in deeper water. In addition, in the summer months, the temperature range in shallow water (6 - 8 m) was relatively higher than the deep water (10 - 12 m), increasing lobsters

metabolism and making them eat more. In the winter in shallow water, the reduction in water temperature depressed metabolism to such a degree that the food they obtained was sufficient for them to maintain body mass (Ennis, 1983).

There was no effect of doubling cage size on growth of adult lobsters. In contrast, juvenile *Homarus americanus* respond to an increase in container size with significant increase in CL and BM (Shleser,1974; Aiken and Waddy, 1978; Van Olst and Carlberg, 1978; Beal and Protopopescu, 2012). The cages used by Beal and Protopopescu's (2012) were large enough for juvenile lobsters to freely move around and the large surface area for settling organisms supplied plenty of food for the lobsters. Crustaceans are able sense and response to an increase in container size by increasing their CL or BM (Aiken and Waddy, 1978; Van Olst and Carlberg, 1978). However, in the present study even doubling the compartment size still restricted the animal's movement and thus the increase in compartment size was not really enough to impact growth of post-molted adult lobsters.

3.5.4 Health and physiological condition

The serum protein concentration is a rapid and effective way of determining the quality and physiological condition of lobsters (Wang and McGaw, 2014). There is a strong positive correlation between serum protein concentration and hepatopancreas size, heart size and edible meat content and a negative correlation with moisture content of the hepatopancreas and muscle tissue (Wang and McGaw, 2014).

Serum protein concentration increased with temperature. Even though the serum protein concentration of fed lobsters maintained in 5˚C did not reach levels of those in 10

and 15˚C, it still increased slightly. As temperature increased, the lobsters ate more food (Wang and McGaw, in prep.). The level of food intake is directly related to serum protein concentration and animals with a higher feeding frequency attain higher serum protein levels compared with those on a restricted diet (Stewart and Li, 1969; McLeese, 1972a; Smith and Dall, 1982; Moore et al., 2000; Pascual et al., 2006; Wang and McGaw, 2014).

The final serum protein concentrations of the lobsters at Triton area were lower than any of the other treatments, although the colder water temperatures may have contributed, the decrease would primarily be related to the lower food input, because lobsters maintained in the laboratory at 5˚C and fed regularly exhibited an increase in serum protein concentration. In support of this assumption, the decrease in serum protein concentration was greater for lobsters set on open sea bottoms where they would not get the potential input of mussels. Interestingly, lobsters held in corner compartments had higher serum protein concentrations than lobsters held in the centre compartments. The corner compartments had a larger surface area in direct contact area with the surrounding environment, allowing more surface area to forage and these lobsters would be the first to come into contact with any food drifting into the cages.

The lobsters held at Triton with very low serum protein concentrations were of a much lower quality (smaller organ sizes, less edible meat content, higher moisture content in organ and tissues) when compared with wild caught lobsters. In addition, when the lobsters from Triton were transported to the Department of Ocean Sciences for analyses at the end of the six month period, many became moribund during the 10 h period of emersion. The majority of the serum protein is hemocyanin, which is used for oxygen

transport (Uglow, 1969; Lin and Chen, 2001). Healthy crustaceans are able to increase their total protein levels, especially hemocyanin, and alter hemocyanin oxygen affinity during aerial exposure (Taylor, 1981; Lorenzon et al., 2007, 2008). Because hemocyanin functions in emergency oxygen transport (Taylor, 1981), the lobsters from Triton with a low serum protein concentration would have a reduced ability to carry oxygen and would succumb more quickly to the effects of systemic hypoxia (Taylor, 1981; Taylor and Whiteley, 1989). Thus it is also important to take into account the level of serum protein of crustaceans destined for live transport, and animals maintained in the field with a low food input might not make it to market (Lorenzon et al., 2007, 2008).

In the Rushoon, lobster serum protein concentrations increased significantly during the first three months which was a function of the regular feeding schedule (twice/week). This pronounced increase was partly due to the fact that the serum protein concentrations were also low to start with because the lobsters had been held in boxes (and not fed) for two weeks prior to experimentation. When the feeding switched to once per month in the last three months, the serum protein concentration dropped slightly. The low water temperature in last three months from September to December (2013) might have also contributed to the drop of the serum protein concentration and in wild lobsters serum protein drops to lower levels in the winter season and this is independent of feeding (Stewart and Li, 1969; Ennis, 1973). Although serum protein concentration dropped slightly for lobsters at Rushoon, it was still 1.5-fold higher than those fed once per month in laboratory (similar water temperature). The difference here might be due to two reasons. Firstly the lobsters in laboratory were fed once per month for six months,

while the lobsters at Rushoon were only switched to one feeding per month in the last three months. Secondly the lobsters at Rushoon were likely feeding on other food items that drifted into the cages.

