THE UNIVERSITY OF HULL

Physiological effects of post-harvest commercial practices on the lobster, *Homarus americanus*

being a Thesis submitted for the Degree of

Doctor of Philosophy

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by

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Glossary

List of abbreviations used in this thesis

ppt	parts per thousand
ppm	parts per million
mml l^{-1}	millimoles per litre
HACCP	Hazard Analysis Critical Control Point
FMEA	Failure Mode and Effects Analysis
CCP	critical control point
CL	Critical Limit
BPM	beats per minute
Hcy	haemocyanin
RI	Refractive Index, in the context of lobsters is actually expressed as %Brix
DFO	Canadian Department of Fisheries and Oceans
FCC	Fisheries Council of Canada
LFA	Lobster Fishing Area
CAD	Canadian dollars
DLP	Dryland Pound

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Abstract

The lobster, *Homarus americanus* (Milne Edwards) is a commercially-important species harvested in Canada and exported live to international markets. Landed condition and post-harvest handling practices play a significant role in the ability of an exporter of live lobster to provide customers with a premium product. A set of standardized quality handling, storage and shipping parameters does not exist, an absence that can lead to quality degradation or premature mortality that impacts both the reputation of the *H. americanus* stakeholders as well as being a potential waste of a limited natural resource. Studies on physiological risk factors for storage, emersion tolerance and cardioventilatory responses to immersion temperature change were conducted to assess their impact on the maintenance of quality in the supply chains of live *H. americanus*.

Based on survival analysis during long term storage, it was found that storage risk factor predictive success was season-dependent, with haemolymph lactate positively correlated with long-term storage mortality in spring-caught lobsters, but not with winter-caught ones. Haemolymph total protein, haemocyanin and refractive index were positively-correlated with long term storage mortality in winter-caught lobsters but not spring-caught ones.

The immediate pre-harvest acclimation temperature affected the lobsters' response to emersion. Haemolymph pH and lactate levels during emersion revealed that the standard 3 °C environmental air temperature, used for commercial live shipments, causes a delay in the onset of anaerobic metabolism in 7.5 °C, but not 2.5 °C pre-acclimated lobsters.

Although *H. americanus* is reportedly sensitive to changes in environmental temperatures less than 0.5 °C, it was found that acute changes in immersed water temperature of 5 °C did not elicit an overall change in cardioventilatory activity. Complete haemolymph temperature change occurred within 30 minutes of immersed, acute environmental temperature change, notably faster than was observed in the absence of cardioventilatory activity, indicating the effectiveness of the cardioventilatory activities in *H. americanus* as a heat exchange system.

The current findings, along with existing relevant research findings in the literature, were used to create a template for model commercial post-harvest practices for *H. americanus*. The template consists of a food quality and safety control program based on the globally-accepted HACCP principles of inductive reasoning that stress proactive versus reactive processes. The current research findings provide critical control points that can be applied throughout the post-harvest distribution chain from first landing to delivery to the end consumer. These critical control points are identified with suggested parameters to use as guidelines for the *H. americanus* live trade in an effort to improve the consistency of quality and reduce the risks that can lead to product and / or quality loss as a result of incorrect practices.

Chapter 1
General Introduction

1.1 The Canadian Fishery

1.1.1 Socioeconomic Value of the Fishery

The *Homarus americanus* commercial fishery is the single most important fishery in Canada with 2013 total landings of 74,686 metric tonnes worth a net value of $680.5 million (CAD) (Fisheries and Oceans Canada, 2014), and annual exports worth over $1 billion (Gardner et al., 2010). Canada is the largest lobster exporting country in the world, accounting for 32% of the total global lobster export trade. Canada's largest export market is the USA in which 62% of their lobster products are imported. China and the EU account for the next largest volumes of exported Canadian lobster for a combined total of 19% of Canada's total lobster exports. The Canadian lobster fishery employs an estimated 25,000 people in the lobster harvest sector and another 10,000 at shore-based lobster processing plants (Gardner et al., 2010). Lobster-related jobs are located mostly within the small communities in which the fishery takes place throughout rural Atlantic Canada. The fishery remains a key component to the condition of the Atlantic Canadian economy.

1.1.2 History

A commercial fishery for *Homarus americanus* began during the late 1700's in the Northwest Atlantic Ocean. Early recorded diaries of European explorers indicated a great abundance of lobsters along the northeast coast of North America (Cobb and Phillips, 1980). Commercial landings have been recorded continuously in Canada since 1894 (Figure 1.1) and in the United States since 1928.

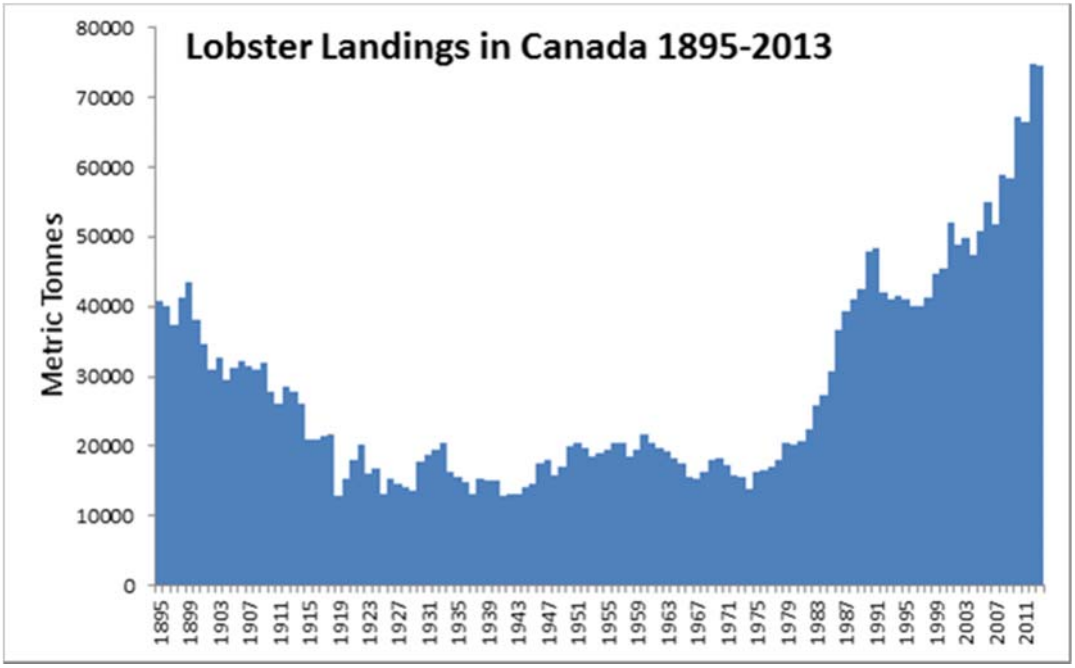

Figure 1.1 *Homarus americanus:* historical lobster landings in the Canadian fishery from 1894 – 2013. Source: Fisheries and Ocean Canada: Facts on Canadian Fisheries. Available online: http://www.dfo-mpo.gc.ca [Accessed 12/5/2014].

Current *H. americanus* landings are at historic highs in both Canada and the USA and both fisheries have been experiencing 10-30% growth per year in the last decade (DFO, 2014).

During the early establishment of a commercial *H. americanus* lobster fishery there was an increasing demand for lobsters, but a lack of infrastructure for proper distribution of live lobsters. This lack of infrastructure led to the growth of a canning industry in the late 19th century to produce products that could be stored for prolonged periods. By the early 20th century, over 900 lobster canneries were in operation in Atlantic Canada. Markets for canned lobster included Europe and areas within the USA too far away from the fishing ports along the North Atlantic coast to purchase the preferred, live lobster. However, minimum landed-size restrictions in both the USA and Canada, newly-enacted canning regulations in the USA and declining canned lobster prices as a result of competition from Japanese canned crab meat, led to the decline of the canning industry

in the mid-20th century and facilitated the development of the live lobster business. With improving rail networks in North America and the development of improved lobster packing techniques, the live lobster business took over in the mid 1900's from the once vibrant canning industry of the late 19th century. However, without the ability to store product and therefore smooth out the supply glut of live lobsters due to the fishery's seasonal nature, much of the live catch was sold in competitive, time sensitive markets in which prices declined throughout the trading day as the quality of the live lobsters deteriorated because of inadequate holding conditions at the markets (Acheson, 2003).

1.1.3 Current Fishery

The Canadian *H. americanus* lobster fishery is divided into lobster fishing areas (LFA) with individualised fishing management regulations to support the maintenance of a healthy stock and reduce the fishing pressure effects of concentrated effort in the high-producing fishing areas (Figure 1.2). The fishery has two separate management strategies for the inshore (LFAs 1-39) and offshore (LFA 41) zones. The inshore zones use input controls (e.g. trap limits and seasons) and the one offshore zone uses output controls in the form of a quota system. The Canadian Federal Department of Fisheries and Oceans (DFO) manages the fishery and outlines the restrictions within an integrated fisheries management plan (Fisheries Act, Government of Canada, 2014).

A harvester is required to own a license specific to an LFA in which they wish to operate. The number of available licenses is limited by the LFA based on the estimated carrying capacity of fishing effort in each area, as determined by the DFO. The fishing license further restricts fishing effort by seasons and each LFA has its own individual season start and end dates, with all LFAs (with the exception of LFA 41) having at least one period in which they are closed.

The fishery is solely a trap-based harvest with each license permitted a specific number of traps depending on the LFA, ranging from 250 to 375 traps. Within each individual

LFA, the specific trap dimensions and trap entrance size are set and regulated by the DFO along with the minimum allowable lobster size based on carapace length, but, within these restrictions, the fisher is permitted to land as much lobster as they wish with the exception of the quota-based LFA 41. Harvesting of ovigerous females is also strictly forbidden in all LFAs.

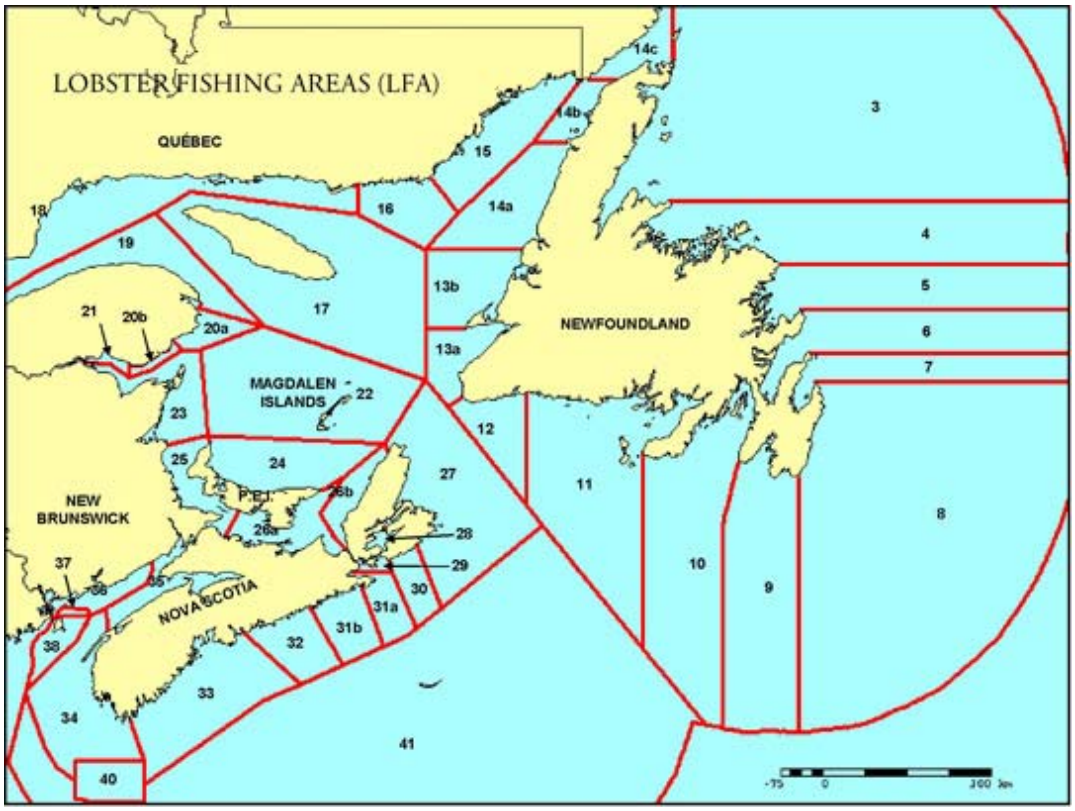

Figure 1.2 *Homarus americanus* Canadian lobster fishing areas. Numbers represent individual lobster management zones. Source: Fisheries and Ocean Canada: Facts on Canadian Fisheries. Available online: http://www.dfo-mpo.gc.ca [Accessed 12/30/2014].

Landing quantities vary by LFA with some of the most lucrative areas achieving individual fisher catches as high as 3 tonnes per day.

Generally there are two overall seasons in which lobsters are actively harvested and are known as the winter and spring seasons. The winter season starts on the last Monday in November in LFAs 33 and 34 situated along the southwest shore of Nova Scotia, two of the most lucrative LFAs in the fishery. Landings from these LFAs during the first few weeks of the fishery in November and December yield the highest catch rates of any

lobster fishery in the world and make up an estimated 66% of the total yearly lobster landings in Canada (Coffen-Smout et al., 2013). As the water begins to cool down close to 0 °C in January, catch rates decline significantly and many fishermen cease fishing until the water warms up above 2 °C once again in April when the spring fishery commences. The winter-opening LFAs continue to fish into the spring season until the end of May after which their fisheries are closed. Overlapping with LFA 33-34's spring closures is the opening of the northeast LFAs 27-32 at the end of April and beginning of May (depending on the individual LFA), and which remain open typically until mid-July. Landings that occur from April until mid-July are known as spring season lobsters. The timing of the seasons is roughly aligned with the seasonality of the moulting process in *H. americanus* in an effort to harvest product during the intermoult (November) through to advanced pre-moult (July) when the exoskeletons are hard and the lobster is fully-meated, and avoiding the harvest of soft shell lobsters that occur in July to September when the majority of the lobster population is undergoing ecdysis, which is described in more detail in section 1.4.1 below. Ecdysis is known as shedding to the industry and relates to the act of the lobster shedding its old shell.

1.1.4 Harvested Size Distribution

For regulatory purposes, lobster sizes are expressed as carapace length (CL), measured from the posterior apex of the eye socket to the posterior edge of the cephalothorax parallel to the body line of the cephalothorax (Figure 1.3). The minimum landed legal size ranges from 82 – 83 mm CL depending on the LFA. The lobster industry, however,

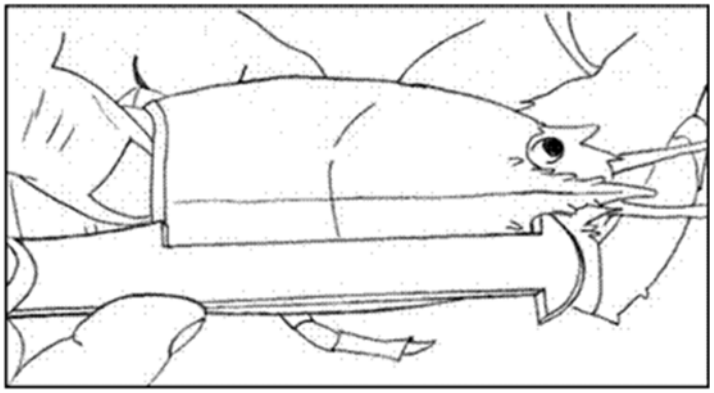

Figure 1.3 Homarus americanus: measurement of the carapace length (CL) using a minimum legal size measure device taken from the posterior apex of the eye socket to the posterior, dorsal edge of the carapace parallel to the carapace midline. Available online:. (http://www.maine.gov/dmr/rm/lobster/guide/#sizes [Accessed July 28, 2015]

divides lobster catch into size classes based on their individual weights, with the majority of the landed catch having an approximate lower and upper end of 400 grams and 4 kilograms respectively, although there are recorded catches of lobsters weighing greater than 10 kilograms. Each lobster supplier has its own set of size class specifications in which the smaller sizes are separated by approximately ¼ pound (lb / 114 g) increments up to 2 pound lobsters (2 lb / 910 g), and 1 – 3 pound (1-3 lbs / 454g to 1.4 kg) increments for larger sizes. Many buyers have approximately 10 different size classes with market names which are related to their weight in pounds for each class starting with Chix (1 lb), Quarters (1 ¼ lb), Halves (1 ½ lb), Three-Quarters (1 ¾ lb), Selects (2 lb), 2.5 to 3's (2.5lb – 3lb), 3 to 4's (3 lb– 4 lb), 4 to 6's (4 lb – 6 lb), 6 to 9's (6 lb – 9 lb) and 9+ or Jumbos (>9 lb). Higher demand for a particular size may cause it to be subdivided, which is the case with the Chix and Quarters that may be subdivided into small, medium and large

Chix and Quarters. The average size of landed catch falls within the Quarters size class and approximately 70% of the landed catch is a Select-sized lobster or smaller.

1.2 Development of the Live Lobster Market

A demand for lobsters delivered live to the end-user promoted significant industry technology development in the 1970s when the potential for higher prices for live lobsters over processed (cooked, frozen or canned) grew as a consequence of the discovery of lucrative overseas markets. However, the early live lobster harvesting, storage and distribution activities in North America were volume-based with little knowledge of how to maintain lobster quality. This resulted in many instances in which mortality rates were as high as 25-40% because of handling, storage and shipment conditions (MacDonald, 2010). The early versions of live lobster storage systems consisted of communally-packed lobsters in wooden crates that were immersed in flow-through seawater systems without the provision of temperature control or supplemental aeration. Chelae immobilization was accomplished with lobster plugs, wooden pegs inserted through the membrane of the dactylus to physically prevent the claw from functioning and so reducing damage caused by aggressive interaction between communally-held lobsters (Figure 1.4).

Figure 1.4 *Homarus americanus* pincer claw showing an inserted wooden lobster plug to immobilise the dactylus of the claw. Image available online: http://www.flikr.com [Accessed 10/1/2014].

Complications with this invasive claw immobilisation method were observed when lobsters were held for extended periods of time and these included muscle tissue melanisation and, in more severe cases, infection with the gram-positive bacterium *Aerococcus viridans (var.) homari,* a causative agent for the fatal bacterial disease, Gaffkaemia (Stewart et al., 1969). The disease is highly infectious and there appears to be no immune response by the host lobster. The bacterium causes a severe septicemia and subsequent decline in haemocytes, leading to a time-temperature dependent death within a matter of days at ambient seawater temperatures. Once it was discovered that Gaffkaemia infection originated via a damaged lobster integument, claw immobilisation with elastic rubber bands replaced the destructive lobster plug method and the incidence of Gaffkaemia was significantly reduced (Wilder and McLeese, 1961).

As it became more common for lobsters to be handled with more care and in an undamaged state, successful storage time was increased from 1-2 weeks to a month or

more. However, Stewart et al. (1972) observed that colder, winter storage conditions in which ambient seawater temperature was typically below 5ºC were better suited to longer-term, communal storage than warmer, spring and summer conditions when ambient seawater temperatures of 12ºC or higher could be observed.

The volume of lobster from the Canadian lobster fishery has two distinct peaks based on the opening of the winter seasons in November, and in April to July when resumption of catches occur once the water temperatures are again warm enough for lobsters to be active foragers entering the harvesters' traps. During these two peak periods there is an oversupply of Canadian *H. americanus* to the market and therefore market prices are at their lowest. Once the supply begins to decline due to low water temperatures in the winter season and due to fishery closures at the end of the spring season, market prices increase, with their maximum market value occurring immediately before the start of the next season. The seasonality of supply and market price is what led to the further development of long-term storage techniques in which the supply could be buffered with increased storage capacity and a controlled, gradual release of lobsters to the market on a year-round basis. Due to the risk of potentially high-cost mortality in these systems, technological refinements in storage techniques were envisioned as necessary to maintain a profitable year-round live lobster business (MacDonald, 2010).

1.3 Segregated Storage

In the late 1970's, a startup seafood company, Clearwater Seafoods (Clearwater), hired a shrimp aquaculture biologist to review the then current storage and shipping practices within the Canadian live lobster industry and to develop improved methods so that the company could compete in the premium Japanese live seafood marketplace. By 1982 Clearwater had designed and implemented a new type of storage system that was based on individually segregated lobster habitats in a stackable tray system so as to reduce the

footprint required for high-density, intensive long-term storage (Figure 1.5 and 1.6). The system was designed to use durable plastic trays that require high flow rates to maintain stable water quality parameters within the acceptable range of immersed *H. americanus,* which includes maintaining dissolved oxygen above 90% saturation and total ammonia below 1 mg/L.

Figure 1.5 *Homarus americanus* segregated tray system for long-term storage (from Clearwater Seafoods)

Figure 1.6 *Homarus americanus* long-term holding system (from Clearwater Seafoods)

Segregated storage eliminated the aggressive interactions between lobsters that were common in communal storage systems and which led to damaged integuments, infections and general loss of quality. Due to the elimination of these deleterious intraspecies interactions, along with the low storage temperature that reduced the lobsters metabolic rate and moult progression, the tray storage system was able to extend lobster storage up to 6 months, eliminating the fishery supply 'boom and bust' events that occur throughout the year, buffering the end-consumer from typical natural resource-related supply issues and it also allowed out-of-season sales to take place.

1.4 Lobster Biology

The lobster, *Homarus americanus*, is a decapod crustacean from the family Nephropidae in which the first three pairs of periopods (thoracic limbs) are biramous, with the first pair having a particularly enlarged pair of chelae used for crushing of their prey's exoskeleton

as well as for defense against predators, which include other lobsters that may be fighting for food, shelter or a mating partner. *H. americanus* is very similar in anatomy and physiology to the European species, *Homarus gammarus,* and these two species, along with a third Nephropidae, *Nephrops norvegicus* found in waters of the northeast Atlantic Ocean, are three of the most commercially-important lobster species in the world (Holthius, 1991).

1.4.1 Aspects of the Moulting Process

The lobster is a crustacean with a hard outer exoskeleton composed mainly of calcium and a chitin-protein matrix layer. The lobster grows by the replacement of its rigid exoskeleton with a new, larger version during a process called moulting, or shedding of the old exoskeleton. The actual process of exoskeleton shedding (ecdysis), takes approximately 30 minutes to complete, and generally occurs in most of the lobster population at the end of August through to September, although actual time to ecdysis is time-temperature dependent and varies with seasonal temperatures (Aiken and Waddy, 1976). Ecdysis is a relatively brief period compared with the time taken with preparations for, and recovery from, the actual event. There are a number of moult stages that a lobster goes through pre- and post-moult, that can be used to determine the timing of the moult with a relatively high degree of accuracy (Aiken, 1973). Approximately 4 months prior to ecdysis, the lobster goes into early premoult in which the development of a new exoskeleton underneath its current one occurs. The process is controlled by a moult-inhibiting hormone (MIH) excreted from the x-organ located in the eyestalk of the lobster (Couch et al., 1976). As the name suggests, MIH prevents the lobster from starting the process of exoskeleton development that ultimately leads to ecdysis. The production and release of MIH is controlled by the x-organ in the eyestalks, and eyestalk ablation has been shown to cause uncontrolled moulting behaviour (Castell et al., 1977). During active premoult, calcium from the old exoskeleton is slowly dissolved and collected into a region

on either side of the stomach in the form of gastroliths (Travis, 1960) and will be used to re-calcify the new exoskeleton, post-ecdysis. Various effects and tolerance levels have been observed as being related to moult stage. For example, changes in behaviour and feeding (Lipcius and Herrnkind, 1982), oxygen consumption rates (Penkoff and Thurberg, 1982) and haemolymph chemistry (Mercaldo-Allen, 1991) as well as the lobster's ability to withstand various types of environmental stressors, such as hypoxia, emersion, temperature change and ambient ammonia levels (Mugnier et al., 2008; Stewart and Li, 1969) and post-harvest handling practices (Basti et al., 2010; Giomi et al., 2008). Approximately 1h prior to ecdysis, the lobster begins consumption of significant quantities of water that exerts hydrostatic pressure on the shell, causing it to expand and release, revealing the newly formed, soft exoskeleton beneath. Water consumption continues for approximately 2h post ecdysis (Mykles, 1980). The exoskeleton will expand to between 112-125% of its previous size before beginning the re-calcification process observed during the post-moult stage (Aiken, 1973). The volume of water involved is hormonally controlled (Jackson et al., 1987).

The post-moult stage lasts approximately 3 months, the actual time dependent on the environmental conditions. At the completion of post-moult, exoskeleton hardness is re-established and the muscle tissue volume recovers to fill the larger volume created by the new exoskeleton. Haemolymph total protein level varies with moult stage (Barlow and Ridgway, 1969; Stewart and Li, 1969) and has been shown to be related to the animal's meat content (Stewart et al., 1967). The protein content in the haemolymph is fixed, and the concentration of protein will vary with the haemolymph volume that changes throughout the moult cycle (Glynn, 1968). Haemolymph protein concentration is a common constituent measurement used within the *H. americanus* industry to assess lobster quality in terms of the proportion of edible meat content and the animal's ability

to withstand stressors associated with the live lobster supply chain (Leavitt and Bayer, 1977; Stewart et al., 1967).

Female lobsters mate after the moulting period from July to September and begin the process of maturing eggs from the ovaries until the following summer. Several weeks before the eggs have finished their internal development, tegmental (cement) glands within the female's pleopods (abdominal limbs) begin to produce a sticky substance that, once mature eggs are extruded onto the tail of the lobster, is used to adhere the eggs to the pleopods for the duration of external maturation. Fecundity, as measured by number of eggs extruded, is approximately linearly correlated with carapace length (Saila et al., 1969). For the smallest legal-sized lobsters landed in Canada, a female clutch of eggs is estimated to be 7,000 to 9,000 eggs and up to 115,000 eggs on larger sizes within the fishery. Fecundity in the closely-related species *H. gammarus* has been reported to be approximately 50 to 70% of *H. americanus* at the same carapace lengths in which the correlation was found to be linear rather than logarithmic (Hepper and Gough, 1978).

During the extrusion process and prior to attachment to the pleopods, the eggs are externally fertilized with male sperm from the spermatophore (2) that were deposited into the female by the male post-ecdysis. The fertilized, externally-attached eggs then continue development for a further 10 to 12 months before hatching. The female will not go through ecdysis during the period in which she has an external clutch of eggs. Once the eggs hatch and larvae are released to the surrounding seawater, they float to the surface and scatter with the wave and wind action during their planktonic phases that last approximately 4 to 6 weeks before transitioning to the benthic phase for the rest of their lives.

1.4.2 Nitrogen Metabolism

Lobsters are ammonotelic animals in which ammonia waste is excreted via passive diffusion across the gill surface to the dilute seawater environment. The gills are located

in a left and right branchial chamber in which constant sea water flow, generated from left and right scaphognathite beating activities helps keep the diffusion gradient and subsequent ammonia transfer from the haemolymph high (Freire et al., 2008; Regnault, 1987). The mechanism for diffusion of ammonia nitrogen is mediated by a branchial Na^+ □ H^+ exchange between the seawater and haemolymph. The rate of nitrogen efflux is affected by various conditions such as environmental temperature (Schmitt and Uglow, 1996), feeding (Kemp et al., 2009; McGaw et al., 2009) and moult stage (Carvalho and Phan, 1998). Emersion causes a cessation of gill ventilation and results in nitrogen efflux impairment that manifests as an accumulation of haemolymph ammonia above resting, ambient levels (Bernasconi and Uglow, 2008a; Danford et al., 1999; Schmitt and Uglow, 1997). Ambient levels are restored once the lobster is re-immersed and gill chamber ventilation re-commences. Elevated haemolymph ammonia levels can become toxic and reduce survival (Basti et al., 2010; Young-Lai et al., 1991), the tolerance levels of which are dependent on the condition of the lobster and its ability to withstand such stressors.

1.4.3 Haemolymph Acid-Base Balance in Lobsters

General ion exchange, (e.g. nitrogen efflux as discussed in section 1.4.2, as well as a Cl^- □HCO_3^- ion exchange) is considered to aid in the maintenance of a relatively constant haemolymph alkalinity (Whiteley and Taylor, 1990) and occurs mainly across the gill surface. Emersion-related gill impairment, or a disturbed ion ratio within an immersed environment (e.g. hypoxia or elevated environmental CO_2), results in a haemolymph acidosis due to the accumulation of haemolymph CO_2 (hypercapnia) as well as an increase in lactic acid, which is known to increase the oxygen affinity of haemocyanin and partially offsets the effects of hypoxia (Danford et al., 2002; Morris and Oliver, 1999a). The acidosis is compensated for by an increase in haemolymph calcium and bicarbonate ions sourced from the exoskeleton (deFur et al., 1980; McMahon, 2001).

1.4.4 Cardioventilatory Behaviour

The ion exchange systems, described here in Sections 1.4.2 & 1.4.3, rely on a functioning ventilation of seawater across the gill surface. This occurs through the beating of the scaphognathites located inside the anterior region of the left and right branchial chamber that brings about a negative pressure and thus acts as the impellors of pumps, pulling water anteriorly (Wilkens and McMahon, 1972) over the gills and exiting through a left and a right-hand opening near the mouthparts (Figure 1.7) The scaphognathites are specialized exopodites that alternate pitch with each ½ stroke to cause water propulsion.

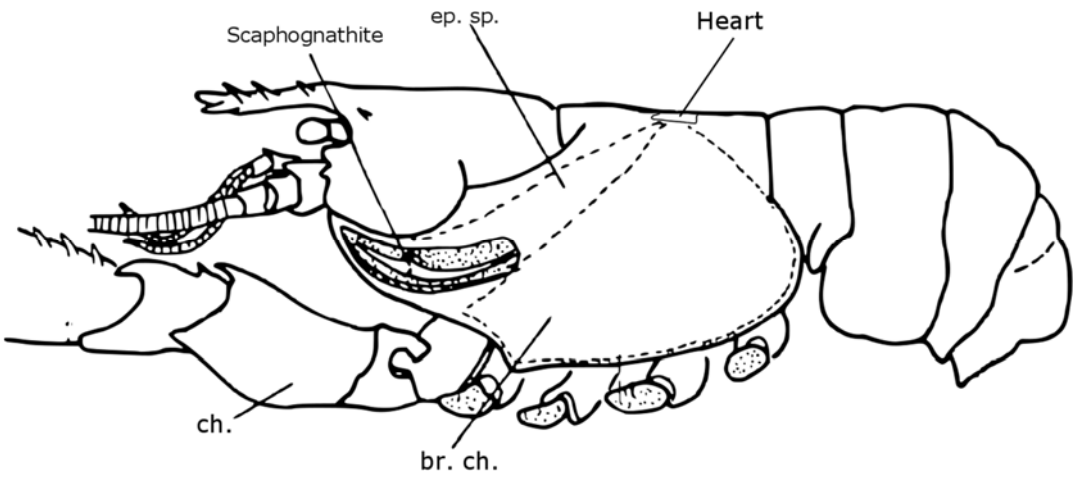

Figure 1.7 *Homarus americanus:* Diagram of a lobster indicating the cardioventilatory system. The scaphognathite is revealed below the exoskeleton. br. ch., branchial chamber; ch., cheliped; ep. sp., epibranchial space. Modified from McMahon (1972).

A myogenic heart in an open circulation system is used to circulate haemolymph in the lobster, with a continuous flow through the gills, bringing a fresh supply of haemolymph to the exchange surfaces. Heart rate in crustaceans is highly variable and temperature-dependent (Frederich et al., 2000) and sensitive to changes in environmental variables, such as salinity (Dufort et al., 2001), hypoxia (Airriess and McMahon, 1994; Frederich and Pörtner, 2000; Morris and Callaghan, 1998), and starvation (Ansell, 1973). Changes in cardioventilatory rates can be used to monitor the effects of environmental stressors on

crustaceans (Cumberlidge and Uglow, 1977; Depledge and Andersen, 1990), the sensitivity of which allows for observation of very minute environmental changes (Dufort et al., 2001; Jury and Watson, 2000).

1.5 Current Post Harvest Handling Practices

What follows is a description of live lobster post-harvest handling, storage and transport practices used by Clearwater Seafoods Limited Partnership (Clearwater), a Canadian live lobster company based in Halifax, Nova Scotia that exports approximately 3000 metric tonnes of premium live lobster annually. Clearwater's live lobster distribution system incorporates many of the standard practices used throughout the industry as well as its own unique quality control and storage systems outlined in the following sections which allow the company to access the lucrative, high-priced premium grade global lobster markets.

1.5.1 Lobster Procurement

Lobsters are harvested by trap, which is a known best practice for harvesting to reduce damage and stress of crustaceans to ensure they are commercially-acceptable for the live trade (Ridgway, Taylor, Atkinson, Chang, et al., 2006). Once removed from the trap at sea, the lobster's chelae are immobilised immediately with rubber bands to prevent injury to fishers or to adjacent lobsters during temporary communal storage. Once banded, the lobsters are placed in standard plastic, vented crates (IPL Plastics, Ltd) (Figure 1.8). Crates hold communally a maximum of 45±5 kg of lobsters and are protected from environmental extremes of sun exposure, temperature, wind and rain whilst they are stored on the deck of the boat during the harvest day.

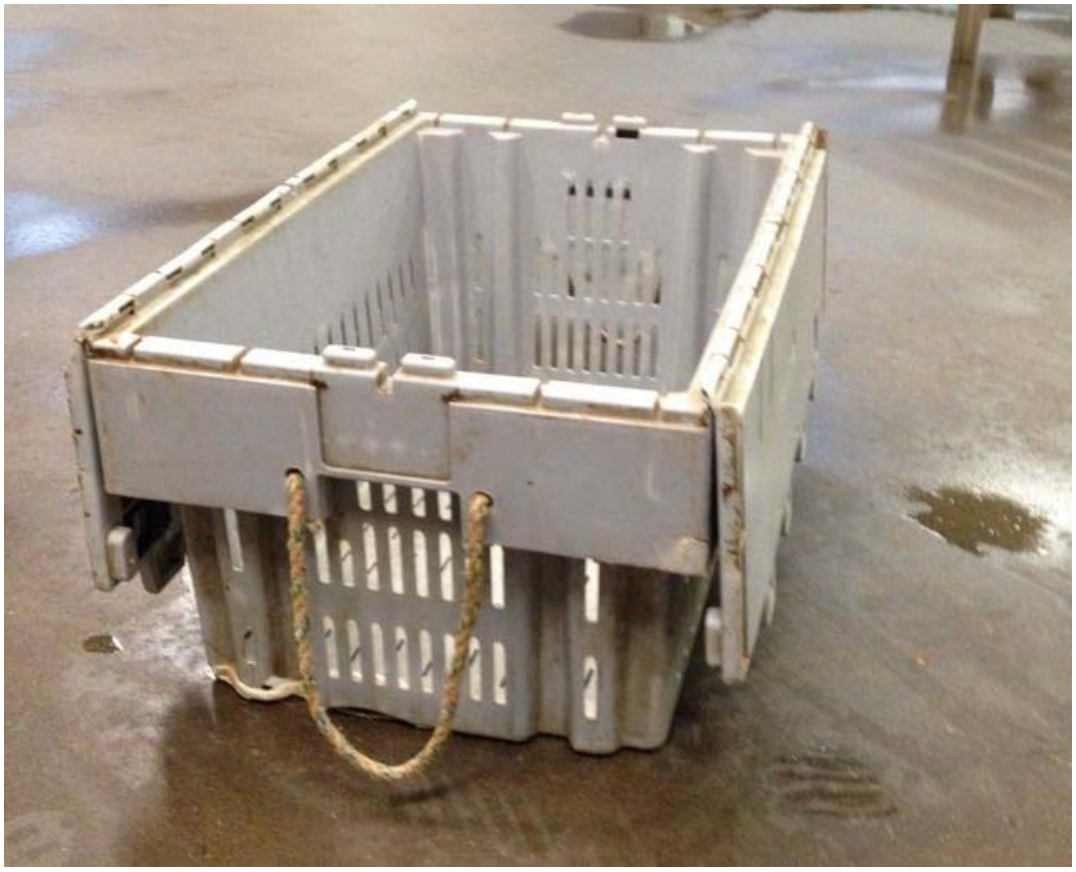

Figure 1.8 Vented plastic lobster crate in common use in the post-harvest transport and short-term storage of the *Homarus americanus* fishery.

An inshore fishing day-trip duration maximum is approximately 12 hours. Once the boat returns to the dock, the crates of lobsters are removed from the boat, inspected and adjusted for maximum weight per crate and immersed at the dock in an aerated, vented wooden lobster holding pen known as a lobster car (Figure 1.9) or in an aerated, ambient temperature flow-through reservoir system (Figure 1.10).

Figure 1.9 Typical shore-side reception temporary storage system in the *Homarus americanus* fishery. This photo shows a version with supplemental aeration. Crates are strung together with nylon rope (Clearwater Seafoods LP Photo).

Figure 1.10 Typical shore-side reception reservoir system in the *Homarus americanus* fishery with a flow-through ambient seawater supply and supplemental aeration (Clearwater Seafoods LP photo).

Lobster cars, shown in Figure 1.9, rely on ocean tidal currents to supply seawater for control of ammonia and, typically, have supplemental aeration to supply the high

dissolved oxygen levels required of freshly-harvested crustaceans (Aldrich, 1975). Lobster reservoirs, shown in Figure 1.10, use a pump to carry seawater to the immersed crates and often have supplemental aeration as well. The day fishing boats will generally land in the late afternoon or early evening and the unloaded, car or reservoir-stored crates of lobsters will remain immersed until the following morning at which time they are shipped, emersed, for 4-6 hours to a long-term storage facility by road transport using a refrigerated, humidity-controlled vehicle programmed to maintain 3˚C +/- 1˚C air temperature in the freight compartment.

On arrival, the crates of lobsters are placed in an ambient flow-through, aerated seawater system for a minimum of 48 h to recover from harvest and transport handling stress. Seawater temperatures vary seasonally from a low of 1 °C in late winter and a high of 15 °C in September. Since the lobster harvest seasons are generally open from November to June, ambient seawater temperature in the recovery tank systems ranged from 2.5 - 7.5 °C.

1.5.2 Commercial Wholesaler Grade, Inspection and Storage

At the wholesaler's facilities, the crates of lobsters are unloaded from the truck and immersed in aerated seawater for approximately 24-36 h in a temperature-controlled, aerated seawater reservoir maintained at 3˚C ± 0.2˚C and >95% O_2 saturation to recover from the handling and shipping activities from the dock. After this recovery step, the crates are removed from the reservoir and individual lobsters removed from the crates for quality and size inspection.

1.5.2.1 Vigour Index

Vigour index assessment is conducted on each animal using a vigour index adapted from Spanoghe (2001) but modified specifically for *H. americanus* and is shown in Table 1-1.

Table 1-1 *Homarus americanus* classes of vigour indices for assessing lobster condition

Index	Title	Description
1	Strong	Strong Limb movement, no drooping of limbs
2	Medium	Limb and claw movement, relatively strong but with slight drooping of limbs
3	Weak	Minimal limb movement, drooping of limbs but slight strength in claws, mouthparts slightly open
4	Moribund	No limb or claw movement, complete hanging of all limbs, eyes sunken, mouthparts open, some movement of antennae
5	Dead	As level 4 but no movement of antennae or eyes after stimulation

Quality inspection consists of selecting only animals with a vigour index of 1, described in Table 1-1 (above), and with no visible physical damage on the carapace, including cracked exoskeletons, missing or damaged periopods or any other form of fresh damage that may have been caused by handling steps up to this point. Fresh damage is defined as any damage with no observable melanisation of the wound site that would indicate that the injury happened prior to harvest, assuming the lobsters were freshly harvested 24 hours earlier. Acceptable lobsters are placed on a continuous flow, conveyor belt (Figure 1.11) and directed over a proprietary quality scanning system that identifies lobsters that are not within the intermoult stage.

Figure 1.11 *Homarus americanus* live lobster automatic size and quality grading line system (from Clearwater Seafoods)

Identified and rejected lobsters from the scanner consist mainly of post-moult animals in the winter fishery and pre moult animals in the spring fishery). Acceptable intermoult lobsters continue to the weigh scale and are then placed into the appropriate bin for their size from a size range consisting of 16 different market-defined groups. Size and quality sorting occurs at a rate of 60 to 90 lobsters per minute. Intermoult animals were selected for the emersion and cardioventilatory trials in Chapters 4 & 5, whereas any moult stage harvested in the fishery was used for the risk factor assessment trials in Chapter 3.

1.5.3 Commercial Quality Control

Successful long-term (> 2 months) storage of live lobsters requires low storage mortality because of the low profit margins for this business sector. Figure 1.12 illustrates that shippers, who store and export purchased lobsters from harvesters, have profit margins that range from approximately $0.50 to $1.50 per pound of product exported (Gardner et al., 2010). At these low margins, exposure to storage or shipping mortality must be kept at very low levels to avoid erosion of net margins.

Figure 1.12 Share of export price between the harvesters and shippers of live *Homarus americanus* exports to the USA, a major export region for the Canadian lobster fishery. Source: Gardner Pinfold Market Research Project Number 1361 from http://www.gardnerpinfold.ca/project_details.php?ProjectNumber=1361 accessed December 3, 2014.

To obtain the required low post-harvest mortality rates, an accurate, objective quality health assessment of purchased shipments of lobsters, by the shippers from harvesters, is key to determining the overall fitness levels of the lobsters necessary for them to withstand the rigours of the post-harvest, live lobster supply chain. Without such quality criteria the shipper takes on considerable risk of increased product mortality if the lobsters are stored beyond their working shelf life and shipped emersed to distant markets. The physiological challenges associated with the prolonged emersion will be well beyond their tolerance capabilities.

At Clearwater Seafoods' commercial live lobster holding facilities, a random subset of approximately 100 lobsters from each lot, identified as coming from a known lobster dealer, is sampled from the size and quality sorting belt, and a health assessment on this subset is carried out by quality control (QC) staff. A 'lot' is defined as a single source (dealer) and single day's catch and is given a unique identification number that is used to trace the shipment throughout the rest of the distribution chain for both quality control and food safety purposes. Parameters measured by the QC staff include haemolymph refractive index, exoskeleton hardness, moult stage and Gaffkemia screening as well as QC verification of vigour index and physical condition. In the present study, a specific suite of physiological parameters were chosen to be evaluated. Their selection was based on previously unpublished studies supported by Clearwater Seafoods—a distributor of live lobster that maintains a department staffed with lobster quality-control biologists for research and development, as well as making ongoing live lobster quality and health assessments with which to determine product suitability for the live trade.

1.5.4 Lobster Storage Conditions

Graded and QC-verified lobsters are placed into a segregated thermoplastic storage tray to prevent cannibalisation and injury associated with communal storage (Figure 1.13).

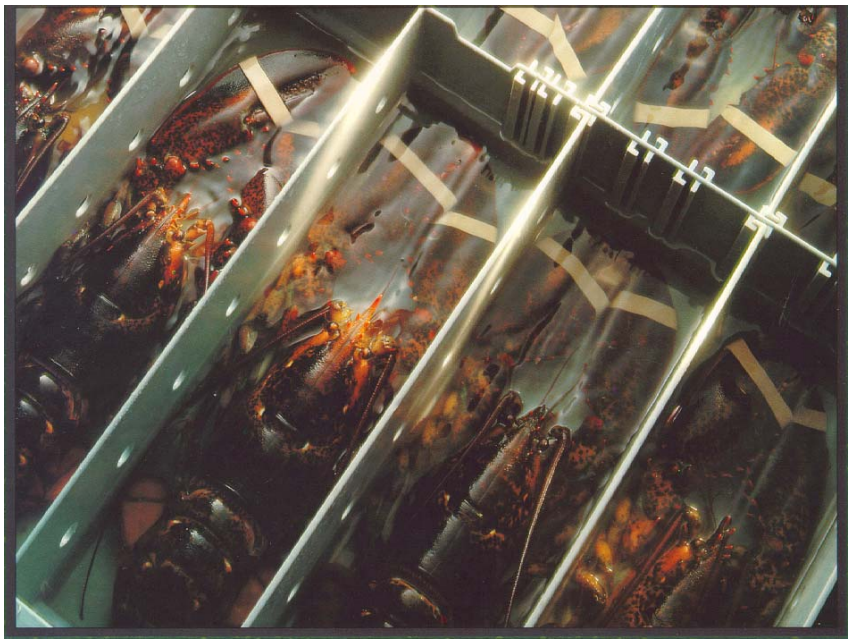

Figure 1.13 *Homarus americanus* segregated tray system for long-term storage (from Clearwater Seafoods)

The storage trays are configured for each size bin to allow for physical space requirements for each lobster. Storage trays are placed into a racking system, 32 trays high and supplied with continuously-flowing, chilled seawater (1.0˚C to 3.0˚C) from a header over each rack of trays. The seawater completely fills each tray and drains down to the lower tray, completely submerging individual lobsters. Chilled seawater is supplied to the trays at a rate of 26 litres / min, pumped from an underground reservoir with partially-recirculated water. New water make-up rates into this reservoir are adjusted on the basis of lobster inventory to maintain seawater total ammonia concentrations below 1.5 mg/L and dissolved oxygen above 95% saturation (actual concentration of oxygen in water is dependent on seawater temperature). Lobsters are held in this storage system from 1-6 months, depending on arrival condition of individual lots and market demand. During this long-term storage period, the lobsters remain unfed. Risk assessment from QC health assessments on individual lots are used to determine maximum storage length and managed by a custom computer database management system.

Lobsters selected for this study have undergone the above treatment and selected from the long term storage facility inventory, based on source to reduce source-specific variability and with a storage time between 1-2 months (to reduce confounding factors of fresh caught bait digestion and extended periods in an unfed state). Trial-specific selection, handling and storage conditions are explained in their respective chapters.

1.6 Aims and Objectives

Commercial practices used in the post-harvest storage and distribution of *Homarus americanus* have varying degrees of impact on the animal's physiology, health and vigour. The effects of such practices have been investigated on several species of crustaceans and under various environmental conditions (Basti et al., 2010; Gomez-Jimenez, 1998; Lavallée et al., 2000) . There are, however, few studies of the relative impact of these practices at the low temperatures that are typically prevalent in the Canadian live lobster fishery. The aim of this thesis was to investigate the effects of specific, industry-relevant practices during transport and storage on lobster quality within the high and low of the temperature range as observed typically in the *H. americanus* post-harvest trade and to provide a toolset of measurements and operating parameters within which lobster quality and health can be maintained.

Chapter 2 describes investigations into the effectiveness of a suite of lobster physiological measurements using survival analysis statistical tools to predict long-term live lobster commercial storage success. The physiological measurements selected were based on parameters that are known to be influenced by environmental conditions known to occur in the commercial trade of live *H. americanus*, such as temperature, emersion duration and exposure to sub-optimal conditions during immersion (e.g. low oxygen and variable water temperature maintenance). The measured parameters were analysed for their potential as a risk factor for loss of vigour from subsequent long-term (3+ months)

immersed storage in an effort to identify key measures for predicting live lobster storage success and quality maintenance. In particular, which post-harvest practices have the most influence on lobster survival during storage? Are they related to the seasonal condition of the lobster due to their moult stage and its related effects on haemolymph protein concentrations, or is it due to environmental conditions or physical handling during post-harvest storage and distribution of the lobsters? Is there a difference in the risk factors' influences by season? By answering these questions one may be able to determine which post-harvest practices are commercially important for maintaining lobster quality.

In Chapter 3, investigations were made to assess the effect of various environmental conditions of typical low and high temperature emersion during a commercial consignment. There are many different practices in the industry related to shipping of live *H. americanus* emersed with a range of pre-acclimation immersion and emersion temperature environments. Do these range of environments affect differently the lobster's ability to withstand short (36h), medium (72h) and long (96h) duration emersion events followed by re-immersion? Specifically, are there effects on emersion success arising from differences in lobster pre-acclimation condition prior to emersion, and is recovery from emersion events influenced by pre-acclimation conditions? The effect of size on the results of emersion events was investigated also. Presumably, a larger lobster would take longer to reach a warmer or colder emersion environment due to their weight differences and associated higher thermal capacity. Does this difference affect the outcome of emersion trial with similar environmental temperature conditions? If so, the impact could be that industry practices should take size into account for shipping emersed live lobsters.

Chapter 4 explores the cardioventilatory mechanism of *H. americanus* and its role as a heat exchange system affecting the rate of change of body temperature during acute immersed temperature changes. During post-harvest handling of *H. americanus*, it may

be exposed to several different temperature environments as they are moved from the shore to the final customer. Observations from various tank systems along the distribution chain reveal that there is little attention paid to the maintenance of immersion temperature to avoid acute changes when the lobsters are moved from one system to another. Such acute body temperature changes would seem to have a deleterious impact on the lobster quality maintenance as they attempt to adjust to these changes. Does the cardioventilatory system act as a heat exchanger to buffer these acute exposures by altering the new water ventilation rates and limiting exposure of the lobster's haemolymph to the new environment? The studies undertaken in Chapter 5 were designed to reveal if any such control is apparent and, if present, to what extent is its effect. If there is control, can the cardioventilatory response to acute temperature changes be used to determine a tolerance range within which no control is observed, and outside of which altered response indicates that the temperature change may be too large? Answers to these questions will help to identify specific ranges and tolerance limits that may be useful to the industry as standard operating practices within which lobster quality is more likely to be maintained.

The final Results Chapter (Chapter 5) focuses on identifying key results from Chapters 2 through 5 that can be used as standard operating practices and control limits during the post-harvest handling and storage of live *H. americanus*. The format of this chapter is based on inductive reasoning principles used in the food safety industry through the use of a Hazard Analysis Critical Control Point (HACCP) approach. Using this approach, each handling step within the post-harvest trade is reviewed to identify hazards that have an effect on the quality of the product. These hazards are defined by their control criteria (critical control points) and outlined in a set of operating parameters and procedures to ensure the lobster is kept within the limits of reasonable care and quality maintenance. It is hoped that this model plan for live lobster post-harvest supply chain logistics will be used as a template for the lobster industry.

Chapter 2
Post-harvest risk factors affecting long-term storage of the American lobster, *Homarus americanus*

Identifying criteria with which to predict storage success.

2.1 Introduction

Each fishing season provides lobsters at different stages in their moult cycle, which in turn leads to catches with animals in different physiological condition. Sea and air temperatures during the two seasons vary as well, providing different environmental conditions in which the lobsters are harvested and landed. These changing variables, along with harvester and dockside handling and shipping practices may lead to different risk factors which can affect the long-term storage success of post-harvest lobsters, which this study aims to identify and evaluate. A summary of risk factors and their possible causes is given in Table 2.1. A more detailed explanation about the mechanisms surrounding the variability of values of the risk factors is given in Section 1.1.1.

Table 2.1 *Homarus americanus*: proposed, measurable risk factors for the assessment of post-harvest live lobsters for suitability of quality maintenance during long-term storage in a commercial holding facility.

Risk Factor	Possible reasons for identification as a risk factor
Haemolymph total protein	Low total protein concentration may affect lobster immune response to stressors and available energy for stressor response and vigour maintenance. High total protein concentration may indicate lobster approaching ecdysis and therefore unsuitable for long term storage and commercial quality maintenance
Haemolymph % Brix	An industry standard method to estimate haemolymph total protein concentration explained above
Haemolymph osmolality	Acid-base disturbances in lobster haemolymph may be a sign of previous exposure of lobster to substandard environmental conditions such as low salinity which may affect their ability to survive long term post-harvest storage and shipping practices
Exoskeleton hardness	Used by industry to determine quality of lobsters based on estimating recovery from ecdysis or late stages of proecdysis. May be correlated with moult-specific risk factors as well
Haemolymph lactate	Lobsters kept in an emersed, or immersed in a low oxygen environment will begin to accumulate haemolymph lactate via anaerobic metabolism.
Pleopod stage	Pleopod stage is used to determine moult stage in crustaceans, and can be used to determine whether a lobster is in a state of recovery from ecdysis, in which its meat content and capacity to withstand the rigours of commercial handling practices, or it is in a state of active preparation for ecdysis in which case it is not suitable for long term storage due to quality implications with moulting during storage
Sex	Reproductive cycle differences between male and female lobsters may have an effect on their ability to be stored in a long term holding system.
Haemolymph haemocyanin concentration	Haemocyanin is the oxygen carrier in lobster haemolymph. If there are seasonal differences or variable levels of haemocyanin within a group of harvested lobsters, this may impact the lobster's ability to survive extended periods emersed, or immersed in low oxygen environments during post harvest handling. Low haemocyanin levels may limit a lobster's ability to withstand these environments and impact their survivability during post-harvest storage and shipping.
Post-harvest immersion recovery	Many lobster buyers will purchase lobsters directly from harvesters and immerse them in a receiving reservoir for varying lengths of time from 0h to several days prior to subsequent packing for storage or emersed shipping to the end consumer. Does this immersion procedure allow the lobster a recovery period from the harvest and dockside handling and environment exposure? It is the assumption of the industry that this practice allows the lobster to recover from harvest handling and shipping stress and improves post-harvest commercial storage and shipment success.

Lobsters (*Homarus americanus*) were chosen for this trial from Lobster Fishing Area (LFA) 34 (Figure 2.1), a seasonal fishery in the southwestern region of Atlantic Canada that begins on the last Monday of November and lasts until May 30th of the following year, with the majority of the animals being landed in the first and last 6 weeks of the season. During the 2012-13 season, the total seasonal landings in LFA 34 were 22,776

metric tonnes (DFO, 2014). This made LFA 34 the single largest fishing area in Atlantic Canada, comprising 30% of the total Canadian lobster landings of 74,790 t in a fishery comprised of 45 separately-managed lobster fishing areas. If risk factors exist from this fishery that can improve post-harvest commercial storage success, it has the potential for significant impact on the reduction of losses due to post-harvest handling and storage practices.

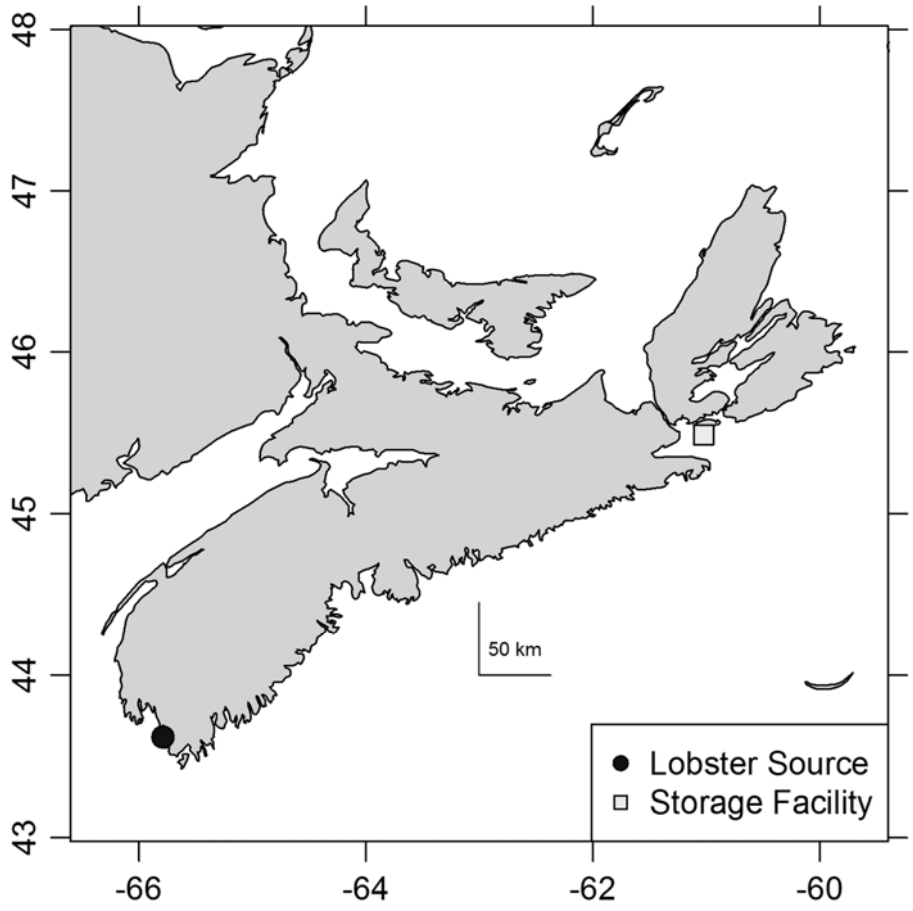

Figure 2.1 *Homarus americanus*: Lobsters were harvested in Lobster Fishing Area 34 in Southwest Nova Scotia and landed at Dennis Point Wharf, East Pubnico, Nova Scotia (●), then transported to Clearwater Seafoods' facilities in Arichat, Nova Scotia (□).

The first phase of the LFA 34 fishery occurs in late November and is known as the 'winter season' during which a significant proportion of the season's total landings will occur before the Christmas period in an effort to meet a high market demand for live lobsters in international markets during this holiday period. The 'spring season' of the fishery occurs approximately during the last 6 weeks of the season - from late April until May 30th, after which harvesting ceases for the closed season until the following November (Fisheries Act, Government of Canada, 2014). Lobsters harvested early in the winter season occur as a mixture of different stages in their moult cycle and the mixture differs from those harvested in the spring season. *Homarus americanus* generally goes through ecdysis in late summer (July and August), followed by post-moult recovery to intermoult during the fall and early winter (September to November) in which pleopod stages range from 1.0 to 2.5 (molt stage C_4 to late D_0) with the majority being no greater than pleopod stage 1.0 (moult stage C_4 to early D_0) (Aiken, 1980; Aiken, 1973; Aiken and Waddy, 1976). Only a subset of the population will go through ecdysis, leading to a mixture of moult stages in the landed catch at the start of the winter season in late November. During the winter when ocean temperatures are close to freezing, moult stage progression is slowed or even plateaus until the spring when ocean temperatures warm up and moult stage progression continues. In the spring fishery, landed lobster moult stage composition is mostly intermoult (C_4) and early pre-moult (stages D_0 to D_1'), characterized by consistent high shell hardness and, above seasonal ocean temperatures, can cause moult stage progression to occur faster and lead to late pre-moult and moulting lobsters (moult stages D_1'' to D_3) to be landed even in the early spring. This variability in moult stage composition of the catch is recognized as the cause of seasonal variations in physiological condition of the lobsters. Sea and air temperatures early and late in the season differ also and expose lobsters to environmental conditions at both ends of the annual temperature extremes in which the lobsters are harvested and landed. These different post-harvest

environmental variables may represent different risk factors for the long-term storage of harvested lobsters, similar to what was reported by Ridgway et al. (2006) for *Nephrops norvegicus*, and which this study aims to identify and evaluate for *Homarus americanus*. The focus of the present study is to assess the immediately observable product quality and health impacts from the harvest vessel procedures and also on the downstream, post-harvest implications of specific risk factors associated with seasonal, environmental and animal handling parameters that impact live lobster quality and survival.

Lobsters are landed during a short period of time relative to market supply requirements. This has led to the development and implementation of short (4-6 week) and long-term (up to 6 month) storage systems to enable the satisfaction of global market demand, despite a short-term supply. These storage facilities rely on a supply of quality lobsters able to withstand and recover from the rigours of harvest, shipment and storage conditions and remain marketable as high quality products at the end of the storage period. Mortality in these holding systems is the single largest operating expense and having an effective, objective measure with which to determine storage and shipment fitness is important therefore to the success of the live lobster trade.

2.2 Aims and objectives

The aim of this study is to evaluate a selected group of measurable risk factors of the post-harvest lobster, *Homarus americanus*, for their effectiveness in predicting long-term (120 days) storage survival in a commercial holding facility, and to determine if a pre-storage immersion recovery treatment improves storage survival. The study is designed to test both the statistical strength of each risk factor in predicting survivability during storage and which factors show a high level of correlation and therefore provide a selection from within a group of related measures and treatments that are suitable for a commercial holding facility to implement for quality control purposes.

2.3 Materials and methods

2.3.1 Animal selection

All animals selected for this trial were strong, vigour index 1 condition as outlined in Chapter 1, with no missing appendages or visible injuries to their exoskeletons. Lobsters were obtained from a single source in Southwest Nova Scotia as described in the Introduction (Section 2.1) Lobsters chosen were from a single commercial size class (Small Chix) that had an average weight of 465 ± 30g. Both males and females were included in a sample randomly-selected for gender and moult stage as determined by the criteria of Aiken (1973). Trials were repeated for each of the two receiving seasons (Winter and Spring) as described in the Introduction (Section 2.1). The reason that a single source was used was to minimise any effects due to variable post-harvest handling practices amongst different lobster harvesters in Nova Scotia. Lobsters were randomly selected from the production line during the size and quality grading process upon receipt to the Clearwater Seafoods storage facility in Arichat, Nova Scotia as shown in Figure 2.1.

2.3.2 Animal Husbandry

Harvested lobsters were delivered to the storage facilities at Clearwater Seafoods in Arichat after 6 hour's transport by road from the harvest region. Lobsters were delivered emersed in plastic crates (Figure 2.3) designed specifically for the *H. americanus* fishery by IPL Inc., Saint-Damien, QC, Canada. Received crates of lobsters were unloaded from a 3.0˚C ± 1˚C temperature-controlled, 12.2m long transport trailer capable of holding approximately 340 crates of lobsters stacked 12 high on pallets (Figure 2.2).

Figure 2.2 *Homarus americanus* lobster crates stacked on a pallet inside a refrigerated, temperature-controlled truck trailer

Figure 2.3 *Homarus americanus* lobster crates used throughout the Canadian and American lobster fishery. Each crate is designed to hold approximately 40kg.

Figure 2.4 Typical *Homarus americanus* lobster crate reception reservoir used to immerse 40kg capacity lobster crates for recovery or short-term storage. Clearwater Seafoods, Clark's Harbour, Nova Scotia.

Received loads were either immersed in a temperature-controlled reservoir (1 – 3˚C ± 0.2˚C, Figure 2.4) for a 24 h recovery post-harvest period or, unpacked, graded for quality and size and put into the long-term storage system, known at Clearwater Seafoods as the Dryland Pound (DLP) System, described in Chapter 1.

Lobsters used in the current studies were kept in this system unfed for the duration of each of the two 140 day trials. Water total ammonia, pH, temperature and dissolved oxygen levels were checked daily. Throughout the trials, the total ammonia levels remained below 1.0 mg•l-1, pH was 7.7± 0.3 pH units and oxygen remained above 90% saturation.

2.3.3 Commercial Storage Trial

Lobsters from incoming shipments of fresh-harvested, single source shipments were selected for the trial during a 2-week period in December 2010 and again in May 2011. As mentioned in section 2.3.1, Small Chix were used for this trial. Incoming lobsters were size- and quality-graded, either post 48 h immersion recovery in a communal reservoir

while still being housed in the 40 kg capacity lobster crates ('Wet' group) or immediately after arrival from the harvest with no immersion recovery period ('Dry' group). Both Wet and Dry reception methods are utilized to receive lobsters in commercial production. For these trials, lobsters were removed from the crates during the grading process and inspected for vigour and damage. Following standard practice at the live lobster storage facility, the obviously-undamaged, vigour index 1 lobsters were selected for long-term storage. Selected lobsters were graded by quality and size on a conveyor belt-type continuous grading system as described in Chapter 1. Quality grading was accomplished with a proprietary, non-invasive technology that was able to estimate the haemolymph protein concentration of the lobster, and that data was then used specifically to identify and select intermoult (C_4, pleopod stage 0) and pre-moult (D_0 to D_3, pleopod stage 1 to 5.5) lobsters for this trial. Each lobster selected from the commercial grading process for this trial was immediately sampled for haemolymph chemistry analyses, data collected on exoskeleton hardness and pleopod stage, and then attaching a uniquely numbered plastic coil tag (National Band and Tag Company, Newport KY, USA) and placed in the dryland pound (DLP) trays. Each animal was sampled only once, at the start of the trial, and handled for approximately 30 seconds to sample and tag lobsters for the trial. The sampled and tagged lobsters were placed into the DLP tray immediately after sampling and each tray of 36 lobsters was placed in the DLP racking system and seawater flow started within 1 hour of collection to begin the DLP storage trial. Lobsters were supplied with filtered, natural seawater, chilled to 3.0˚C ± 0.2˚C in the winter trial, and to 1.5˚C ± 0.2˚C in the spring trial – in accordance with standard operating procedures at this facility. The colder, spring temperature set point is used to inhibit the animals' progression through to ecdysis during long-term storage (Aiken, 1973).

A total of 144 animals (four full DLP trays) per week for four weeks were collected (Table 2.2) during the normal course of grading of incoming product for each of the two seasons

(Winter and Spring). Two of the four trays per week were filled with lobsters from the 48h pre-recovery treatment (Wet group), and the other 2 trays were filled with lobsters from taken directly from the receiving truck (Dry group). A summary of the groups that were selected for the storage trial is shown in Table 2.2. These groups were selected to compare risk factor predictive strength of storage success (% survival) of pre-treatment type (Wet or Dry) and season (Winter or Spring).

Table 2.2 *Homarus americanus*: Number of lobsters sampled for each treatment and season group.

	Season		
Treatment	**Winter**	**Spring**	**Total**
Wet	288	288	**576**
Dry	288	288	**576**
Total	**576**	**576**	**1152**

Lobsters were checked for mortality during the DLP storage trial once per week using minimal disturbance by gently pulling the water-filled tray out of the rack and touching each lobster to determine whether they were alive. Dead lobsters were removed and the tray gently reinserted into the DLP racking system. At the end of the 120 day trial all remaining lobsters were removed and a final mortality assessment completed. Overall mortality within the system was within the standard expected proportion for lobsters from the two seasons that were graded and stored in DLP for commercial purposes, eliminating the question of trial handling practices as a factor in the storage period lobster survival performance.

1.1.1 Risk Factor Parameters

The parameters listed in this section have been observed previously to indicate seasonal differences in moult stage, such as setal staging (Aiken, 1973), haemolymph refractive index (Leavitt and Bayer, 1977; Ozbay and Riley, 2002), exoskeleton hardness (Grubert et al., 2012; Hicks and Johnson, 1999), and haemolymph total protein levels (Stewart et

al., 1967; Stewart and Li, 1969), differences in emersion and handling stress, such as haemolymph lactate (Ocampo, 2003) and osmolality (Dall, 1970) and nutritional or physiological state (haemolymph haemocyanin) (Engel et al., 2001).

1.1.1.1 Haemolymph preparation

Volumes of 1.6 – 1.8 mL of whole haemolymph were extracted from each lobster's infrabranchial sinus using a 3 ml syringe with a 26-gauge needle inserted through the arthrodial membrane of a periopod. The whole haemolymph was proportioned for each of the analyses which are described below in sections 2.1.1.2 to 2.1.1.6. Whole haemolymph sub samples were transferred from the withdrawn parent sample of each lobster using a pipette and analysed for osmolality (50 µl), refractive index (100 µl), haemoycyanin (200 µl) and total protein (100 µl). 500 µl of the parent sample was deproteinised by dilution 1:1 with chilled 6% perchloric acid solution, centrifuged at 8,000 rpm for 5 minutes and the supernatant drawn off the resulting pellet. 500 µl of the parent whole haemolymph sample was diluted 50:50 with Milli-Q^{TM} ultrapure water into a microcentrifuge tube and shaken vigorously by hand for 5 seconds to ensure complete mixing and to reduce clotting of the sample prior to centrifuging at 8,000 rpm for 5 minutes. Supernatant was withdrawn off the resulting pellet and transferred to a new microcentrifuge tube and immediately frozen at <-20 ºC until analysis of the samples occurred the following day.

1.1.1.2 Haemolymph Refractive Index

Haemolymph refractive index was measured using an Atago PAL-1 temperature-compensated, digital refractometer (Atago USA, Inc.) onto which approximately 50 µL of whole haemolymph were placed for recording. Readings were expressed as % Brix, an *H. americanus* industry-adopted standard scale used by harvesters, dealers and shippers

to assess haemolymph protein levels and also to determine relative meat content associated with moult stage (Leavitt and Bayer, 1977).

1.1.1.3 Haemocyanin

Freshly-collected lobster haemolymph samples were diluted by 1:1 with ultrapure water and frozen and stored at <-20 ºC (as detailed in section 1.1.1.1) until ready for analysis the following day. The thawed samples were further diluted with ultrapure water to reach a final dilution factor of 1:19. Diluted samples were analysed with a UV spectrophotometer at 334 nm. Haemocyanin is quickly oxygenated *in vitro*, causing a bluish-purple coloured haemolymph, the colour intensity of which is relative to the concentration of haemocyanin in the sample. The copper-bound pigment concentration was calculated from the measured % absorption values using an $\mathcal{E}^{1\%}$ value of 17.26 from an extinction coefficient at 334nm ($\mathcal{E}_{334}$) of 2.30 (Engel et al., 1993; Nickerson and Van Holde, 1971) shown in the following formula :

$$Haemolymph\ Hcy\ (mM) = \frac{A_{334} \times dilution\ factor}{\mathcal{E}^{mM}\ (17.26)}$$

1.1.1.4 Lactate

Haemolymph lactate concentration was measured from deproteinised haemolymph using a L-Lactate colourimetric analysis kit (catalogue number 735-10, Trinity Biotech) and analysed using a Novaspec Plus spectrophotometer (Biochrom US, Holliston, MA, USA) with % absorbance set to 540 nm. Haemolymph was deproteinised using a 1:1 dilution of haemolymph:chilled 6% perchloric acid. Haemolymph was centrifuged at 7200 rpm for 5 minutes and the supernatant extracted and used for the analysis.

1.1.1.5 Total Protein

Haemolymph total protein was measured from approximately 100 µl haemolymph samples using a similar procedure to that used by Stewart (1967), using the modified biuret method of Layne (1957) in which bovine serum protein is used as a standard. The biuret method involves the introduction of stabilized copper with NaOH that reacts with serum proteins to produce a blue to purple colour solution with a peak absorbance at 540nm. The concentration of haemolymph protein is proportional to the % absorbance of the reacted sampled at 540nm.

1.1.1.6 Haemolymph Osmolality

Whole haemolymph osmolality was analysed by placing 50 µl of haemolymph in a micro centrifuge tube which was then inserted into a micro-osmette osmometer (Precision Systems Inc., MA, USA) for direct measurement of haemolymph osmolality. The osmometer was turned on 1 hour prior to use to allow the unit to reach operating temperature stability and was calibrated at the start of each day's sampling. The calibration was measured and verified with osmometer standards every 30 minutes to ensure osmometer operation stability and re-calibrated as required. Measurements were recorded in units of $mOsm \bullet kg^{-1}$.

1.1.1.7 Exoskeleton Hardness

A durometer, designed specifically for lobster exoskeletons (Type 307LOB, PTC Durometers, CA, USA) was used to determine exoskeleton hardness of lobsters. The durometer has a spring-loaded force dial calibrated within the hardness and exoskeleton flex range of the *H. americanus* carapace. The readings can vary depending on the exoskeleton location where tested and the action of the user pushing the durometer against the exoskeleton. To minimise variability, a single location on the side of the cephalothorax exterior to the gill chamber and between the 1^{st} and 2^{nd} pair of periopods

was used, and a single user measured and recorded exoskeleton hardness throughout the trials.

1.1.1.8 Moult Stage

Moult stage classification by pleopod setogenesis staging from Aiken (1973) was used for this trial with proecdysis moult classifications that ranged from intermoult (stage C_4) to early and late pre-moult (stages D_0 to D_3). Aiken's pleopod staging subdivides proecdysis into distinct stages based on the observations of the epidermis and setae presence and characteristics. Table 2.3 describes the relationship between pleopod and moult stages used for this study as adapted from Aiken (1973).

Table 2.3 *Homarus americanus*: Relationship between pleopod stage as identified using pleopod setogenesis and actual moult stage within the intermoult (C_4) and proecdysis (D_0-D_3) moult stages. Adapted from Aiken (1973).

Pleopod Stage	Moult Stage
0	C_4
1.0	D_0
1.5	D_0
2.0	D_0
2.5	D_0
3.0	D_1'
3.5	D_1''
4.0	D_1'''
4.5	D_2'
5.0	D_2''
5.5	D_3

To determine pleopod stage, the terminal 1/3 of one of the 2nd pair of the pleopod exopodites was severed with a pair of scissors, placed on a glass slide and observed using the low power (40x) lens configuration of a compound, visible light microscope (Olympus BH-2, Olympus Corporation, Tokyo, Japan). The unique features that categorise lobsters within each pleopod stage are shown in Figure 2.5 and described below.

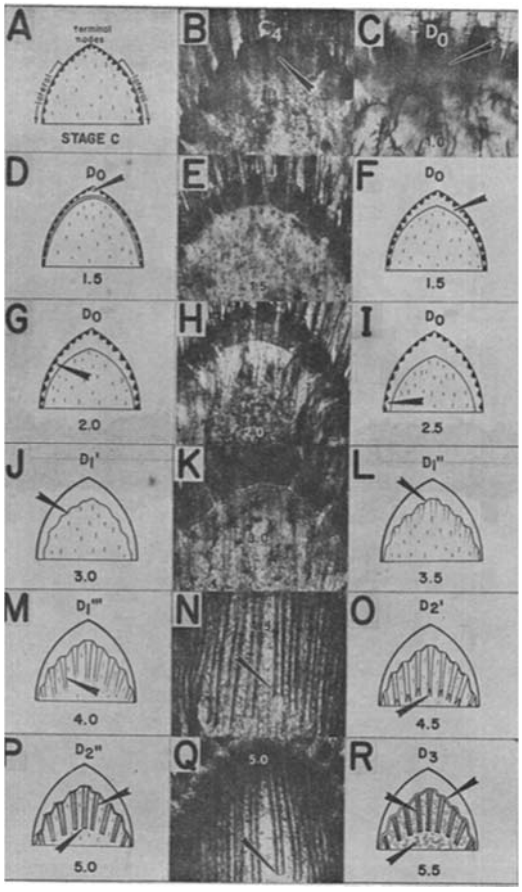

Figure 2.5 *Homarus americanus*: Moult stage determination by pleopod epidermal and setae morphology differences during intermoult (A,B) and proecdysis (C-R). From: (Aiken, 1973).

Pleopod stage 0 (moult stage C_4) is indicated on the exopodites by complete contact of the epidermis to the distal, cuticular tips with no epidermal retraction visible (apolysis) whereas the various pre-moult D (1.5-5.5) stages have specific epidermal morphology and setae development characteristics in which the epidermis begins to contract and new setal formations appear and become more defined leading eventually to ecdysis.

2.3.4 Statistical Analysis

Risk factors were consolidated by season (Winter and Spring) and pre-treatment group (Wet, 48h immersion recovery and Dry, non-recovered) and the total cumulative numbers of lobsters that were assessed as alive (vigour index 1-3) and dead (vigour index 4 or 5) at the end of the long term storage trial were compared using the independent 2-group t-

test for parametric data or the independent Mann-Whitney U test for non-parametric data. Results were considered significantly different if $p < 0.05$ unless specifically stated in the text.

Correlation analysis of all risk factor parameters was completed using the Pearson correlation coefficient for parametric data and the Spearman rank correlation coefficient for non-parametric data. Correlations were classified by strength using the criteria outlined in Table 2.4.

Table 2.4 Correlation Coefficient classification of correlation strength.

Correlation coefficient value (*r*)	Strength of Correlation
1	Perfect
0.8 – 0.9	Very Strong
0.5 – 0.8	Strong
0.3 – 0.5	Moderate
0.1 – 0.3	Modest
< 0.1	Weak

Acceptance of the correlation occurred only with correlation values in which there was a significant result ($p < 0.05$). The correlation analyses were used to compare each measured risk factor with every other one used in this study to determine if strong correlations exist between factors and therefore make conclusions on the ability to eliminate the assessment of redundant measures and to understand the interrelationship between the risk factors.

Survival analysis was then completed on the measured risk factors using Kaplan-Meyer survival curves and the Mantel-Cox log-rank test for differences amongst the curves. The

continuous variable data sets (haemolymph total protein, lactate and osmolality as well as moult stage) were divided into three groups of increasing values. Haemolymph total protein results were divided into low (< 25 mg•ml^{-1}), mid (25 – 40 mg•ml^{-1}) and high (>40 mg•ml^{-1}) groups. Haemolymph lactate results were divided into low (< 2 mml l^{-1}), mid (2 - 4 mml l^{-1}) and high (< 4 mml l^{-1}) groups. Haemolymph osmolality results were divided into low (< 850 mml l^{-1}), mid (850 - 950 mml l^{-1}) and high (< 950 mml l^{-1}) groups. Moult stage was divided into intermoult (pleopod stage 0), early premoult (stages 1 – 2.5) and active premoult (stages > 2.5). Active premoult was used to subdivide proecdysis and represents the beginning stage at which the lobster is considered committed to moult and will continue towards ecdysis regardless of environmental temperature. Early premoult lobsters up to and including pleopod stage 2.5 (the last pleopod stage prior to D_1') can reach development plateaus based on environmental conditions such as low seawater temperatures as experienced in the winter months (Aiken, 1980) and it is therefore assumed that a development plateau during proecdysis occurs within pleopod stages 0 to 2.5 if the storage temperature is maintained below 5 °C.

Log-rank test for trends were reported in comparisons of three survival curves (haemolymph total protein, lactate and osmolality as well as moult stage) in which the log-rank test was significant ($p < 0.05$). The dichotomous risk factors of sex (male and female), exoskeleton hardness (soft-medium and hard) and pre-treatment type (Wet and Dry) were analysed for differences in survival using Mantel-Haenszel hazard ratios in which the log-rank test was significant ($p < 0.05$).

Reported values are given as means +/- SE unless specifically stated otherwise in the text. Plotted data in all figures are represented as means with +/- SE bars.

Overall risk factor versus storage time and correlation analysis and plots were completed using R v 3.1.2 for Mac OS X, R Core Team (2014). R: A language and environment for

statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. Survival analyses and Kaplan-Meier survival curve plots were completed using both R v 3.1.2 for Mac OS X and GraphPad Prism version 6.0f for Mac OS X, GraphPad Software, La Jolla California USA, http://www.graphpad.com/.

2.4 Results

2.4.1 Risk Factors versus Storage Outcome

There was a significant difference ($p < 0.0001$) in the overall mean haemolymph % brix values between the Winter and Spring groups. The Winter and Spring mean values were 9.77 ± 2.94 % and 10.89 ± 2.00 % respectively. The Winter non-recovered (Dry) and recovered (Wet) groups had mean haemolymph % brix values of 9.99 ± 2.9 %and 9.56 ± 2.96 % respectively and the Spring equivalent groups had mean haemolymph % brix values of 11.35 ± 2.07 % and 10.42 ± 1.82 % respectively. Storage success, as measured by the 120 - day storage sum of alive and dead lobsters for haemolymph % brix, is shown in Figure 2.6 for both the Winter and Spring season groups. There was a significant difference ($p < 0.001$ in both groups) between the alive and dead group haemolymph mean % brix in both the Dry and Wet Winter groups but no significant differences between the equivalent mean values of the alive or dead groups in either the Spring pre-treated groups ($p > 0.05$ in both cases).

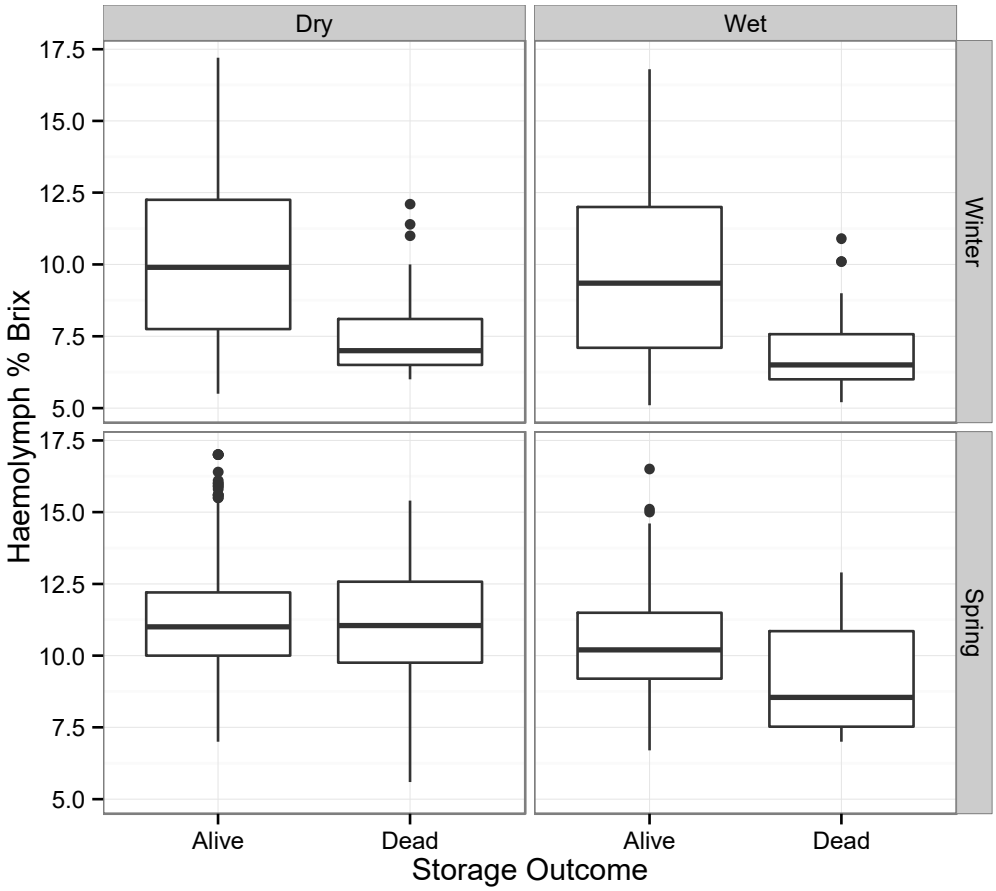

Figure 2.6 *Homarus americanus*: Pre - storage haemolymph % Brix for 48 h recovered (Wet) treatment and immediate post-harvest (Dry) treatment groups of Winter and Spring season lobsters. Alive and dead groups represent lobster condition after 120 days of Dryland pound storage. n = 288 per treatment group per season. Values given as means with associated 95% confidence limits. Individual data points refer to values falling outside the 95% confidence limits.

Overall Winter and Spring exoskeleton hardness indices were significantly different ($p < 0.0001$) with means of 92.38 ± 4.75 and 91.18 ± 4.66 respectively. A summary of the storage success outcomes by season and pre – treatment (Dry, non-recovered and Wet, 48 h recovered) groups is shown in Figure 2.7. The Winter, wet pre – treatment storage success outcomes were significantly different ($p < 0.05$) but there was no significant difference between the dry pre – treatment group means ($p > 0.05$). There were no differences between the storage success outcomes for either of the Spring season dry or wet pre – treatment groups ($p = 0.52$ and 0.68 respectively).

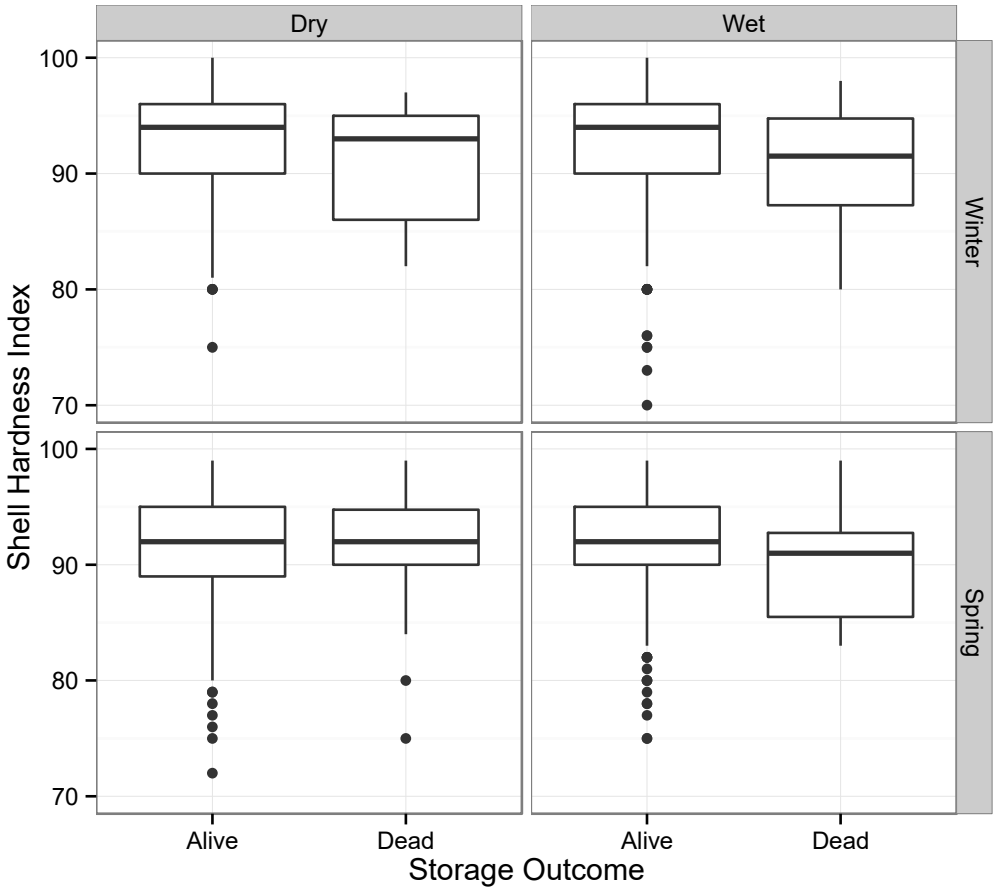

Figure 2.7 *Homarus americanus*: Pre - storage exoskeleton hardness indices for 48 h recovered (Wet) treatment and immediate post harvest (Dry) treatment lobsters for groups of Winter and Spring season lobsters. Alive and dead groups represent lobster condition after 120 days of Dryland pound storage. n = 288 per treatment group per season. Values given are means with associated 95% confidence limits. Individual data points refer to values falling outside the 95% confidence limits.

The overall Winter and Summer mean haemolymph osmolality values were 893.46 ± 69.55 mOsm•l^{-1} and 874.48 ± 43.00 mOsm•l^{-1} respectively. A summary of the haemolymph osmolality by season and pre – storage treatment group (dry, non-recovered and wet, 48 h-recovered treatments) is shown in Figure 2.8. There were no significant within-season differences of storage success between the pre-treatment groups in either the Winter and Spring seasons.

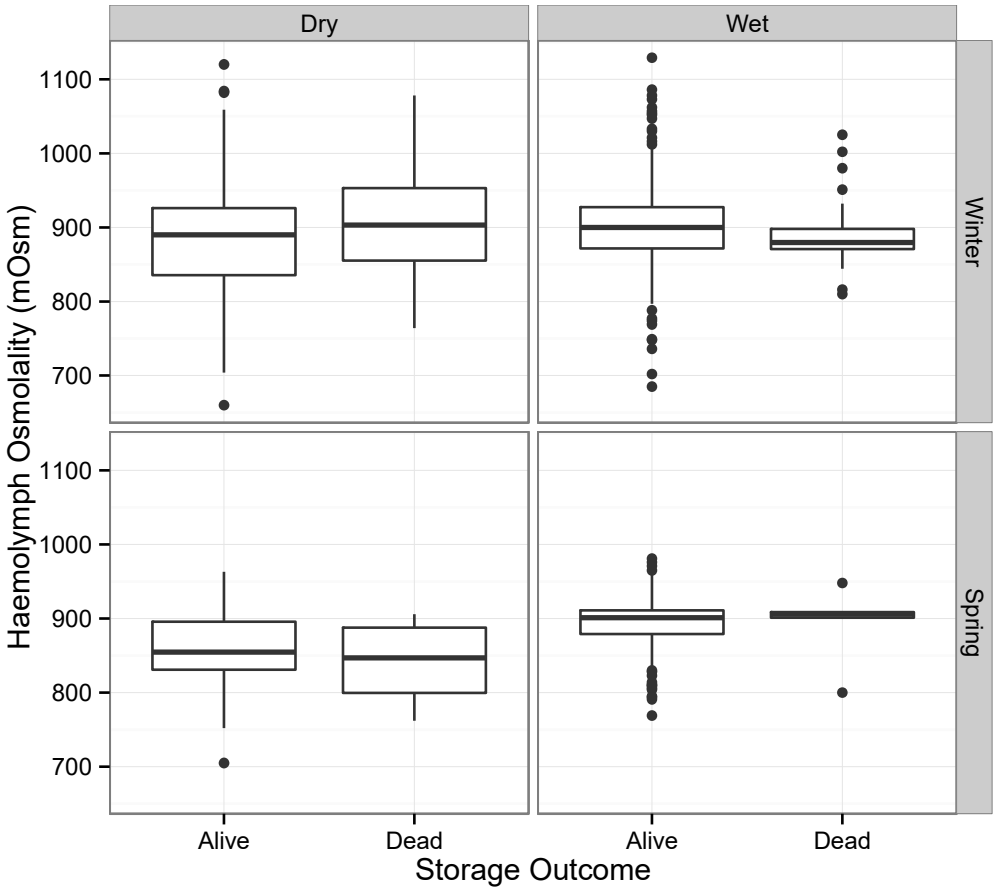

Figure 2.8 *Homarus americanus*: Pre - storage lobster haemolymph osmolality (mOsm•l^{-1}) for 48 h recovered (Wet) treatment and immediate post harvest (Dry) treatment lobsters for groups of winter and spring season lobsters. Alive and dead groups represent lobster condition after 120 days of Dryland pound storage. n = 288 per treatment group per season. Values given as means with associated 95% confidence limits. Individual data points refer to values falling outside the 95% confidence limits.

Overall Winter and Spring season haemolymph total protein concentrations were significantly different ($p < 0.0001$) at 45.16 ± 20.78 and 52.85 ± 15.23 mg•l^{-1} respectively. Within season comparisons of storage success by pre-storage treatments (Figure 2.9) indicate that there were highly significant differences in both the wet and dry pre-treatment Winter groups ($p < 0.001$ in both groups) but none were found in the Spring groups ($p = 0.65$ for the wet group and $p = 0.2$ for the dry group).

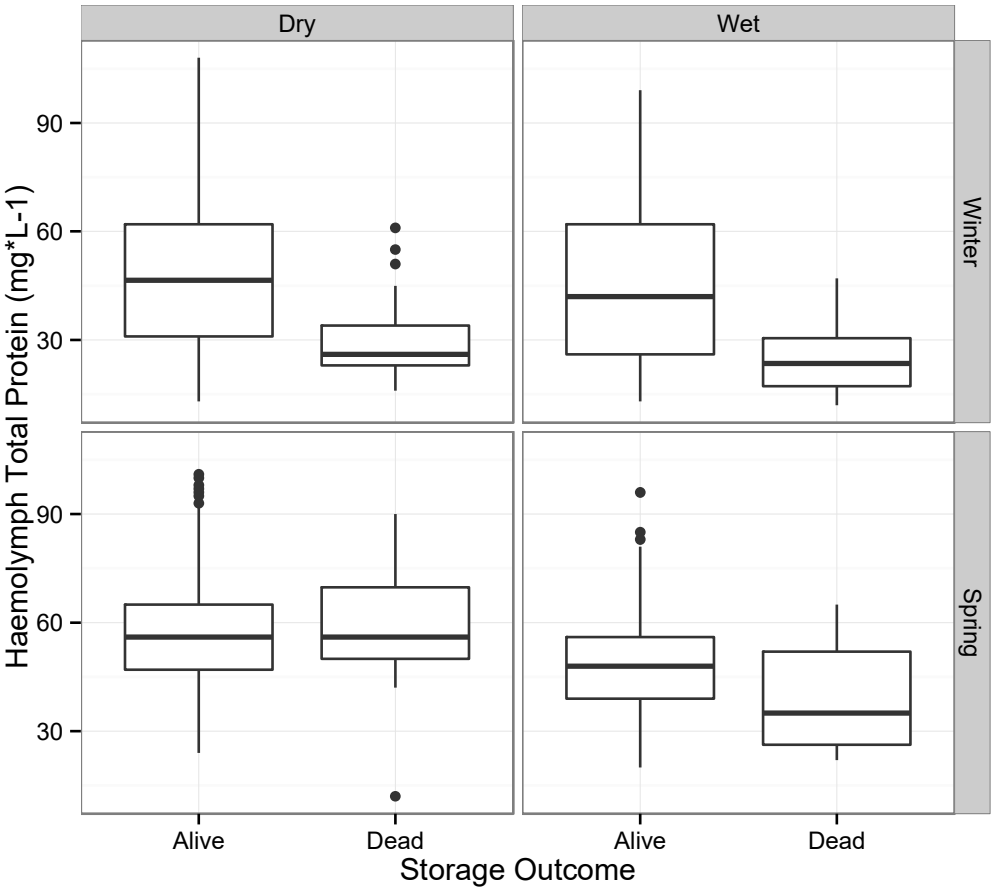

Figure 2.9 *Homarus americanus*: Pre - storage lobster haemolymph total protein ($mg \bullet l^{-1}$) for 48 h recovered (Wet) treatment and immediate post harvest (Dry) treatment lobsters for groups of Winter and Spring season lobsters. Alive and dead groups represent lobster condition after 120 days of Dryland pound storage. n = 288 per treatment group per season. Values given as means with associated 95% confidence limits. Individual data points refer to values falling outside the 95% confidence limits.

A summary of the pre – storage haemolymph lactate levels is shown in Figure 2.10. Overall Winter and Spring mean lactate levels differed significantly ($p < 0.0001$) with means of 1.87 ± 2.48 and 3.29 ± 3.56 mml l^{-1} respectively. The seasonal differences between lactate levels in both the wet and dry pre – season recovery groups were highly significant (ps < 0.0001 for both pre – treatment groups). There was a significant difference of lactate levels between the Winter lobsters that had died during long-term storage and those that survived ($p < 0.05$). There was a significant difference between the mean haemolymph lactate levels of the Spring non-recovered groups' storage outcomes

($p < 0.01$) but no significant differences were observed between the Spring 48 h recovered pre – treatment groups ($p = 0.22$).

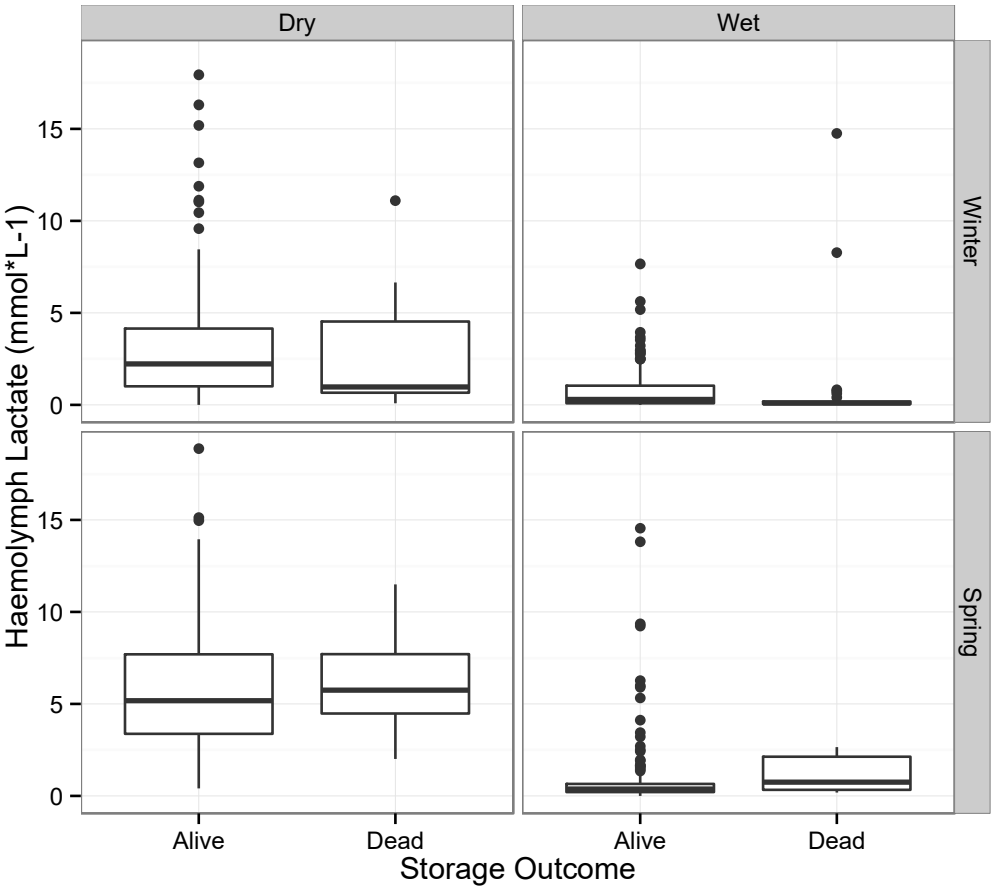

Figure 2.10 *Homarus americanus*: Pre - storage lobster haemolymph lactate (mml l^{-1}) for 48 h recovered (Wet) treatment and immediate post harvest (Dry) treatment lobsters for groups of Winter and Spring season lobsters. Alive and dead groups represent lobster condition after 120 days of Dryland pound storage. n = 288 per treatment group per season. Values are given as means with associated 95% confidence limits. Individual data points refer to values falling outside the 95% confidence limits.