The serum protein concentrations were also impacted by diet. Serum protein concentrations of non-molted lobsters at 5, 10 and 15˚C, were slightly higher when fed mixed diets compared with those fed the mussel only diet. The mixed diet was similar to the lobsters natural prey and supplied essential nutrients for maintenance and growth (McLeese, 1972a, b; Hagerman, 1983).

Mean serum concentration also changed over the molt cycle; the sharp drop of serum protein concentration following molting is supported by previous studies (Barlow and Ridgway, 1969; Djangmah, 1970; Hepper, 1977; Hagerman, 1983; Philp and Marteinsdottír, 2013). This decline is a result of dilution associated with water uptake which is used for expansion of the body, as well as the incorporation of hemolymph proteins into the new shell (Terwilliger, 1999). The slow increase of serum protein during postmolt represents body tissue growth, which replaces the water (Smith and Dall, 1982; Oliver and MacDiarmid, 2002). Serum protein concentration declined in post-molted lobsters in 15˚C fed mussels, but increased in those fed the mixed diet. Post-molted lobsters appeared to have a poor appetite for the mussel diet, while those fed the mixed diet continued feeding. Juvenile European lobster fed diet of bivalves also have lower levels of serum protein than others fed shrimp, echinoderms and pellets (Hagerman, 1983). Low serum protein concentration from the mussel diet might be attributed the lower energetic content of molluscs when compared with other benthic invertebrates

(Brawn et al., 1968). This suggests that although bivalve molluscs are readily eaten by lobsters (Weiss, 1970; Ennis, 1973) and are a good source of calcium for exoskeleton hardening, they lack all the essential nutrients needed for survival (Brawn et al., 1968; Smith et al., 2005; Barclay et al., 2006; Mente, 2010). This may be an important consideration when attempting to hold lobsters under mussel aquaculture operations and additional feeding may be required for post-molted lobsters.

Although there appeared to be a link between mortality rates of post molted lobsters and their low serum protein concentration, this mortality as a function of low serum concentration was not found in intermolt lobsters. Despite the fact that there was a high mortality rate in animals which exhibited a substantial decrease in serum protein concentration, there was no direct evidence that animals became moribund when it reached a certain level, or that the decrease in serum protein concentration itself was the cause of death. Certainly low serum protein concentrations are associated with animals that are less tolerant of stress (Wang and McGaw, 2014). This is taken into account in commercial storage operations in Nova Scotia where only lobsters with high serum protein are selected for storage. Lobsters from Newfoundland are a poor candidate for such storage methods because their serum protein concentrations are fairly low to start with (S. Lamont, pers. comm.). Thus rather than inducing chill coma and fasting, inshore benthic cages with artificial or natural feeding may be a more reliable and cost-effective way for longer term storage of adult lobsters in rural Newfoundland.

3.6 Conclusions

The use of benthic inshore cages would appear to be a feasible method to store or grow-out adult lobsters. Taste tests showed that although people could discern a difference between cage-held and store bought lobsters, there was no preference for either type (Thompson, 2014).

Inshore benthic cages would be useful for remote areas with short fishing seasons, enabling harvesters to hold lobsters and release them onto the market when they have grown, or when market price dictates. Survival rates in the field will likely be high, at least for animals stored for up to six months. Deep colder water reduces a lobster's metabolism and need for food, thus extending their storage time (Nelson et al., 1977; Childress et al., 1990). Although cold water enhances survival, it reduces molting (growth rate) and overall quality (edible meat content), probably due to the reduced intake of food. These lobsters have a lower serum protein concentration and are more susceptible to the effects of emersion during transport to market (Taylor, 1981; Taylor and Whiteley, 1989; Lorenzon et al., 2007). Animals stored in warmer shallow water are more likely to molt and grow bigger. This larger animal will fetch more money at market. However, mortality rates may be somewhat higher during the molting process and some of the product may be lost at this time (McLeese, 1956; Mykles, 1980; Bowser and Rosemark, 1981). Although the lobsters in the benthic cages are likely feeding on material drifting into cages, supplemental feeding of the animals leads to an increase in size of post-molted lobsters and produces a healthier, higher quality animal that is more tolerant of stress. However, one then has to factor in the costs and logistics of feeding the animals. Cage

size does not have an appreciable effect on growth or survival of lobsters. Nevertheless, the animals held in compartments with a large surface area exposed to the surrounding environment tend to be healthier because they may be able to forage opportunistically on food that drifts into the cages.

The best way to maximize growth and quality while reducing overhead costs associated with maintenance would be to hold the lobsters in cages in shallow warm water under aquaculture facilities. Here they would get the potential input of food falling off culture lines or out of the net pens, without the logistical problems associated with manual feeding. This research not only has determined a feasible method to store lobsters and increase their sale price, it also suggests that lobsters could be used in multitrophic aquaculture operations as a means to remove food or dead animal material that would otherwise rot and stagnate on the seafloor.