Overall mean pre – storage lobster haemocyanin levels were significantly different ($p < 0.0001$) for the Winter and Spring season with means of 0.48 ± 0.17 and 0.52 ± 0.13 mml l^{-1} respectively. A summary of the storage success outcomes by lobster haemocyanin levels is shown in Figure 2.11. Within - season differences between the alive and dead storage outcome haemocyanin levels were observed only in the Winter, wet and dry pre – treatment groups ($p < 0.0001$ in each case). No significant haemocyanin level

differences between storage success outcomes were found between the Spring pre – treatment groups ($p > 0.05$ in each case).

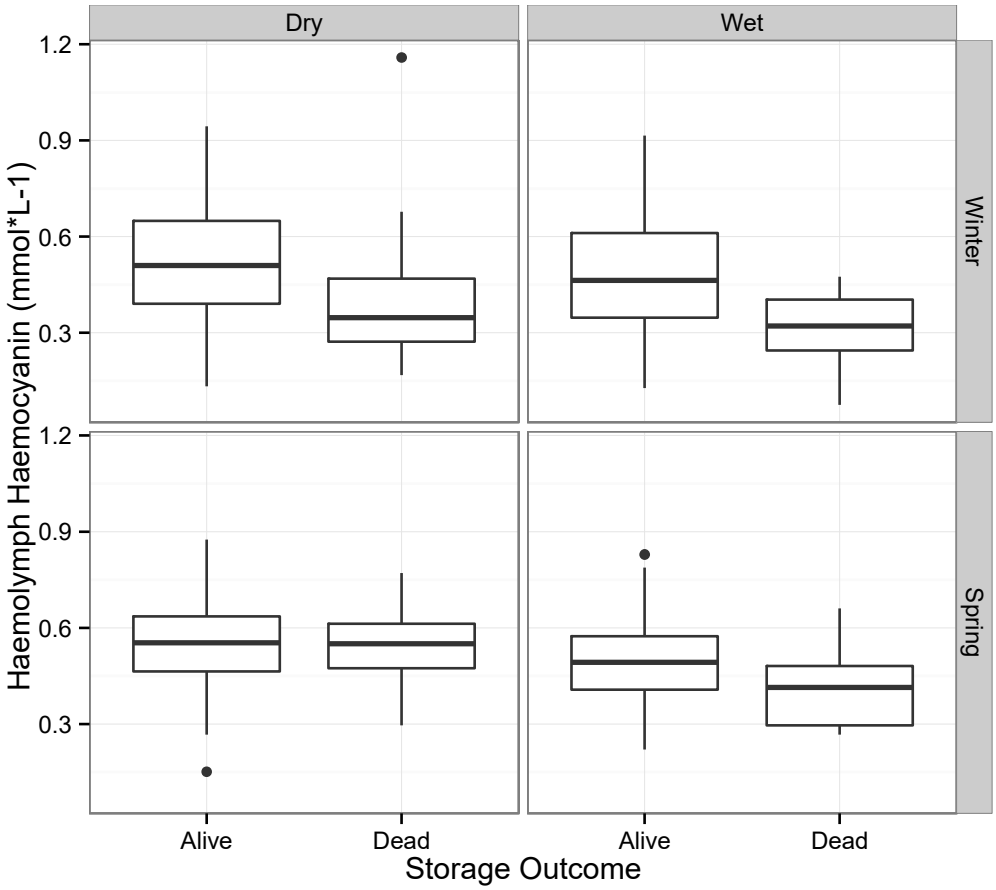

Figure 2.11 *Homarus americanus*: Pre - storage lobster haemocyanin (mml l^{-1}) for 48 h recovered (Wet) treatment and immediate post harvest (Dry) treatment lobsters for groups of Winter and Spring season lobsters. Alive and dead groups represent lobster condition after 120 days of Dryland pound storage. n = 288 per treatment group per season. Values given as means with associated 95% confidence limits. Individual data points refer to values falling outside the 95% confidence limits.

A summary of the statistical comparisons of the risk factors by season and pre – treatment group is shown in Table 2.5. In the Winter season trial, all but the haemolymph osmolality showed significant differences in the mean values between successful (Alive) and unsuccessful (Dead) 120 day DLP storage groups, with haemolymph % brix, pleopod stage, haemolymph total protein and haemocyanin differences that were highly significant ($p < 0.001$), and exoskeleton hardness and haemolymph lactate differences that were significant ($p < 0.05$). In the Spring season trial, only mean haemolymph lactate

showed significant differences in DLP storage outcomes between the Wet and Dry pre – treatment groups ($p < 0.01$).

Table 2.5 *Homarus americanus*: *p* – values for significant differences between measured parameters of successful (Alive) and unsuccessful (Dead) groups after long term storage (120 days) in the Winter and Spring season groups. n = 144 per treatment group for a study total of n = 576 animals.

	Winter Season		Spring Season	
Parameter	48 h Recovery	No Recovery	48 h Recovery	No Recovery
Haemolymph % Brix	*< 0.001*	*< 0.001*	0.18	0.88
Exoskeleton Hardness	*<0.05*	0.05	0.52	0.68
Pleopod Stage	*< 0.001*	*< 0.05*	0.49	0.49
Haemolymph Osmolality	0.08	0.14	0.55	0.15
Haemolymph Total Protein	*< 0.001*	*< 0.001*	0.20	0.65
Haemolymph Lactate	*<0.05*	0.08	0.22	*< 0.01*
Haemocyanin	*< 0.001*	*< 0.001*	0.16	0.74

The results of the risk factor versus storage outcome outlined above indicate that there were significant differences in outcome of storage success between many of the factors analysed in the winter season, but only one strong correlation (haemolymph lactate) in the spring season group from both pre-treatment groups. Further analysis of the risk factors to determine their effectiveness as an assessment tool is presented in subsequent results Sections 2.4.2, 2.4.3 and 2.4.4.

2.4.2 Risk Factor Correlation

A summary of the correlations between the risk factors is shown in the correlation matrices in Figure 2.12 and Figure 2.13 for the Wet, pre-recovery and Dry, no recovery groups respectively. The correlations in the matrices are based on the data from individually sampled lobsters in which each risk factor is compared with every other factor. The correlation coefficients from these risk factor analyses are further reported with levels of significance (p) in Table 2.6 and

Table 2.7 for the Wet, pre-recovery and Dry, no recovery groups respectively. The Winter and Spring data were combined to provide the full spectrum of moult stage data that occur in a typical harvest season in the Canadian lobster fishery from November until June the following year. Analysing seasonal correlations separately did not reveal any significant differences from the combined Winter and Spring dataset. Pre-treatment groups of 48 h recovery (Wet) and no recovery (Dry) were analysed separately due to the changes in haemolymph chemistries, such as lactate and total protein, that are known to occur in crustaceans during emersion and re-immersion (Bernasconi and Uglow, 2008a; Danford et al., 1999).

The Wet pre-recovery treatment group correlation matrix is shown in Figure 2.12 and summarized in Table 2.6 with corresponding levels of significance. The Wet pre-recovery treatment group (Figure 2.12 and Table 2.6) shows a very strong correlation between % Brix and total protein (TP) ($r = 0.971$, $p < 0.001$). Haemocyanin (Hcy) also showed a very strong correlation with total protein and % Brix ($r = 0.818$, $p < 0.001$ and $r = 0.813$, $p < 0.001$ respectively). Moult stage showed strong correlations with both total protein and % Brix ($r = 0.709$ and 0.718 respectively, $p < 0.001$ in both cases) and a lower, but still strong, correlation with haemocyanin concentration ($r = 0.548$, $p < 0.001$). The rest of the paired comparisons showed moderate to trivial correlation values ($r < 0.4$).

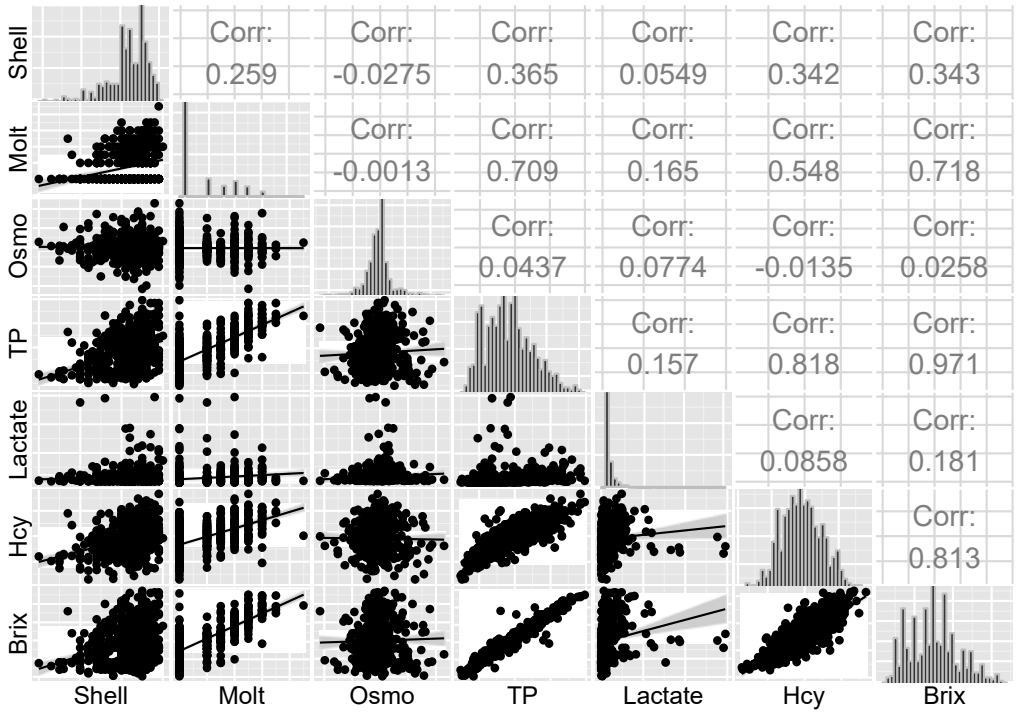

Figure 2.12 *Homarus americanus*: correlation matrix of possible storage risk factors for seasoned ("Wet") post-harvest lobsters from Winter and Spring season combined. Parameters include exoskeleton hardness (Shell), pleopod stage (Moult), haemolymph osmolality (Osmo), haemolymph total protein (TP), haemolymph lactate (Lactate), haemocyanin (Hcy) and haemolymph %Brix (Brix). Corr = Spearman product moment correlation coefficients (*r*). n=576.

Table 2.6 *Homarus americanus*: Spearman product moment correlation coefficients (*r*) for storage risk factors of seasoned ("Wet") post-harvest combined Winter and Spring season lobsters. n = 576. Italicised values are strongly correlated and bold, italicized values are very strongly correlated. Significance levels: * p < 0.05, ** p < 0.01, *** p< 0.001

	Moult	Osmolality	Total Protein	Lactate	Haemocyanin	% Brix
Exoskeleton Hardness	0.259***	-0.0275	0.365***	0.0549**	0.342***	0.343***
Moult		-0.0013	*0.709****	0.165***	*0.548****	*0.718****
Osmolality			0.0437	0.0774***	-0.014	0.026
Total Protein				0.157***	**0.818****	**0.971****
Lactate					**0.858****	0.181***
Haemocyanin						**0.813****

The Dry pre-recovery treatment group correlation matrix, shown in Figure 2.13 and summarized in

Table 2.7 with corresponding levels of significance, show a similarly very strong correlation of haemolymph total protein (TP) with % Brix (RI) ($r = 0.969$, $p < 0.001$) to that of the Dry group described earlier. Haemocyanin concentration was strongly correlated with haemolymph total protein and % Brix ($r = 0.706$, $p < 0.001$ and $r = 0.702$, $p < 0.001$ respectively). Moult stage showed strong correlations with both total protein and % Brix ($r = 0.722$ and 0.759 respectively, $p < 0.001$ in both cases) and strong, but lower correlation with haemocyanin concentration ($r = 0.509$, $p < 0.001$). All other risk factors showed moderate to weak correlations with each other ($r < 0.42$).

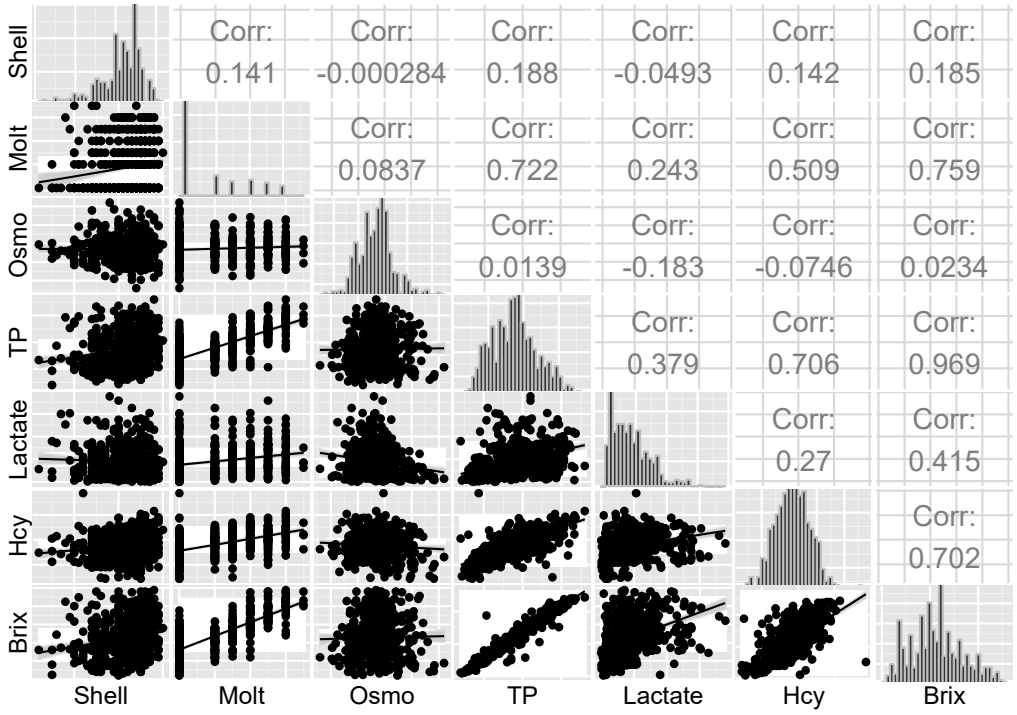

Figure 2.13 *Homarus americanus*: correlation matrix of possible storage risk factors for immediately received (Dry) post-harvest combined Winter and Spring season lobsters. Parameters include exoskeleton hardness (Shell), pleopod stage (Moult), haemolymph osmolality (Osmo), haemolymph total protein (TP), haemolymph lactate (Lactate), haemocyanin (Hcy) and haemolymph %Brix (Brix). Corr = Spearman product moment correlation coefficients (*r*). n=576

Table 2.7 *Homarus americanus*: Spearman product moment correlation coefficients (*r*) for storage risk factors of immediately received (Dry) post-harvest combined Winter and Spring season lobsters. n = 576. Italicised values are strongly correlated and bold, italicized values are very strongly correlated. Significance levels: * p < 0.05, ** p < 0.01, *** p< 0.001

	Moult	Osmolality	Total Protein	Lactate	Haemocyanin	% Brix
Exoskeleton Hardness	0.141***	-0.0003	0.188***	-0.0493	0.142**	0.185***
Moult		0.0837*	*0.722****	0.243***	*0.509****	*0.759****
Osmolality			0.0139	-0.183***	-0.0746	0.0234
Total Protein				0.379***	*0.706****	***0.969****
Lactate					0.27***	0.415***
Haemocyanin						*0.702****

2.4.3 Survival Analysis and Log-Rank Test for Trend of Risk Factors

Based on the measured risk factor comparison correlation coefficients in Section 2.4.2, analysis of all of the parameters would be redundant due to the strong correlation amongst the haemolymph protein-related groups of haemolymph total protein, % Brix and haemocyanin. Therefore, total protein was chosen to represent these three factors in the survival analyses. Parameters of haemolymph total protein, exoskeleton hardness, haemolymph osmolality, haemolymph lactate, pleopod stage and gender as well as pre-treatment type (recovery or no-recovery) were selected for a more detailed Kaplan-Meier survival analysis. In each case, factors were plotted against DLP storage time, noting any change in status (dead or missing) during the length of storage. Survival curves are shown for each parameter for Winter and Spring in each figure further described below.

Survival curves for Winter and Spring haemolymph total protein groups are shown in figures Figure 2.14a and b respectively. Total protein data were divided into three groups based on known levels of risk for other storage-related complications in *H. americanus* (Theriault et al., 2008). As described earlier, these haemolymph total protein categories were 'Low' (< 25 mg•ml^{-1}), 'Medium' (25 – 40 mg•ml^{-1}) and 'High' (> 40 mg•ml^{-1}). There was a significant difference between the three total protein groups in the Winter

season ($p < 0.001$) with a significant trend also noted with decreasing mortality from low to high group ($p < 0.0001$). There was no significant difference in survival between the total protein groups in the Spring trial ($p = 0.352$).

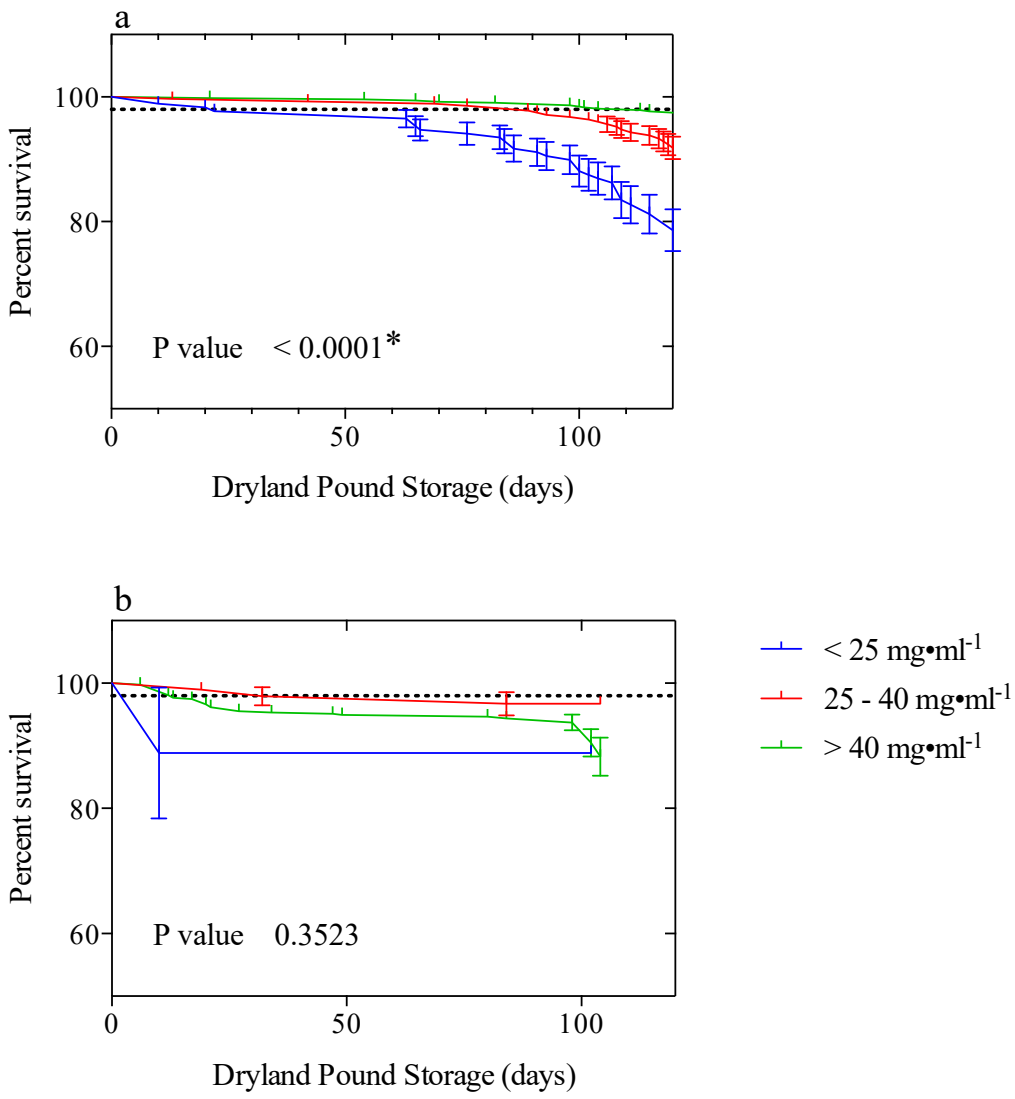

Figure 2.14 *Homarus americanus*: Kaplan-Meier survival curve of haemolymph total protein (mg•ml^{-1}) versus DLP storage in days for Winter (Figure 2.14a) and Spring (Figure 2.14b) season lobsters. Total protein levels are divided into three categories: low (< 25 mg•ml^{-1}), medium (25 - 40 mg•ml^{-1}) and high (> 40 mg•ml^{-1}). Percent survival curves are shown with SEM bars. Dashed horizontal line represents 2% cumulative mortality level. n = 576 per season. Log-rank (Mantel-Cox) P values are indicated in each figure. * denotes significant differences between categories ($p < 0.05$).

The exoskeleton hardness survival analyses for Winter and Spring are shown in Figure 2.15a and b respectively. Exoskeleton hardness was dichotomised into two groups; 'Soft-Medium' comprising exoskeleton hardness index from 0 – 89 and 'Hard' comprising

values >= 90. There was a significant difference between the two exoskeleton hardness index groups in the Winter season ($p < 0.0001$), but no significant difference was observed in the Spring season trial ($p = 0.1951$). The Mantel-Haenszel hazard ratio indicated that the Soft-Medium exoskeleton group has a mortality rate that was 3.51 times that of the Hard exoskeleton group in the Winter season trial.

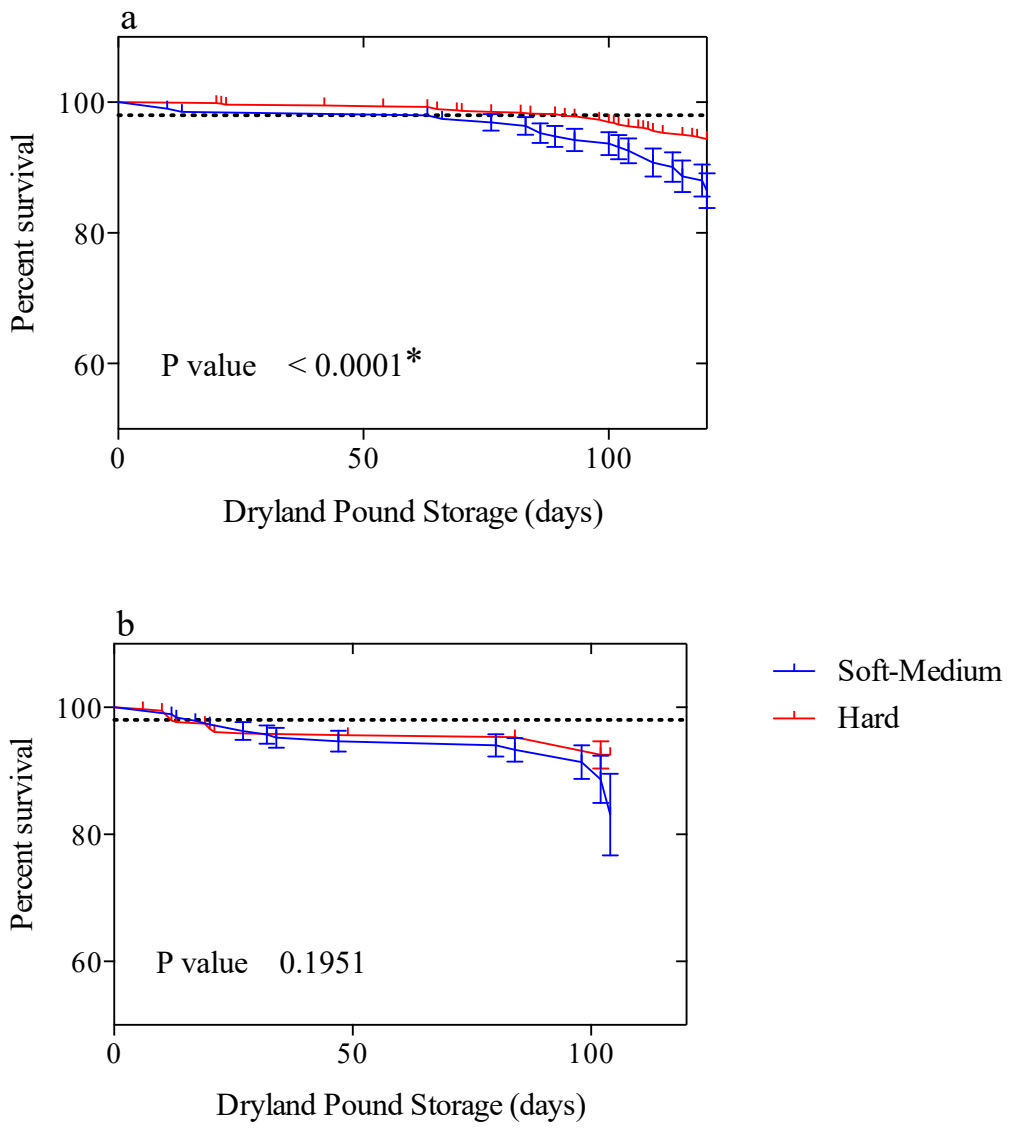

Figure 2.15 *Homarus americanus*: Kaplan-Meier survival curve of exoskeleton hardness index versus DLP storage in days for Winter (Figure 2.15a) and Spring (Figure 2.15b) season lobsters. Exoskeleton hardness indices are divided into two categories: Soft-Medium (70 - 89) and Hard (90 - 100). Percent survival curves are shown with SEM bars. Dashed horizontal line represents 2% cumulative mortality level. n = 576 per season . Log-rank (Mantel-Cox) P values are indicated in each figure. * denotes significant differences between categories ($p < 0.05$).

Haemolymph osmolality survival curves, shown in Figure 2.16a and b, have osmolality divided into three categories of increasing levels as the 'Low' (<850 mOsm•l^{-1}), 'Medium' (850 – 950 mOsmol•l^{-1}) and 'High' (> 950 mOsm•l^{-1}). There were no significant differences between the groups in either the Winter or Spring season trial (*p*s 0.254 and 0.449 respectively).

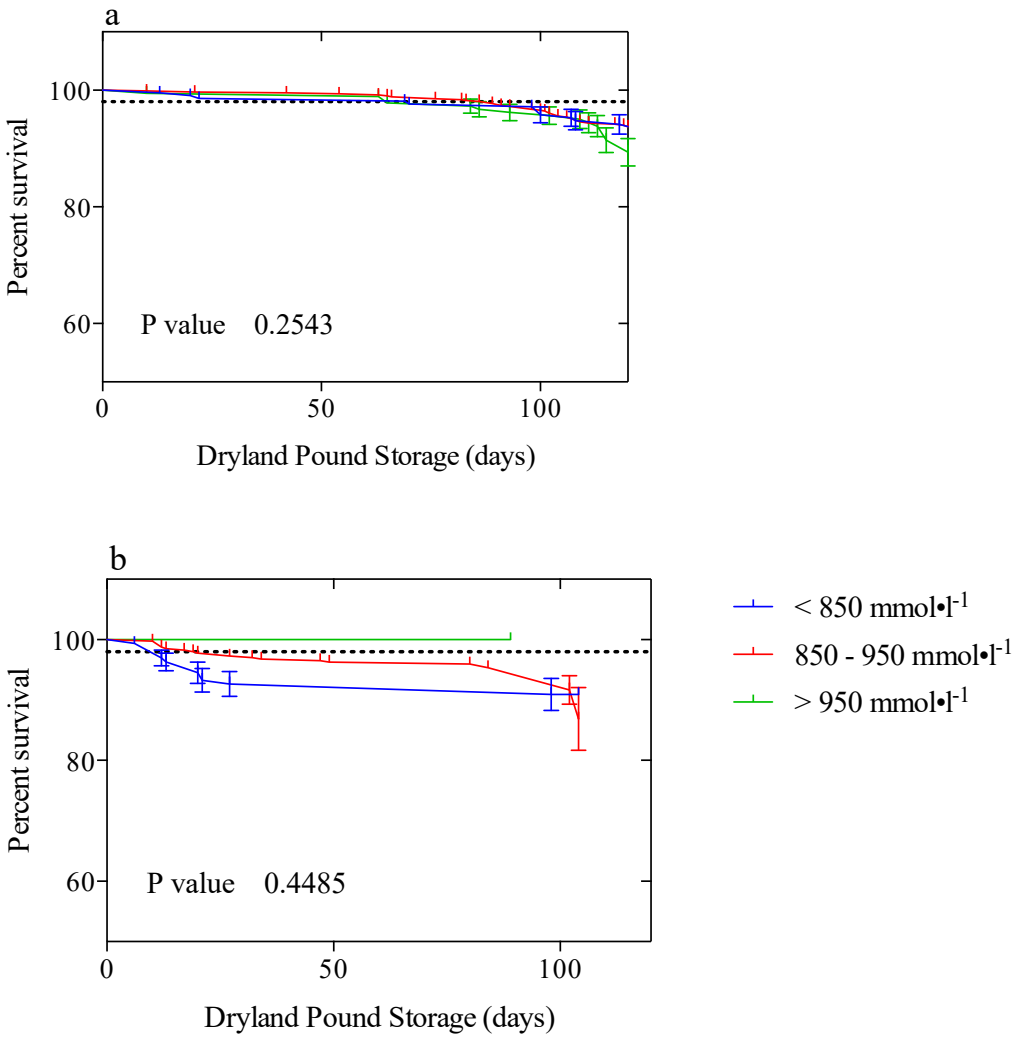

Figure 2.16 *Homarus americanus*: Kaplan-Meier survival curve of haemolymph osmolality (mml l^{-1}) versus DLP storage in days for Winter (Figure 2.16a) and Spring (Figure 2.16b) season lobsters. Osmolality levels are divided into three categories: low (< 850 mml l^{-1}), medium (850 - 950 mml l^{-1}) and high (> 950 mml l^{-1}). Percent survival curves are shown with SEM bars. Dashed horizontal line represents 2% cumulative mortality level. n = 576 per season . Log-rank (Mantel-Cox) P values are indicated in each figure. * denotes significant differences between categories (p < 0.05).

A comparison of survival for haemolymph lactate for the Winter and Spring season trials is shown in Figure 2.17a and b respectively. Haemolymph lactate data were divided into three categories of increasing concentration as 'Low' (< 2 mml l^{-1}), 'Medium' (2 - 4 mml l^{-1}), and 'High' (> 4 mml l^{-1}). There was a significant difference between the three levels in both the Winter and Spring season ($p = 0.0039$ and <0.0001 respectively). Log-rank test for trend results from low to high groups indicate significant differences for both the Winter and Spring season trials ($p = 0.0146$ and < 0.0001 respectively), which indicate a trend of decreasing survival with initial haemolymph lactate concentration on arrival.

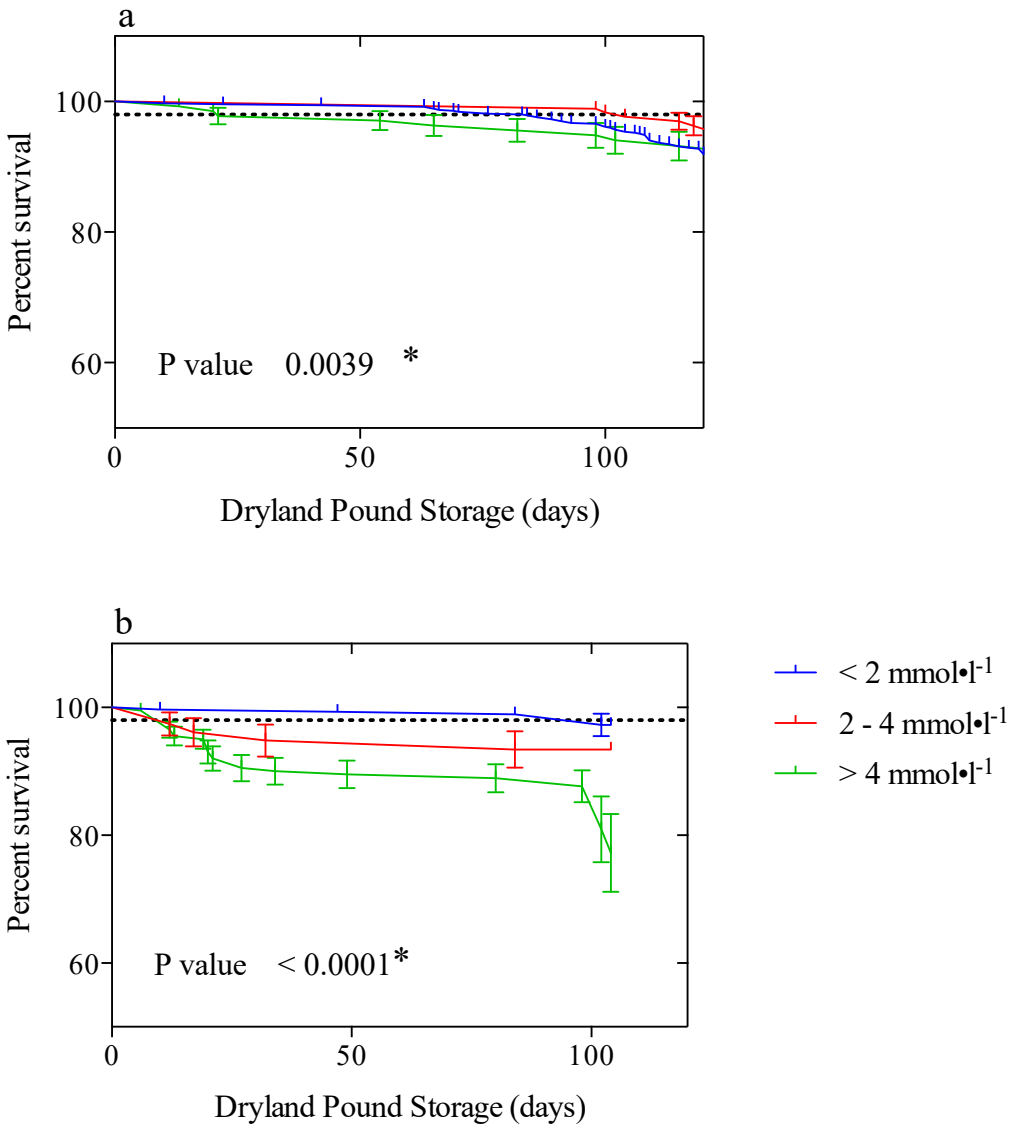

Figure 2.17 *Homarus americanus*: Kaplan-Meier survival curve of haemolymph lactate (mml l^{-1}) versus DLP storage in days for Winter (Figure 2.17a) and Spring (Figure 2.17b) season lobsters. Lactate levels are divided into three categories: low (< mml l^{-1}), medium (2 - 4 mml l^{-1}) and high (> 4 mml l^{-1}). Percent survival curves are shown with SEM bars. Dashed horizontal line represents 2% cumulative mortality level. n = 576 per season. Log-rank (Mantel-Cox) P values are indicated in each figure. * denotes significant differences between categories ($p < 0.05$).

The moult stage survival analysis results of the Winter and Spring season trials are shown in Figure 2.18a and Figure 2.18b respectively. Moult stages were divided into three groups in order of progression from intermoult (C_4 or pleopod stage 0) to early premoult (D_0 or pleopod stages 1 – 2.5) and onto active premoult (D_1' to D_3 or pleopod stages > 2.5) based on broad categories suggested in Aiken (1973) and used by others for risk

analysis of other health parameters (Fotedar and Evans, 2011; Theriault et al., 2008). There was a significant difference in survival between the three moult stage groups for the Winter season ($p < 0.0001$) but no significant difference in the Spring trial group.

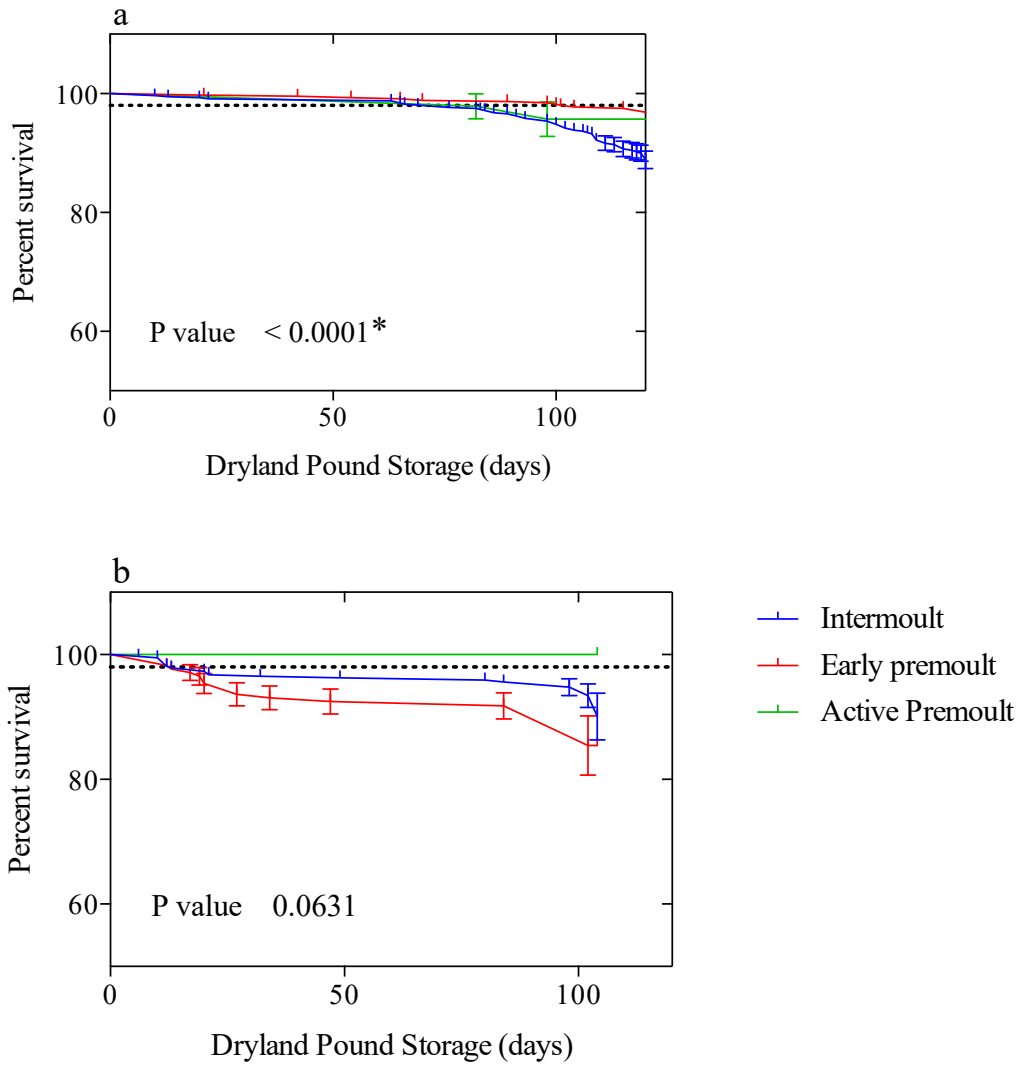

Figure 2.18 *Homarus americanus*: Kaplan-Meier survival curve of pleopod stage versus DLP storage in days for Winter (Figure 2.18a) and Spring (Figure 2.18b) season lobsters. Pleopod stage is divided into three groups: Intermoult (Stage C), early pre-moult (Pleopod stages 1 – 2.5) and active pre-moult (Pleopod stages > 2.5) as described in section 2.3.4. Percent survival curves are shown with SEM bars. Dashed horizontal line represents 2% cumulative mortality level. n = 576 per season . Log-rank (Mantel-Cox) P values are indicated in each figure. * denotes significant differences between categories ($p < 0.05$).

2.4.4 Survival Analysis with Hazard Ratios

For groups that contained dichotomous parameters, survival analysis with hazard ratios must be used to compare groups since Kaplan-Meier survival analysis must contain at least three comparison groups. The survival analysis summary of pre-treatment types (48h immersion “Wet” and no immersion “Dry” on arrival groups) for both the Winter and Spring season trials is shown in Figure 2.19a and Figure 2.19b respectively. There was no difference in survival for 120 days of DLP storage during the winter trial ($p = 0.4636$) whereas there was a significant difference in survival between the Wet and Dry pre-treatments in the Spring season trial ($p = 0.006$). The Spring season Dry group had a 2.58 times higher mortality rate based on the Mantel-Haenszel hazard ratio.

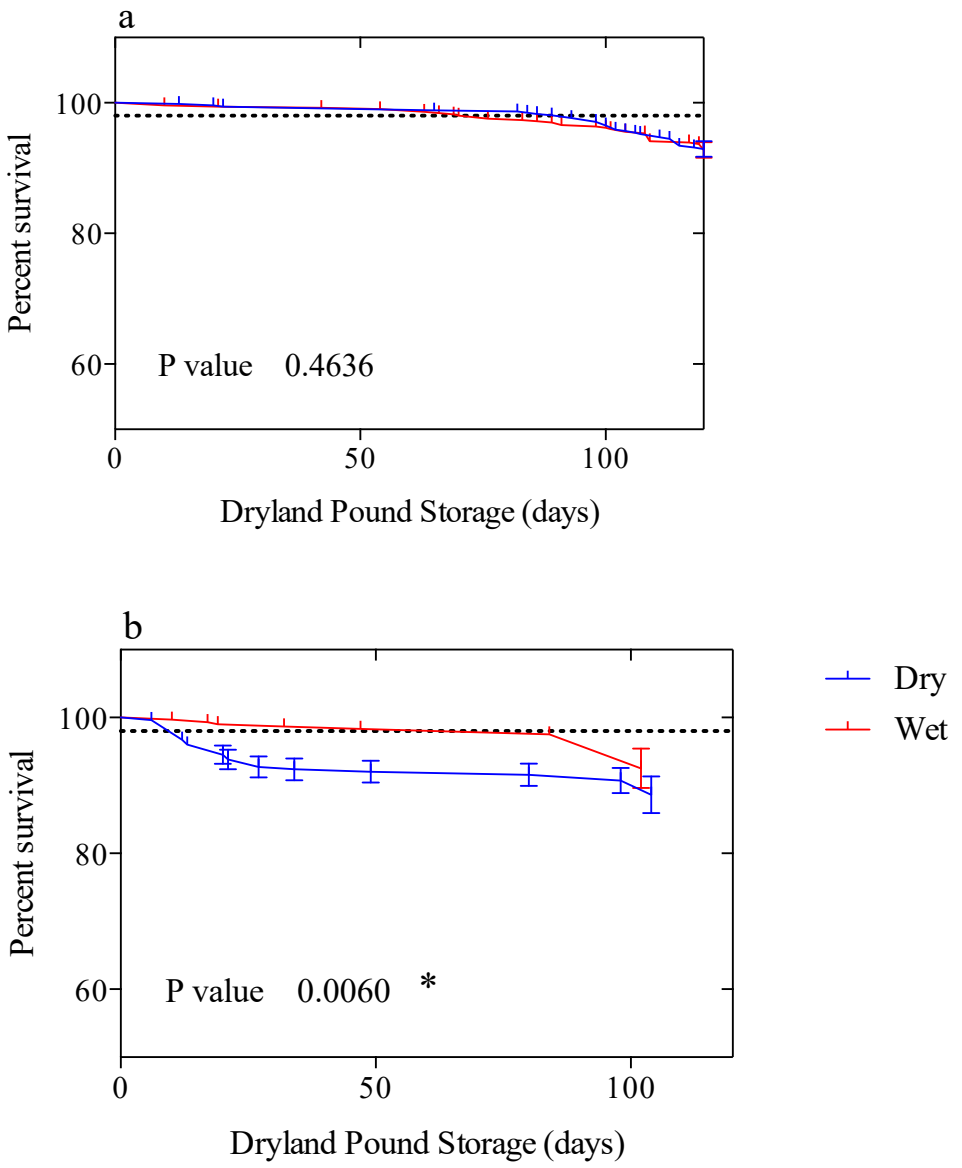

Figure 2.19 *Homarus americanus*: Kaplan-Meier survival curve of pre-treatment type versus DLP storage in days for Winter (Figure 2.19a) and Spring (Figure 2.19b) season lobsters. Pre-treatment groups consist of immediately sampled (Dry) and 48 h immersion (Wet). Percent survival curves are shown with SEM bars. Dashed horizontal line represents 2% cumulative mortality level. n = 576 per season . Log-rank (Mantel-Cox) P values are indicated in each figure. * denotes significant differences between categories ($p < 0.05$).

The survival curves based on gender in the Winter and Spring season trials are shown in Figure 2.20a and b respectively. There was a significant difference in survival of males compared with females in the Winter season trials ($p = 0.0001$) but no significant gender-related difference was observed in the Spring season trial ($p = 0.4155$). The Mantel-

Haenszel hazard ratio indicated that female mortality rate was 2.24 times higher than that of the males in the Winter season trial.

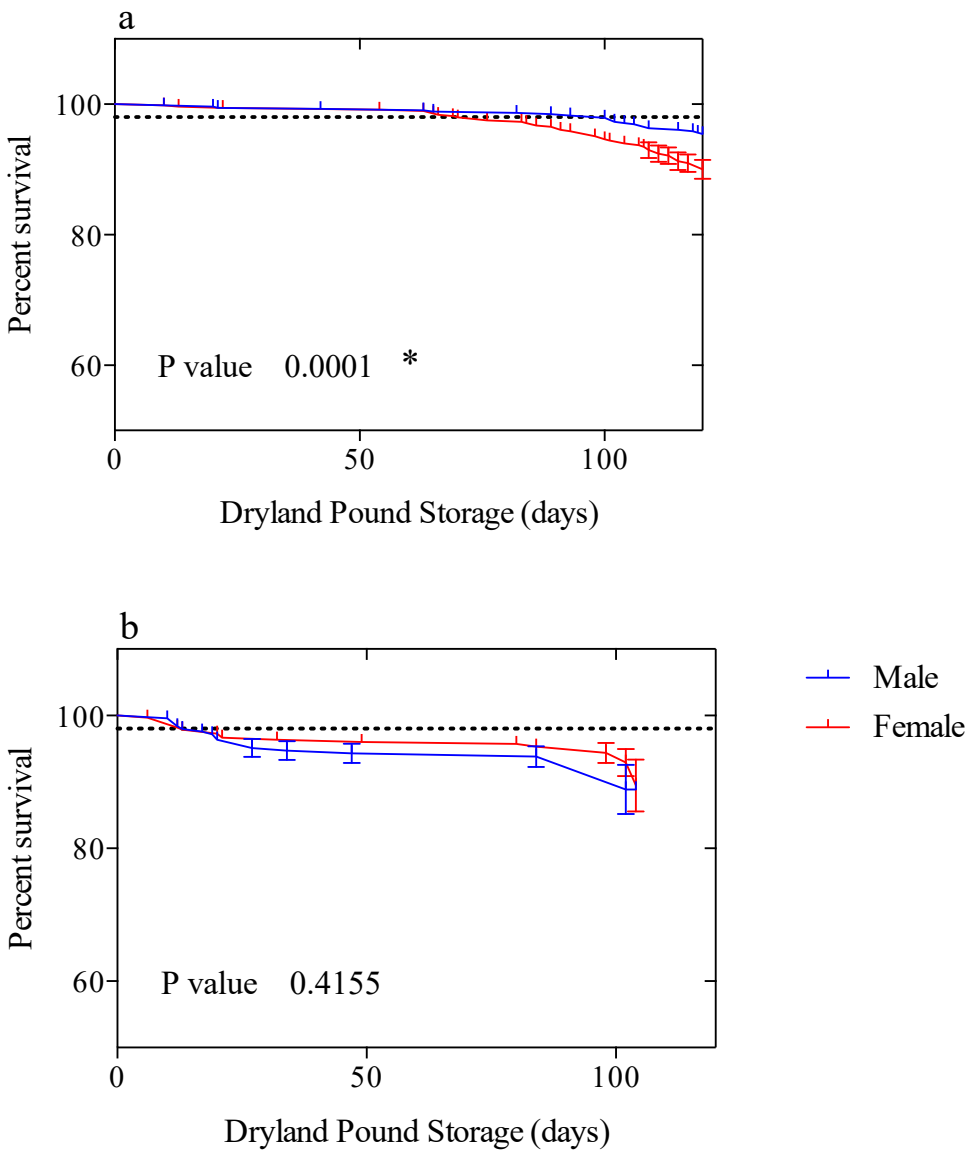

Figure 2.20 *Homarus americanus*: Kaplan-Meier survival curve of Sex (Male & Female) versus DLP storage in days for Winter (Figure 2.20a) and Spring (Figure 2.20b) season lobsters. Percent survival curves are shown with SEM bars. Dashed horizontal line represents 2% cumulative mortality level. n = 576 per season . Log-rank (Mantel-Cox) P values are indicated in each figure. * denotes significant differences between categories ($p < 0.05$).