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CHAPTER 4: Summary

4. Summary of findings: Uses for industry

The aim of the thesis was to explore how cage maintenance might affect the general health and growth of lobsters. If successful this would provide a method for individual lobsters harvesters to hold their catch for prolonged periods and release it onto the market when prices are high. In order to test this idea, I assessed health and growth performance of adult lobster *H. americanus* in both the laboratory and field. The following parameters were recorded: survival rate, molting rate, growth of molted lobsters by increment change in body mass and carapace length and change in body mass of non-molted lobsters. The general health and physiological condition of the animals was determined by measuring the concentration of protein in the hemolymph using a hand held refractometer.

In Chapter 2, I was able to verify that serum protein concentration was not only an indicator for lobster nutritional status, but also an indicator of quality and physiological condition over a wide range of experimental conditions. I found strong positive correlations between serum protein concentration and hepatopancreas and heart size and edible meat content. As the size of the organs decreased, their water content increased and there was a strong negative relationship between serum protein concentration and moisture content of the hepatopancreas and body muscle tissue. Use of serum protein concentration as an indicator of quality and physiological condition in the lobster, *Homarus americanus* (Milne-Edwards, 1837). Journal of Shellfish Research."

In Chapter 3, I performed four different sets of experiments, each over a six month period. Two series of experiments in the laboratory enabled different factors (temperature, diet, feeding frequency, cage size) to be manipulated. In the field I investigated survival and growth in self-feeding and artificial feeding treatments. Temperature had a noticeable effect, in the laboratory molting and growth were observed to increase with increasing temperature. Diet was also important: although blue mussels are a favoured prey item of lobsters they do not provide all the essential nutrients, and a mixed diet is required, especially during the post-molt period. Animals can survive, and still molt and grow when food is restricted. However, those with access to an adequate supply of food grew larger after molting and were healthier and in a better physiological condition. The size of the cage did not appear to have an appreciable effect on growth or survival. Field studies showed that lobsters can be maintained in benthic cages for a period as long as six months. The lobsters showed a high survival rate and vigor in both the self-feeding and artificial feeding treatment. Although lobsters ranched in the Triton area had no artificial input of food materials, they maintained their body mass, although the quality (organ sizes and edible meat content) was fairly low. In contrast, the lobsters that were held in the Rushoon and fed by hand not only maintained body mass, or molted, but also exhibited a slight increase in their overall quality with increasing serum protein concentration. The findings of Chapter 3 are being submitted as a paper to the journal Aquaculture.

In light of current findings the following recommendations can be made on the use of benthic storage cages.

1. Survival rate in the field will be high in benthic cages, especially if animals are stored for six months or less

2. Deep colder water reduces a lobsters metabolism and need for food, thus extending their storage time.

3. Although cold water enhances survival, it reduces molting (growth rate) and overall quality, due to lack of food. These lobsters may be more susceptible to the effects of emersion during transport to market.

4. Animals stored in warmer shallow water are more likely to molt and grow bigger. This bigger animal will fetch more money at market. However, mortality rates may be somewhat higher during the molting process and some of the product may be lost at this time. A cost analysis for extended storage in benthic cages is needed to determine the financial feasibility of this method of holding lobsters.

5. Feeding of the animals improves the increase in size after molting and produces a healthier, higher quality animal that is more tolerant of stress. This would likely produce a more valuable animal. However, one then has to factor in the costs and logistics of feeding the animals.

6. Cage size does not have an appreciable effect on growth or survival of lobsters. Nevertheless the animals with a large surface area exposed to the surrounding environment tend to be healthier because they may be able to forage opportunistically on food that enters the cages.

7. The best way to maximize growth, and quality but to reduce overhead costs associated with maintenance would be to hold the lobsters in cages, in shallow warm water under aquaculture facilities. Here they would get the potential input of food for growth.

8. In Newfoundland the lobster fishing is confined to a short summer season. 95% of Newfoundland lobsters are exported off the island for sale in the Maritimes and Maine. The glut of lobsters usually dictates a lower price. This method could be used by harvesters to store lobsters caught in the summer and release a larger animal for the Christmas time market when it would fetch a higher price.

9. This research not only has determined a feasible method to store lobsters and increase their sale price, it also suggests that these animals could be used in multitrophic aquaculture as a means to remove food or dead animal material coming off aquaculture facilities that would otherwise rot and stagnate on the seafloor.

10. Feral lobsters held near mussel farms fared as well or better than lobster held outside the mussel farms. The suggestion from harvesters that mussel farms impact adult wild lobsters in a detrimental fashion, is not supported by these findings.