2.5 Discussion

Not all landed *H. americanus* are able to withstand the rigours of post-harvest handling and long-term storage. Intrinsic, natural conditions, mainly related to moult stage, play a major part in establishing the nutritional state and biological condition—hence commercial value—of the quality of a freshly-landed lobster. Some of these biological factors require direct measures of moult stage and exoskeleton hardness to provide estimations of moult stage (Aiken, 1973; Grubert et al., 2012), or measurements of haemolymph total protein (Ozbay and Riley, 2002; Stewart et al., 1967), haemocyanin (Morris and Oliver, 1999b), and muscle and hepatopancreas tissue proportions (Neil, 2012; Stewart et al., 1967) as estimates of nutritional indices.

In addition to these biological factors, environmental variables also play a significant role in the determination of the success of post-harvest handling practices. These factors have the effect of either preserving or reducing the amount of time lobsters can be held out of water or stored until demand in the market increases, and they include environmental measures (e.g. temperature, dissolved oxygen and salinity) for immersed animals and temperature, humidity and handling for emersed animals. Some physiological measures can be used also to determine whether lobsters have received inappropriate post-harvest conditions in their recent past. For example, crustaceans held emersed or immersed under low oxygen conditions will show signs of haemolymph acidosis (deFur and McMahon, 1984; Taylor and Whiteley, 1989; Tyler-Jones and Taylor, 1988). Also, the onset of anaerobic metabolism elicits an increase in the concentration of haemolymph lactate (Hervant et al., 1999; Ocampo, 2003) and environmental salinity changes may elicit changes in haemolymph osmolality (Van Horn and Tolley, 2009). These metabolic events are reversed when ambient conditions are re-established, although recovery rates will vary depending on physiological variable and circumstances. For example, emersion-induced, recovered haemolymph ammonia levels return within minutes of re-immersion

(Danford et al., 2002), whereas the increased haemolymph lactate levels may take several days to return to pre-emersion values (Barrento et al., 2010; Bernasconi and Uglow, 2008b; Lorenzon et al., 2007).

In the present study, various risk factors were selected with the purpose of estimating initial quality of post-harvested *H. americanus* or to identify cases of mishandling or environmental condition abuse occurring prior to arrival to the storage facility. The resulting data were used to determine whether there was a causal link between factor values and successful long-term (120 day) storage and, therefore, whether such assessments provided a possible pre-screening measure of the commercial quality of incoming shipments of lobsters. The objective of this study was to ascertain the means to enable the identification of animals at risk of dying during long-term storage and thus to reduce such losses by using these higher risk animals to supply other, short-term market requirements. All animals that were selected for this study met the criteria of a high quality animal based on vigour and condition index and, therefore, were perfectly suitable for the live trade supply chain. However, not all animals that meet the highest quality criteria when freshly-landed are able to maintain that level of quality during protracted storage and it is the identification of those with the most potential for long term storage that is of particular commercial value and the main goal of this study.

The risk factors studied here showed differences in their effectiveness of predicting storage success with season. In the Winter season trial, haemolymph total protein, % Brix and, to a lesser degree, haemocyanin concentration were all factors identified as potentially strong indicators of long-term storage success, with factor concentration directly related to decreased long-term storage mortality in each case. Haemolymph lactate concentration was also shown to have a significant effect on the outcome of long-term storage in both the Winter and Spring trials, with lactate levels directly and

positively related to increased storage time mortality rates. The effect, however, was more pronounced in the Spring season in which low lactate levels had good overall survival for the duration of the trial whereas unacceptably high mortality rates were observed early on in the Spring trial with the mid (2 – 4 mml l^{-1}) and high (>4 mml l^{-1}) lactate groups. These findings suggest that should lactate levels have become elevated prior to receipt at the storage facility, this may have caused deleterious, non-recoverable damage to the lobsters that may have resulted in elevated numbers of mortalities after as little as 15 to 20 days of storage.

Haemolymph osmolality, predicted to be associated with exposure to salinities above or below the suitable range for *H. americanus* (Van Horn and Tolley, 2009), did not show any significant relationship with storage success. This may be due to not having received any animals that had been subjected to salinity stress because the prior history of the animals at the start of the trial was not known - other than controlling for source to reduce the variability of results due to different handling regimes and environmental conditions with different lobster suppliers.

Gender-related differences of survival outcome were significant in the Winter trial, but not in the Spring one. In the Winter trial, female lobsters had a 2.24-fold higher mortality rate than males. It is not known why this difference should occur and further trials will be made because the significance of this outcome that, if verified, represents a significant financial loss during storage that currently is not accounted for at lobster storage facilities.

Pre-treatment recovery type showed significant differences in the Spring but not the Winter trials. In the Spring trial, lobsters that did not receive the 48-hour immersion recovery period on arrival at the facilities (Dry) had a 2.6-fold higher rate of mortality compared with the recovered, Wet group. Spring trial lobsters were harvested from warm seawater compared with those in the Winter trial, and these lobsters are at a higher risk

of being subjected to adverse post-harvest conditions of prolonged, elevated emersion temperatures due to holding conditions on the boats during the harvest. The standard spring harvest fishing vessel is not equipped with adequate immersed storage on board, and trap caught lobsters are either held emersed in warm spring air temperatures that are typically warmer than the seawater temperature on the bottom from which they were harvested, or the boats will have rudimentary live wells to keep lobsters immersed with flowing water that rapidly becomes fouled with elevated ammonia levels and depleted of dissolved oxygen. The water for the boat's live wells is sourced from the sea surface and is typically at a higher temperature than the ocean bottom seawater temperature as well as being at risk of reduced salinity due to rain water floating at the sea surface. The likelihood of exposure to these adverse conditions in the spring-harvested catch is high. Consequently, a post-arrival recovery phase comprising 48 h of immersion prior to subsequent put away activities that included handling during size and quality grading, may have given lobsters the renewed tolerance of these further handling steps. Presumably the 48 hour immersion step allowed the lobsters to reduce elevated levels of haemolymph ammonia and lactate and to consume an unrestricted oxygen supply and allow for recovery prior to further handling within the facility.

Finally, moult stage had a significant effect on survival in the Winter trial but not in the Spring one. The Winter, intermoult lobsters had a significantly higher mortality rate than both the early and active pre-moult group lobsters. Possibly this is due to the intermoult lobsters in the Winter trial being comprised of mostly early intermoult animals, or perhaps at a stage of post-moult (metecdysis) in which the shells are mostly hardened but the lobster tissue has not fully established in the new, larger shell. Pleopod staging is not able to distinguish metecdysis from anecdysis, since more parameters must be considered for this stage, including exoskeleton hardness and muscle tissue concentration. Muscle tissue concentration is correlated with haemolymph total protein concentration (Stewart et al.,

1967), therefore the additional information on haemolymph total protein proved to be more useful as a risk factor predictor of storage success during the winter season when the harvest may contain a mixture of post-moult and intermoult lobsters. Based on the haemolymph total protein level distribution within the Winter group, which was at the lower end of the range of total protein at this time of year, and having already established earlier that haemolymph total protein was highly significant in its effect on Winter storage mortality, it is likely that the higher risk lobsters with low blood protein were in post-moult or very early intermoult stages. Within the intermoult (C_4) stage from the data collected for this study there was a range of haemolymph total protein that included the lowest recorded values of < 25 mg/ml overall. Theriault et al. (2008) found that intermoult *H. americanus* from the winter fishery were at a higher risk of developing impoundment shell disease during long term storage, which supports the present study outcome that these lobsters do not have the capacity to withstand commercial storage. The absence of the influence of moult on mortality in the Spring trial could be explained by the moult-stage composition of lobsters in this trial, which comprised mainly early pre-moult animals. At the low Spring storage temperature of 1.5 ºC, suppression of the moult for the duration of the storage period would have occurred in lobsters at pleopod stages < 3.0 (Aiken, 1980). However, the timing of the moult will differ slightly each year based in seasonal variability, with the result that the average composition of active pre-moult lobsters in any month may also be an annual variable. It is expected that the active pre-moult group would have a higher risk of mortality during long-term storage despite the low storage temperatures. However, studies that can focus specifically on the survival rate of the active pre-moult lobster group will be required to confirm this.

In conclusion, the present studies reveal that the Winter and Spring seasons within the Canadian *H. americanus* fishery present different risk factors that can be used to predict fitness of catch for long term, immersed storage. Winter season results indicate that

moult-related risk factors such as pleopod stage, haemolymph total protein, % Brix and haemocyanin as well as exoskeleton hardness are effective measures to predict storage success. The Spring season results indicate that handling-related risk factors such as haemolymph lactate, osmolality and arrival pre-treatment with 48 hour immersion have significant influence on long term storage success, and the moult-related factors were not reliable predictors of storage outcome. Although there are a number of risk factors identified for each season's harvest that are possible predictors of post-harvest commercial storage success, there were strong correlations present between factors in some cases that would allow one to choose a preferred method based on their capacity to conduct the risk factor analyses. For example, haemolymph % Brix was strongly correlated with haemolymph total protein and haemocyanin, both of which require more sophisticated equipment and training to assess compared to haemolymph % Brix. The present findings indicate that the monitoring of a carefully-selected suite of physiological variables relating to freshly-harvested lobsters offer a means by which the risks associated with quality (hence commercial) losses during post harvest activities such as long term immersed storage can be predicted and evaluated. Based on the present findings, it would be beneficial for the commercial trade to adapt a carefully selected set of risk factors to assess incoming post-harvest lobsters in order to prevent the losses that can occur from long term storage. Such a sampling program would need to be adjusted based on the season of the landed catch, and in some cases such as in the spring fishery, the results may be an effective tool in evaluating harvest handling and storage practices that lead to a lobster with a higher risk of post-harvest mortality.

Chapter 3
The effects of commercially-mediated, acute temperature changes on the lobster, *Homarus americanus* and their downstream implications: changes in the emersed lobster

[Θ]Part of this study was given as an oral contribution to the conference: International Conference and Workshop on Lobster Biology and Management, Bergen, Norway 2011.

3.1 Introduction

Although the lobster *Homarus americanus* is predominantly a subtidal species, it possesses the ability to live emersed for extended periods of time, as generally observed in intertidal species that are routinely exposed to air during low tide or subtidal species that may be exposed to hypoxic conditions (Bridges and Brand, 1980; Airriess and McMahon, 1994; Grieshaber et al., 1994; Schmitt and Uglow, 1998). The effects of emersion on crustaceans have been extensively studied and the physiological adjustments involved are well known (Airriess and McMahon, 1994; Danford et al., 1999; Lorenzon et al., 2007; Morris and Oliver, 1999a; Taylor and Whiteley, 1987). During emersion the lobster gill lamellae collapse which reduces the exposed surface area available and increases diffusion distances for gas and ion exchange and subsequently leads to impairment of gill function. This impairment results in an internal hypoxia and a shift from aerobic to anaerobic metabolism in which byproducts such as ammonia, carbon dioxide and lactate increase in the haemolymph. These changes affect haemolymph acid-base regulation and bring about an increase in haemocyanin oxygen affinity (Danford et al., 2002).

The reaction rates of the responses to emersion are dependent on body temperature (Whiteley and Taylor, 1990; Barrento et al., 2011). Being an ectothermic species, the body temperature of an immersed lobster closely matches that of its surrounding seawater and an acute change to the seawater temperature during immersion causes a similar acute change to the lobster's body temperature. However, when a lobster is emersed, the effects of environmental (air) temperature change on its body temperature are reliant on the rate

of latent heat transfer and are thus slower to occur because of the lower associated heat-exchange thermodynamics compared with the transbranchial heat exchange system that operates during immersion (Whiteley et al., 1995).

The rates of body temperature adjustments appear to be little known but, presumably, are directly dependent on the prevailing temperature that, for an immersed lobster, will be that of the ambient seawater. Temperature adjustment is probably more rapid in an immersed rather than an emersed lobster but the difference in adjustment rates remain to be tested in *H. americanus*, although studies at 10 ˚C to 20 ˚C range have been conducted by Whiteley et al. (1995) in the European lobster, *H. gammarus* in which it was found that an acute external temperature change was followed by a body temperature change in 6 minutes whilst immersed, but 1 – 2 h during emersion.

Improved knowledge of the effects that acute temperature changes have on immersed or emersed lobsters is valuable from both ecological and commercial perspectives. Although acute temperature change events of a magnitude of several degrees may be uncommon in the natural environment, they do occur frequently in the post-harvest environment. Lobsters, and shellfish generally, are expensive food items that are traded alive in supply chains that are complex and may be prolonged in terms of both distance and duration. The commercial objective is to deliver live products consistently and cost-effectively and this entails the application of husbandry procedures that include temperature manipulation (Gomez-Jimenez et al., 2001).

3.2 Aims and Objectives

The present study was designed to evaluate the responses of immersed and emersed lobsters to measured, acute changes of temperature commonly used in the post-harvest environment of lobsters in Canada and as evidenced by alterations to a suite of haemolymph variables that are acknowledged to reflect the animals' metabolic responses and, more subjectively, using a scale that represents the general vigour of the animals.

Previous studies, in which different emersion temperatures have been compared using *H. americanus* (Lorenzon et al., 2007; Qadri et al., 2007), were made at temperatures outside that of the standard range used in the Canadian lobster fishery. The two pre-treatment temperatures (2.6 ˚C and 5.6 ˚C) used here represent actual environmental condition ranges within commercial Canadian lobster holding systems. The lower temperature is a typical winter season mechanical refrigeration control system temperature and the higher temperature is a typical winter season ambient seawater flow-through system temperature. Both of these temperatures are used within various facilities and a landed lobster may be subjected to one or both of these, when immersed and emersed, during typical commercial activities to get lobsters from the shore to the end consumer. Two lobster sizes were evaluated to determine whether a known size-dependent rates of oxygen consumption in crustaceans (Bridges and Brand, 1980; Crear and Forteath, 2000) would have a measureable effect on the factors measured during this emersion trial. The two sizes represent the smallest legal size lobster from the Canadian fishery (Small Chix weighing 484 ± 26 g) and a typical medium size (Halves at approximately 706 ± 30 g) used for export to premium Asian markets in China and Japan. Such information could be useful for determining whether there are size-specific emersion tolerance considerations for the successful long duration, emersed shipment of lobsters to foreign markets.

3.3 Materials and Methods

3.3.1 Animal Husbandry

Lobsters were selected from a single source, harvested from a trap fishery located in lobster fishing area 34 in southwest Nova Scotia, Canada and landed in West Pubnico (Figure 3.1). From the point of landing, lobsters were shipped to Bedford, Nova Scotia

where the storage and emersion trials were conducted. Harvest and post-harvest handling and storage procedures are described in Chapter 2 .

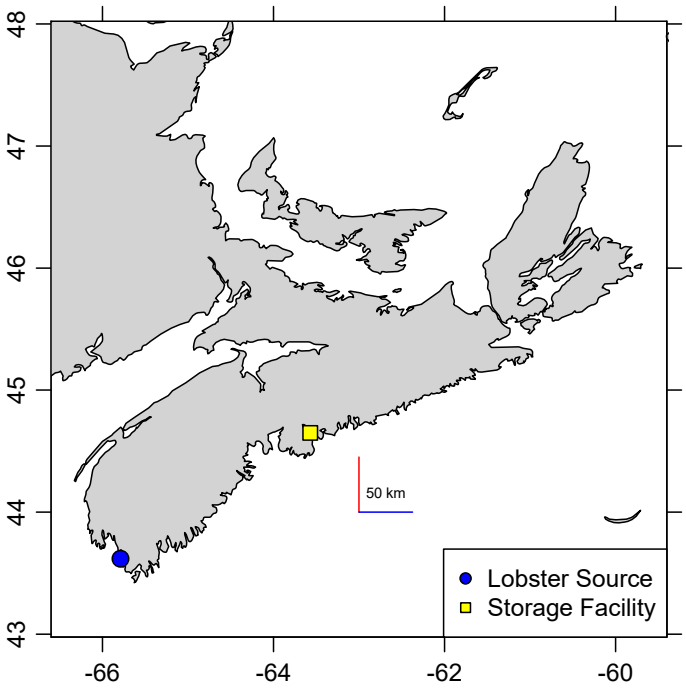

Figure 3.1 Lobster source from a port in Lobster Fishing Area 34 highlighted (•) on the map along the southwestern coast of Nova Scotia, Canada as well as the commercial facilities in which the storage and emersion trials were conducted (□). One degree of vertical latitude = 111 km. Map made with R version 3.1.2 and Natural Earth. Free vector and raster map data @ naturalearthdata.com.

Lobsters selected for this trial were held immersed for 2 weeks prior to the start of the emersion trial in a pre-treatment system set at one or other of the two temperatures (2.6 ˚C and 5.6 ˚C) to ensure adequate temperature acclimation. The lobsters were not fed during this time in order to reduce adverse emersion performance effects attributable to feeding (McGaw, 2005; McGaw et al., 2009).

3.3.2 Simulated Consignment Emersion Trials

Two sizes and two temperature treatments were selected for comparisons. The two sizes consisted of commercially relevant, small- and medium- sized lobsters known as Small Chix and Halves, respectively. The Small Chix had a mean weight of 484 ± 26 g and the Halves had a mean weight of 706 ± 30 g. The two temperature treatments used were 2.6

˚C and 5.6 ˚C, which are considered as accepted levels within the lower and upper range of industry-relevant temperatures encountered during post-harvest handling, storage and shipping of live *H. americanus*. Six emersed group sample times were used—0 h (Control), 24, 48, 60, 72 and 96 h and three post-emersion, re-immersed sample times (12, 24 and 48h re-immersion) from each of the two emersed groups of 48 and 72h. In order to minimise sampling error associated with handling (Jussila et al., 1999; Taylor and Whiteley, 1989) and temperature fluctuations during the sampling procedures, each sampling occasion had its own associated box of lobsters from which to sample. Eight samples were selected from each of the two sizes and two temperature groups on each sampling occasion from an unopened box, and selected lobsters were only sampled once during the trial. This necessitated packing 4 boxes of lobsters for each sample period (one for each of two test temperatures for each of two size classes) for a total of 48 packed boxes and 384 sampled lobsters (n = 32 controls at T = 0, n = 160 during emersion and n = 192 during re-immersion).

Lobsters were sampled at T = 0 h as pre-emersion controls for each of the two temperature treatments and size classes (n = 32). Packed boxes were maintained at an air temperature of 3 ˚C ± 0.5 ˚C in a temperature-controlled cooler. As a precaution against desiccation, lobsters were packed in sealed polystyrene boxes with seawater-soaked paper absorbent pads placed over the top of the lobsters and four 900g gel-ice packs placed over top of the wet pads prior to securing the polystyrene lid (Figure 3.2).

Figure 3.2 Commercial consignment shipping system for *Homarus americanus* that was used for the present study emersion trial. The box consists of an inner polystyrene box with segregated vertical storage dividers. Lobsters are packed vertically on their tails and a seawater soaked absorbent paper pad placed over top of the claws followed by two 900 g gel ice packs designed to maintain lobster body temperature.

At the end of each of two standard commercial trial durations (T=48 and 72 h), 3 groups of 8 lobsters were re-immersed for each of 12, 24 and 48 h in an industry standard, temperature-controlled reception reservoir with seawater maintained at 3.0 ± 0.2 ˚C. Reservoir water quality was maintained with an adequate supply of filtered, new seawater that maintained immersion seawater total ammonia below 5 µml l^{-1}, pH at 7.8 ± 0.2 and dissolved oxygen > 90% saturation. Figure 3.3 displays a summary of the trial protocol.

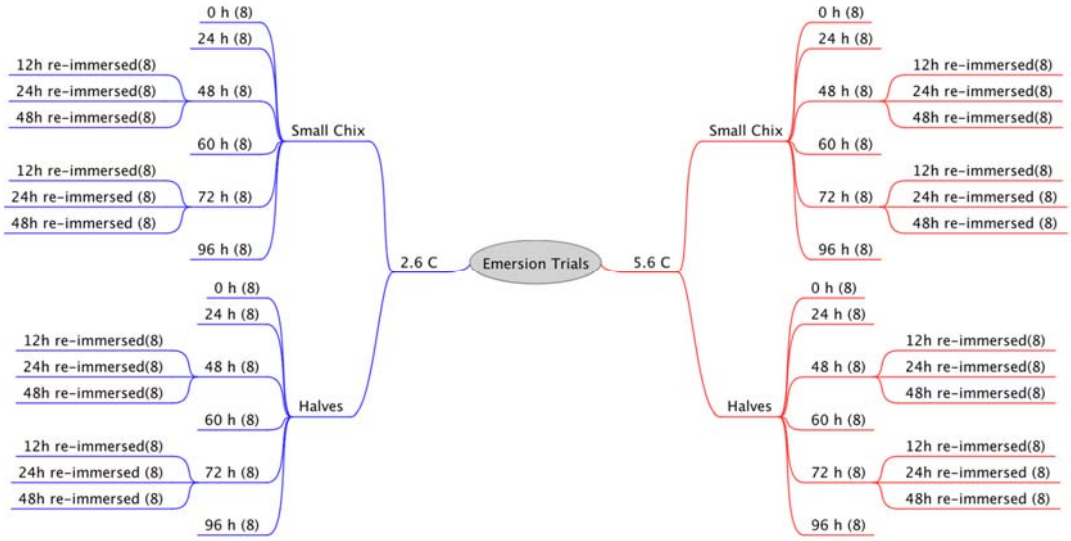

Figure 3.3 Emersion trial plan diagram for *Homarus americanus* emersion trials at two pre-acclimation temperatures (2.6 and 5.6 C). Groups of n=8 lobsters for each of two size classes (Small Chix and Halves) were held emersed for each of 6 prescribed times between 0 h (control) and 96 h. Groups of n=8 lobsters emersed for 48 h and 72 h were re-immersed at 3 C and sampled at 12, 24 and 48 h post emersion. Each sample n in parentheses (n) indicates a group of lobsters (sampled only once) in the trial.

Once each group of eight lobsters had been sampled at each time point they were immersed in 45kg capacity lobster crates and returned to the production plant for commercial use.

3.3.3 Haemolymph Chemistry

At each sample time (T = 0, 24, 48, 60, 72, 96 h emersed and T = 12, 24 and 48 h re-immersed for both the 48 h and 72 h emersed groups), 2 - 2.5 mL of haemolymph were taken from the prebranchial sinus via the arthrodial membrane at the base of the 4th periopod from each of the n = 8 animals sampled for each temperature and size group. 500 µL aliquots of each haemolymph sample were diluted 1:1 with 500 µL of ultra-pure water for ammonia analyses and 500 µL of haemolymph were deproteinised with 500 µL of 6% perchloric acid for glucose and lactate concentration assays. The remaining whole haemolymph sample was assayed for pH using a Fisher Scientific Acumet semi micro combination pH electrode (Thermo Fisher Scientific Inc., MA, USA). All haemolymph sample pH assays were processed within 1 minute of collection in order to avoid excessive CO_2 flux with aerial exposure. Haemolymph temperature within the lobster

was also recorded within one minute of haemolymph collection using a 36 gauge type K piercing thermocouple probe (Omega Engineering, Canada) inserted into the prebranchial sinus via the arthrodial membrane at the base of the 4th periopod and this measurement was used to represent core body temperature. Vigour index was also judged at the time of sampling (General Materials and Methods Chapter 2). Only obvious undamaged, strong lobsters (Vigour Index 1) were selected and packed for the emersion box trials at the start of the experiments.

3.3.3.1 Total Haemolymph Ammonia (NH_4)

Total haemolymph ammonia (NH_4) was measured using a flow injection/gas diffusion (FIGD) system capable of measuring haemolymph sample volumes as low as 250 μL, as described in Hunter and Uglow (1993). Linear correlation of the standard peak heights was always very high ($R^2 > 0.97$). Because of the high clotting properties of lobster haemolymph, standards of known ammonia concentrations were run prior to, and immediately after, each run of a maximum of 32 samples to verify that the performance of the solutions and gas exchange membrane remained consistent throughout the sample injection analysis period.

In this study, the DC voltage signal output from the FIGD system was read by a Powerlab data acquisition system (AD Instruments) and converted to a digital trace that was analysed by LabChart, the accompanying software for the Powerlab. Analysis consisted of performing automated peak analysis on both standard and sample peak traces to extract the maximum peak heights from each injection. These data were then inputted into GraphPad Prism statistical software (GraphPad Software, La Jolla California, USA) in which a linear regression equation was created from the standards and applied to the haemolymph samples.

3.3.3.2 Haemolymph Lactate

Haemolymph lactate concentration was measured from deproteinised haemolymph using an L-Lactate colourimetric analysis kit (catalogue number 735-10, Trinity Biotech) and analysed using a Novaspec Plus spectrophotometer (Biochrom US, Holliston, MA, USA) with % absorbance set to 540 nm. The analysis kit reagent powder is comprised of lactate oxidase enzyme to convert lactic acid to pyruvate and hydrogen peroxide (H_2O_2). The oxidative condensation of chromogen precursors that are included in the reagent are oxidized by H_2O_2 to produce a coloured dye with an absorption maximum at 540 nm. Lactate levels in the samples correlate linearly with these absorption readings.

The lactate reagent powder was reconstituted with 10 ml of Milli-Q™ water in the provided reagent vial, gently inverting until fully dissolved. Reconstituted reagent was mixed within 1 hour of use, and is stable for up to 8 hours at room temperature. 1.0 ml of reagent solution was pipetted into individually labelled test tubes, one for each sample, as well as a blank and a standard. To the standard, 10 µl of lactate standard solution (40 mg/dl) was added. To the samples, 10 µl of deproteinised haemolymph was added. The test tubes were left to incubate at 25 ℃ for 10 minutes. Absorbance of the standard and sample solutions were then read with the blank as a reference at 540 nm. Readings were completed within 10 minutes following incubation as instructed by the test kit. Lactate concentration of the samples was calculated using the following equation:

$$Lactate\ (\text{mmol}\ l^{-1}) = \frac{Absorbance\ of\ Sample}{Absorbance\ of\ Standard}\ x\ 40\ x\ 0.111$$

The multiplication factor of 0.111 in the above equation was used to convert the standard from mg/dl to mmol l^{-1}.

3.3.3.3 Haemolymph pH

Lobster haemolymph pH was measured with a semi-micro combination pH electrode (Acumet, Fisher Scientific) and handheld automatic temperature compensated pH meter (Orion model 890, Thermo Fisher Scientific, Canada). Extracted haemolymph was measured for pH within 20 seconds of sampling to avoid excessive gas exchange of the haemolymph that could affect the reading results. The pH meter was calibrated between the range of pH 4.0 and 7.0 to ensure accuracy of *H. americanus* haemolymph readings that have previously been observed within this range (Stewart et al., 1966).

3.3.3.4 Haemolymph Glucose

D-Glucose was measured from deproteinised lobster haemolymph using a Sigma colourimetric glucose assay kit (GAGO-20, Sigma Aldrich) and analysed using a Novaspec Plus spectrophotometer with % absorbance set to 540 nm. The assay principle includes the following three reactions:

1. $$D-Glucose + H_2O + O_2 \xrightarrow{\substack{Glucose \\ Oxidase}} D-Gluconic\ Acid + H_2O_2$$

2. $$H_2O_2 + \underset{(colourless)}{\underset{Reduced}{o-Dianisidine}} \xrightarrow{Peroxidase} \underset{(brown)}{\underset{Oxidized}{o-Dianisidine}}$$

3. $$\underset{(brown)}{Oxidized\ o-Dianisidine} \xrightarrow{H_2SO_4} \underset{(pink)}{Oxidised\ o-Dianisidine}$$

Glucose oxidase and peroxidase enzymes are used to cause a series of oxidation reactions, the end product being a stable, coloured liquid. The liquid's colour is linearly proportional to the original glucose concentration in the haemolymph sample. In practical terms as per

the Sigma GAGO test kit instructions, a Glucose Oxidase/Peroxidase reagent capsule (G-3660, Sigma Aldrich) was dissolved in 39.2 ml of Milli-Q™ water. A pre-weighed, 5 mg vial of o-Dianisidine reagent (D-2769, Sigma Aldrich) was reconstituted with 1.0 ml of Milli-Q™ water, inverting several times to dissolve the reagent powder. 0.8 ml of the reconstituted o-Dianisidine is combined with the 39.2 ml of Glucose Oxidase/Peroxidase reagent. The resulting assay reagent was stored refrigerated (2-4 ℃) in darkness until use, but no longer than 1 week. The solution is stable for up to 1 month refrigerated.

To determine glucose concentration of the extracted haemolymph samples, 500 µl of the deproteinised haemolymph was added to 1 ml of the assay reagent in a test tube and mixed thoroughly. The samples were incubated in a 37 ℃ water bath for exactly 30 minutes, after which the reaction was stopped abruptly by the addition of 1 ml of 12M H_2SO_4, causing the samples to turn pink (as described in equation 3 above). The absorbance of the pink samples was measured at 540 nm using a Novaspec Plus spectrophotometer (Biochrom US, Holliston, MA, USA) against a distilled water blank and an 0.05 mg/ml glucose standard. Sample glucose concentrations were calculated with the following equation:

$$mg\ Glucose = \frac{(\Delta A540\ of\ Test)\ (0.05\ )}{\Delta A540\ of\ Standard}$$

3.3.4 Statistical Analysis

Haemolymph chemistry data were tested for normality using the D'Agostino & Pearson omnibus normality test (alpha = 0.05). Data that did not pass this normality test were log transformed to obtain normality. The data were then analysed using one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparisons test for data being compared to the T = 0 h control (emersion + re-immersion trials) and Tukey's multiple comparisons test for data compared to every other time in each treatment group (0 – 96 h

emersion trials). Reported *p* values are adjusted for multiplicity for each comparison with a family-wise significance and confidence level of p = 0.05 (95% Confidence Interval). Within-trial treatments were compared using linear regression for each temperature group with comparisons of slopes conducted using linear regression covariance comparison tests against pooled data with significance set at 95% confidence intervals (p = 0.05) or 2-way ANOVA with interactions between emersion + re-immersion time and either size or pre-treatment acclimation temperature. Bonferroni's multiple comparisons test was used when significant ($p < 0.05$) interactions were observed. Multiplicity-adjusted P values were used for each comparison with the family–wise significance and confidence level set at 0.05. Unless specifically stated in the text, no significant differences were found within the control groups (T = 0 h).

3.4 Results

3.4.1 96h Emersion

3.4.1.1 Haemolymph Ammonia

Within both the low (2.6 ˚C) and high (5.6 ˚C) acclimation temperatures, both the small (Chix) and large (Halves) size lobsters had a similar trend of increasing haemolymph ammonia concentration with duration of emersion (Figure 3.4a,b). Significant differences ($p < 0.001$) from the controls (T = 0) occurred at 24 h in both the 2.6 ˚C and 5.6 ˚C acclimation temperature treatments for both the small and large size lobsters. In the 2.6 ˚C acclimation temperature treatment trial there was no significant difference between the two sizes until 96 h emersion whereas at no time period were there significant differences between the two sizes in the high acclimation temperature treatment trial.

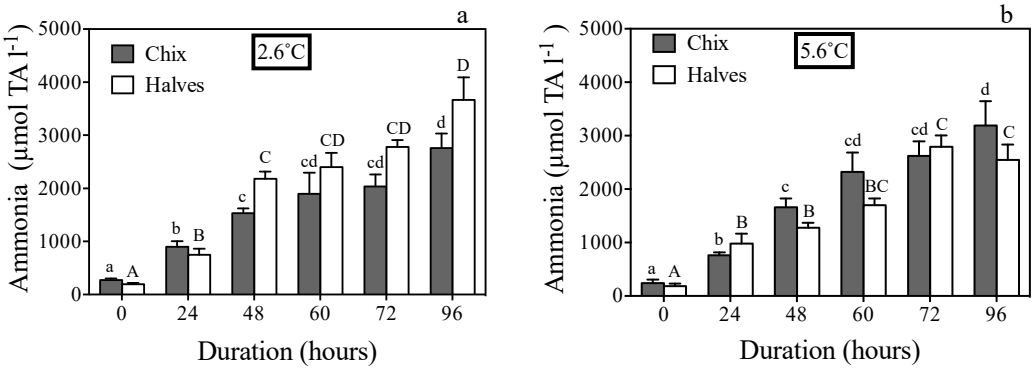

Figure 3.4a,b *Homarus americanus*: Changes to haemolymph total ammonia of small (Chix) and large (Halves) size lobsters after 0, 24, 48, 60, 72 and 96h emersion at 3.0 ˚C. Figure 3.4a represents cold (2.6 ˚C) acclimated lobsters and Figure 3.4b represents warm (5.6 ˚C) acclimated lobsters. Values represent means ± SEM of n = 8 animals. Different letters above the bars (a,b,c,d & A,B,C,D) represent significant differences between different letters of the same case (p < 0.05). n = 192 total.

Observing the data from the two size groups within a single temperature treatment, the small (Chix) size lobsters showed a similar trend of increasing haemolymph ammonia within the 2.6 ˚C and 5.6 ˚C acclimated animals (Figure 3.5a,b) whereas the large (Halves) size lobsters showed a slightly elevated level of haemolymph ammonia in the cold group versus the warm group, although neither of the intra-temperature treatment groups were significantly different at each measurement time (p > 0.05).

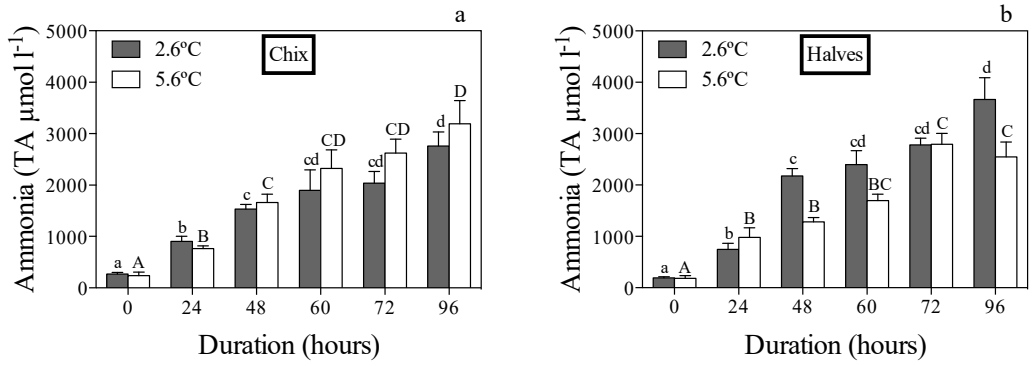

Figure 3.5a,b *Homarus americanus*: Changes to haemolymph total ammonia at two lobster pre-acclimation temperatures of 2.6 ˚C and 5.6 ˚C after 0, 24, 48, 60, 72 and 96h emersion at 3.0 ˚C. Figure 3.5a represents small (Chix) size lobsters and Figure 3.5b represents large (Halves) size lobsters. Values represent means +/- SEM of n = 8 animals. Different letters above the bars (a,b,c,d & A,B,C,D) represent significant differences between different letters of the same case (p < 0.05). n = 192 total.

Linear regression of the inter-size haemolymph ammonia comparisons at two temperatures reveal a difference in slopes by temperature for both the small and large size

groups of lobsters, but each with opposite trend results with respect to temperature treatment (Figure 3.6a,b). The smaller sized Chix lobsters had a steeper slope of increasing haemolymph ammonia in the 2.6 ˚C (compared with the 5.6 ˚C acclimated group), but the difference was not significant (p = 0.1152). The larger-sized Halves lobsters had a significantly steeper slope of increasing haemolymph ammonia in the 2.6 ˚C compared with the 5.6 ˚C acclimated group (p = 0.0084).

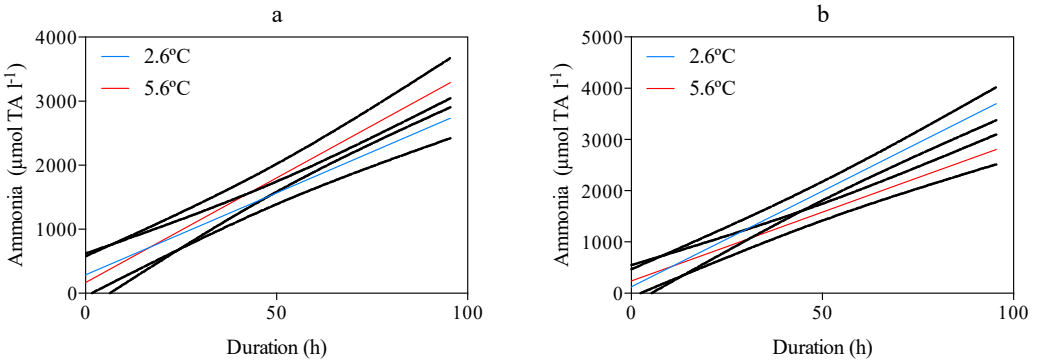

Figure 3.6a,b *Homarus americanus*: linear regression trend of haemolymph ammonia concentration increase with emersion time during 0 to 96h emersion at 3.0 ˚C at two pre-trial acclimation temperatures. Figure 3.6a represents a group of small lobsters (Chix) and Figure 3.6b represents a group of large lobsters (Halves). Linear regression lines are coloured corresponding to their pre-acclimation treatment temperature for n = 8 animals per group. Black dashed lines indicate 95% confidence intervals. n = 192 animals total.

3.4.1.2 Haemolymph Lactate

In both sizes and both temperature treatments there was a general trend of increasing haemolymph lactate concentration with increased emersion duration (Figure 3.7a,b). In the small size group (Chix) at the 2.6 ˚C temperature treatment, mean haemolymph lactate levels increased significantly above control values after 48 h (p = 0.0060), then decreased slightly at 60 h before continuing to increase until the end of the emersion trial at 96 h. Haemolymph lactate concentration increased in the large size group (Halves) during the 96 h emersion period, although absolute values were consistently lower in this size group relative to the small size group at the same treatment temperature. This was also the case in the 5.6 ˚C temperature treatment trial (Figure 3.7b) although differences in

concentrations from the control were statistically significant only at the 72 h emersion time (p = 0.0057). The increase in lactate concentration in the 2.6 ˚C temperature treatment trial became significant at 60 h for the large group (p = 0.0002) compared to 48 h for the small group as mentioned earlier.

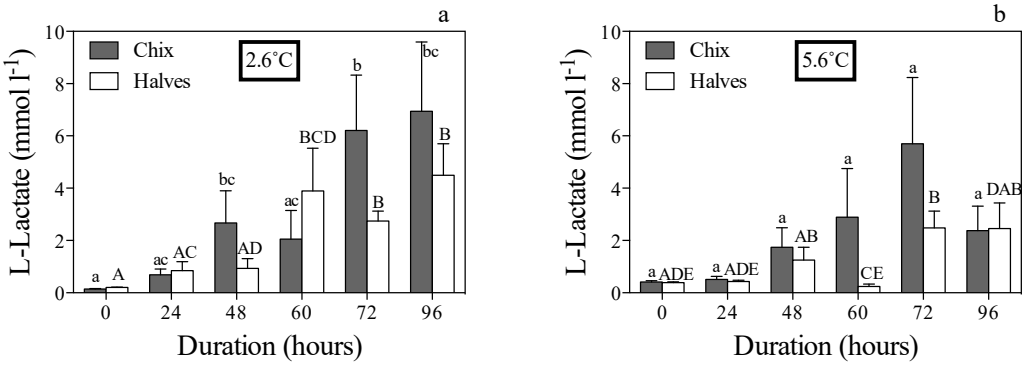

Figure 3.7a,b *Homarus americanus*: Changes to haemolymph lactate of small (Chix) and large (Halves) size lobsters after 0, 24, 48, 60, 72 and 96h emersion at 3.0 ˚C. Figure 3.7a represents cold (2.6 ˚C) acclimated lobsters and Figure 3.7b represents warm (5.6 ˚C) acclimated lobsters. Values represent means ± SEM of n = 8 animals. Different letters above the bars (a,b,c,d & A,B,C,D) represent significant differences between different letters of the same case (p < 0.05). n = 192 total.

A comparison of temperature treatment groups for each size, as shown in Figure 3.8a,b, reveals a similar trend of increasing haemolymph lactate concentration with similar absolute values, although in the smaller size group there was a noticeable, but non-significant, reduction in lactate levels in the higher temperature group at 96 h emersion and a significant decrease from 48 h to 60 h in the large size group (p = 0095). Post T = 0 h (control), there was a high degree of within-group variability at each time point, as is suggested by the relative size of the SEM error bars in Figure 3.8a,b.

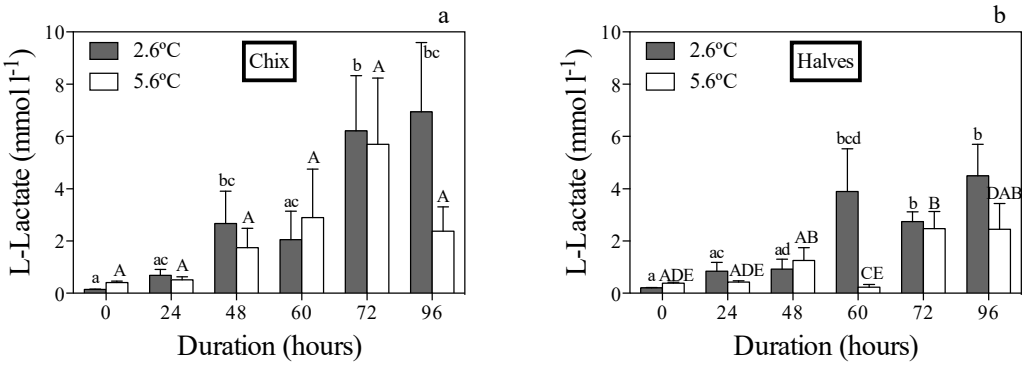

Figure 3.8a,b *Homarus americanus*: Changes to haemolymph lactate at two lobster pre-acclimation temperatures of 2.6 ˚C and 5.6 ˚C after 0, 24, 48, 60, 72 and 96h emersion at 3.0 ˚C. Figure 3.8a represents small (Chix) size lobsters and Figure 3.8b represents large (Halves) size lobsters. Values represent means ± SEM of n = 8 animals. Different letters above the bars (a,b,c,d & A,B,C,D) represent significant differences between different letters of the same case ($p < 0.05$). n = 192 total.

3.4.1.3 Haemolymph pH

The haemolymph pH at the 24 h emersion sampling time in the 2.6 ˚C acclimated Chix dropped, relative to the control value, from pH 8.1 to pH 7.3 (compared with a drop from pH 7.9 to pH 7.1 for the corresponding Halves). Haemolymph pH values then remained significantly lower than the control values throughout the sampling periods from 24 h to 96 h despite a slight but significant increase in between the 60 h and 72 h emersion period in both size groups in the 2.6 ˚C temperature treatment trial (Figure 3.9a). A similar decline in haemolymph pH from control values was shown by both size groups in the 5.6 ˚C acclimation treatment trials (Figure 3.9b) - although control haemolymph pH values were significantly lower in the 5.6 ˚C acclimated groups (compared with the colder, 2.6 ˚C, acclimated groups) (Figure 3.9b). In the 5.6 ˚C acclimated group trials, the haemolymph pH had increased significantly from the control ($p < 0.0001$) at the 60 h sampling period - earlier than described above for the 2.6 ˚C treatment trials, in which the comparable increase occurred at 72 h emersion (Figure 3.10a,b).

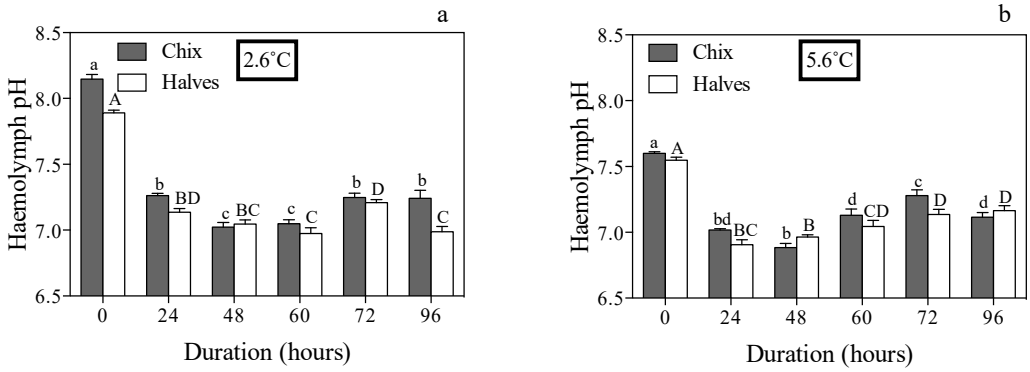

Figure 3.9a,b *Homarus americanus*: Changes to haemolymph pH of small (Chix) and large (Halves) size lobsters after 0, 24, 48, 60, 72 and 96 h emersion at 3.0 ˚C. Figure 3.9a represents cold (2.6 ˚C) acclimated lobsters and Figure 3.9b represents warm (5.6 ˚C) acclimated lobsters. Values represent means ± SEM for *n* = 8 animals. Different letters above the bars (a,b,c,d & A,B,C,D) represent significant differences between different letters of the same case ($p < 0.05$). *n* = 192 total.

Although control haemolymph pH values of the two temperature treatment groups differed, the relative trend of pH changes observed during emersion were similar for both sizes and for both temperature groups (Figure 3.10a,b). In all size and temperature treatment groups, significant changes from the relevant control groups of the haemolymph pH occurred at the first emersion sampling time of 24 h and mean values then remained below the relevant control values throughout the duration of the 96 h emersion trial.

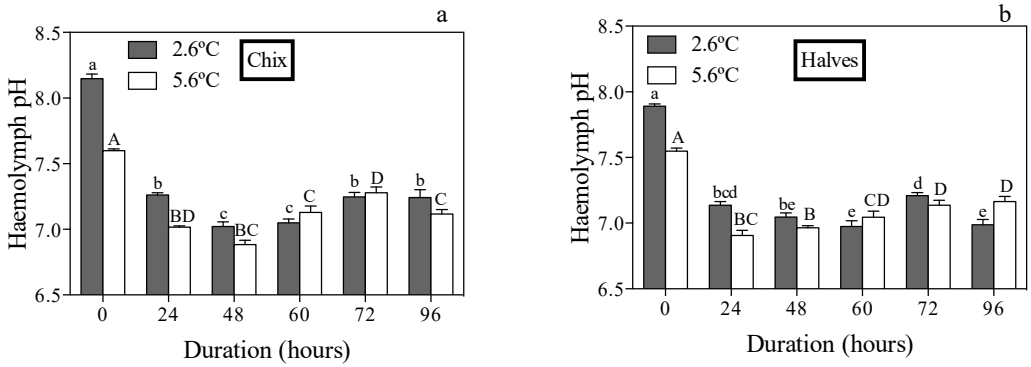

Figure 3.10a,b *Homarus americanus*: Changes to haemolymph pH at two lobster pre-acclimation temperatures of 2.6 ˚C and 5.6 ˚C after 0, 24, 48, 60, 72 and 96h emersion at 3.0 ˚C. Figure 3.10a represents small (Chix) size lobsters and Figure 3.10b represents large (Halves) size lobsters. Values represent means ± SEM. Different letters above the bars (a,b,c,d & A,B,C,D) represent significant differences between different letters of the same case ($p < 0.05$). n = 192 total.

3.4.1.4 Haemolymph Glucose

The data obtained on lobster haemolymph glucose concentrations at the tested temperatures and emersion times are summarized in Figure 3.11a,b and Figure 3.12a,b. The 2.6 ˚C acclimated lobsters had initial (control) haemolymph glucose concentrations of 0.24 mml l^{-1} for the smaller size (Chix) and 0.45 mml l^{-1} for the larger size (Halves) (Figure 3.11a). There was a general trend of progressively-increased haemolymph glucose concentration with increased emersion time that became significantly different from control levels at 48 h in the Chix size group ($p = 0.0006$). This contrasted with the findings for the Halves that did not attain concentrations significantly higher than the control and, at T = 48 h and 72 h, their mean values were less than the control value - although these decreases were not significant ($p > 0.05$ in each case). The 5.6 ˚C acclimated animals had mean haemolymph glucose concentrations that showed a trend of increase with emersion duration to 48 h for the Chix and 60 h for the Halves, after which, mean glucose levels did not vary significantly in either size group. In the warmer-acclimated Chix, no mean haemolymph glucose value was significantly different from the control value but that measured for the T = 96 h, warm-acclimated Halves did become significantly different from the control value ($p = 0.0014$).

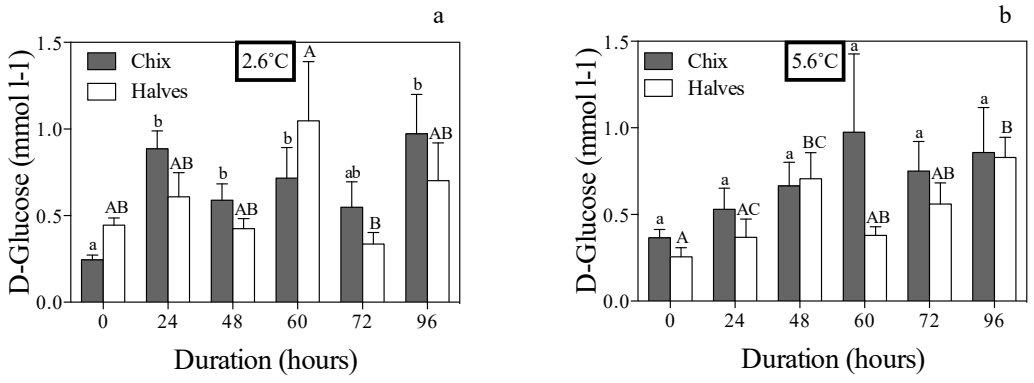

Figure 3.11a,b *Homarus americanus*: Changes to haemolymph glucose of small (Chix) and large (Halves) size lobsters after 0, 24, 48, 60, 72 and 96h emersion at 3.0 ˚C. Figure 3.11a represents cold (2.6 ˚C) acclimated lobsters and Figure 3.11b represents warm (5.6 ˚C) acclimated lobsters. Values represent means ± SEM for n = 8 animals. Different letters above the bars (a,b,c,d & A,B,C,D) represent significant differences between different letters of the same case ($p < 0.05$). n = 192 total.

A comparison of the acclimation temperature treatment groups' haemolymph glucose trial data (Figure 3.12a,b) showed an increase from the control (T = 0 h) value in both size groups and both temperature treatments. The 2.6 ˚C temperature-acclimated Chix showed a 3.7-fold haemolymph glucose increase from the control value at 24 h, an increase from 0.24 mml l^{-1} d-glucose to 0.89 mml l^{-1} at 24 h. The 5.6 ˚C temperature-acclimated Chix showed a progressively increasing haemolymph glucose concentration with emersion duration and reached a maximum mean level of 0.98 mml l^{-1} at 60 h emersion and then maintained levels above the control concentration for the remainder of the 96 h emersion trial.

The mean haemolymph glucose concentration of the 2.6 ˚C acclimated Halves increased progressively during emersion up to a maximum of 1.05 mml l^{-1} d-glucose at 60 h, 2.3 times higher than the control concentration of 0.45 mml l^{-1} - but this difference was not statistically significant (p = 0.3821). Levels subsequently decreased in this trial group to those of the control values for the remainder of the emersion time at 72 h and 96 h (Figure 3.12b). The 5.6 ˚C acclimated Halves also showed a progressively increased mean haemolymph glucose concentration with emersion duration, with levels declining slightly at 60 h and then increased at each sampling period for the remainder of the 96 h emersion trial (Figure 3.12b).

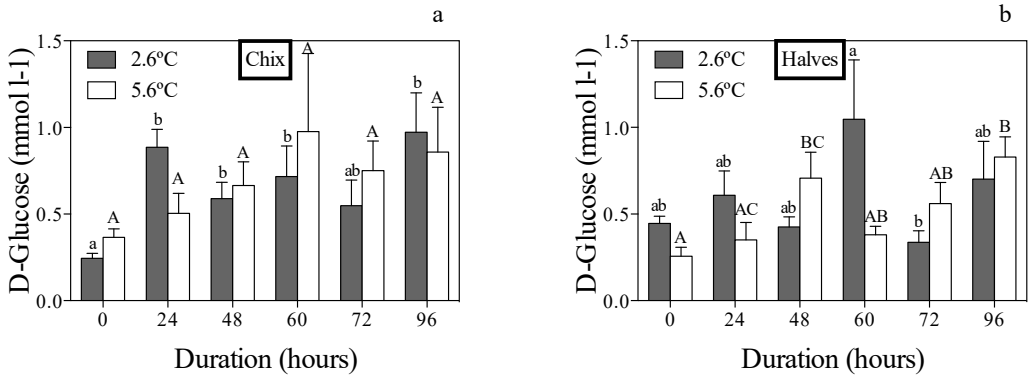

Figure 3.12a,b *Homarus americanus*: Changes to haemolymph glucose at two lobster pre-acclimation temperatures of 2.6 ˚C and 5.6 ˚C after 0, 24, 48, 60, 72 and 96h emersion at 3.0 ˚C. Figure 3.12a represents

small (Chix) sized lobsters and Figure 3.12b represents large (Halves) size lobsters. Values represent means ± SEM. Different letters above the bars (a,b,c,d & A,B,C,D) represent significant differences between different letters of the same case ($p < 0.05$). n = 192 total.

3.4.1.5 Vigour Index

Vigour index (VI) facilitated qualitative comparisons of the outcomes of each treatment. The VI for the 2.6 ˚C, small size (Chix) group for the 96 h emersion trial (Figure 3.13a) showed that vigour index changes from the control (100% Strong) were observed to begin at 48 h emersion sampling time, after which the relative proportion of strong specimens declined to 2 out of 8 lobsters. The comparable 5.6 ˚C acclimated group (Figure 3.13b) showed index changes from strong as beginning at the 60 h emersion sampling time and declined to 4 out of 8 lobsters at 96 h. Only one mortality occurred in the small lobster groups for the duration of the 96 h trials and this occurred at 60 h in the 2.6 ˚C acclimated group (Figure 3.13a).

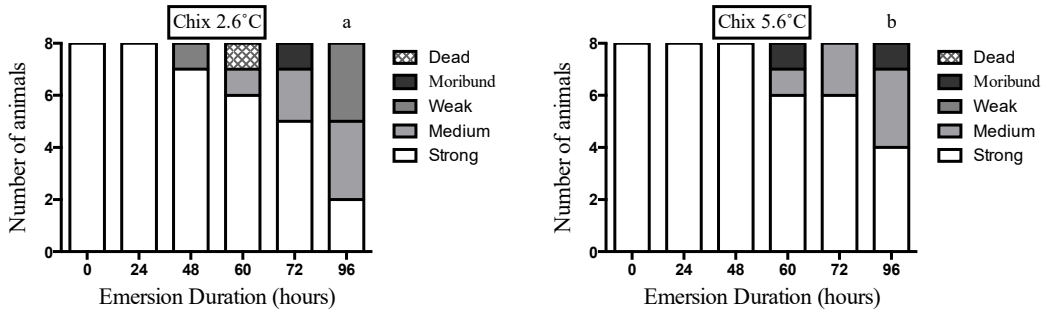

Figure 3.13a,b *Homarus americanus*: Vigour index comparison of small size (Chix) lobsters after 0, 24, 48, 60, 72 and 96 h emersion at 3.0 ˚C. Figure 3.13a represents the cold acclimation treatment group and Figure 3.13b represents the warm acclimation treatment group. *n* = 96 total.

A decline in VI from the 100% strong control group at T = 0 h was first observed to have occurred in the Halves at the 48 h emersion sampling time in both the 2.6 ˚C and 5.6 ˚C acclimated groups (Figure 3.14a,b). The 2.6 ˚C acclimated groups, however, had the larger declines in VI at 72 h and 96 h compared with the 5.6 ˚C acclimated groups for these larger-sized lobsters. A single mortality occurred within the groups of Halves and

it was observed at the 96 h emersion sampling time for the 2.6 ˚C temperature acclimated group treatment (Figure 3.14a).

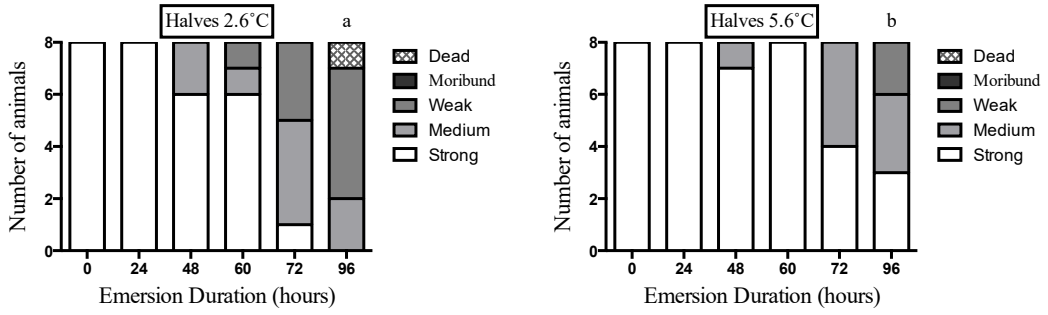

Figure 3.14a,b *Homarus americanus*: Vigour index comparison of large size (Halves) lobsters after 0, 24, 48, 60, 72 and 96 h emersion at 3.0 ˚C. Figure 3.14a represents the 2.6 ˚C acclimation treatment group and Figure 3.14b represents the 5.6 ˚C acclimation treatment group. *n* = 96 total.

3.4.1.6 Body Temperature

A comparison of lobster body temperature with emersion duration by acclimation treatment group is shown in Figure 3.15. Both the small (Chix) and large (Halves) sizes were combined for each acclimation treatment group since there were no observed differences in mean body temperature between the size groups at each sampling period. The two temperature treatment groups were significantly different at all sampling periods ($p < 0.05$) during the 96 h emersion trial except for the 60 h sampling period ($p > 0.9999$) at which time both temperature groups showed a temporary decrease in temperature from the 48 h emersion sampling period. In both temperature groups, body temperatures declined from the control at the 24 h sampling period followed by an increase to the temperature-controlled ambient air temperature of 3.0 ± 0.5 ˚C at the 48 h sampling period and remained at this level for the rest of the emersion sampling periods.

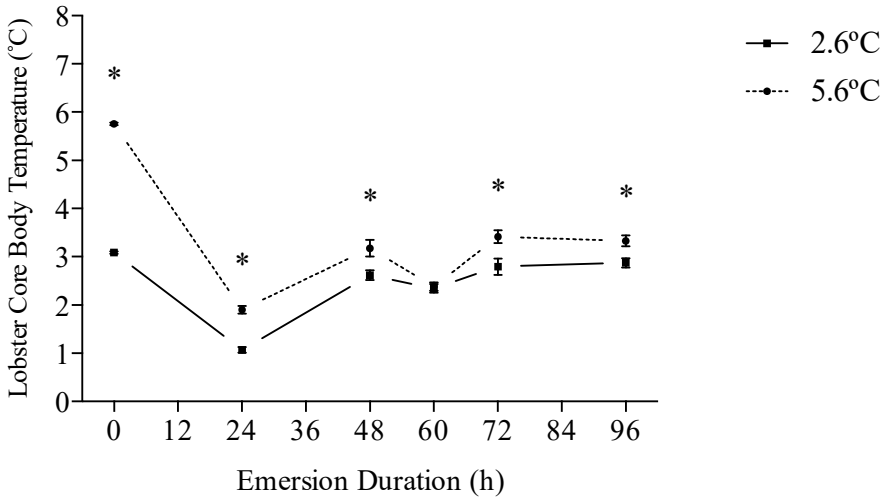

Figure 3.15 *Homarus americanus*: Core body temperature comparison of two acclimation temperature treatment groups (2.6 ˚C and 5.6 ˚C) after 0, 24, 48, 60, 72 and 96 h emersion at 3.0 ˚C. Values represent means ± SD for *n* = 16 animals. * represents significant difference between temperature treatment groups ($p < 0.05$). *n* = 96 total.

In the case of the 2.6 ˚C acclimation treatment group, the animals' mean body temperature remained at, or above, their acclimation treatment temperature for all sampling periods after 24 h. In the case of the 5.6 ˚C acclimation treatment group, the animals' mean body temperature declined from their acclimation treatment temperature at the first emersion sampling period (T = 24 h) and remained below their acclimation temperature for the remainder of the trial.

3.4.1.7 96 h emersion summary

Table 3.1 contains a summary of the 96 h emersion trial data.

Table 3.1 *Homarus americanus*: summary of results from 96 h emersion trial comparing two lobster sizes (small: Chix and large: Halves) with two pre-treatment acclimations at 2.6 ºC and 5.6 ºC.

Parameter	Summary of findings
Haemolymph Ammonia	Haemolymph ammonia increased with emersion time. No significant difference with size or temperature until 96 h at which time the Chix haemolymph ammonia was higher than the Halves.

Haemolymph Lactate	Haemolymph lactate increased with emersion time. Chix lactate was higher at each time point than Halves for both treatment temperatures, becoming significantly different from the control at 48 h (Chix) and 60 h (Halves) at 2.6 ºC. For 5.6 ºC, only the Chix increased significantly from control levels (at 60 h) although levels decreased below control at that point and then above control > 60 h.
Haemolymph pH	Haemolymph pH declined with emersion time until 72 h for 2.6 ºC and 60 h at 5.6 ºC. All were significantly different from controls within 24 h in all sizes and temperature treatments. pH by sizes were significantly different in the controls between temperature groups (higher pH in lower temperature groups in both sizes). Both sizes and temperatures showed similar trends of decreasing pH to 60 – 72 h emersion followed by slight pH recovery that was significant in all but the Halves 5.6 ºC group.
Haemolymph Glucose	Haemolymph glucose became higher than control in the 2.6 ºC Chix at 24 h. Halves were only different from control at 72 h in which they were lower than all other readings. Generally, glucose increased with emersion time until 72 h where levels decreased in most cases followed by a subsequent increase at 96 h.
Vigour	Chix and Halves retained lower vigour index values (better) at 5.6 ºC than for 2.6 ºC treatments. The greatest loss of vigour was in the 2.6 ºC Halves group at 96 h in which the only death was recorded in the trial.
Haemolymph Temperature	Both acclimation temperature treatment groups declined significantly from controls in the first 24 h sampling period followed by a general matching of external air temperature for the remainder of the trial. The 5.6 ºC treatment groups remained significantly higher than the 2.6 ºC group at all sample times except for 60 h.

3.4.2 48 h Emersion + Re-immersion

3.4.2.1 Haemolymph Ammonia

Following 48 h emersion, haemolymph ammonia from the 2.6 ˚C acclimated small (Chix) and large (Halves)-sized lobsters showed a similar trend of increasing ammonia levels in emersed animals, attaining significantly higher levels than control (T = 0 h) animals at 24 h ($p < 0.0001$) (Figure 3.16a). Chix reached a maximum haemolymph ammonia level of 1,661 µml l^{-1} at 48 h emersion and the Halves reached a higher ammonia level of 2,178 µml l^{-1} at the same sampling time, although the ammonia levels were not significantly different between the two size groups throughout the emersion + re-immersion trial ($p > 0.05$). Both size groups had returned to control ammonia levels at the 12 h re-immersion sampling period and remained at this level throughout the 48 h of re-immersion.

A similar trend of increasing haemolymph ammonia with emersion duration occurred with the 5.6 ˚C acclimation treatment trial (Figure 3.16b) in which no significant difference existed between size groups, although both the Chix and Halves had significantly higher haemolymph ammonia levels than the relevant control ammonia values at the 24 h and 48 h emersion sampling periods ($p < 0.0001$ for both size groups at both sampling periods).

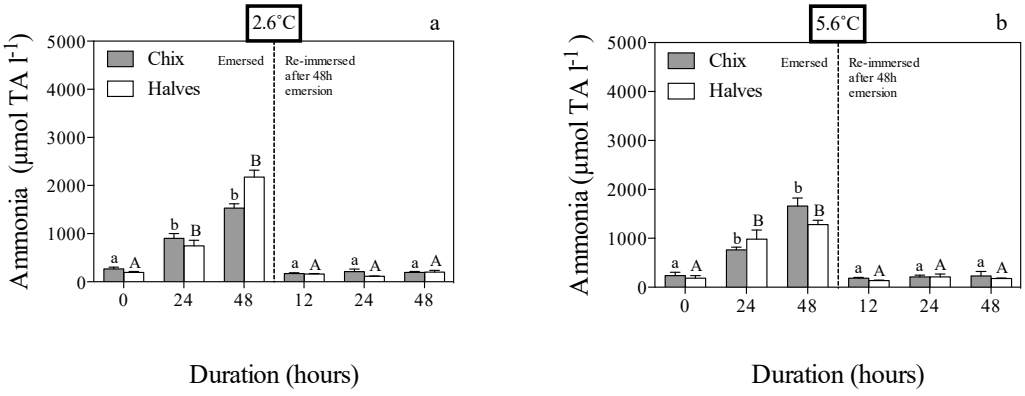

Figure 3.16a,b *Homarus americanus*: Changes to haemolymph total ammonia level for small (Chix) and large (Halves) size lobsters after 0, 24 and 48h emersion at 3.0 ˚C followed by 12, 24 and 48h re-immersion at 3.0 ˚C. Figure 3.16a represents cold (2.6 ˚C) acclimated lobsters and Figure 3.16b represents warm (5.6 ˚C) acclimated lobsters. Values presented as means ± SEM for $n = 8$ animals. a, b and A,B represent significant differences between different letters of the same case ($p < 0.05$) in which differences were compared to the control (T = 0 group). $n = 192$ total.

A comparison of the results at the two acclimation temperatures (Figure 3.17a,b) showed similar levels of group mean haemolymph ammonia at each sampling time, except for a higher, but non-significant level in the 2.6 ˚C treatment (compared with the 5.6 ˚C treatment) of the Halves at the 48 h emersion sampling period. All size and temperature treatment groups showed a return to control haemolymph ammonia levels at the first 12 h re-immersion sampling period, with no significant differences between the size and temperature groups ($p > 0.05$ in all cases).

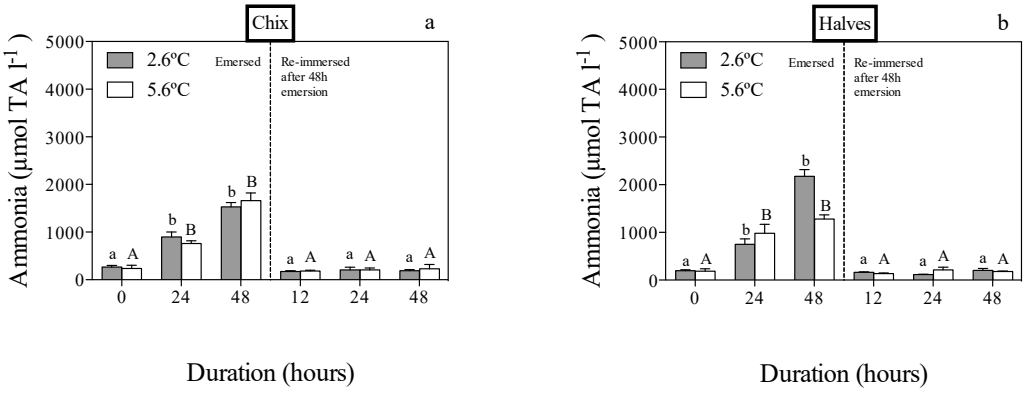

Figure 3.17a,b *Homarus americanus*: Changes to haemolymph total ammonia for cold (2.6 ˚C) and warm (5.6 ˚C) acclimated lobsters after 0, 24 and 48 h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Figure 3.17a represents small (Chix) size lobsters and Figure 3.17b represents large (Halves) size lobsters. Values presented as means ± SEM for *n* = 8 animals. a, b and A,B represent significant differences between different letters of the same case (p < 0.05) in which differences were compared to the control (T = 0 group). *n* = 192 total.

3.4.2.2 Haemolymph Lactate

Both 2.6 ˚C acclimated size groups showed significantly increasing lactate with emersion time (Figure 3.18a), (p = 0.0028 and p = 0.0021 for Chix and Halves, respectively, at 24 h emersion). The re-immersion haemolymph lactate levels did not return to control levels until the 24 h re-immersion period in the Chix and 48 h re-immersion in the Halves. The haemolymph lactate levels of the 5.6 ˚C acclimated groups (Figure 3.18b) did not increase significantly above control levels until the 48 h emersion period sampling for both the Chix and Halves. Haemolymph lactate remained significantly higher than control levels during the initial 12 h re-immersion period but had returned to control levels at 24 h re-immersion in both size groups.

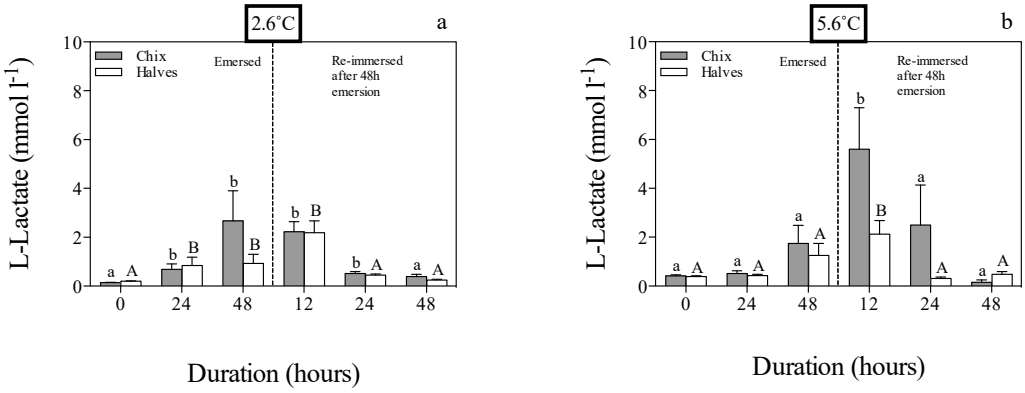

Figure 3.18a,b *Homarus americanus*: Changes to haemolymph lactate for small (Chix) and large (Halves) size lobsters after 0, 24 and 48 h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Figure 3.18a represents cold (2.6 ˚C) acclimated lobsters and Figure 3.18b represents warm (5.6 ˚C) acclimated lobsters. Values presented as means ± SEM for *n* = 8 animals. a, b and A,B represent significant differences between different letters of the same case ($p < 0.05$) in which differences were compared to the control (T = 0 group). *n* = 192 total.

Comparisons of the haemolymph lactate levels, in terms of the temperature treatments, revealed they increased progressively during emersion and had reached levels significantly higher than the control levels at the 24 h emersion sampling time in the 2.6 ˚C treated groups. This trend continued to attain maximum levels at both the 48 h emersion sampling time and the subsequent 12 h re-immersion sampling time and eventually dropped back to control levels at the 48 h re-immersion sampling time. A similar trend was observed in the 5.6 ˚C treated groups with the smaller Chix. In this group, however, the maximum lactate levels were reached only at the 12 h re-immersion time and had returned to control levels by the 24 h re-immersion sampling time. Within the larger-sized Halves group, the haemolymph lactate levels increased progressively during emersion, and attained a lower maximum value than the smaller sized Chix. Both temperature-treated groups of Halves had returned to control haemolymph lactate levels at the 24 h re-immersion sampling time.

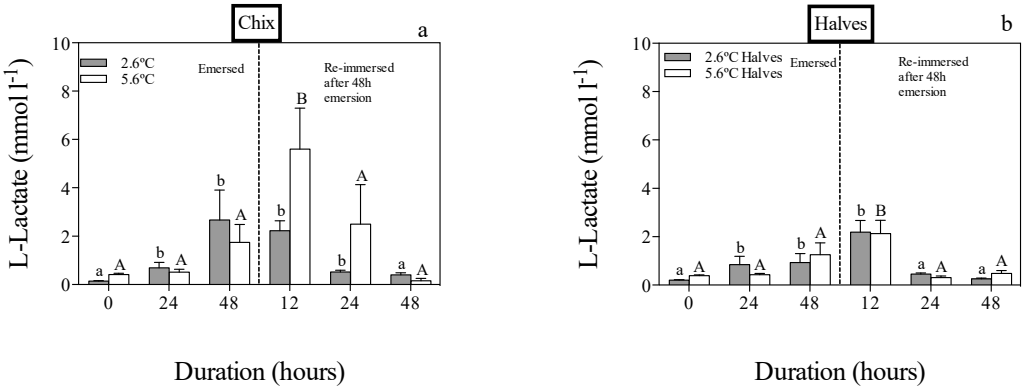

Figure 3.19a,b *Homarus americanus*: Changes to haemolymph lactate for cold (2.6 ˚C) and warm (5.6 ˚C) acclimated lobsters after 0, 24 and 48 h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Figure 3.19a represents small (Chix) size lobsters and Figure 3.19b represents large (Halves) size lobsters. Values presented as means ± SEM for *n* = 8 animals. a, b and A,B represent significant differences between different letters of the same case (p < 0.05) in which differences were compared to the control (T = 0 group). *n* = 192 total.

3.4.2.3 Haemolymph pH

Haemolymph pH became significantly lower than control values at the 24 h emersion sampling period in all the groups (Figure 3.20a,b). However, both of the 5.6 ˚C treated groups recorded the lower starting haemolymph pH values (Figure 3.20b), the smaller size (Chix) returned to control haemolymph pH values by the 12 h re-immersion sampling time and by the 48 h re-immersion sampling time for the larger Halves. Neither of the 2.6 ˚C treated groups recovered to control pH levels during the entire re-immersion period (Figure 3.20a).

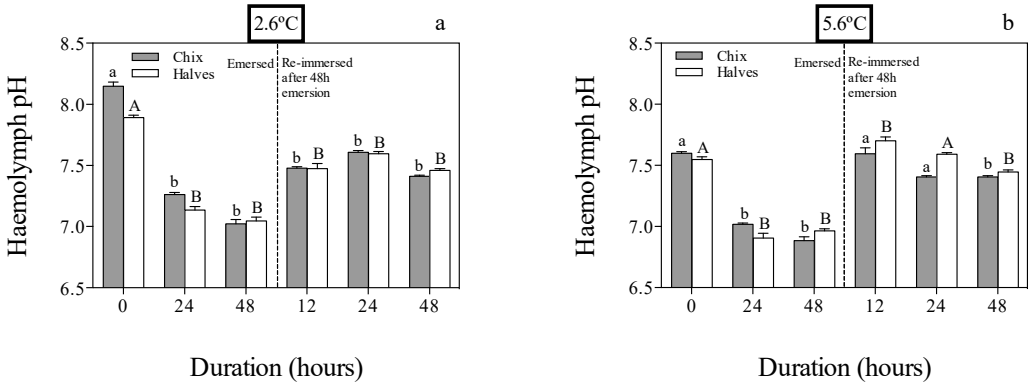

Figure 3.20a,b *Homarus americanus*: Changes to haemolymph pH for small (Chix) and large (Halves) size lobsters after 0, 24 and 48 h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Figure 3.20a represents cold (2.6 ˚C) acclimated lobsters and Figure 3.20b represents warm (5.6 ˚C) acclimated lobsters.

Values presented as means ± SEM for *n* = 8 animals. a, b and A,B represent significant differences between different letters of the same case (*p* < 0.05) in which differences were compared to the control (T = 0 group). *n* = 192 total.

A comparison of haemolymph pH by acclimation temperature treatment (Figure 3.21a,b) revealed a significant difference between the two control groups in both the Chix and Halves ($p < 0.0001$ in both size groups). The mean haemolymph pH of the two temperature-acclimated groups of Chix remained different at all sample times except at 24 h and 48 h post-emersion. A comparable comparison of the Halves revealed the mean haemolymph pH levels had reached similar values for both temperature-acclimated groups at 48 h immersion, then both groups continued to increase above the 48 h emersion levels during the entire re-immersion period.

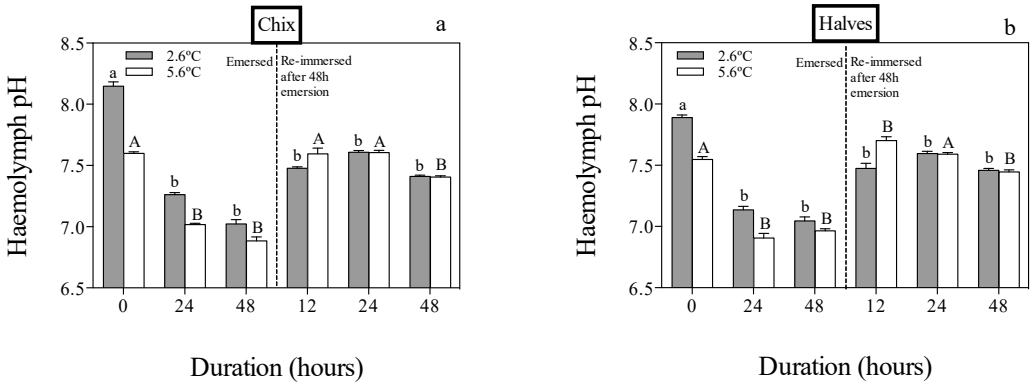

Figure 3.21a,b *Homarus americanus*: Changes to haemolymph pH for cold (2.6 ˚C) and warm (5.6 ˚C) acclimated lobsters after 0, 24 and 48 h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Figure 3.21a represents small (Chix) size lobsters and Figure 3.21b represents large (Halves) size lobsters. Values presented as means ± SEM for *n* = 8 animals. a, b and A,B represent significant differences between different letters of the same case (p < 0.05) in which differences were compared to the control (T = 0 group). *n* = 192 total.

3.4.2.4 Haemolymph Glucose

The haemolymph glucose comparisons by size for each acclimation temperature are shown in Figure 3.22a,b. Within the 2.6 ˚C acclimation temperature trial, the control haemolymph glucose values of the Chix and the Halves were significantly different ($p = 0.0233$). Glucose values increased above the control values at the 24 h sampling time (p

< 0.0001) and remained above control values until the 24 h re-immersion sampling time. There was no significant change from the control value of the halves, however, until the 24 h re-immersion sampling time - at which point it was significantly below control levels (p = 0.0360).

In the 5.6 ˚C trials (Figure 3.22b), both size groups showed a general trend of increasing haemolymph glucose with emersion and a subsequent decline at the 24 h re-immersion sampling time. The Chix showed no significant change from control glucose levels until the 12 h re-immersion sampling period (p = 0.0012) whereas the Halves showed no significant change from the control values until the 48 h emersion sampling period (p = 0.0015), after which values dropped to control levels at the 24 h re-immersion sampling time. An increase above control glucose levels was found at the 48 h re-immersion sampling period in the Halves (p = 0.0277), but a similar increase was not found in the Chix.

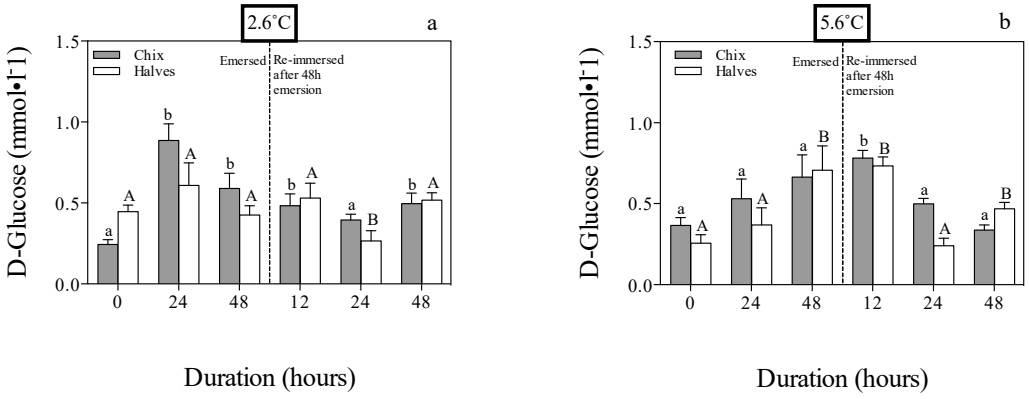

Figure 3.22a,b *Homarus americanus*: Changes to haemolymph glucose for small (Chix) and large (Halves) size lobsters after 0, 24 and 48 h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Figure 3.22a represents cold (2.6 ˚C) acclimated lobsters and Figure 3.22b represents warm (5.6 ˚C) acclimated lobsters. Values presented as means ± SEM for *n* = 8 animals. a, b and A, B represent significant differences between different letters of the same case (p < 0.05) in which differences were compared to the control (T = 0 group). *n* = 192 total.

Haemolymph glucose concentrations within the temperature acclimation treatment trial comparisons are shown in Figure 3.23. In the Chix group trials, there were no differences between the acclimation temperature treatments, except at the 24 h emersion sampling

period (p = 0.0156), at which time the 2.6 ˚C group reached a maximum of 0.89 µml l-1. Mean levels then dropped over the remaining emersion and re-immersion periods (Figure 3.23a). In the Halves, there was a significant difference between the control groups (p = 0.398), but not for the rest of the sampling times of the emersion and subsequent re-immersion trial. Glucose levels increased progressively during emersion and continued to increase at the 12 h re-immersion period in the warmer temperature group but this was not found in the colder, 2.6 ˚C group. In both treatment groups, however, there was a decline to control levels (Halves) and below control levels (Chix) at 24 h re-immersion.

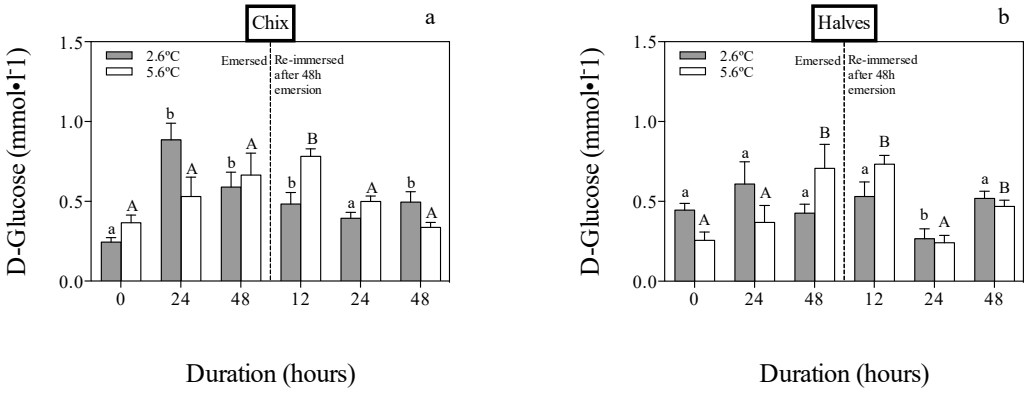

Figure 3.23a,b *Homarus americanus*: Changes to haemolymph glucose for cold (2.6 ˚C) and warm (5.6 ˚C) acclimated lobsters after 0, 24 and 48 h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Figure 3.23a represents small (Chix) size lobsters and Figure 3.23b represents large (Halves) size lobsters. Values presented as means ± SEM for *n* = 8 animals. a, b and A,B represent significant differences between different letters of the same case (p < 0.05) in which differences were compared to the control (T = 0 group). *n* = 192 total.

3.4.2.5 Vigour Index

Vigour Index (VI) for the 48 h emersion and 48 h re-immersion period by acclimation treatment temperature are shown in Figure 3.24 for the Chix and Figure 3.25 for the Halves. A decline from 100% strong VI = 1 lobsters in the control at T = 0 h occurred at 48 h emersion in the 2.6 ˚C trial, with a further decline occurring at the 12 h re-immersion period whereas the first observed decline from VI = 1 in the 5.6 ˚C trial occurred at the 12 h re-immersion period. Two of the eight lobsters at the final sampling time at 48h re-immersion were observed as medium VI in the 5.6 ˚C acclimation treatment trial

compared with the same size group for the 2.6 ˚C treatment trial in which 100% VI = 1 were observed at the 48 h re-immersion time.

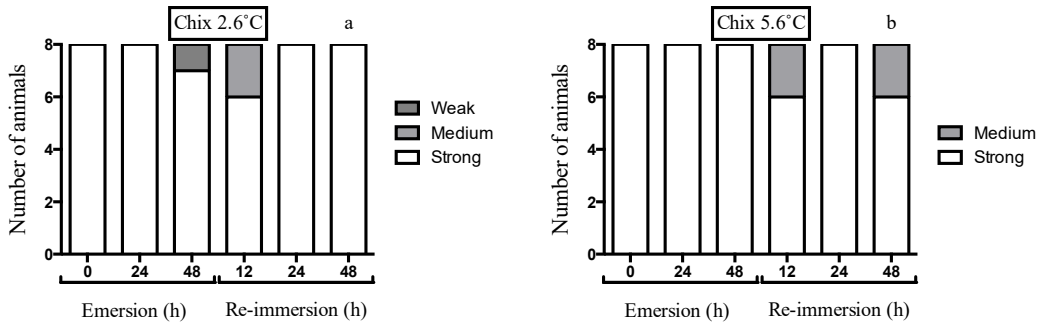

Figure 3.24a,b *Homarus americanus*: Vigour index comparison of small size (Chix) lobsters after 0, 24 and 48h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Figure 3.24a represents the cold acclimation treatment group and Figure 3.24b represents the warm acclimation treatment group. *n* = 48 total.

Within the larger – sized halves trial in the 2.6 ˚C acclimation treatment group (Figure 3.25a), a VI change had occurred in the 48 h emersion group (compared with the control of *n* = 8 strong) with 2 of 8 animals observed as VI = 2 (medium), and each subsequent sampling period at 12, 24 and 48 h emersion had between 2 to 3 of 8 animals changed from strong to medium VI. The same size group in the 5.6 ˚C acclimation group also had less than 100% strong VI animals at 48 h emersion and showed subsequent decreases in strong animals at the 12 and 24 h sampling times - although there were 100% strong lobsters at the 48h re-immersion sampling time (Figure 3.25b).

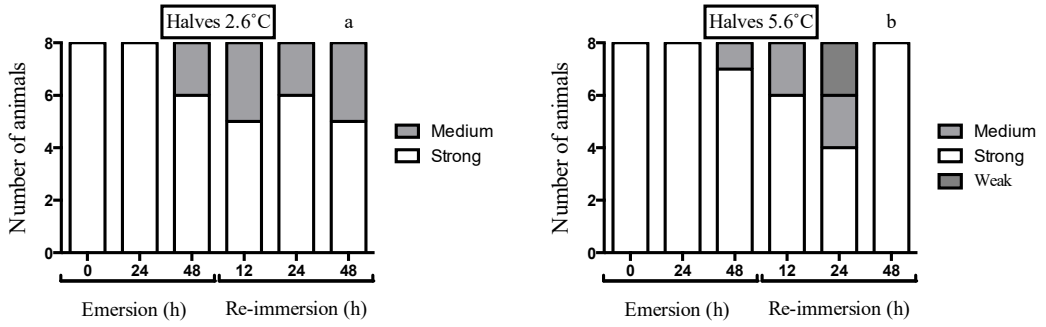

Figure 3.25a,b *Homarus americanus*: Vigour index comparison of large size (Halves) lobsters after 0, 24 and 48h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Figure 3.25a represents the cold acclimation treatment group and Figure 3.25b represents the warm acclimation treatment group. *n* = 48 total.

3.4.2.6 Body Temperature

Lobster core body temperature results for 48 h emersion followed by 48 h re-immersion are shown in Figure 3.26. The two temperature treatment groups were significantly different ($p < 0.0001$) from each other at all emersion sampling times, but there were no significant differences in mean body temperature between the two groups at each of the three re-immersion sampling times of 12, 24 and 48 h ($p > 0.05$ at each re-immersion period). Lobsters remained within the re-immersion seawater control temperature for the duration of the re-immersion period of 48 h.

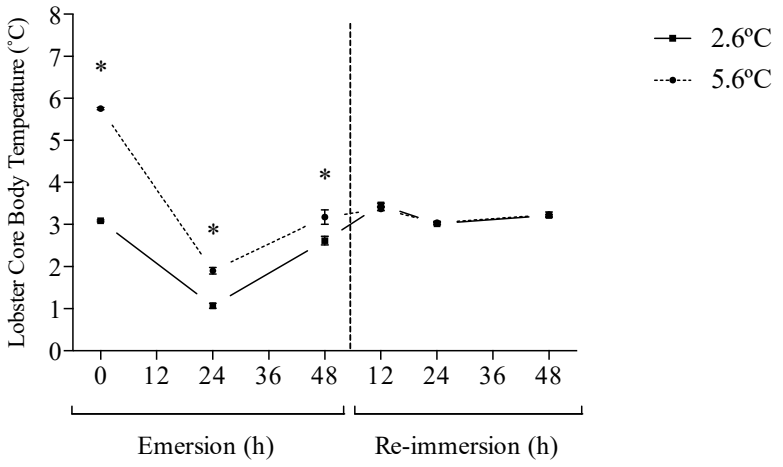

Figure 3.26 *Homarus americanus*: Core body temperature comparison of two acclimation temperature treatment groups (2.6 ˚C and 5.6 ˚C) after 0, 24 and 48 h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Values represent means ± SD for *n* = 16 animals. * indicates a significant difference between temperature treatment groups ($p < 0.05$). *n* = 96 total.

In the case of the 2.6 ˚C acclimation treatment group, mean body temperature was 0.4 ˚C above their pre-trial acclimation temperature and the 5.6 ˚C treatment group was 2.6 ˚C below their pre-trial acclimation temperature during all three re-immersion sampling periods.

3.4.2.7 48 h emersion + re-immersion summary

Table 3.2 provides a summary of the results for the 48 h emersion and re-immersion trial

Table 3.2 *Homarus americanus*: summary of results from 48 h emersion and 12, 24 and 48 h re-immersion trials comparing two lobster sizes (small: Chix and large: Halves) with two pre-treatment acclimations at 2.6 ºC and 5.6 ºC.

Parameter	Summary of findings
Haemolymph Ammonia	Haemolymph ammonia increased with emersion time in all cases, but to higher levels in both sizes from 2.6 ºC groups compared with 5.6 ºC. All size and treatments returned to control levels at 12 h re-immersion. The 2.6 ºC Halves size had significantly elevated ammonia levels compared to 2.6 ºC Chix at 48 h emersion. No significant differences between temperature treatment groups except at 48 h emersion for the 2.6 vs 5.6 ºC Halves.
Haemolymph Lactate	Levels increased with emersion and continued to increased during re-immersion until 24 h in both treatments and sizes. levels remained elevated above control values up to 48 h re-immersion, but only significantly so at 12 h re-immersion.
Haemolymph pH	Haemolymph pH declined with emersion for the 48 h emersion, and returned to control values only in the 5.6 ºC group, although a relatively slow recovery was observed with re-immersion time.
Haemolymph Glucose	Haemolymph glucose became elevated with emersion time, but not significantly in most cases (only 2.6 ºC Chix and 5.6 ºC Halves). Glucose did not recover with re-immersion until 24 h in all cases that were elevated above control at 48 h emersion sample time.
Vigour	There was a slightly higher loss of vigour in the 2.6 C treatment temp (cf the 5.6 ºC group) although recovery was observed in all sizes and temperature treatments except for the 2.6 ºC Halves. No lobsters died in this trial.
Haemolymph Temperature	Haemolymph temperature declined towards emersion temperature in both temperature pre-treatments, although the 5.6 ºC group remained significantly higher until re-immersion after which all groups were observed at the same haemolymph temperature which matched the immersion environment temperature.

3.4.3 72 h Emersion + Re-immersion

3.4.3.1 Haemolymph Ammonia

Haemolymph ammonia levels progressively increased above control values in both size groups and acclimation temperature treatment trials (Figure 3.27). All groups had mean haemolymph ammonia levels significantly above the control values at the 24 h sampling time ($p < 0.05$). Although the 2.6 ˚C treatment trial (Figure 3.27a) appeared to show the Chix as having slightly higher ammonia levels than the Halves, there were no significant differences between size groups except at the 12 h re-immersion sampling time when the Chix had a slightly higher haemolymph ammonia level than the Halves ($p = 0.0130$). In both temperature trials, haemolymph ammonia had returned to control levels at the first re-immersion sampling time (12 h).

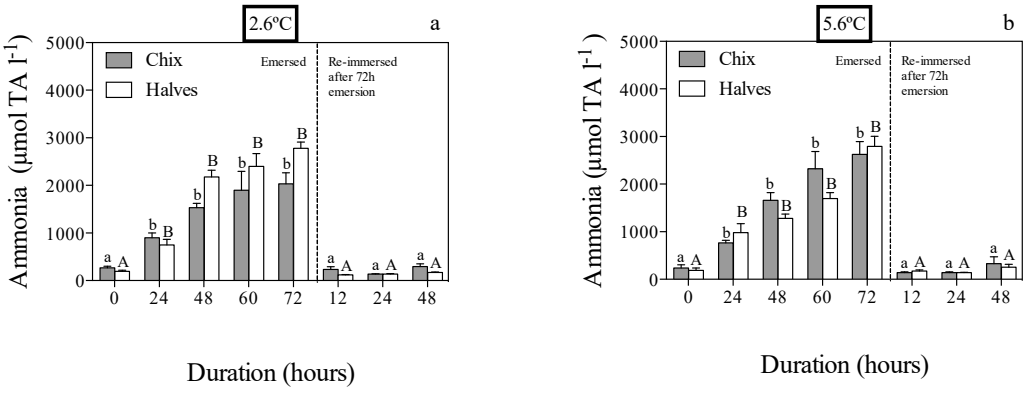

Figure 3.27a,b *Homarus americanus*: Changes to haemolymph total ammonia for small (Chix) and large (Halves) size lobsters after 0, 24, 48, 60 and 72 h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Figure 3.27a represents cold (2.6 ˚C) acclimated lobsters and Figure 3.27b represents warm (5.6 ˚C) acclimated lobsters. Values presented as means ± SEM for *n* = 8 animals. a, b and A,B represent significant differences between different letters of the same case (p < 0.05) in which differences were compared to the control (T = 0 group). *n* = 256 total.

Comparisons of the haemolymph ammonia levels between the two temperature treatments are shown in Figure 3.28 and show that there were also no differences between temperature groups in both size groups - with the exception of the 48 h sampling period of the Halves trial (Figure 3.28b) in which the 5.6 ˚C group had a significantly lower ammonia level than the 2.6 ˚C group (p = 0.0173).

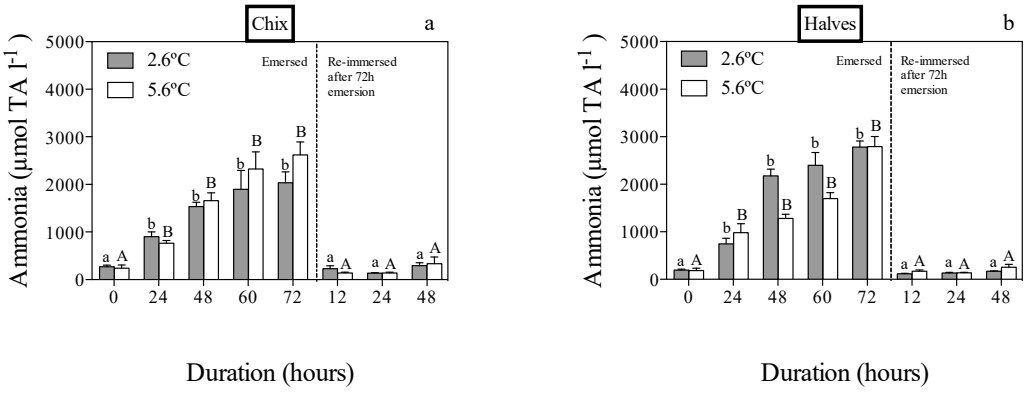

Figure 3.28a,b *Homarus americanus*: Changes to haemolymph total ammonia for cold (2.6 ˚C) and warm (5.6 ˚C) acclimated lobsters after 0, 24, 48, 60 and 72h emersion at 3.0 ˚C followed by 12, 24 and 48h re-immersion at 3.0 ˚C. Figure 3.28a represents small (Chix) size lobsters and Figure 3.28b represents large (Halves) size lobsters. Values presented as means ± SEM for *n* = 8 animals. a, b and A,B represent significant differences between different letters of the same case (p < 0.05) in which differences were compared to the control (T = 0 group). *n* = 256 total.

3.4.3.2 Haemolymph Lactate

In the 2.6 ˚C temperature trials, both size groups showed increasing haemolymph lactate levels during emersion and into the subsequent re-immersion period (Figure 3.29a). The levels in the Chix became significantly higher than control levels at the 48 h sampling time whereas those of Halves were higher than control levels at the 24 h sampling period ($p = 0.0002$ & 0.0307 respectively). The Chix showed a return to control levels at the 24 h re-immersion sampling time whereas the Halves did not show a return to control mean levels for the duration of the trial. There were no significant differences between the two size groups in the 2.6 ˚C trials - other than at the 24 h re-immersion period when the level of 5.68 µml lactate•l^{-1} was significantly higher than that of the Halves (0.81 µml lactate•l^{-1} ; $p = 0.0009$) at this time. There was also a general trend of progressively increasing haemolymph lactate levels in the 5.6 ˚C trials (Figure 3.29b), with the exception that, during emersion, the Halves showed no increase above control levels until the 72 h emersion sampling time. During the re-immersion period both size groups showed haemolymph lactate levels decreasing with re-immersion duration, with both size groups having reached or dropped below control values by the 48 h re-immersion sampling time. There were no significant differences between the two size groups at each sampling time in the 5.6 ˚C trial - other than at 24 h re-immersion, when the mean lactate level of 0.65 µml lactate•l^{-1} was significantly lower than the 3.21 µml lactate•l^{-1} of the Halves ($p = 0.0429$).

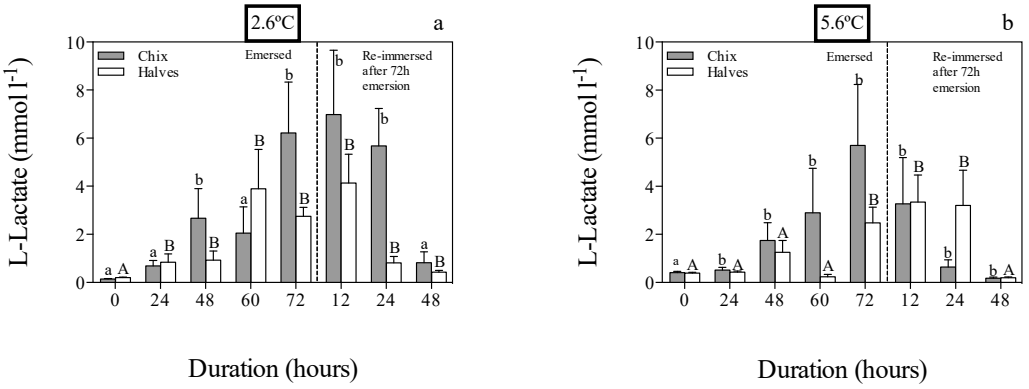

Figure 3.29a,b *Homarus americanus*: Changes to haemolymph lactate for (Chix) and (Halves) sized lobsters after 0, 24, 48, 60 and 72h emersion at 3.0 ˚C followed by 12, 24 and 48h re-immersion at 3.0 ˚C. Figure 3.29a represents cold (2.6 ˚C) acclimated lobsters and Figure 3.29b represents warm (5.6 ˚C) acclimated lobsters. Values presented as means ± SEM for *n* = 8 animals. a, b and A,B represent significant differences between different letters of the same case (p < 0.05) in which differences were compared to the control (T = 0 group). *n* = 256 total.

Both temperature comparison trials revealed a progressive increase in haemolymph mean lactate levels during the 72 h emersion period followed by a decline back to control levels during the subsequent 48 h re-immersion period (Figure 3.30). There were no significant differences between the two acclimation temperature groups for the Chix at any sampling time and only one sampling time (60 h) showed a significant difference for the Halves when the 2.6 ˚C group had a mean lactate level of 3.89 µml lactate•l^{-1} compared with the warmer group mean lactate level of 0.24 µml lactate•l^{-1} ($p < 0.0001$).

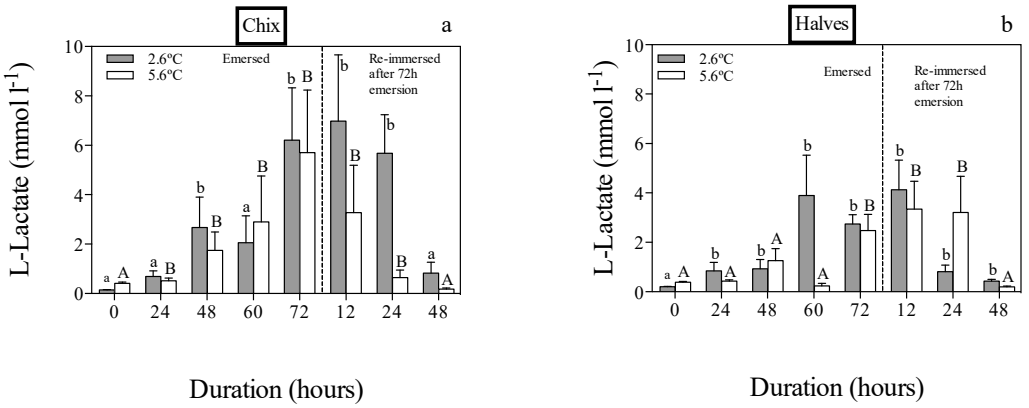

Figure 3.30a,b *Homarus americanus*: Changes to haemolymph lactate for cold (2.6 ˚C) and warm (5.6 ˚C) acclimated lobsters after 0, 24, 48, 60 and 72h emersion at 3.0 ˚C followed by 12, 24 and 48h re-immersion at 3.0 ˚C. Figure 3.30a represents small (Chix) size lobsters and Figure 3.30b represents large (Halves) size lobsters. Values presented as means ± SEM. a, b and A,B represent significant differences between different letters of the same case (p < 0.05) in which differences were compared to the control (T = 0 group). *n* = 256 total.

3.4.3.3 Haemolymph pH

The haemolymph pH comparisons within the acclimation treatment trials can be found in Figure 3.31. In the 2.6 ˚C acclimated group, pH declined to below ambient levels at the 24 h time in both size groups and did not return to control levels for the remainder of the emersion and re-immersion sampling periods. Between-size comparisons at the 2.6 ˚C group (Figure 3.31a) was significantly lower in the larger-sized Halves group at the 24 h sampling time ($p = 0.0157$) but was similar to the smaller Chix at all other sampling times. The haemolymph pH of both size groups in the 5.6 ˚C trials had dropped below control levels at the 24 h sampling time, and remained at levels between pH 6.88 and 7.28 during the remaining emersion sampling times of 48 h, 60 h and 72 h. Subsequently, they increased during the re-immersion sampling period to reach control values at 24 h re-immersion in both size groups (Figure 3.31b).

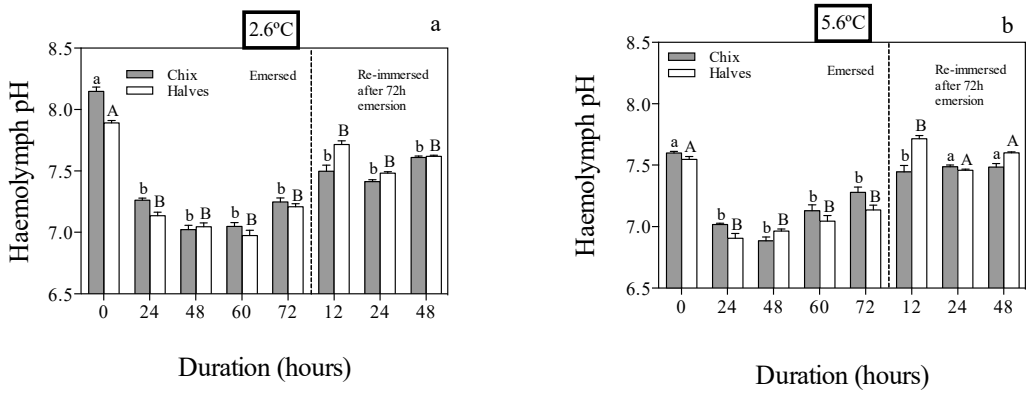

Figure 3.31a,b *Homarus americanus*: Changes to haemolymph pH for small (Chix) and large (Halves) size lobsters after 0, 24, 48, 60 and 72 h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Figure 3.31a represents cold (2.6 ˚C) acclimated lobsters and Figure 3.31b represents warm (5.6 ˚C) acclimated lobsters. Values presented as means ± SEM for $n = 8$ animals. a, b and A,B represent significant differences between different letters of the same case ($p < 0.05$) in which differences were compared to the control (T = 0 group). $n = 256$ total.

There were significant differences between the control values of the 2.6 ˚C and 5.6 ˚C acclimation temperature treatment groups in both the Chix and Halves sized lobster trials

($p < 0.0001$ in both size group trials). This difference remained at the 24 h and 48 h sampling times in the Chix trial and at the 24 h sampling period for the Halves trial. Within the Chix group trials (Figure 3.32a), the mean haemolymph pH of the 2.6 ˚C group remained below control values at every sampling time of the 72 h emersion and 48 h re-immersion periods. The 5.6 ˚C group showed a recovery to control pH levels by the 24 h re-immersion sampling time and remained at this level for the rest of the trial. The Halves group temperature comparison trials (Figure 3.32b) revealed significant decreases from control haemolymph pH levels in both the 5.6 ˚C and 2.6 ˚C groups at the 24 h sampling time ($p < 0.0001$ in both temperature treatment groups). The pH levels remained below control values for the remainder of the trial in the 2.6 ˚C group whereas the 5.6 ˚C group had returned to control values by the 24 h re-immersion sampling period.

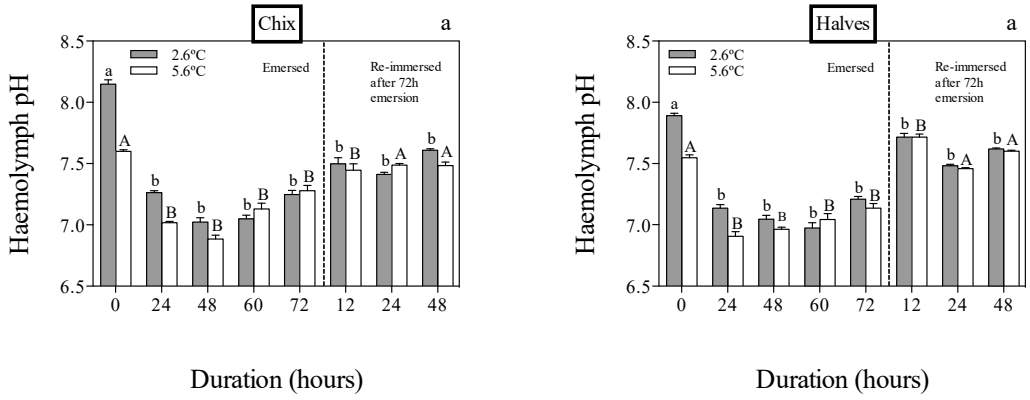

Figure 3.32a,b *Homarus americanus*: Changes to haemolymph pH for cold (2.6 ˚C) and warm (5.6 ˚C) acclimated lobsters after 0, 24, 48, 60 and 72 h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Figure 3.32a represents small (Chix) size lobsters and Figure 3.32b represents large (Halves) size lobsters. Values presented as means ± SEM for *n* = 8 animals. a, b and A,B represent significant differences between different letters of the same case ($p < 0.05$) in which differences were compared to the control (T = 0 group). *n* = 256 total.

3.4.3.4 Haemolymph Glucose

In the 2.6 ˚C trials, the Chix showed an increased haemolymph glucose level from the control at the 24 h emersion sampling time and maintained levels above control for the remainder of the emersion and re-immersion periods (Figure 3.33a). In this same trial

however, the Halves showed a general trend of hyperglycaemia up to the 72 h sampling time but to levels not significantly higher than the control value (Figure 3.33b). There were no significant differences between the two size groups in the 2.6 ˚C trial. The 5.6 ˚C acclimation treatment trial showed progressively increased hyperglycaemia with emersion duration until the 72 h emersion sampling time for the Chix and until the 60 h sampling time for the Halves. Mean haemolymph glucose levels in the Chix did not increase significantly above control levels throughout the entire emersion and re-immersion trial whereas haemolymph glucose levels in the Halves had increased significantly above control levels by the 48 h sampling time ($p = 0.0023$) and remained above control levels for all subsequent emersion and re-immersion sampling times except at the 60 h emersion sampling time (Figure 3.33b). Other than at the 24 h re-immersion sampling time ($p = 0.0102$), there were no significant differences between the two size groups in the 5.6 ˚C trials.

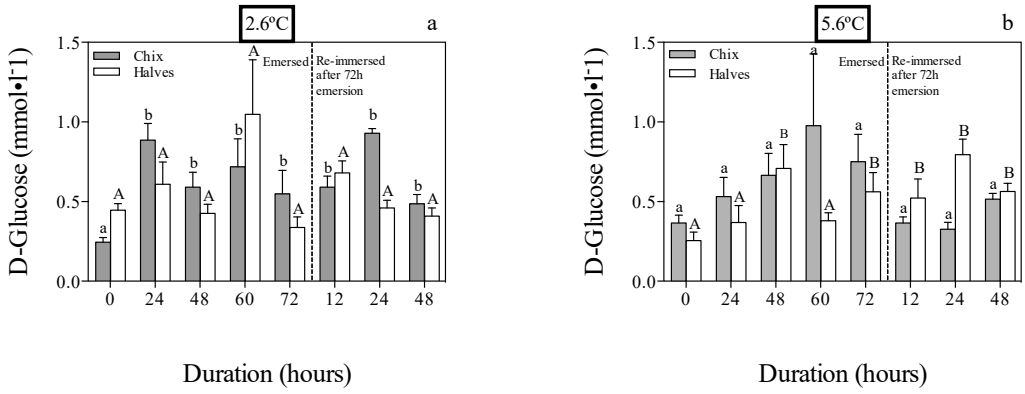

Figure 3.33a,b *Homarus americanus*: Changes to haemolymph glucose for small (Chix) and large (Halves) size lobsters after 0, 24, 48, 60 and 72 h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Figure 3.33a represents cold (2.6 ˚C) acclimated lobsters and Figure 3.33b represents warm (5.6 ˚C) acclimated lobsters. Values presented as means ± SEM for *n* = 8 animals. a, b and A,B represent significant differences between different letters of the same case ($p < 0.05$) in which differences were compared to the control (T = 0 group). *n* = 256 total.

The summarized acclimation treatment group comparison trial haemolymph glucose data are shown in Figure 3.34. In the Chix trials, both the 2.6 ˚C and 5.6 ˚C groups showed progressively-increased haemolymph glucose levels with emersion duration until the 72

h emersion sampling time, although the 5.6 ˚C group did not reach significantly elevated levels above control values at any sampling time during the emersion or re-immersion period (Figure 3.34a). The 2.6 ˚C acclimation treatment group reached significantly higher haemolymph glucose levels at the 24 h emersion sampling period ($p < 0.0001$) and remained above control levels for the rest of the emersion and re-immersion sampling periods. There were no significant differences between the two temperature groups within the small-sized trial except at the 24 h re-immersion sampling time ($p = 0.0421$). Within the Halves trial, there were no significant differences between the two temperature treatment groups, except at the 60 h emersion sampling time ($p = 0.0014$). The haemolymph glucose levels in the 2.6 ˚C group did not increase significantly above control levels during the emersion or re-immersion trial periods whereas the 5.6 ˚C group had glucose above control values at all sampling periods, except for 24 h and 60 h emersion sampling times (Figure 3.34b).

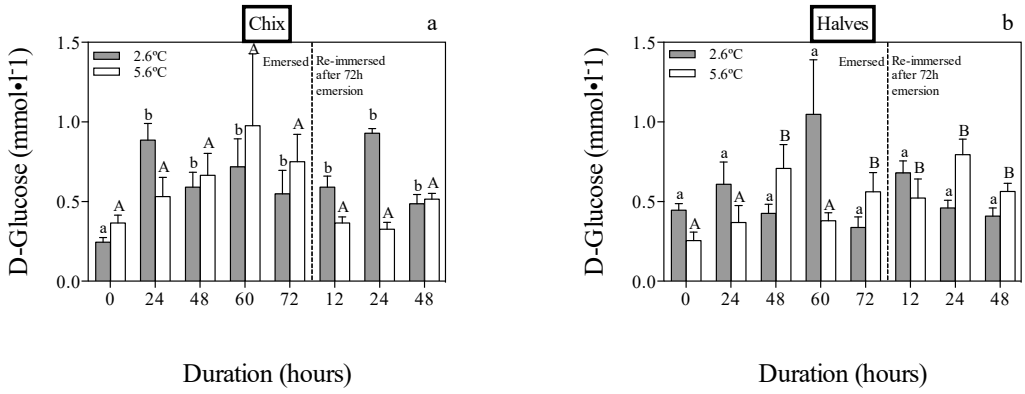

Figure 3.34a,b *Homarus americanus*: Changes to haemolymph glucose for cold (2.6 ˚C) and warm (5.6 ˚C) acclimated lobsters after 0, 24, 48, 60 and 72h emersion at 3.0 ˚C followed by 12, 24 and 48h re-immersion at 3.0 ˚C. Figure 3.34a represents small (Chix) size lobsters and Figure 3.34b represents large (Halves) size lobsters. Values presented as means ± SEM for *n* = 8 animals. a, b and A,B represent significant differences between different letters of the same case ($p < 0.05$) in which differences were compared to the control (T = 0 group). *n* = 256 total.

3.4.3.5 Vigour Index

Beginning at the 48 h emersion sampling time, vigour index began to decline in the 2.6 ˚C / Chix group and at the 60h emersion sampling time for the 5.6 ˚C / Chix group (Figure 3.35). Vigour index continued to decline with successive sampling times in the 2.6 ˚C treatment group, until the 48h re-immersion time at which the number of animals at VI 1 increased from a trial low of 2 to 5 out of 8 animals. One animal was dead at the 60 h emersion time in this trial. Mean VI also continued to decline from that first observed at the 60 h emersion time in the 5.6 ˚C group, with the number of VI = 1 animals per sampling period reaching a minimum of 4 out of 8 animals in the 12, 24 and 48 h re-immersion periods. One animal per temperature treatment was dead at the 60 h emersion sampling time.

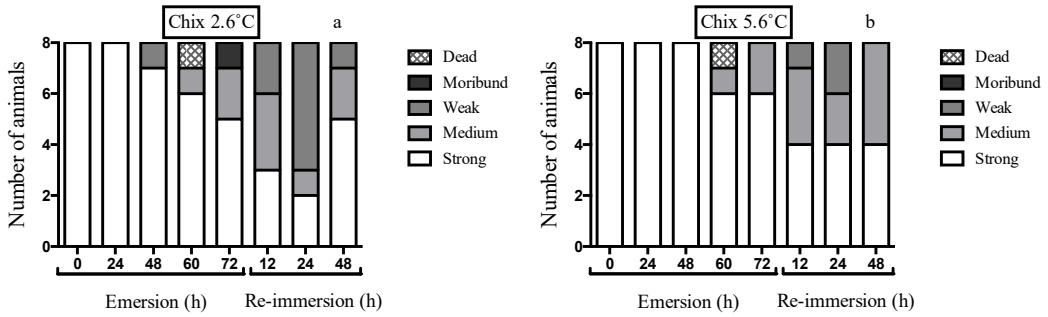

Figure 3.35a,b *Homarus americanus*: Vigour index comparison of small size (Chix) lobsters after 0, 24, 48, 60 and 72h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Figure 3.35a represents the cold acclimation treatment group and Figure 3.35b represents the warm acclimation treatment group. *n* = 8 animals per sampling period for a total of 128 animals.

In the Halves trials, the number of VI = 1 animals began to decline at the 48h emersion sampling time within both the 2.6 ˚C and 5.6 ˚C groups, with *n* = 6 and *n* = 7, VI = 1 animals observed in the 2.6 ˚C and 5.6 ˚C groups respectively (Figure 3.36). The 2.6 ˚C treatment group reached a low number of VI = 1 animals at the 72 h emersion sampling time (1 out of 8 animals) and recovered to *n* = 6 animals at VI = 1 by the 48h re-immersion time. The 5.6 ˚C group reached its lowest number of VI = 1 animals at the 24 h re-

immersion time (2 out of 8 animals) but this group also recovered to n = 6 animals at VI = 1 by the 48 h re-immersion time. No Halve lobsters died during the trials.

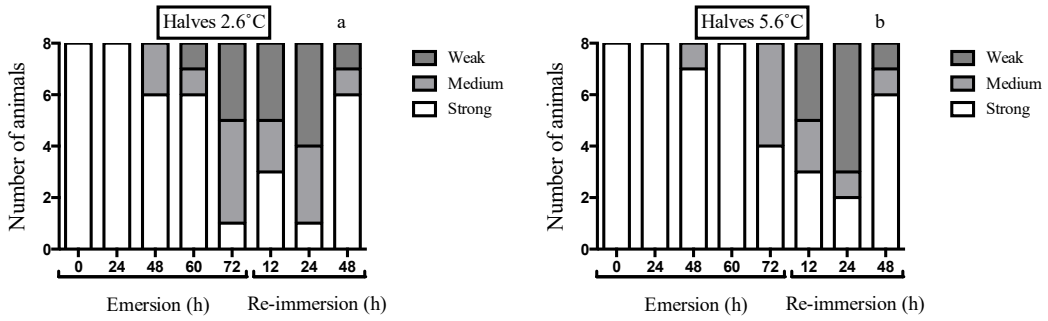

Figure 3.36a,b *Homarus americanus*: Vigour index comparison of large size (Halves) lobsters after 0, 24, 48, 60 and 72h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Figure 3.36a represents the cold acclimation treatment group and Figure 3.36b represents the warm acclimation treatment group. *n* = 8 animals per sampling period for a total of 128 animals.

3.4.3.6 Body Temperature

Lobster core body temperature data for the 72 h emersion / 48 h re-immersion trials are shown in Figure 3.37. The two groups' mean body temperatures were significantly different at all emersion sampling times, with the exception of that at 60 h. Both groups showed a drop from their mean control values (T = 0 h) at the first sampling time of 24 h, followed by an increase to the controlled ambient external room air temperature of 3.0 ± 0.5 ˚C by 48 h. Subsequently, both groups remained at within this temperature range for the remainder of the emersion time. Upon re-immersion, mean body temperature of both groups were within range of the reception water system temperature of 3.0 ± 0.2 ˚C, and remained at this level for the duration of the re-immersion period.

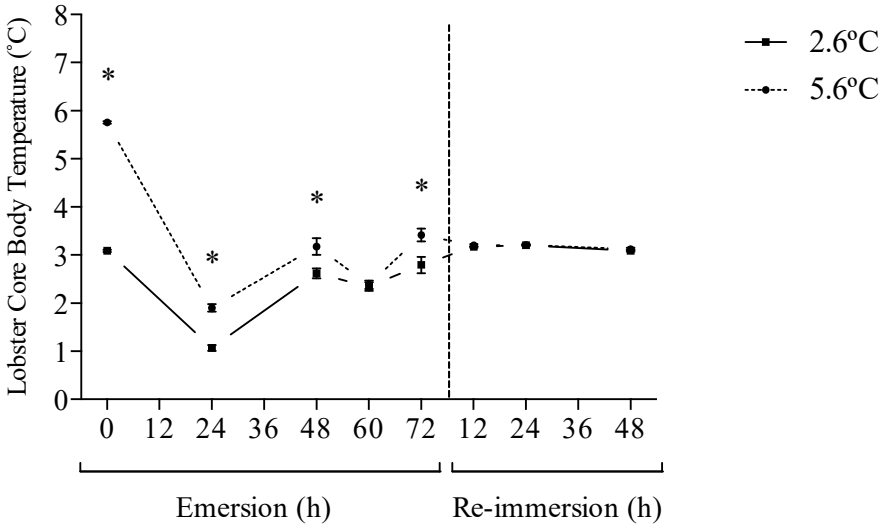

Figure 3.37 *Homarus americanus*: Core body temperature comparison of two acclimation temperature treatment groups (2.6 ˚C and 5.6 ˚C) after 0, 24, 48, 60 and 72 h emersion at 3.0 ˚C followed by 12, 24 and 48 h re-immersion at 3.0 ˚C. Values represent means ± SD for n = 16 animals. * indicate significant difference between temperature treatment groups ($p < 0.05$). n = 128 total.

3.4.3.7 72 h emersion and re-immersion summary

Table 3.3 provides a summary of the results for the 72 h emersion and re-immersion trial. Table 3.3 *Homarus americanus*: summary of results from 72 h emersion and 12, 24 and 48 h re-immersion trials comparing two lobsters sizes (small: Chix and large: Halves) with two pre-treatment acclimations at 2.6 ºC and 5.6 ºC.

Parameter	Summary of findings
Haemolymph Ammonia	Haemolymph ammonia increased overall with emersion time and returned to control levels by 12 h re-immersion in all cases. Halves showed elevated emersed levels compared to Chix with 2.6 ºC treatments but generally below Chix values with 5.6 ºC treatments.
Haemolymph Lactate	Haemolymph lactate increased overall with emersion in both groups and sizes and generally did not return to control levels until 48 h re-immersion. The 2.6 ºC treatment groups had generally elevated lactate levels in both sizes compared to the 5.6 ºC group.
Haemolymph pH	2.6 C treatment lobsters from both sizes declined below control values at 24 h emersion and did not recover to control values for the entire trial period. Recover to control levels occurred with 5.6 ºC treatment groups for both sizes at 24 h re-immersion.
Haemolymph Glucose	Haemolymph glucose levels increased above control levels during emersion in 2.6 C Chix, but not Halves. 5.6 ºC Halves increased above control levels during emersion at 48 and 72 h, but not Chix. Both temperature treatments and sizes recovered slightly with immersion, but only significantly in the 2.6 ºC Halves and 5.6 ºC Chix.
Vigour	Vigour declined more during 72 h emersion in the 2.6 ºC treatments than with 5.6 ºC, although the 2.6 ºC Halves size recorded more Vigour 1 lobsters at 48 h than the 2.6 C Chix. There was one recorded death, in the 2.6 ºC Chix at 60 h emersion, during this trial.
Haemolymph Temperature	Both temperature treatments' lobster haemolymph temperature declined to emersion environment temperature within 24 h and remained at emersion temperature for the duration of the emersion segment. The 5.6 ºC group maintained significantly higher body temperatures during emersion than the 2.6 ºC group, but both groups were not significantly different during all re-immersion sampling times.

3.5 Discussion

These studies were made in order to gain a better understanding of some of the physiological implications of procedures used routinely in the post-harvest environment of *H. americanus*. These implications involve the combined effects of temperature and emersion on the respiratory gas and ion exchange across the gills. The gill epithelium is the principal site for the fluxes of respiratory gases, ions and temperature (Henry et al., 2012; Lin et al., 2002; Lovett et al., 2006; Nanba et al., 2012; Weihrauch et al., 2002).

Because of their filamentous structure, they tend to collapse during emersion and their normal functioning becomes impaired (Taylor and Whiteley, 1989).

The supply chains for live lobsters need to be kept cost effective whilst being effective in terms of delivering live, quality products consistently. Airfreight is the principal mode of commercial bulk distribution of *H. americanus* and airfreight costs and airline companies' regulations prohibit the inclusion of seawater with consignments. Consequently, studies such as these are vital for the seafood industry because lobsters are aquatic species that seldom, if ever, experience emersion in their natural environment. A high humidity maintained at the gill surface does appear to allow some transbranchial epithelium exchanges in a related homarid, *Nephrops norvegicus* (Schmitt, 1995) and any water retained in the gill chamber may facilitate minimal levels of a variety of gill functions. The final gill chamber fluid leaked from 24 h emersed *Cancer pagurus* was found to be rich in ammonia (Uglow and Danford, *pers. com*) but, in view of the small volumes involved, this is likely not to be a significant route for ammonia loss during emersion.

The impaired transbranchial epithelial function during emersion results in the retention of the ammonia produced at this time, much of it accumulated in the haemolymph. In the present study, the ammonia production appears to be constant and temperature dependent, as evidenced by the linear increases in the relative haemolymph ammonia concentration with emersion duration. It may have been expected that the lower temperature-acclimated group would have had the lower rate of ammonia production such as was observed in *Panulirus japonicus* at 5 and 15 ˚C (Chen and Huang, 2001), but in this study the reverse was found to occur. It is known that ectotherms utilise different strategies to compensate for decreasing environmental temperature, and in some cases protein degradation occurs due to protein phase changes as the temperature approaches 0 ˚C (Ramløy, 2000; Issartel et al., 2005). Such protein degradation may account for an elevated haemolymph

ammonia observed in the current study. The elevated haemolymph ammonia levels may also have been a consequence of a mass-dependent difference in the metabolic rate and will be the subject of future studies.

The removal of haemolymph ammonia following re-immersion was much more rapid than the preceding accumulation during emersion. In addition to the haemolymph ammonia accumulation, a haemolymph acidosis and a haemolymph accumulation of L-lactate were found to occur during the emersion period of these studies, which thus support the findings of others. [e.g. *Cancer productus* (deFur and McMahon, 1984); *Cancer pagurus* (Regnault, 1992); *Nephrops norvegicus* (Bernasconi and Uglow, 2008b; Schmitt and Uglow, 1997); *Necora puber* (Durand and Regnault, 1998)]. The generally lower acclimation temperatures used in the present studies will have resulted in the *H. americanus* having generally lower ammonia production rates than those studied by Danford et al (1999). Schmitt and Uglow (1998) suggested that the acidosis found in emersed *N. norvegicus* was caused mainly by CO_2 accumulation rather than by the accompanying lactate accumulation. Re-immersion resulted in a rapid lowering of the haemolymph lactate concentration accumulated during the emersion period as found also by Bernasconi and Uglow (2006).

L-Lactate is the main end-product of glycolysis during environmental and functional anoxia in crustaceans (Gäde, 1983) and, in these studies, haemolymph lactate concentrations increased progressively during the emersion period, thus indicating a switch to anaerobic metabolism and supporting the findings of studies relating to a range of marine crustacean species [e.g. those of Bernasconi (2006); Johnson and Uglow (1985); Schmitt and Uglow (1997); Spicer et al. (1990); Taylor and Whiteley (1989; 1987); Tyler-Jones and Taylor (1988); Vermeer (1987)].

Ellington (1983) showed that lactate is removed from the haemolymph during recovery from anaerobiosis. Its metabolic fate appears to be uncertain (Grieshaber et al., 1994) and,

generally, its removal in crustaceans is a protracted process (Albert and Ellington, 1985; Bridges and Brand, 1980; Hervant et al., 1999; Phillips et al., 1977; Zou et al., 1996). Lorenzon et al (2007) found that *H. americanus* mean haemolymph lactate levels following air transport from North America to Italy (typically a *ca* 36 h consignment) only returned to within control values after 24 h re-immersion. Bernasconi and Uglow (2006) found that mean haemolymph lactate levels of *Nephrops norvegicus* after 2h of re-immersion remained higher than control values, and a return to control (pre-emersion) values was found to occur after 12h for *Panulirus interruptus* (Gomez-Jimenez, 1998); 14h for *Homarus gammarus* (Whiteley and Taylor, 1992) and 24h for *Orconectes lomosus* (Gäde, 1984). This general trend has been observed also in the present studies in which haemolymph lactate levels were found to continue increasing to above the final emersion levels for the first 12h of the subsequent re-immersion period before returning to control values after 24h following re-immersion. Jackson et al. (2001) observed a buffering effect of lactate production during emersion in the crayfish *Austropotamobius pallipes* in which lactate was being sequestered in the carapace as well as accumulated in the haemolymph. Upon re-immersion the lactate sequestered in the carapace is released back to the haemolymph causing the prolonged increase in haemolymph lactate post immersion despite availability of oxygen and cessation of anaerobic metabolism. This lactate wash out effect on post-emersion haemolymph lactate accumulation was observed in the present study for periods of up to 48 hours re-immersion.

Emersion and physical disturbances such as handling are known to cause a release of crustacean hyperglycaemic hormone (CHH) into the haemolymph that, in turn, mediates the release of glucose from carbohydrate stores in crustaceans (Chung et al., 2015; Johnson and Uglow, 1985; Kleinholz et al., 1950; Schmitt and Uglow, 1997; Speed et al., 2001). In the present studies, both size groups showed an emersion-induced hyperglycaemia after 24h, and this was particularly pronounced in the lower temperature

groups. The lower temperature groups showed a partial recovery after 24 h, unlike the warmer temperature groups, but then returned to the pronounced hyperglycaemic values at 60 h emersion, then decreased to a low hyperglycaemic value at 72h emersion before increasing to a higher value at 96 h emersion and increased for the first 24 h of the re-immersion period for both the 48 and 72 h emersion / 48 h re-immersion trials before returning to control values after 24 h re-immersion.

Although the time course of change of the haemolymph glucose levels in the lower temperature group appears to be random, it parallels the changes found for another homarid, *Nephrops norvegicus*, under very similar experimental conditions (Bernasconi, 2006) and compares well with a study by Morris and Oliver (1999a) in which > 30h emersion at < 5 ˚C induced a haemolymph hyperglycaemia from 0.4 to 0.85 mml glucose l^{-1} in *Jasus edwardsii*. Possibly, the stored glycogen reserves could not be accessed because of an internal hypoxia, which would also help explain the increased haemolymph lactate levels that occurred during emersion. Maximum haemolymph glucose levels during emersion were similar to those observed by Danford et al. (1999) in emersed *H. americanus* during a commercial consignment in which glucose levels reached a maximum of approximately 0.6 mml l^{-1} during emersion and 0.87 mml l^{-1} 18 days post emersion whilst immersed.

Animals acclimated at different temperatures are known to have concomitant thermal tolerance relationship to changes in their environmental temperature (Kumlu, Türkmen, et al., 2010; McLeese, 1956; Re et al., 2012). Mathur (1982) observed a linear relationship between acclimation temperature and the preferred and avoided temperature range in *Orconectes obscurus*. A similar relationship in *Macrobrachium acanthurus* reveals that an animal acclimated to higher temperatures has a higher thermal preference range *compared with* the same species acclimated to lower temperatures (Díaz et al., 2002), and that these acclimation-related differences affect the optimal cultivation parameters which

are then dictated by the seasonal seawater temperature variations. In the present study, acclimation temperature had a significant effect on the haemolymph parameters associated with metabolic rate during emersion, but not in the simple, absolute comparison of lower and higher acclimation temperature treatments (i.e. 2.6 ˚C group lobsters responded with higher metabolic rates than 5.6 ˚C acclimated lobsters during temperature-controlled emersion). The observed responses with *H. americanus* in the present emersion trials indicated a larger increase in haemolymph ammonia and lactate in the 2.6 ˚C acclimated treatment group versus the 5.6 ˚C group. Body temperature observations during emersion revealed that the 5.6 ˚C acclimated lobsters were effectively chilled down by 3.9 ˚C to 1.9 ˚C body temperature at the first sampling period of 24 h emersion, which probably caused the observed reduction in metabolic rate and subsequent reduced accumulation of haemolymph ammonia and lactate (compared with the 2.6 ˚C group in which the mean body temperature groups were chilled down by 2.0 ˚C at 24 h emersion from pre-trial acclimation temperature values). The decrease in body temperature below the 3.0 ± 0.5 ˚C controlled air temperature in the emersion storage space was likely caused by the gel ice packs included in the packed boxes of lobsters. The gel ice packs have a starting temperature of -18 ˚C due to being stored in a commercial freezer as per standard industry practice. The gel ice packs are designed specifically to reach and maintain a melting point of 3 ˚C, and presumably absorbed excess heat within the first 24 h of emersion due to their low starting temperature which effectively reduced the lobsters' mean body temperature during this period. Whiteley et al. (1995) observed that during acute air temperature change, *Homarus gammarus* showed a body temperature adjustment to the emersion air temperature within 1 – 2 h of emersion. This was likely the case in the present study and it can be assumed that lobsters in the emersion trials had reached emersion environmental temperatures by the first sampling time of 24 h emersion. The gel ice packs likely reached their melting point of 3 ˚C between the 24 h

and 48 h emersion sampling times—based on the observed increase in mean lobster body temperature at the 48 h emersion sampling time, after which mean body temperatures matched the controlled environmental trial temperatures.

Vigour index declined in both temperature groups and sizes with increasing emersion time, but contrary to expectations, the cold (2.6 ˚C) acclimated lobsters declined in vigour index more rapidly than the warm (5.6 ˚C) acclimated lobsters. The emersion temperature of 3 ˚C may have been a factor in the unexpected results based on premise that the cold acclimated group body temperatures would have warmed up to the 3 ˚C external environment whereas the warm acclimated group would have been chilled down to the 3 ˚C. Cooling is a known practice for pre-conditioning lobsters for emersed commercial consignments (Morris and Oliver, 1999a) to reduce the effects of handling stress and to slow metabolic rate and therefore prolong aerobic metabolism while emersed. This chill conditioning effect would only have occurred in the warm acclimated group in the present study. This suggests that pre-consignment acclimation temperature may be an important consideration in determining the emersion temperature environment during commercial shipping. In the present study, colder pre-conditioning of the lobsters appeared to be detrimental to the long term emersion success based on current industry practices of a 3 ˚C emersion environment. Within the *H. americanus* live trade it is commonly understood that cold storage and shipping conditions are required to ensure a high success rate of landed, post consignment strong (Vigour Index 1) animals. However, based on the observations from the current studies, one must take into consideration acclimation history so as to ensure that the temperature set point chosen for conditioning pre-consignment animals and that the environmental conditions during emersion should remain at or below acclimation temperatures. This will have the effect of reducing the animals' resting metabolic rate and cause a delay in the onset of the effects of emersion that lead to degradation of quality during commercial airfreight shipments.

3.5.1 Summary of findings

- Haemolymph ammonia may possibly be useful as an indicator of metabolic rate during emersion, with steeper increases in animals observed at higher emersion body temperature than their acclimation temperature compared with those held emersed with a body temperature lower than acclimation temperatures.
- Unlike ammonia, haemolymph lactate does not decrease as quickly during re-immersion from prolonged emersion events, with levels not reaching control values until 48 h re-immersion. This apparent slow recovery response may provide a useful tell-tale indicator for commercial firms that wish to know the pre-purchase history of incoming live lobsters in which emersion time and temperature abuse that may occur will be detectable through haemolymph lactate analysis.
- pH appears to be another good indicator of the onset of anaerobic metabolism in which haemolymph acidosis occurs with emersion time. However, a slight recovery from acidosis appears to occur during extended periods of emersion as well as during re-immersion and therefore may not be a particularly reliable measurement for understanding handling or environmental storage condition abuse that may have occurred during emersion.
- Size had little influence over any of the emersion parameters. It may, however, play a more significant role when large loads of emersed animals are above refrigeration set points within the transport container system and may take even longer to reduce body temperatures down, if at all, during the consignment.
- Acclimation temperature had a significant effect on the measured emersion parameters. A lobster's acclimation history should be taken into consideration

when determining ideal shipping temperatures and times. The general consensus in the *H. americanus* industry is that a temperature range of 3-5 ˚C is ideal to ship lobsters emersed, but this may be an over-generalization that works only if lobsters are acclimated at or above this temperature range prior to emersion. Otherwise, long term, low temperature stored lobsters may have the opposite effect of an increased metabolic rate at this emersion temperature range that could lead to degradation of quality during emersion.

Chapter 4
The Cardioventilatory Response to Environmental Temperature of the Immersed Lobster, *Homarus americanus*

4.1 Introduction

A lobster's natural environment is the seabed, which although a relatively stable environment compared to the terrestrial environment, is nevertheless in a state of constant change with respect to some environmental variables (e.g. temperature). Such changes are the results mainly of small, gradual, seasonal changes as well as those brought about by natural ocean bottom currents. Intertidal animals may experience extreme environmental temperature changes within a single tidal cycle, particularly if the animal becomes emersed during low tide. Lobsters are capable of being a highly mobile species and are able to travel as much as 11km per day (Campbell and Stasko, 1986). Thus, potentially, they could encounter significant temperature changes in their environment during their daily movements. The responsiveness of lobsters to the range of temperatures and the rates of temperature change that they experience is of considerable interest from both an ecological and a commercial perspective. Ideally, the responsiveness of an animal to a measured change in the intensity of an external variable (e.g. temperature) is measured in terms of an altered activity of a part, or the whole, of the organism in response to measured changes of the variable. The activity measured may be overt (e.g. an escape response of the animal away from the source of the change (Mathur et al., 1982; Crossin et al., 1998) or covert such as using an altered metabolic rate resulting in an altered respiration and metabolite production rates (Airriess and McMahon, 1994; Goudkamp et al., 2004).

Cardioventilatory activities of crustaceans, as represented by heart and scaphognathite beat behaviours, have been studied extensively (Uglow, 1973; deFur et al., 1988; McMahon, 2001) and reveal responses related to environmental changes such as

increasing or decreasing oxygen concentration (Uglow, 1973), temperature (Jury and Watson, 2000) and salinity (Dufort et al., 2001). As such activities can be monitored continuously, they represent a sensitive means by which the metabolic responses of lobsters to changes in the intensity of some environmental variables (e.g. water temperature or oxygen saturation) can be observed and quantified. Lobsters are ectothermic, with a core body temperature that closely approximates that of the surrounding ambient seawater or air. As described in Chapter 3, changes to the circulating levels of aerobic or anaerobic metabolites are elicited as responses to changes to the intensity of environmental variables (e.g. temperature or oxygen availability). Such events, however, can be monitored principally by periodic sampling, which itself can provoke alterations to the animal's metabolic rate. Cardioventilatory activities can be monitored using non-invasive techniques that impose little or no physical burden to animals the size of commercial lobsters (> 450g) and thus are particularly useful as an aid for evaluating the metabolic effects of changes to the intensity of environmental variables that occur commonly in the commercial supply chains of live lobsters – particularly as such changes may result in commercial quality loss of the lobsters. Monitoring techniques like these have the added advantage that they facilitate the optimisation of supply chain procedures such that the prospects of product quality maintenance can be enhanced.

4.1.1 Aims and objectives

The principal aims of this Chapter were to measure cardioventilatory activities of immersed lobsters during temperature change events and their resulting body temperature changes to determine whether there is evidence of behavioural regulation of body temperature change in an attempt to moderate the acute changes to the temperature of

their environment, and whether there are size-related differences in their behaviours or outcomes.

4.2 Materials and Methods

4.2.1 Animal Handling and Husbandry

Two commercial size classes of lobsters (Chix at 464 ± 35g and Selects at 956 ± 95g) were used for this trial (n=8 per group for a total of 48 lobsters, including two control groups). Chix represent the smallest legal size landed in the fishery and the Selects provided a body weight approximately twice the size of the Chix and therefore any expected size-related differences in observations should be present with this span in weight category. The lobsters were trap-harvested from Canadian Lobster Fishing Area (LFA) 34 and landed in West Pubnico, Nova Scotia by inshore lobster fishermen (Figure 4.1).

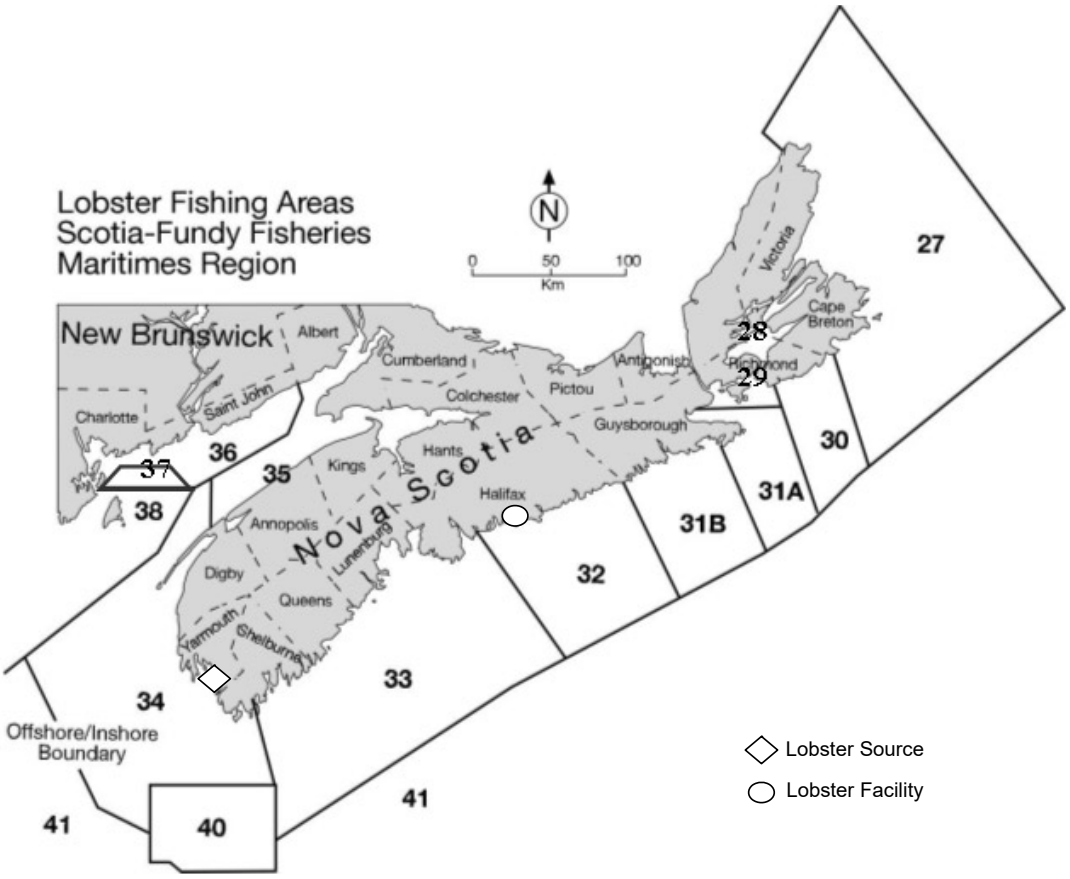

Figure 4.1 Atlantic Canadian Lobster Fishing Area Map indicating source of landed lobsters (◊) as well as the facility in which the trials were conducted (◯). Lobster Fishing Areas are divided by solid lines. Adapted from Canadian Department of Fisheries and Oceans website: http://www.dfo-mpo.gc.ca/fm-gp/peches-fisheries/ifmp-gmp/maritimes/img/lobfisareas-eng.jpg. Accessed on December 28, 2014).

The lobsters were handled as per typical industry practice and described in Chapter 1. Lobsters for this trial were transported emersed for 4 hours to Clearwater Seafoods' lobster facilities in Halifax, Nova Scotia and immediately placed into 3.0 ºC ambient flow-through seawater for 48 hours prior to the start of the trials.

4.2.2 Cardioventilatory Measurement

Heart and scaphognathite activities were recorded using a non-invasive, infrared photoplethysmography technique (Depledge and Andersen, 1990). In the present study, two types of infrared optocouplers (CNY70 & TCRT1000, Vishay Intertechnology, Inc.)

were connected to a PowerLab® 8/35 data acquisition system and an ML228 Bridge Amplifier (ADInstruments, Inc.) via an 8-pin DIN plug that provided 5VDC excitation voltage to power the optocoupler when a 470Ω programming resistor was connected to pins 5 and 8 and recorded the sensor output voltage (Figure 4.2). The CNY70 required a 120Ω resistor and the TCRT1000 a 150Ω resistor soldered inline to the +5VDC anode of the IR LED and detector (Figure 4.3). The optocoupler terminals were potted in waterproof epoxy putty (LePage® Putty Epoxy, Henkel Canada) to protect the wiring during immersion trials. The sensor output signals were displayed on a Macintosh MacBook portable computer and recorded for later analysis using LabChart® 8.0.5 (ADInstruments Inc.).

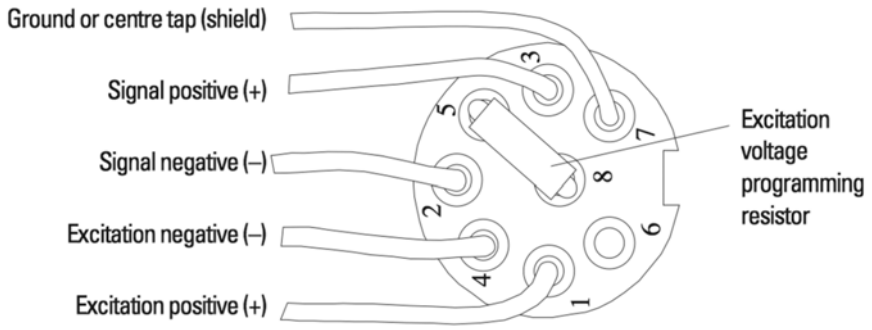

Figure 4.2 ADInstruments 8-pin DIN plug configuration for a typical transducer setup. The current optocouplers used pins 1, 3 and 4 as well as 5 and 8 for the programming resistor to emit +5VDC on pin 1 (From ADInstruments ML228 Octal Bridge Amp Owner's Manual, ADInstruments Incorporated).

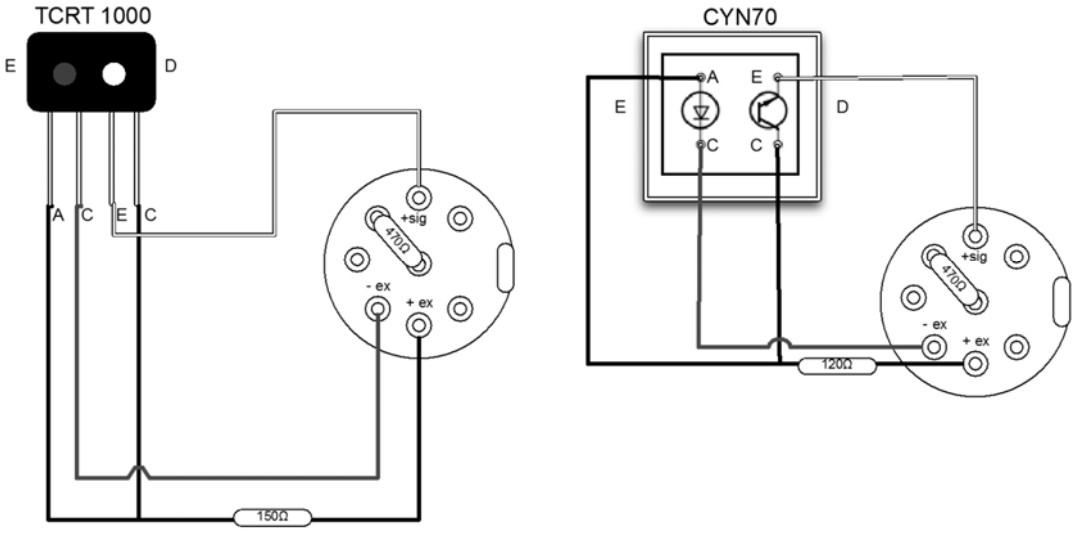

Figure 4.3 Two optocoupler wiring diagrams for use with the ADInstruments ML228 Octal Bridge Amp. The TCRT1000 was used for lobster scaphognathite activity and the CNY70 was used for the heart activity.

The optocouplers were attached temporarily to the lobster's exoskeleton surface using cyanoacrylate adhesive (LePage® Gel Control® Super Glue, Henkel Canada). This rapid-drying adhesive was used to reduce the handling stress known to influence the heart rate response (Wilkens et al., 1985; Schapker et al., 2002). The CYN70 sensor was attached on the dorsal carapace surface above the pericardial sinus for heart rate measurements and the lower-profile, flat TCRT1000 sensors were attached on the left and right anterior ventral region above the scaphognathites (Figure 4.4). After each trial the sensors were removed easily without damage to the lobster's exoskeleton or the sensor.

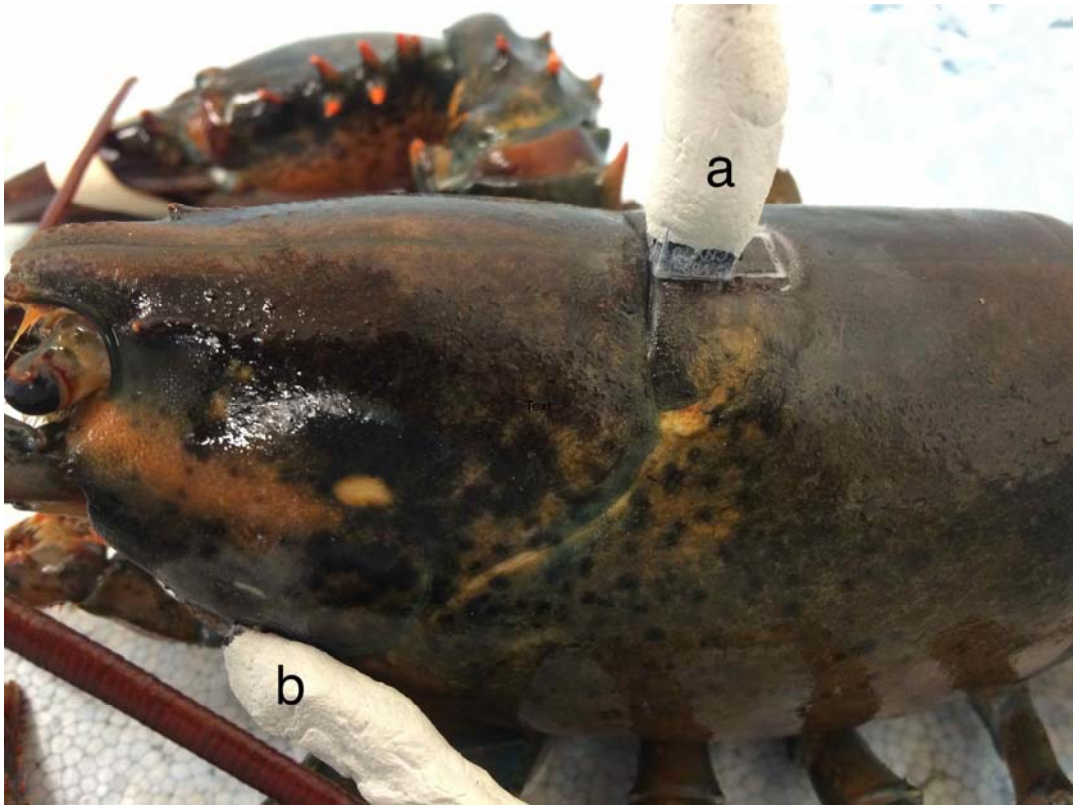

Figure 4.4 Infrared optocoupler sensors attached with cyanoacrylate glue on the carapace of *Homarus americanus* directly over the heart (a) and left scaphognathite (b) for cardioventilatory rate measures (right scaphognathite sensor not shown in figure).

4.2.3 Haemolymph Temperature Measurement

Lobster haemolymph temperature was measured with a 36-gauge thermocouple wire (Omega Engineering Canada, Inc., QC, Canada) inserted approximately 1 cm through the arthrodial membrane of one of the 4th periopods and secured with a nylon tie wrap fastener (Figure 4.5). Once the thermocouple was inserted, the lobster was replaced to its acclimation temperature tank for 24 h prior to the start of the temperature change trials—the delay to allow recovery from any effects of the invasive handling to attach the thermocouple. The thermocouples were connected to a TC-08, 8-channel USB thermocouple data logger (Pico Technologies Limited, UK) and the data were logged to a PC running the Pico Technologies data logging software.

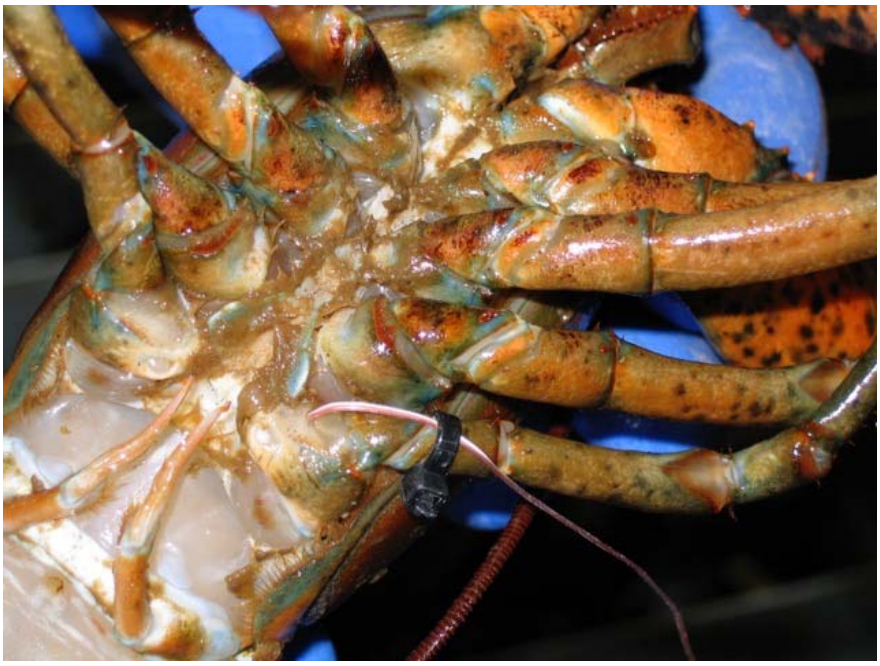

Figure 4.5 An example of a thermocouple inserted through the arthrodial membrane of the 4th periopod of a *Homarus americanus*. The thermocouple wire was secured with a black tie-wrap clasp shown above.

4.2.4 Cardioventilatory Rate Trials

For the cardioventilatory rate trials, two independent TSL-250, 950 L refrigerated lobster storage systems (Bionovations, Inc., NS Canada) were connected to a rectangular, 30 litre insulated flow-through opaque chamber with close fitting lid with internal dimensions of 42 cm x 28 cm x 15 cm (L x W x H) (The Coleman Company, Inc.). The chamber contained an inlet and outlet connected by ½" internal diameter flexible, food grade silicone tubing at opposite ends of the chamber (Figure 4.6).

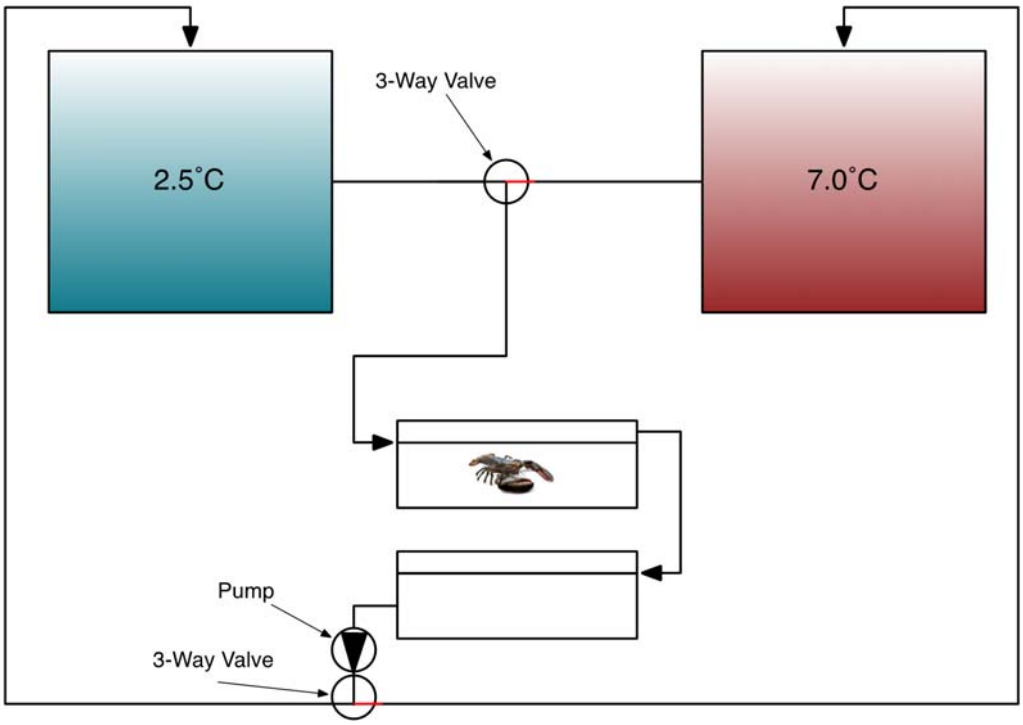

Figure 4.6 *Homarus americanus* temperature change system setup. Two independent tanks (2.5ºC and 7.0ºC) supply water to the trial chamber (pictured with lobster). Pump and control valves allow for rapid adjustment of water temperature from one level to the other.

The inlet hose was connected directly to each TSL-250 tank system via an automated 3-way ball valve to direct the flow from either the cold (2.5˚C) or warm (7˚C) system. Water exited the trial chamber via the outlet and was gravity fed into a 2nd 30 litre insulated reservoir chamber that contained a submersible pump with the outlet connected to a 2nd automated 3-way ball valve to direct the exit water back to the appropriate temperature control system via ½" internal diameter flexible food grade silicone tubing. The 2nd reservoir chamber was used to isolate the pump system from the chamber containing the lobster and avoid interaction between the water circulation equipment and the lobster.

Lobsters were taken from the acclimation temperature tank (at either 2.5 or 7.0 ºC) and IR sensors attached to the carapace directly over the heart and left and right scaphognathites. They were then immediately placed in the temperature change chamber with water recirculating from the same source and temperature. Due to the minimal handling required to attach the IR sensors, lobsters were left for 30 minutes after sensor

attachment to recover prior to starting the recording. After the 30 minutes, recording of the heart, scaphognathites and haemolymph temperature was started. A sampling period of 30 minutes of resting state cardioventilatory activities was recorded prior to the temperature change event. After the initial 30-minute resting state recording was complete, the inlet water source to the chamber was switched to the other treatment temperature (2.5˚C to 7˚C and 7˚C to 2.5˚C) using the automatic 3-way ball valve assemblies. Recording continued until the lobster haemolymph temperature stabilised at the new treatment temperature.

Once all temperature change trials were complete, one of the two controlled temperature tanks was adjusted to match the temperature of the other tank (2.5ºC / 2.5ºC and 7.0ºC / 7.0ºC). Control animals being held in at 2.5ºC were equipped with the scaphognathite and heart IR sensors and put through the same cardioventilatory recording trials as above, but the switching of water source from one tank to the other occurred without a temperature change to observe any effects of the water source change in the absence of any water temperature change. No differences in mean heart or scaphognathite beat rates were observed in either size or temperature acclimation group in the control trials indicating that the trial data results can be attributed solely to a temperature change event.

4.2.5 Treatments

From the landed shipment, vigour and condition index 1 Small Chix- and Selects-sized lobsters (strong and undamaged as described in Chapter 1) were chosen for the trial. The 2 size groups were further divided into two groups by temperature treatment with the "Cold" and "Warm" groups of lobsters placed in recirculating, temperature-controlled systems maintained at 2.5°C and 7.0˚C, respectively. These temperatures are typical low and high seawater temperatures experienced in an ambient, flow through commercial lobster storage systems during the fishing seasons. Natural seawater was used in the

systems with carbon filtration integrated in-line with the water flow to the experimental chamber to reduce the effect of different chemosensory detections that may cause cardioventilatory rate changes independent of temperature (Larimer, 1964; Burnovicz et al., 2009). A validation trial, in which both systems were at the same temperature for the trial, was conducted to confirm that the novel chemosensory effect was not present. Lobsters were held unfed for a minimum of 14 days at each temperature treatment prior to the temperature change trials to allow for acclimation to the starting temperature.

For the cardioventilatory trials, each lobster had three IR sensors attached to its carapace with rapid-drying cyanoacrylate glue approximately 30 minutes prior to the trial. These were used to measure heart and left and right scaphognathite beat rates simultaneously.

4.2.6 Statistical Analysis

Haemolymph temperature curves were compared between size groups using a sigmoidal curve-fit model for the temperature increase and decrease trials in which independent fits were compared to a global fit and the maximum slope was compared between groups using a t-test. The D'Agostino-Pearson omnibus normality test was used to determine whether the residuals were Gaussian and the goodness of fit was quantified with r^2. Replicates tests were used to determine if the curves systematically deviated from the data points. The rate of temperature change data had non-linear Gaussian fit models applied to compare separate fitted curves to a single global fitted curve. Normality tests as stated above were used and goodness of fit quantified with r^2 and noted in the Results text. The decreasing temperature data were transformed to absolute values to allow for proper application of the Gaussian fit model. All model curves were considered significantly different at $p < 0.05$ and separate models were rejected if they did not pass the normality test.

4.3 Results

4.3.1 Haemolymph Temperature

Results for immersed haemolymph temperature change trials, for both the 4.5 ºC increase and decrease, are shown in Figure 4.7 a & b respectively. Sigmoidal curve-fit models of the same data are shown in Figure 4.8 a & b. In each trial, both the small- (Chix) and large- (Selects) sized lobsters had similar haemolymph temperature change curve profiles throughout the 50-minute trial ($p > 0.05$). A 5-minute delay was observed before the onset of haemolymph temperature change in both size groups in the increasing temperature trial and a 3-minute delay observed in both size groups in the decreasing temperature trial. In the increasing temperature trial, the Chix reached their maximum mean haemolymph temperature after 22 minutes whereas the larger-sized Selects reached the same maximum mean haemolymph temperature after 37 minutes. The curves for the Chix and Selects temperature increase trial both had strong goodness of fit with r^2 values of 0.9477 and 0.9473 respectively. In the decreasing water temperature trial, both the Chix and Selects mean haemolymph temperature reached their lowest level at approximately 33 minutes after the temperature change start and the sigmoidal curve-fitted models were not significantly different ($p > 0.05$), but had strong r^2 goodness of fit values of 0.9971 and 0.9977 for the Chix and Selects, respectively. The transition midpoint times at which the mean lobster haemolymph temperature was at 50% of the new acclimation temperature was 12.38 minutes for the Chix and 13.22 minutes for the Selects, although they were not significantly different ($p = 0.0725$). However, there was a significant difference between the Chix and Selects transition midpoint times during the increasing temperature trial ($p = 0.023$), in which the values were 10.51 minutes and 11.28 minutes respectively.

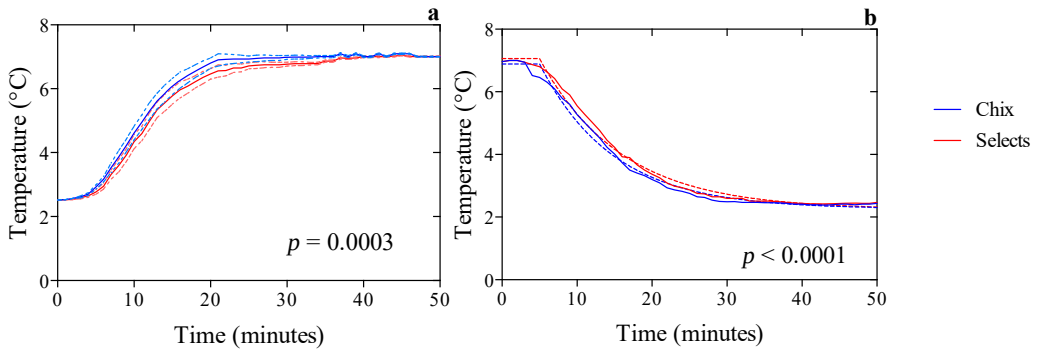

Figure 4.7 *Homarus americanus*: Haemolymph temperature change in small (Chix, blue line) and large (Selects, red line) sized lobsters exposed to an acute increase in water temperature from 2.5ºC to 7.0ºC (Figure a) and an acute decrease in water temperature from 7.0ºC to 2.5ºC (Figure b). Matched colour broken lines indicate SEM. n = 16 animals per temperature change trial; *p*-values < 0.05 indicate significant differences between curve-fit slopes of size groups in each trial as shown in Figure 4.8a & b.

The haemolymph temperature data in the curve-fit models in Figure 4.8b for the decreasing temperature trial were transformed to 1/Haemolymph temperature to give a positive sigmoidal curve for data analysis.

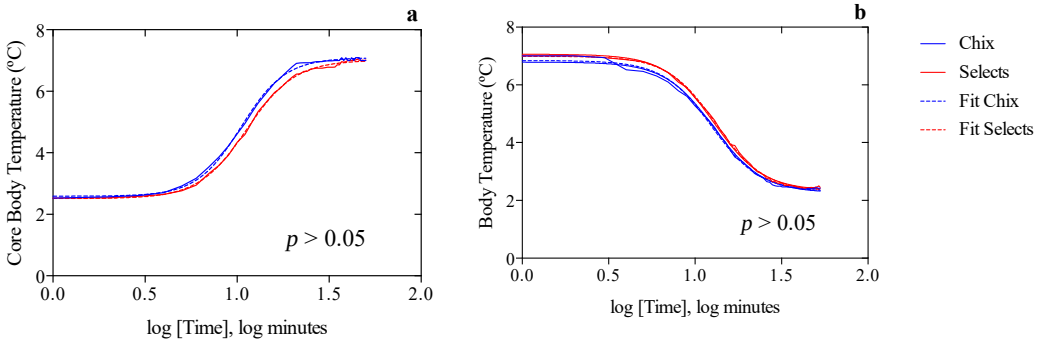

Figure 4.8 *Homarus americanus*: Haemolymph temperature change in small (Chix, blue line) and large (Selects, red line) sized lobsters exposed to an acute increase in water temperature from 2.5ºC to 7.0ºC (Figure a) and an acute decrease in water temperature from 7.0ºC to 2.5ºC (Figure b). Matched colour broken lines indicate sigmoidal variable slope curve fit lines. Decrease temperature data expressed as 1/Y to use the curve-fit model. n = 16 animals per temperature change trial; *p*-values indicate significant differences between curve-fit slopes of size groups in each trial (p < 0.05).

The rates of change of haemolymph temperature were calculated and compared with the immersion water temperature for each trial as shown in Figure 4.9a & b. An observed maximum positive rate of change of 0.375 ºC•min^{-1} occurred at 8.5 minutes for the Chix

and 0.362 ºC•min^{-1} at 9.5 minutes for the Selects compared with a rate of change of 0.480 ºC•min^{-1} at 7.5 minutes for the water bath data (Figure 4.9a). Increasing temperature-change curve models were fitted to each size group and water temperature and revealed significant differences between the two size group mean haemolymph temperatures and the water temperature ($p < 0.0001$), but not between the two size groups, independent of the water temperature curve ($p > 0.05$). Decreasing temperature-change curve models showed similar trends of results with no significant differences between the size groups of Selects, Chix and water temperature data ($p > 0.05$), but a significant difference existed between the two size groups and the water temperature curve ($p < 0.0001$). There was an observed maximum negative rate of change of −0.274 ºC•min^{-1} at 10.5 minutes for the Chix compared with −0.271 ºC•min^{-1} at the same time point for the Selects (Figure 4.9b). This contrasts with the water bath data that showed a maximum negative rate of change of −0.378 ºC•min^{-1} at 6.5 minutes into the temperature change event. Fitted non-linear model curves for the increasing temperature trial showed strong goodness of fit for both the Chix and Selects data (r^2 values of 0.9765 and 0.9728 respectively) as well as the water bath data ($r^2 = 0.9606$) (Figure 4.9a). Fitted non-linear model curves for the decreasing temperature trial also showed strong goodness of fit for both the Chix and Selects data (r^2 values of 0.9532 and 0.9750 respectively) as well as the decreasing water data ($r^2 = 0.8715$) (Figure 4.9b).

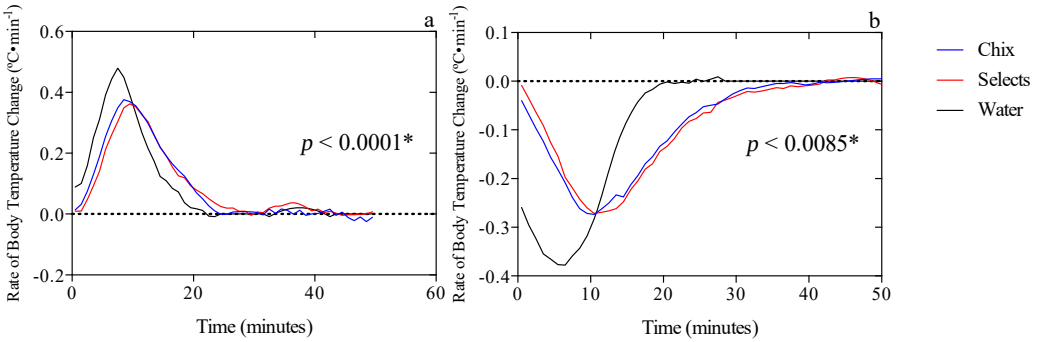

Figure 4.9 *Homarus americanus*: Haemolymph temperature rate of change (in ºC•min^{-1}) for small (Chix, blue line) and large (Selects, red line) for 50 minutes immersion during an acute temperature change at Time = 0. Figure a and b represent a 4.5ºC water temperature increase and decrease respectively. Black line represents immersion temperature rate of change. p-values represent test for differences between size class and water bath curve slopes. * indicates significant differences (p < 0.05).

For illustrative purposes, the haemolymph temperature change of a Vigour Index 5 (dead) lobster was collected under the same conditions as those used to collect the temperature change data of the active, Vigour Index 1 animals from the temperature change trials. The results obtained for an acute, 4.5 ºC increase in immersion temperature (Figure 4.10) reveal that the haemolymph temperature increase of the Vigour Index 5, Select-sized lobster increased linearly whereas a sigmoid pattern rate of increase in the vigour index 1 Select was observed. The core temperature increase of the Vigour Index 5 animal was also much slower than that of the Vigour Index 1 animal and, at the end of the 50-minute trial, had not attained the maximum immersion bath temperature.

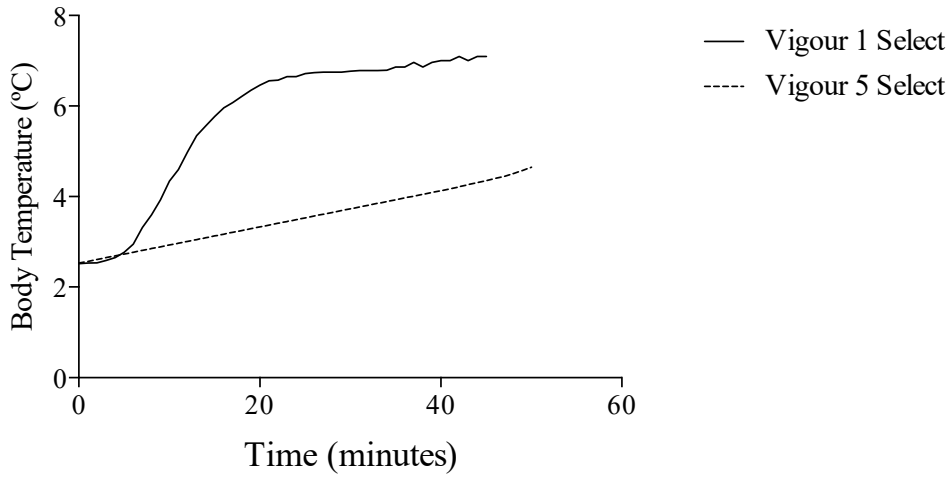

Figure 4.10 *Homarus americanus*: Comparison of mean haemolymph temperature (ºC) in active (Vigour Index 1) with a dead (Vigour Index 5) Select-sized lobster during an acute water temperature change from 2.5ºC to 7.0 ºC. n = 8 animals Vigour Index 1 and n = 1 Vigour Index 5 animal.

4.3.2 Cardioventilatory Behaviour

A summary of the acute, decreasing temperature recordings for heart and scaphognathite trials is shown in Figure 4.11a & b, respectively. Both size groups showed a decline of both heart and scaphognathite beat rates after approximately a 1-minute delay. The Chix size group showed a steeper decrease in heart rate (compared with the Selects) but the scaphognathite rates of both size groups declined at the same rate for the first 5 minutes following exposure to the colder, 2.5ºC water. The Chix had the higher initial heart rate but showed the higher decrease in mean heart rate to reach a final value below that of the Selects mean heart rate. Contrastingly, the mean scaphognathite rate of both size groups remained broadly similar throughout the trial (Figure 4.11b) although the Chix maintained a final mean beat rate of between 6-14 bpm higher than the Selects. Both heart and scaphognathite beat rate curves showed a similar shape to the mean haemolymph

temperature change, although the haemolymph temperature displays an offset delay of approximately 5 minutes.

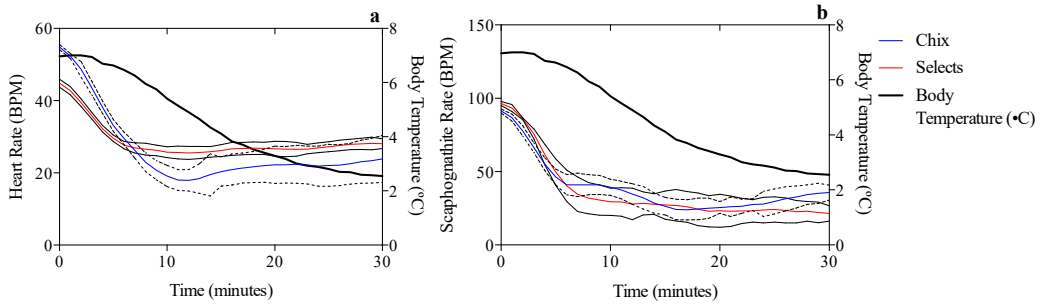

Figure 4.11 *Homarus americanus*: Mean heart (Figure 4.11a) and scaphognathite (mean of left & right) (Figure 4.11b) beat rate in beats per minute for small- (Chix, blue line) and large- (Selects, red line) sized lobsters after an acute, immersed temperature decrease from 7.0°C-2.5°C. The bold black line is the combined mean haemolymph temperature change. Rates are in beats per minute with black lines indicating standard error. n = 8 animals per size group.

A summary of the acute, increasing temperature recordings for heart and scaphognathite trials is shown in Figure 4.12a & b, respectively. At the start of the temperature increase, heart rates began increasing in both size groups. However, the Chix group increased at a faster rate and reached a maximum peak mean heart rate of 48 bpm before leveling out to a rate of 46-48 bpm for the remainder of the trial period, 16 bpm higher than the resting heart rate at the start of the trial. The Selects size group showed a slower increase in heart rate compared with the Chix, but eventually reached a similar maximum rate of 47 bpm before leveling out to a rate of 46-48 bpm for the remainder of the trial period.

The Chix size mean scaphognathite beat rate showed an initial 3 minute delay in change from resting rates before declining slightly and then increasing to a maximum of 96 bpm. The Selects mean scaphognathite rate showed an initial 5 minute delay in change from resting rates, declining slightly before increasing to a maximum of 99 bpm, 30 bpm above the resting beat rate at the start of the trial.

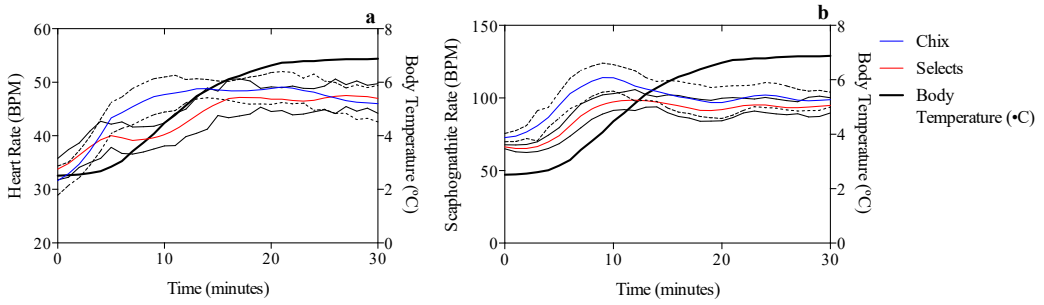

Figure 4.12 *Homarus americanus*: Mean heart (Figure 4.12a) and scaphognathite (mean of left & right) (Figure 4.12b) beat rate in beats per minute for small- (Chix, blue line) and large- (Selects, red line) sized lobsters after an acute, immersed temperature increase from 2.5ºC-7.0ºC. The bold black line is the combined mean haemolymph temperature change. Rates are in beats per minute with black lines indicating standard error. n = 8 animals per size group.

A comparison of starting, maximum/minimum and final mean heart and scaphognathite beat rates for both the acute temperature increase and decrease trial are shown in Figure 4.13 a & b and Figure 4.14 a & b respectively. The mean heart and scaphognathite beat rates in both the acute temperature increase and decrease trials, the Peak and Minimum rates respectively are significantly higher than their respective trial Start resting heart rate ($p < 0.05$ in all cases). However, there were no significant differences between any of the Peak and trial End rates for either the mean heart or scaphognathite beat rates, but both organs showed a change in beat rate that closely matched their haemolymph temperature change during the acute water temperature change as was shown in Figure 4.11 a & b and Figure 4.12 a & b.

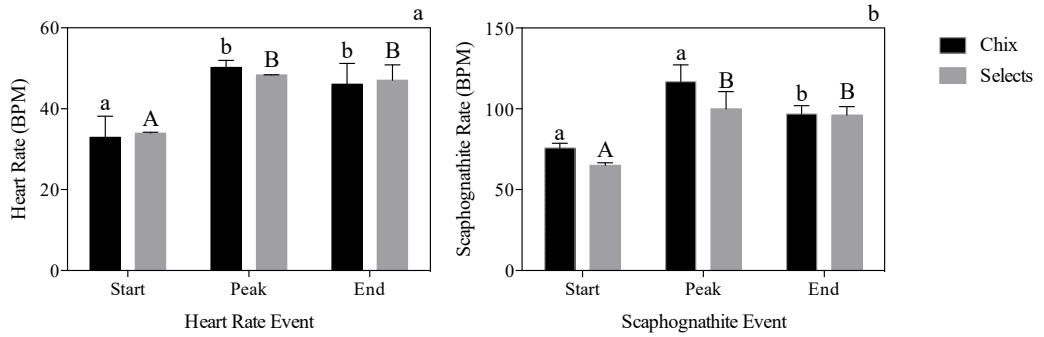

Figure 4.13 *Homarus americanus*: Mean heart (Figure 4.13a) and scaphognathite (Figure 4.13b) (mean of left & right) beat rate at the Start, maximum Peak rate and the End of a 30 minute acute, immersed temperature increase trial from 2.5ºC-7.0ºC for Chix- (black) and Selects- (grey) sized lobsters. n = 8 animals per group. Different letters of the same case above series indicate significant differences between groups ($p < 0.05$).

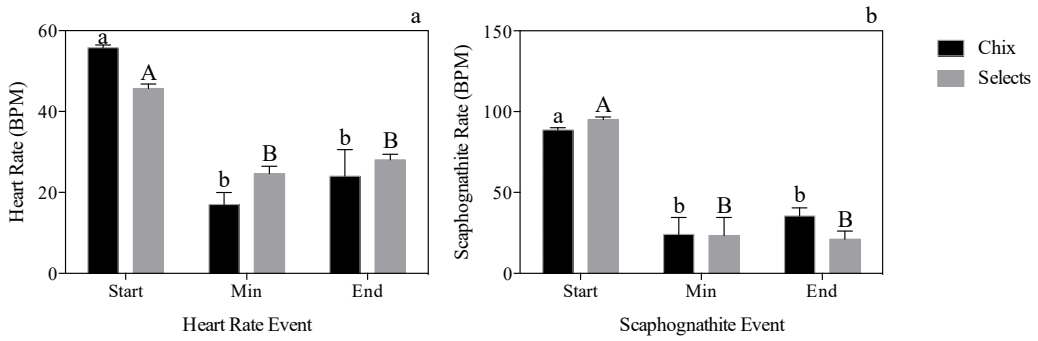

Figure 4.14 *Homarus americanus*: Mean heart (Figure 4.14a) and scaphognathite (Figure 4.14b) (mean of left & right) beat rate at the Start, maximum Peak rate and the End of a 30 minute acute, immersed temperature decrease trial from 7.0ºC-2.5ºC for Chix- (black) and Selects- (grey) sized lobsters. n = 8 animals per group. Different letters of the same case above series indicate significant differences between groups ($p < 0.05$).

4.4 Discussion

The present findings reveal that the lobster transbranchial heat exchange system, as controlled by the beat behaviour of the heart and scaphognathites, comprises an efficient and effective means by which the animal can adjust its internal temperature to an external temperature change within its ambient water temperature tolerance envelope. In its natural, seabed habitat, the lobster will rarely experience an acute, seawater temperature change as large as those used in the present trials but such temperature change experiences in the post-harvest environment occur frequently as deliberate procedures or inadvertent events. Temperature manipulation is a widely-used, long-established, but poorly-understood, post-harvest practice. The present studies were designed to provide more information on what happens when a lobster experiences an acute change in ambient water temperature and what the commercial implications of these changes are.

The present study illustrates that an immersed lobster, with its branchial heat exchange system functioning, can effect a complete haemolymph temperature equilibrium change to occur in less than 10 minutes (compared with > 40 minutes when emersed or when the

cardioventilatory system is not functioning). Clearly, *Homarus americanus* is tolerant of the 4.5 ºC temperature change within the temperature range used in these trials and showed only slight delays before the onset of change in haemolymph temperature. Several studies have shown that *Homarus* spp. are capable of withstanding much higher changes in immersion temperature than those used in the present trials (McLeese, 1956; Whiteley et al., 1995; Camacho et al., 2006; Worden, 2006). The actual limiting temperatures are dependent on the animal's prior thermal history and whether the animal has reached a stable acclimation temperature (García-Esquivel et al., 2010; Lewis and Ayers, 2014). Other environmental factors (e.g. salinity and oxygen saturation levels) are modifying influences that can impact negatively on a crustacean's temperature tolerance (McLeese, 1956; Kumlu, Kumlu, et al., 2010). The current findings illustrate that, to a certain extent, internal temperature adjustments to external temperature changes are under the control of the behaviour of the circulatory and ventilatory systems which implies the current monitoring techniques may be used to determine, with some precision, the scope of body temperature changes that are compatible with preserving the commercial quality of lobsters in supply chains. Such information would be of particular utility during the receipt of shipments when lobsters are re-tanked in systems supplied with ambient or refrigerated seawater at a substantially higher or lower temperature than the haemolymph temperature of the newly - delivered animals. Where a maximum rate of change exists, beyond which the lobster may suffer irreversible damage and loss of commercial value due to its high risk of mortality, an assessment of incoming shipment lobster haemolymph temperatures could be verified and adjustments made to the reception tank system water temperatures in which they will be immersed to avoid thermal shock. These reception tank system temperatures would likely require continuous adjustment as the seasonal ambient temperature changes throughout the fishing season, a practice that is not

currently a standard in the post-harvest commercial trade and may be an unknown contributor to mortality and loss of commercial quality and fitness.

It is interesting to note from these studies that despite the weight differences between the size groups (the Selects were double the size of the Chix), the temperature change curves were not significantly different in the temperature change trials. Although a larger animal has a smaller surface area-to-volume ratio that will reduce the body heat loss or gain in a passive system, both size groups showed similar rates of change of haemolymph temperature in the present study and suggests there is a larger rate of exchange of heat in the active system of the larger Selects-sized lobsters. Fick's law of diffusion states that the rate of exchange (in this case transbranchial heat flux) is dependent on the surface area of the gills, the distance between the source and destination of the heat and a temperature gradient. A study on gill surface area of brachyurans indicates that there is a negative relationship of gill surface area with weight (Gray, 1957), which suggests that the larger-sized Selects lobsters should have a relatively decreased heat exchange ability compared with the smaller Chix. The data from the current study revealed that there were no significant differences in scaphognathite or heart beat mean frequencies between the two sizes, therefore the application of Fick's law suggests that the gradient of exchange must be maintained at a higher level in the larger lobsters, possibly by an increased water ventilation volume across the gills and haemolymph flow volume through the gills.

These studies revealed differences in the details of temperature adjustment between two different size classes of lobsters used within the *H. americanus* fishery. Little is known about the other size groups that make up the full complement of size classes harvested and which encompasses a range from an approximate minimum of 450g to a maximum of 10kg. From a commercial perspective, it would be of interest to know much more detail of potential size-related differences in acute temperature tolerance of lobsters. Current supply chains use handling and storage practices that result in equal treatment

amongst all size classes and this may not be an appropriate option. Consequently, follow-up studies involving the full size range of *H. americanus* are scheduled for the near future. In order for these studies to be adapted effectively within the current commercial practices of the *Homarus americanus* trade, the follow-up studies will need to focus on determining specifically the lobsters' temperature change limits as they relate to actual commercial scenarios that exist as such temperature regimes will be of concern in terms of the maintenance of the lobsters' commercial quality. In this context, cardioventilatory behaviour data could be a useful tool to use as an aid to determining what are the most appropriate temperatures to use when reception and storage tank water systems require adjustments to allow the lobster to maintain vigour and to reduce the impact of handling and other commercial procedures. Such information requires a temporally dynamic approach because of the gradual changes arising from seasonal differences in the received lobster's pre-harvest acclimation history.

Chapter 5
The Application of Hazard Analysis Critical Control Point Principles to the Live Commercial Trade of *Homarus americanus*

5.1 Introduction

The trade in live *Homarus americanus* comprises a complex series of handling, storage and transport steps involving a large number of stakeholders including fishermen, shore-side buyers, storage/shipping companies, ground and air cargo companies, destination market wholesalers, retailers and end consumers (Gardner et al., 2010). Given the complexity of the live *H. americanus* supply chain logistics, there are many opportunities for the quality of the first-landed catch to be compromised, with the potential of reducing the financial value of the lobster as a product as it passes from one step to the next. Typically, individual stakeholders have a range of measures and control steps that are used to deliver the lobster to the next individual in the supply chain, but these measures range in their effectiveness for preserving product quality and, in some cases, may be based only on an observation assessment of whether the animal is still alive, regardless of whether it is actually suitable or acceptable to the end-user further along in the supply chain (Spanoghe and Bourne, 2001). This can lead to degradation of the initial catch quality (intrinsic quality) of the lobster to the point at which it dies and all resources used to harvest, transport and store the lobster up to that point become a wasted effort. If the end result of death and product loss occurs in the final consumer's marketplace, this may also lead to the reduction of reputation as a premium product, a reputation that is often needed to garner the prices in the market that fund the expense of getting the lobster from the ocean to a consumer's plate. In order for a premium live lobster trade to obtain maximum value from the marketplace, there needs to be a consistent supply of high quality animals that meet the expectations of the end consumer. For this to occur in such

a highly complex industry with so many stakeholders, a program designed to help maintain a consistent level of quality control standards would need to be implemented at the various stages within the supply chain. Such programs exist in the control of food safety in production plants through the wide-spread, globally-accepted methods known as Hazard Analysis Critical Control Point (HACCP).

5.1.1 History of HACCP

HACCP was developed in the 1960s through a joint effort of the Pillsbury Corporation and the US National Aeronautics and Space Administration (NASA) to ensure the food safety of the products that Pillsbury was supplying to NASA's astronauts would not be a concern during the first human-piloted space missions (Sperber and Stier, 2009). Rather than use final product testing to determine whether a food product was safe, a reactive approach, the HACCP system focused on the processes that were used to produce the food and determine what operating limits were required to ensure that a safe end product was produced (a proactive approach). Canada was one of the first countries to adopt the HACCP system in its seafood processing sector following a high profile recall in 1985 of approximately 1 million cans of rancid and decomposed tuna deemed unfit for human consumption (Harris, 1985). At the time, a reactive approach to food safety control was used in which only final product inspection by the Canadian Fisheries Inspection Branch occurred prior to release for sale. The problem with this final product testing approach is that significant investment in labour and ingredients are used to produce the end product that may subsequently get rejected. Given the large scale of the recall, the federal agency responsible for seafood product safety decided that a change in the current reactive system was necessary and thus formed a group that decided on a new system of control measures for the industry in which HACCP principles were adopted. Since this time, HACCP has been integrated into food safety in nearly every country worldwide that exports fish products.

There are 7 universal principles of HACCP, used by every food regulatory authority that implements a HACCP-based system (Codex Alimentarius Commission, 2003).

These principles are:

1. Hazard Analysis
2. Identification of Critical Control Points
3. Determine Critical Limits
4. Establish Monitoring
5. Establish a Corrective Action Plan
6. Verification Procedures
7. Record Keeping

The 7 principles are used to control potential food safety hazards before they become manifest into the production of an unsafe final product and are designed to identify and correct potential food safety hazards during the production process. The process of assessing potential risks in HACCP is similar to Failure Mode and Effects Analysis (FMEA) used predominantly in the engineering industry (Teng and Ho, 1996) in that inductive reasoning determines the premises that may relate to a failure mode (forward logic). This type of analysis assesses the likelihood of a failure based on current data prior to a failure actually occurring.

5.1.2 HACCP in the Live Animal Trade

Although the HACCP principles were designed specifically for use in food production facilities to identify and control processes that are linked to potential food safety risks, the concept of identifying hazards and defining critical control points and critical limits has a low adoption to other industry sectors, but examples exist, including in aquaculture (Reilly and Kaferstein, 1997) and even the live crustacean trade (Gomez-Jimenez et al., 2001). However, there are technical challenges involved in the

effective implementation of such a system outside of the food safety field because of the required development of a set of parameters that can be used to control processes. Sperber (2005) and Panisello (2001) noted this technical issue and stated that prerequisite programs or good manufacturing practices are alternatives to critical control points when critical limits are difficult to, or have not yet been, established. Although prerequisite programs do contain best practices, their application to controlling potential issues based on operating parameters are limited to guidelines, and do not receive the rigorous process monitoring that makes the HACCP plan so effective. Another problem with the food supply chain adaptation of HACCP for quality control instead of food safety is that the parameters require definitions of quality, and these can be different, depending on the sector. For instance, in the lobster industry, a high meat yield, hard-exoskeleton lobster, landed in a weakened state, would be considered a low quality animal for the live trade, but may still be acceptable for the production of a premium cooked or raw processed product. To limit these problems related to defining lobster quality, the definition of quality in the present study is from the perspective of a live lobster exporter.

5.1.3 Fundamentals for an Effective Live Lobster HACCP program

A quality lobster for the live trade should have the following characteristics:

1. Acceptable meat yield, in which the claws and tail muscle fill the entire volume of the exoskeleton such as occurs in lobsters in intermoult and premoult.
2. Vigour index 1 (vigour index is described in Chapter 1) which includes a strong reaction to handling, with claws and tail fully extended.
3. Possesses the ability to tolerate the rigours of a short - or a long - term storage and subsequent extended emersion during shipment to the final market destination.

To possess these three characteristics, a lobster must first be sourced from a fishery in which the lobsters are of an acceptable meat yield (intrinsic quality). Without this fundamental characteristic, it would be difficult to preserve the other two characteristics in subsequent steps in the supply chain, and the end-user expectations would not be fulfilled. Assuming the first characteristic is achieved, post-harvest handling and storage procedures and parameters have to be controlled throughout the rest of the supply chain to maintain characteristics 2 and 3.

5.1.4 Aims and objectives

The aim of this study was to develop a model HACCP plan to be used in the live lobster supply chain; one in which data from the previous Chapters were used in the development of critical limits where critical control points were identified. The objective was to identify all of the handling, storage and shipment steps present in the Canadian *H. americanus* industry that may require control of conditions or procedures to prevent the loss of lobster quality. The HACCP plan process would then be used to identify whether any of these steps were critical points that could be controlled through procedures or operating parameters which, by use of the findings from Chapters 2 to 4 as well as data from published literature, were then specified in an operating HACCP plan to provide guidance to industry participants that may not necessarily have the knowledge to develop such a program on their own.

The HACCP plan is a working document that requires ongoing research and development to refine the critical limits when new discoveries are made relating to identification of post-harvest risk factors that affect lobster quality parameters. This current exercise was meant to establish the foundation and starting operating parameters that can be further developed by industry if it is accepted and implemented by the industry partners involved.

5.2 Materials and Methods

5.2.1 HACCP Principles

The development of a HACCP plan involves the 7 principles outlined in the introduction of this Chapter. A more detailed explanation of these principles is given below:

1. Hazard Analysis: a review of all of the steps in the process and identifying whether there is a potential food safety hazard and what steps can be used to control the hazard(s).
2. Identify Critical Control Points: determine points in the process in which steps can be taken to eliminate or reduce the potential food safety hazards to acceptable levels.
3. Establish Critical Limits (CL): define the values for each critical control point that can be used as measurements to determine whether or not the process or step will cause a potential food safety issue.
4. Establish Monitoring of Critical Control Points: define a monitoring plan that outlines the frequency and methods for measuring the critical control points to ensure they are kept within the established critical limits and avoid potential food safety hazards.
5. Establish a Corrective Action Plan: Define procedures to use when monitoring procedures identify that a critical control point deviates outside of the established critical limits, and what steps are taken to bring the process step back within acceptable limits so that food is not produced in an unsafe manner.
6. Verification: A process in which the steps established to control hazards are verified and deemed to be effective. This may include calibration procedures, monitoring of steps in addition to the monitoring program and evaluating whether the steps being taken are effective.

7. Record Keeping: Effective record keeping of the monitoring of critical control points to demonstrate consistent, effective application of the critical control points and to provide an audit trail so that outside parties can properly review and assess the effectiveness of the plan to control potential food safety hazards.

In order for these principles to be fulfilled, a definition of quality is required. To accomplish this, development of a product specification document was undertaken. This exercise had to take into consideration end-user expectations and the requirements that would have allowed the product to meet these expectations. Next, the seven principles for the development of a HACCP plan were used to create a model HACCP plan for live lobsters. The first step required that all possible hazards be identified and assessed, and this was undertaken using a hazard analysis worksheet. The second step involved determining which of the possible hazards identified in the hazard analysis worksheet would be considered critical control points (CCP) (Principle 2). A CCP decision tree was used for this exercise. Critical limits for identified CCPs were established next (Principle 3) and a monitoring program developed (Principle 4). A corrective action plan template was developed (Principle 5) to bring processes back within the critical limits when a deviation occurred, and verification procedures were suggested (Principle 6), ensuring that effective reviews of the HACCP plan occur. Finally, a system of record keeping was suggested (Principle 7) so that outside parties can review the HACCP plan for its effectiveness, and for the participants to use for trend analysis and further refinement of the HACCP plan control measures.

5.3 Results

5.3.1 Final product specification

Table 5.1 outlines the final product specifications that the end-user will require and expect upon successful delivery of live lobster. Many of the required control points and critical limits identified in this study have been determined in isolation from previous work, such as that for the tolerance levels of lobsters to various environmental conditions such as the effects of disturbance of oxygen (Whiteley and Taylor, 1992), salinity (Smyth, 2011), emersion tolerance (Fotedar et al., 2001), temperature (Morris and Oliver, 1999a; Lorenzon et al., 2007) and ammonia (Young-Lai et al., 1991; Bernasconi, 2006). The data for these specifications were provided from a live lobster exporter and include characteristics that are expected in the Japanese premium live lobster wholesale marketplace. The packing, shelf life, storage and handling instructions were adapted from Estrella (2002), but adjusted to be more stringent based on current best practices of the exporter.

Table 5.1 *Homarus americanus*: final product specifications to meet the end-user expectations in a premium live lobster market.

Characteristic	Product Description
Product Name	Live Lobster, *Homarus americanus*
Source of Raw Material	Product of Canada, harvested in FAO Area 21 (Northwest Atlantic)
Important Final Product Characteristics	Lobster is alive, vigour index 1, hard, undamaged exoskeleton (Durometer index >70), and all limbs present. Weight of cooked edible portion > 25% of live weight.
Packaging	Air freight: water resistant box capable of maintaining >95% relative humidity and temperature < 5ºC and above freezing.
How the end product is to be used	Intended for general public, sold alive.
Shelf life	4 days in air (emersed), 2 weeks immersed if kept alive within proper storage conditions. Must maintain lobsters as undamaged, vigour index 1.
Storage Instructions	Store above freezing and below 5 ºC. Emersed: • 95% relative humidity • low vibration or movement Immersed: • Dissolved O_2 > 90% saturation • Total Ammonia < 1.0 ppm (NH_4) • Salinity between 29 – 35 ppt • pH between 7.7 – 8.1 • Individually-segregated to avoid cannibalism, if stored for more than 1 week.
Handling Instructions	Fragile: handle with care. Treat packages of lobsters like cartons of eggs. Handle individual lobsters by the body to avoid claw loss. Do not drop or throw lobsters.

5.3.2 Hazard Analysis and Critical Control Point Assessment

The hazard analysis is used to identify all possible steps and processes involved in the distribution of live lobsters to the end-user. This involves creation of a process flow

diagram, a description of the process flow elements and a hazard analysis worksheet based on the process flow diagram contents.

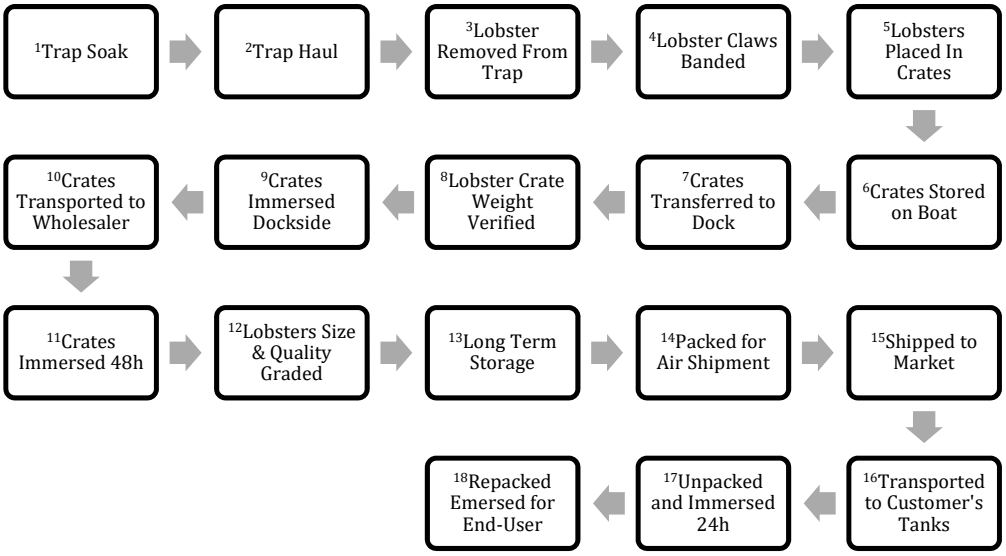

Figure 5.1 *Homarus americanus*: Process flow diagram for the Canadian live lobster distribution chain starting from the trap harvest and ending with live lobster delivered to end-user in target market. Process flows are described in Section 5.3.2.1.

5.3.2.1 Process Flow Description

The specification given within the process flow descriptions given below are based on the current literature where cited. Otherwise, where no literature exists, live exporter standard operating procedures are used.

1. Trap Soak

   Traps placed in harvest area waters. Inshore traps soak time should be minimised to avoid excessive interaction between trapped lobsters that can lead to damage (Matthews, 2001). Traps can reach saturation of lobsters in less than 12 h (Miller and Rodger, 1996).

2. Trap Haul

Traps should be hauled at a rate that does not cause weakening of the lobster (Basti et al., 2010). Offshore fishers that harvest in deeper water should reduce the hauling speed or immerse lobsters in a live well on board the boat with bottom temperature-matched seawater to give lobsters a chance for recovery. Traps with lobsters inside should not be allowed to bang against the side of the boat during hauling onto the deck or rails.

3. Lobster Removed From Trap

Lobsters should be removed carefully from the trap by the body to avoid claw loss from excessive force that results from handling by the claws (Scarratt, 1973). Removed lobsters should be placed gently onto the claw-banding table surface and try to avoid excessive tail flipping that can exhaust muscle glycogen reserves in the lobster (Stentiford and Neil, 2000; Basti et al., 2010).

4. Lobster Chelipeds (Claws) Immobilised

Lobster claws are capable of causing significant damage to another lobster's exoskeleton. A strain-gauge recorded force of 256 Newtons was observed with the crusher claw of a 172mm carapace length *H. americanus* (Elner and Campbell, 1981). Consequently, the lobster's claws should be banded immediately upon removal from the trap to avoid damage due to interaction among lobsters in close proximity. When banding lobsters, hold the lobster by the body or the proximal sections of the chelipeds together to avoid excessive stress on the cheliped attachment to the body of the lobster that can lead to the claws autotomising (Scarratt, 1973).

5. Placed In Crates

Lobsters should be placed into crates immediately upon banding to avoid loss of vigour (Lavallée, 1999), taking care to tuck the tails and pack the lobsters parallel to one another. Keep tail flipping and interaction to a minimum (Spanoghe and Bourne, 2001; van Tamelen, 2005) by ensuring crates are packed to full capacity to restrict movement (45kg per lobster crate as per stated capacity of IPL lobster crates). Pack to a maximum of 45 kg and avoid over packing crates as such will cause crate-handling to become difficult and therefore vulnerable to rough handling. Over-packing crates will also restrict water flow through the crate during immersed storage, leading to reduced oxygen supply and elevated environmental ammonia levels.

6. Crates Stored On Boat

Crates stored emersed on the boat should be kept away from conditions outside the recommended temperature range of 'above freezing and below 5ºC' stated earlier in Table 5.1. Avoid wind exposure as that increases the risk of desiccation and possible irreversible gill damage (van Tamelen, 2005). Crates stored immersed should be supplied with seawater temperatures of 1-5 ºC, oxygen > 90% saturation, salinity of 30 – 34 ppt, and enough water flow through to keep water ammonia levels < 1.5 ppm (Estrella, 2002).

7. Crates Transferred To Dock

Lobster crates should be handled with care to avoid excessive stress and jostling that may produce lobster integument or internal tissue damage during communal storage in crates (Paterson et al., 1997; Jussila et al., 1999). Handle the crates as if they were filled with eggs.

8. Lobster Crate Weight Verified

Lobster crates, verified for proper weight (45kg) as mentioned in #5 (above). Handling of crates should be kept to a minimum and with care, as mentioned in #6 (above).

9. Crates Immersed Dockside

   Crates should be immersed immediately upon completion of crate weight verification. Immersed water conditions should follow the parameters outlined in #6 (above).

10. Crates Transported To Wholesaler

    Crates of lobsters transferred to the wholesaler should be secured inside the transport trailer to avoid shifting of the load during the trip as such movements may cause crates to fall over and damage the packed lobsters. The truck trailer should have a temperature control system to maintain a set point temperature that matches the lobsters' body temperature. This will reduce moisture loss during transport that can result from the refrigeration system running excessively to reach a set point that is too low.

11. Crates Immersed 48h

    Upon arrival to the wholesale facilities, the crates should be removed immediately from the transport trailer and immersed in a reservoir system that maintains water quality parameters outlined in #6 (above). The reservoir water temperature should be set to a maximum of 5ºC (Estrella, 2002) and supplemental aeration used to ensure oxygen is maintained > 90% saturation based on the findings of McLeese (1956). If emersion during transport to the facilities has been used it is likely to have caused the lobsters to have low haemolymph oxygen levels and, therefore, immersion post-emersion will result in significant demands on the oxygen availability in the reservoir

system. This recovery period is necessary for lobsters to recover from the post-harvest handling events up to this point and has been identified in Chapter 3 as a method to reduce the risk of quality loss during subsequent steps in this study.

12. Lobsters' Size & Quality Graded

After step #11's 48 h immersion recovery period (above), lobsters are removed and inspected by hand for vigour and condition. Lobsters that are > vigour index 1, or which have a cracked exoskeleton or missing appendages, are rejected, collected and their weight recorded. If lobsters are harvested during the winter season, a sample of lobsters should be analysed for their haemolymph parameters that are related to meat content, such as haemolymph % Brix and total protein, to determine whether the shipment contains lobsters that do not have an acceptable minimum level of meat content and which may be at risk of excessive mortality during long term storage - a risk factor determined in Chapter 2.

13. Long-Term Storage

Lobsters identified as suitable for long-term storage should be placed in a holding system that maintains the lobsters segregated to prevent injury and infection due to aggressive, cannibalistic interactions. If lobsters are being stored unfed, a common practice in most systems because of the difficulty in maintaining water quality when lobsters are being fed, water temperature should be kept low to reduce their metabolic rate. The survival rate of unfed lobster during storage varies inversely with storage water temperature (Bartley et al., 1980).

14. Packed For Air Shipment

Lobsters selected for air shipment should undergo a final inspection to ensure vigour index is not > 1 and that no injuries are present. Lobsters should be packed in a waterproof box capable of maintaining < 5ºC and above freezing air temperatures within the box during standard, uncontrolled cold-chain logistics present in the air freight industry (Estrella, 2002). If the airfreight supply chain will involve extreme heat, the box should be adequately insulated and incorporate ice packs that can maintain the < 5ºC internal box temperature for the duration of the shipment. The box should be equipped to maintain > 95% relative humidity, typically with the use of a dampened material such as wood shavings or absorbent pads.

15. Shipped To Market

Boxes should be maintained within refrigeration temperatures of 3-5ºC as much as possible, avoiding extremes of temperature that could compromise the internal box temperature. Shipments should be handled with care (Jussila et al., 1999; Lavallée et al., 2000; Crear and Forteath, 2001), and total emersion times kept to below 60 h based on the loss of vigour observed, > 60 h in the emersion trials from Chapter 3 and which likely represents the practical maximum emersion time from which a lobster can recover - taking into account the jostling that occurs during air shipment.

16. Transported To Customer's Tanks

On arrival, boxes of lobsters should be transferred with care to the transfer vehicle. If necessary, temperature control in the vehicle should be used to maintain the boxes between 3-5ºC, and delivered directly to the receiving facilities.

17. Unpacked And Immersed 24h

Received shipments of lobsters will often have been shipped emersed for prolonged periods, and, if so, should be unpacked immediately and immersed in the reception tank system. This system should maintain water quality parameters that match those of the facilities from which the lobsters were shipped and should be maintained subsequently at adequate parameter levels as outlined in step #6 (above). During the unpacking process, an assessment of vigour and condition index should be completed and recorded. Dead lobsters should be discarded, and weak lobsters segregated from strong, vigour index 1 ones so that any that are weak can be used immediately to avoid excessive mortality during reception storage. Before to being shipped to a local end-user, lobsters should remain immersed for a minimum of 24h to allow recovery from the preceding prolonged emersion event. If the lobsters are to be shipped emersed for more than 24h, a minimum of 48h recovery in the reception tank should occur first in order to effect complete recovery from the previous long-distance emersion event.

18. Re-Packed And Shipped Emersed To End-User

Lobsters should be handled by the body and carefully packed into a leak-proof box capable of maintaining emersion temperatures < 5ºC and above freezing, and with relative humidity > 95% saturation.

Table 5.2 *Homarus americanus*: Hazard Analysis Worksheet for the live trade logistics of the Canadian lobster fishery from harvest to end-consumer.

HAZARD ANALYSIS WORKSHEET					
(1)	(2)	(3)	(4)	(5)	(6)
PROCESSING STEP	IDENTIFY POTENTIAL BIOLOGICAL, CHEMICAL, AND PHYSICAL HAZARDS ASSOCIATED WITH THIS PRODUCT AND PROCESS	ARE ANY POTENTIAL PRODUCT QUALITY HAZARDS SIGNIFICANT AT THIS STEP? (YES/NO)	JUSTIFY YOUR DECISION FOR COLUMN 3	WHAT PREVENTATIVE MEASURE(S) CAN BE APPLIED FOR THE SIGNIFICANT HAZARD?	IS THIS STEP A CRITICAL CONTROL POINT? (YES/NO)
Trap Soak	Physical: prolonged trap soak may lead to aggressive interaction and exoskeleton damage.	YES	Exoskeleton damage is unacceptable for live lobster specification	Keep trap soak times within the time that reduces lobster aggressive interaction	NO: controlled by prerequisite program that determines maximum soak time.
Trap Haul	Physical: trap haul speeds may impact lobster vigour index	YES	Excessive trap haul speed is a significant risk factor in quality deterioration	Maintain trap haul speeds appropriate for the water depth in which the lobsters are being harvested	NO: controlled by prerequisite program that determines appropriate trap haul speed.
Lobster Removed From Trap	Physical: excessively harsh handling may cause exoskeleton damage and reduce lobster fitness.	YES	Exoskeleton damage is unacceptable for live lobster specification.	Train lobster-handling staff to properly handle lobsters to prevent injuries to lobsters during removal from traps.	NO: controlled by prerequisite program for training on proper handling techniques.
Lobster Claws Banded	Physical: unbanded claws can cause severe injury to other lobsters when held communally.	YES	Severe injury and exoskeleton damage is unacceptable for live lobster specification.	Lobsters should be banded immediately after taken from the trap. Boat design should allow for keeping lobsters separated from one another prior to being banded to prevent lobster claw crushing damage.	NO: controlled by prerequisite program for banding procedures and boat layout.

HAZARD ANALYSIS WORKSHEET					
(1)	(2)	(3)	(4)	(5)	(6)
PROCESSING STEP	IDENTIFY POTENTIAL BIOLOGICAL, CHEMICAL, AND PHYSICAL HAZARDS ASSOCIATED WITH THIS PRODUCT AND PROCESS	ARE ANY POTENTIAL PRODUCT QUALITY HAZARDS SIGNIFICANT AT THIS STEP? (YES/NO)	JUSTIFY YOUR DECISION FOR COLUMN 3	WHAT PREVENTATIVE MEASURE(S) CAN BE APPLIED FOR THE SIGNIFICANT HAZARD?	IS THIS STEP A CRITICAL CONTROL POINT? (YES/NO)
Placed in Crates	Physical: excessively harsh handling may cause exoskeleton damage and reduce lobster fitness.	YES	Exoskeleton damage is unacceptable for live lobster specification.	Train lobster-handling staff to properly handle lobsters to prevent injuries to lobsters during placement in the crates.	NO: controlled by prerequisite program for training on proper handling techniques.
Crates Stored on Boat	Chemical: boat live well water quality. Physical: live well water temperature.	Chemical: YES Physical: YES	Water quality parameters outside of tolerable limits of lobsters.	Use water quality monitoring equipment to ensure live well is kept within the tolerable limits of live lobsters.	YES: a significant hazard exists and can be controlled.
Crates Transferred to Dock	Physical: excessively harsh handling may cause exoskeleton damage and reduce lobster fitness.	YES	Exoskeleton damage is unacceptable for live lobster specification.	Train lobster-handling staff to properly handle lobster crates to prevent injuries to lobsters during transfer from boat to dock.	NO: controlled by prerequisite program for training on proper handling techniques.
Lobster Crate Weight Verified	Physical: handling during weight verification, top up and reduction.	YES	Exoskeleton damage is unacceptable for live lobster specification.	Train lobster-handling staff to properly handle lobsters to prevent injuries to lobsters during transfer between crates.	NO: controlled by prerequisite program for training on proper handling techniques.

HAZARD ANALYSIS WORKSHEET					
(1)	(2)	(3)	(4)	(5)	(6)
PROCESSING STEP	IDENTIFY POTENTIAL BIOLOGICAL, CHEMICAL, AND PHYSICAL HAZARDS ASSOCIATED WITH THIS PRODUCT AND PROCESS	ARE ANY POTENTIAL PRODUCT QUALITY HAZARDS SIGNIFICANT AT THIS STEP? (YES/NO)	JUSTIFY YOUR DECISION FOR COLUMN 3	WHAT PREVENTATIVE MEASURE(S) CAN BE APPLIED FOR THE SIGNIFICANT HAZARD?	IS THIS STEP A CRITICAL CONTROL POINT? (YES/NO)
Crates Immersed Dockside	Chemical: water chemistry of dockside water. Physical: dockside water temperature.	Chemical: YES Physical: YES	Water quality parameters outside of tolerable limits of lobsters.	Use water quality monitoring equipment to ensure dockside reservoir is kept within the tolerable limits of live lobsters.	YES: a significant hazard exists and can be controlled.
Crates Transported to Wholesaler	Physical: excessive movement of crates during transit and prolonged operation of air refrigeration unit may cause exoskeleton damage and desiccation-related reduction in lobster quality respectively.	YES	Exoskeleton damage and loss of quality is unacceptable for live lobster specification. Loss of vigour and ability to recover from emersion due to low humidity exposure are outside of product specifications.	Training of transport staff for proper securing of load to prevent crate movement during transit. Setting truck refrigeration unit to match lobster body temperature to reduce refrigeration-related product desiccation.	NO: for securing crates: controlled by prerequisite program for training on proper loading of lobster crates. YES for refrigeration: Desiccation can be controlled by proper monitoring of lobster body core temperature to set refrigeration unit set point.

HAZARD ANALYSIS WORKSHEET					
(1)	(2)	(3)	(4)	(5)	(6)
PROCESSING STEP	IDENTIFY POTENTIAL BIOLOGICAL, CHEMICAL, AND PHYSICAL HAZARDS ASSOCIATED WITH THIS PRODUCT AND PROCESS	ARE ANY POTENTIAL PRODUCT QUALITY HAZARDS SIGNIFICANT AT THIS STEP? (YES/NO)	JUSTIFY YOUR DECISION FOR COLUMN 3	WHAT PREVENTATIVE MEASURE(S) CAN BE APPLIED FOR THE SIGNIFICANT HAZARD?	IS THIS STEP A CRITICAL CONTROL POINT? (YES/NO)
Crates Immersed at Wholesaler	Chemical: water chemistry of reception water. Physical: temperature of reception water. Biological: minimum of 48h required for lobsters to recover from prior emersion	Chemical: YES Physical: YES Biological: YES	Water quality parameters outside of tolerable limits of lobsters. Insufficient recovery time degrades vigour and increases risk of storage mortality.	Use water quality monitoring equipment to ensure reception reservoir is kept within the tolerable limits of live lobsters. Use 48h minimum immersion period as standard operating procedure	Chemical, Physical and Biological: YES: a significant hazard exists and can be controlled.

HAZARD ANALYSIS WORKSHEET					
(1)	(2)	(3)	(4)	(5)	(6)
PROCESSING STEP	IDENTIFY POTENTIAL BIOLOGICAL, CHEMICAL, AND PHYSICAL HAZARDS ASSOCIATED WITH THIS PRODUCT AND PROCESS	ARE ANY POTENTIAL PRODUCT QUALITY HAZARDS SIGNIFICANT AT THIS STEP? (YES/NO)	JUSTIFY YOUR DECISION FOR COLUMN 3	WHAT PREVENTATIVE MEASURE(S) CAN BE APPLIED FOR THE SIGNIFICANT HAZARD?	IS THIS STEP A CRITICAL CONTROL POINT? (YES/NO)
Lobsters Size & Quality Graded	Physical: excessively harsh handling may cause exoskeleton damage and reduce lobster fitness. Biological: Selection of animals that meet the requirements of the end-consumer and with the ability to withstand the rigours of long-term storage whilst maintaining vigour index 1.	Physical: YES Biological: YES	Physical: Exoskeleton damage is unacceptable for live lobster specification. Biological: Risk factors exist that may reduce survival of lobsters during long-term storage and degrade quality below end-user expectations.	Physical: Train lobster-handling staff in proper handling techniques to prevent injuries to lobsters during transfer from crates to grading line. Biological: control the quality of product being selected for long-term storage and use in the live lobster trade.	Physical: NO: controlled by prerequisite program for training on proper handling techniques. Biological: YES: significant hazards exist that can be controlled with monitoring of risk factors.
Long-Term Storage	Chemical: water chemistry of long-term storage system water. Physical: temperature of reception water.	Chemical: YES Physical: YES	Water quality parameters outside of tolerable limits of lobsters.	Use water quality monitoring equipment to ensure long-term storage system water is kept within the tolerable limits of live lobsters.	YES: a significant hazard exists and can be controlled.

HAZARD ANALYSIS WORKSHEET					
(1)	(2)	(3)	(4)	(5)	(6)
PROCESSING STEP	IDENTIFY POTENTIAL BIOLOGICAL, CHEMICAL, AND PHYSICAL HAZARDS ASSOCIATED WITH THIS PRODUCT AND PROCESS	ARE ANY POTENTIAL PRODUCT QUALITY HAZARDS SIGNIFICANT AT THIS STEP? (YES/NO)	JUSTIFY YOUR DECISION FOR COLUMN 3	WHAT PREVENTATIVE MEASURE(S) CAN BE APPLIED FOR THE SIGNIFICANT HAZARD?	IS THIS STEP A CRITICAL CONTROL POINT? (YES/NO)
Packed for Air Shipment	Physical: excessively harsh handling may cause exoskeleton damage and reduce lobster fitness. Incorrect use of ice packs and packing materials may lead to inadequate environmental conditions in box. Biological: Vigour index > 1 packed in box has a high risk for mortality during shipment to end-user.	Physical: YES Biological: YES	Physical: Exoskeleton damage is unacceptable for live lobster specification. Improper packaging increases risk of environmental parameters outside the tolerance limits of live lobster and cause degradation of product quality. Biological: Packing vigour index > 1 will lead to further degradation and is unacceptable for live lobster specification.	Physical: train lobster-handling staff in proper handling techniques to prevent injuries to lobsters during packing from crates into air shipment box. Use properly identified materials for shipment of lobsters to the market. Biological: train lobster-packing staff in proper assessment of vigour index to ensure packing of only vigour index 1 lobsters for customers.	Physical: No: prerequisite program for training of staff to handle lobsters and identify suitable packaging and vigour index 1 for the air shipment.

HAZARD ANALYSIS WORKSHEET					
(1)	(2)	(3)	(4)	(5)	(6)
PROCESSING STEP	IDENTIFY POTENTIAL BIOLOGICAL, CHEMICAL, AND PHYSICAL HAZARDS ASSOCIATED WITH THIS PRODUCT AND PROCESS	ARE ANY POTENTIAL PRODUCT QUALITY HAZARDS SIGNIFICANT AT THIS STEP? (YES/NO)	JUSTIFY YOUR DECISION FOR COLUMN 3	WHAT PREVENTATIVE MEASURE(S) CAN BE APPLIED FOR THE SIGNIFICANT HAZARD?	IS THIS STEP A CRITICAL CONTROL POINT? (YES/NO)
Shipped to Market	Physical: excessive movement of boxes during transit may cause physical injury and reduction in lobster quality. Excessive temperatures during transit may cause environmental conditions inside box deviate outside of a lobster's tolerable range during emersion.	YES	Degradation of vigour due to excessive handling of boxes during transit will put the lobster quality outside of specifications. Temperature abuse during transit may reduce the tolerable emersion time and cause irreversible degradation of quality outside of the live lobster specifications.	Training of air cargo handling staff in the proper handling and storage of packed lobsters boxes.	Physical: NO: controlled by prerequisite program for training on proper handling and storage techniques during emersion.

HAZARD ANALYSIS WORKSHEET					
(1)	(2)	(3)	(4)	(5)	(6)
PROCESSING STEP	IDENTIFY POTENTIAL BIOLOGICAL, CHEMICAL, AND PHYSICAL HAZARDS ASSOCIATED WITH THIS PRODUCT AND PROCESS	ARE ANY POTENTIAL PRODUCT QUALITY HAZARDS SIGNIFICANT AT THIS STEP? (YES/NO)	JUSTIFY YOUR DECISION FOR COLUMN 3	WHAT PREVENTATIVE MEASURE(S) CAN BE APPLIED FOR THE SIGNIFICANT HAZARD?	IS THIS STEP A CRITICAL CONTROL POINT? (YES/NO)
Transported to Customer's Tanks	Physical: excessive movement of crates during transit and storage of boxes in excessive heat or cold may cause exoskeleton damage and accelerated emersion-related reduction in lobster quality respectively.	YES	Exoskeleton damage and loss of quality is unacceptable for live lobster specification. Loss of vigour and ability to recover from emersion due to extreme temperature exposure are outside of product specifications.	Training of transport staff for proper securing of load to prevent box movement during transit. Setting truck refrigeration unit to match lobster body temperature to reduce excessive temperature related loss of lobster quality.	NO: for securing boxes: controlled by prerequisite program for training on proper handling and loading of lobster boxes. NO: for refrigeration: excessive emersion temperatures can be controlled by proper monitoring of refrigeration unit set point.

HAZARD ANALYSIS WORKSHEET					
(1)	(2)	(3)	(4)	(5)	(6)
PROCESSING STEP	IDENTIFY POTENTIAL BIOLOGICAL, CHEMICAL, AND PHYSICAL HAZARDS ASSOCIATED WITH THIS PRODUCT AND PROCESS	ARE ANY POTENTIAL PRODUCT QUALITY HAZARDS SIGNIFICANT AT THIS STEP? (YES/NO)	JUSTIFY YOUR DECISION FOR COLUMN 3	WHAT PREVENTATIVE MEASURE(S) CAN BE APPLIED FOR THE SIGNIFICANT HAZARD?	IS THIS STEP A CRITICAL CONTROL POINT? (YES/NO)
Unpacked and Immersed 24h	Physical: excessively harsh handling may cause exoskeleton damage and reduce lobster fitness. Temperature of reception water may be out of tolerable range of lobster. Chemical: water chemistry of customer's storage system water. Biological: Determination of vigour in animals to appropriately allocate the end-product to the expectations of the end-user.	Physical: YES Chemical: YES Biological: YES	Physical: Exoskeleton damage is unacceptable for live lobster specification. Excessive temperature causes unacceptable vigour loss. Chemical: water chemistry outside of tolerable limits of lobster may cause loss of quality and vigour. Biological: inappropriate end-consumer allocation of vigour index may lead to not meeting end-user quality expectations.	Physical: Train lobster-handling staff in proper handling techniques to prevent injuries to lobsters during transfer from shipping boxes to reception tank system. Chemical: Use water quality monitoring equipment to ensure long-term storage system water is kept within the tolerable limits of live lobsters. Biological: Train lobster-handling staff in the proper identification of vigour index.	Physical: NO: controlled by prerequisite program for training on proper handling techniques. Chemical: YES: significant hazards exist that can be controlled with monitoring of water quality. Biological: NO: controlled by prerequisite program for training on proper assessment of vigour index.

HAZARD ANALYSIS WORKSHEET					
(1)	(2)	(3)	(4)	(5)	(6)
PROCESSING STEP	IDENTIFY POTENTIAL BIOLOGICAL, CHEMICAL, AND PHYSICAL HAZARDS ASSOCIATED WITH THIS PRODUCT AND PROCESS	ARE ANY POTENTIAL PRODUCT QUALITY HAZARDS SIGNIFICANT AT THIS STEP? (YES/NO)	JUSTIFY YOUR DECISION FOR COLUMN 3	WHAT PREVENTATIVE MEASURE(S) CAN BE APPLIED FOR THE SIGNIFICANT HAZARD?	IS THIS STEP A CRITICAL CONTROL POINT? (YES/NO)
Re-packed emersed to end-user	Physical: excessively harsh handling may cause exoskeleton damage and reduce lobster fitness. Incorrect use of ice packs and packing materials may lead to inadequate environmental conditions in box. Biological: Vigour index > 1 packed in box has a high risk for mortality during shipment to end-user.	Physical: YES Biological: YES	Physical: Exoskeleton damage is unacceptable for live lobster specification. Improper packaging will lead to increased risk of environmental parameters outside the tolerance limits of live lobster and cause degradation of product quality. Biological: Packing vigour index > 1 will lead to further degradation and is unacceptable for live lobster specification.	Physical: train lobster-handling staff in proper handling techniques to prevent injuries to lobsters during packing from crates into air shipment box. Use properly identified materials for shipment of lobsters to the market. Biological: train lobster-packing staff in proper assessment of vigour index to ensure packing of only vigour index 1 lobsters for customers.	Physical: No: prerequisite program for training of staff to handle lobsters and identify suitable packaging and vigour index 1 for the air shipment.

5.3.3 Establishing critical limits

The critical limits for the identified critical control points from Table 5.2 are shown below in Table 5.3.

Table 5.3 *Homarus americanus*: Critical control point critical limits of identified hazards within the Canadian live lobster trade.

CCP No.	Process Step	Identified Hazard	Control Measures	Critical Limit
1a	Crates stored on boat	High live - well water temperature	Control of temperature	< 5ºC Nov-May, < 10ºC May-July
1b	Crates stored on boat	Low live - well water dissolved O_2 levels	Control of O_2, increase aeration or incoming new make-up water	>90% saturation
1c	Crates stored on boat	High live - well water ammonia levels	Control of ammonia levels, increase incoming new make-up water	< 3 ppm
1d	Crates stored on boat	Low live - well water salinity levels	Control of incoming supply salinity	30-34 ppt
2a	Crates immersed dockside	High dockside holding water temperature	Control of temperature	< 5ºC all season
2b	Crates immersed dockside	Low dockside holding water dissolved O_2 levels	Control of O_2, increase aeration or incoming new make-up water	>=90% saturation
2c	Crates immersed dockside	High dockside holding water ammonia levels	Control of ammonia levels, increase incoming new make-up water	<3.0 ppm
2d	Crates immersed dockside	Low dockside holding water salinity levels	Control of incoming water supply salinity	30-34 ppt
3	Crates transported to wholesaler	Low emersion environment humidity	Control of refrigeration temperature set point to avoid excessive equipment cycling and subsequent lobster desiccation	Refrigeration temperature set point = or <1º below lobster body temperature
4a	Crates Immersed at Wholesaler	High reservoir water temperature	Control of temperature	< 5ºC all season
4b	Crates Immersed at Wholesaler	Low reservoir water O_2	Control of O_2, increase aeration or incoming new water make-up rate	>=90% saturation
4c	Crates Immersed at Wholesaler	High reservoir water ammonia levels	Control of ammonia levels, increase new water make-up supply rate, supplemental biofiltration	<1.5 ppm

4d	Crates Immersed at Wholesaler	Low reservoir water salinity levels	Control of incoming water supply salinity levels	30 – 34 ppt
4e	Crates immersed at Wholesaler	Insufficient recovery immersion period	Control of immersion time in reservoir	Minimum of 48h
5a	Lobsters size & quality graded	Low lobster haemolymph %Brix indicates lobster meat yield not within specification limits and can also lead to excessive long-term storage mortality	Control of product acceptance & rejection based on haemolymph % Brix levels	Minimum of 7.0 %Brix. 7.0-8.5 %Brix for short term storage and >8.5%Brix for long-term storage
5b	Lobsters size & quality graded	High lobster haemolymph lactate levels associated with high risk of mortality during long term storage	Control of product storage time based on lobster haemolymph lactate levels	< 2 mml l^{-1} for long term storage, all others for short term storage
6a	Long-term storage	High long-term system water temperature	Control of temperature	< 3ºC all season
6b	Long-term storage	Low long-term system water O_2	Control of O_2, increase aeration or incoming new water make-up rate	>=90% saturation
6c	Long-term storage	High long-term system water ammonia levels	Control of ammonia levels, increase new water make-up supply rate, supplemental biofiltration	<1.0 ppm
6d	Long-term storage	Low reservoir system water salinity levels	Control of incoming water supply salinity levels	30-34 ppt
7a	Unpacked and Immersed 24h at customer	High reception tank system water temperature	Control of water temperature	<5ºC
7b	Unpacked and Immersed 24h at customer	Low reception tank system water O_2	Control of O_2, increase aeration or incoming new water make-up rate	>=90% saturation
7c	Unpacked and Immersed 24h at customer	High reception tank system water ammonia levels	Control of ammonia levels, increase new water make-up supply rate, supplemental biofiltration	<1.5 ppm

5.3.4 Monitoring, corrective actions, records, and verification procedures

From the established critical limits (CL) in Section 5.3.3 (above), monitoring of the CLs, corrective actions when monitoring indicates a deviation from the CLs, as well as record keeping and verification, are shown in Table 5.4.

Table 5.4 *Homarus americanus*: Critical control point monitoring, corrective actions, record keeping and verification procedures for the Canadian live lobster supply chain.

CCP No.	Monitoring What, how, when (frequency)	Corrective actions	Records	Verification
1a	Temperature: monitor with thermometer at least hourly, preferably continuously with alarm	Adjust temperature control, remove lobsters from water and keep in controlled temperature area matching lobster body temperature	Boat monitoring record form	Every boat trip at landing, verify lobster body temperature
1b	Oxygen: monitor with sensor or test kit at least once per hour during trip, preferably continuously with monitoring system and alarm	Adjust water flows to improve water exchange and / or aeration rate, remove lobsters from water and keep in controlled temperature area matching lobster body temperature	Boat monitoring record form	Every boat trip at landing, verify records
1c	Ammonia: monitor with test kit at least once at end of harvest prior to return to port, or preferably continuously with sensor and alarm	Adjust water flows to improve water exchange rate, remove lobsters from water and keep in controlled temperature area matching lobster body temperature	Boat monitoring record form	Every boat trip at landing, verify ammonia records
1d	Salinity: monitor incoming water flow with hydrometer hourly or preferably with sensor continuously with alarm	Stop incoming flow of low salinity water, drain live well and keep lobsters in controlled temperature area matching lobster body temperature	Boat monitoring record form	Every boat trip at landing, verify salinity records, check lobsters for distended membranes for signs of low salinity exposure
2a	Temperature: monitor with thermometer at least hourly, preferably continuously with alarm	Adjust temperature control, remove lobsters from water and keep in controlled temperature area matching lobster body temperature	Dockside facility monitoring record form	At each delivery pickup, monitor temperature records
2b	Oxygen: monitor with sensor or test kit at least once per hour during trip, preferably continuously with monitoring system and alarm	Adjust water flows to improve water exchange rate, remove lobsters from water and keep in controlled temperature area matching lobster body temperature	Dockside facility monitoring record form	At each delivery pickup, monitor temperature records

CCP No.	Monitoring What, how, when (frequency)	Corrective actions	Records	Verification
2c	Ammonia: monitor with test kit at least daily or preferably continuously with sensor and alarm	Adjust water flows to improve water exchange rate, remove lobsters from water or drain reservoir and keep in controlled temperature area matching lobster body temperature	Dockside facility monitoring record form	At each delivery pickup, monitor temperature records
2d	Salinity: monitor incoming water flow with hydrometer hourly or preferably with sensor continuously with alarm	Stop incoming flow of low salinity water, drain reservoir and keep lobsters in controlled temperature area matching lobster body temperature or move to proper salinity reservoir system	Dockside facility monitoring record form	At each delivery pickup, monitor temperature records
3	Lobster body temperature and set point of refrigeration control. Use IR thermometer on ventral surface of tail to estimate core body temperature. Adjust refrigeration system to match.	Adjust temperature set point to match lobster body core temperature	Transport truck monitoring record form	Review monitoring record form at time of delivery of shipment to wholesaler
4a	Temperature: monitor with thermometer at least hourly, preferably continuously with alarm	Adjust temperature control, remove lobsters from water and keep in controlled temperature area matching lobster body temperature	Wholesale facility monitoring record form	Monitor facility monitoring records at least daily
4b	Oxygen: monitor with sensor or test kit at least twice daily, preferably continuously with monitoring system and alarm	Adjust water flows to improve water exchange rate, remove lobsters from water and keep in controlled temperature area matching lobster body temperature	Wholesale facility monitoring record form	Monitor facility monitoring records at least daily
4c	Ammonia: monitor with test kit at least daily or preferably continuously with sensor and alarm	Adjust water flows to improve water exchange rate, remove lobsters from water or drain reservoir and keep in controlled temperature area matching lobster body temperature	Wholesale facility monitoring record form	Monitor facility monitoring records at least daily
4d	Salinity: monitor incoming water flow with hydrometer hourly or preferably with sensor continuously with alarm	Stop incoming flow of low salinity water, drain reservoir and keep lobsters in controlled temperature area matching lobster body temperature or move to proper salinity reservoir system	Wholesale facility monitoring record form	Monitor facility monitoring records at least daily
4e	Time: checking receipt time, determine amount of time immersed in receipt reservoir for each shipment	Replace lobsters back to reservoir system until 48h immersion period is complete	Wholesale facility inventory record form	Review facility inventory records at end of each shift to ensure compliance

CCP No.	Monitoring What, how, when (frequency)	Corrective actions	Records	Verification
5a	Lobster haemolymph %Brix, representative sample of shipment (minimum 100 animals), measure with refractometer for each shipment	Reject lobsters < 7.0%Brix, separate lobsters with haemolymph <8.5%Brix(short-term storage) from >=8.5%Brix(long-term storage)	Wholesale facility lobster health assessment form, lobster inventory report	Review health assessment forms daily.
5b	Lobster haemolymph lactate levels, representative sample of shipment (minimum of 15 animals), measure with test kit or meter for each shipment	Separate lobster shipments with haemolymph lactate >2mml l^{-1} and identify for short-term storage	Wholesale facility lobster health assessment form, lobster inventory report	Review health assessment daily, match with inventory report for identification of short- and long-term storage
6a	Temperature: monitor with thermometer at least hourly, preferably continuously with alarm	Adjust temperature control, remove lobsters from water and keep in controlled temperature area matching lobster body temperature	Wholesale facility monitoring record form	Review facility monitoring records at least daily
6b	Oxygen: monitor with sensor or test kit at least twice daily, preferably continuously with monitoring system and alarm	Adjust water flows to improve water exchange rate, remove lobsters from water and keep in controlled temperature area matching lobster body temperature	Wholesale facility monitoring record form	Review facility monitoring records at least daily
6c	Ammonia: monitor with test kit at least daily or preferably continuously with sensor and alarm	Adjust water flows to improve water exchange rate, remove lobsters from water or drain reservoir and keep in controlled temperature area matching lobster body temperature	Wholesale facility monitoring record form	Review facility monitoring records at least daily
6d	Salinity: monitor incoming water flow with hydrometer hourly or preferably with sensor continuously with alarm	Stop incoming flow of low salinity water, drain reservoir and keep lobsters in controlled temperature area matching lobster body temperature or move to proper salinity reservoir system	Wholesale facility monitoring record form	Review facility monitoring records at least daily
7a	Temperature: monitor with thermometer at least hourly, preferably continuously with alarm	Adjust temperature control, remove lobsters from water and keep in controlled temperature area matching lobster body temperature	Customer facility tank monitoring record form	Review facility tank monitoring records at least daily
7b	Oxygen: monitor with sensor or test kit at least twice daily, preferably continuously with monitoring system and alarm	Adjust water flows to improve water exchange rate, remove lobsters from water and keep in controlled temperature area matching lobster body temperature	Customer facility tank monitoring record form	Review facility tank monitoring records at least daily

CCP No.	Monitoring What, how, when (frequency)	Corrective actions	Records	Verification
7c	Ammonia: monitor with test kit at least daily or preferably continuously with sensor and alarm	Adjust water flows to improve water exchange rate, remove lobsters from water or drain reservoir and keep in controlled temperature area matching lobster body temperature	Customer facility tank monitoring record form	Review facility tank monitoring records at least daily

5.4 Discussion

The identified critical control points for the live lobster supply chain in this Chapter represent activities that span from harvester, dockside buyer, wholesaler and end-user in the marketplace. Each of these stakeholders will require only a subset of the controls outlined in Section 5.3 (above) but, in order for the effective control of all the hazards identified, a coordination of efforts and cooperation amongst the stakeholders must exist. No one unit in the supply chain is capable of controlling the outcome of quality of the end-product without the contribution of controls required downstream once the product leaves their facility. Also, for one stakeholder to try and correct the problems related to a lack of controls upstream prior to receipt of their live lobster shipments, would result in considerable volumes of rejected lobsters that would not be commercially feasible. Therefore, close-working relationships with all parties involved in the lobster supply chain must be established to ensure consistent supply of high quality product to the end-user.

As mentioned earlier in the Chapter, many of the identified control points and critical limits in this study were investigated in isolation from previous investigations on the tolerance levels of lobsters to various environmental conditions including the effects of disturbance of oxygen (Whiteley and Taylor, 1992), salinity (Smyth, 2011), emersion tolerance (Fotedar et al., 2001), temperature (Morris and Oliver, 1999a; Lorenzon et al., 2007) and ammonia (Young-Lai et al., 1991; Bernasconi, 2006). Also, the risk factors

identified in Chapter 2 for long-term storage such as haemolymph total protein (and correlated measures), lactate and pre-treatment recovery, represent useful data for the development of an effective quality control - based HACCP system. However, these critical limits are not widely known amongst the various levels of the commercial supply chain. Significant effort in the form of specifications and instructional documentation, workshops and field – based auditing of live lobster handling and storage practices will be required to ensure that adequate institutional knowledge is present at all levels of the lobster distribution chain is established to execute an effective HACCP plan.

The critical control point identification in Section 5.3.2 (above) focuses mainly on post-harvest environmental conditions and, to a lesser degree, on the intrinsic quality of the animal. Until such time that quality sorting can occur on the boat, this will remain the case and presents a deviation from the inductive logic that is the basis of the HACCP system. However, until such time that technology exists to that will allow the harvesters to identify quality lobsters with adequate meat content and capacity to withstand the rigours of the live lobster supply chain activities easily and objectively, proper training on best practices for lobster handling and environmental condition requirements is the most effective method to reduce the degradation of intrinsic quality of the landed catch.

5.5 Conclusions

In conclusion, the present exercise to develop a model HACCP plan has highlighted the fact that there is still a need for considerable collective effort amongst the various participants of the lobster post - harvest distribution trade to implement an effective industry-coordinated quality control system that would deliver a consistent end-user premium quality live lobster. It is likely to be beyond control the of individual stakeholders within the live lobster supply chain to champion this effort and therefore it may require the assistance of a larger organisation (e.g. a government department or industry-led coalition group) that would have both the authority to impose control

measures as well as the funds necessary to administer the education and support that would be required. With this support, implementation of such a HACCP control system would likely prove to be an effective approach to maintaining a premium reputation in the global live seafood marketplace.

Chapter 6
General Discussion and Conclusions

6.1 Long-term storage risk factors

Seasonal differences exist within in the risk factors measured in Chapter 2 and these were mostly associated with moult stage. Haemolymph total protein, % Brix, and haemocyanin concentration which are related to the haemolymph volume changes during progressive moult stages from post-moult to active pre-moult (Stewart and Li, 1969), were identified as risk factors for increased long-term storage mortality in the Winter season lobsters, but not for the Spring season ones. Winter lobsters, harvested in November and December, may have a portion of the catch that is still in the post-moult stage and not yet fully recovered from ecdysis. These would be identified in measures related to meat content (e.g. haemolymph total protein) and, therefore, haemolymph protein and related measurements may be an effective method to identify the animals that have not yet reached true intermoult stage, such as is the case with early Winter season lobsters.

Exoskeleton hardness as a risk factor was also significant in the Winter season but not in the Spring, which further confirms that it is useful to be able to identify post-moult lobsters with soft-medium exoskeletons. However, exoskeleton hardness measurement is often a subjective process, even with the use of a measuring device like the durometer used in Chapter 2 (Grubert et al., 2012) and more objective measures (e.g. haemolymph % Brix, total protein or haemocyanin concentration) would be more useful, consistent methods.

Spring lobster, long-term storage mortality was not linked to haemolymph protein, % Brix, haemocyanin concentration or exoskeleton hardness, which is further evidence that these parameters are most useful for the identification of post-moult lobsters, since no

post-moult lobsters were observed in the Moult stages found in the Spring season group. Moult stages that were found included intermoult, passive and active premoult, but there were very few active premoult lobsters in Chapter 2 because of the timing of the particular year's seasonal moult cycle. It would be expected that active premoult lobsters would have a higher influence as a risk factor for long-term storage mortality because of the added energy requirements and physiological demands of ecdysis preparation observed in crustaceans (Alikhan, 1972; Regnault, 1979; Carvalho and Phan, 1998).

Changes in relative haemolymph lactate due to hypoxic events and haemolymph osmolality differences that possibly may be linked to osmotic stress events (e.g. hyposaline salinity exposure) were measured as possible risk factors to determine whether pre-shipment, history-related handling problems could be detected and assessed for their influence on subsequent long-term storage mortality. Neither of these factors had significant influence as a risk factor for mortality in the Winter season lobster group, but high haemolymph lactate concentration was identified as a significant factor in the Spring season group. Haemolymph lactate accumulates during hypoxic events such as when emersed or immersed in low oxygen water (Zou et al., 1996; Danford et al., 1999; Bernasconi and Uglow, 2008b). In Chapter 3, the rate of accumulation of haemolymph lactate was found to be positively correlated with lobster core body temperature and elevated haemolymph lactate levels in purchased lots of freshly harvested lobsters and is therefore a good indication that a hypoxic event occurred somewhere along the distribution chain between the harvest day and the time the lobsters are received to the wholesale storage facilities. These possible time-temperature abuse events may be more prevalent in the Spring season due to an increased risk of exposure to high ambient air and water temperatures that normally occur at this time of year in Atlantic Canada, and which were suggested as a risk factor in other crustacean fisheries (Ridgway, Taylor, Atkinson, Stentiford, et al., 2006; Giomi et al., 2008). However, these studies measured

extremely high haemolymph risk factor levels and environmental conditions relative to the trials in Chapter 2. For example Ridgeway et al. (2006) observed captured, emersed *N. norvegicus* haemolymph lactate levels between 7.07 and 15.64 mml l^{-1}, whereas lactate levels observed in Chapter 2 for the medium risk for mortality group were below 4 mml l^{-1} and the overall mean spring haemolymph lactate level observed was 3.29 ± 3.56 mml l^{-1}, considerably lower than the high levels observed in *N. norvegicus*. Extreme conditions and haemolymph lactate levels may not be present in the Canadian lobster fishery based on the low overall haemolymph lactate levels observed in Chapter 2, but slightly elevated lactate levels which were likely to have been associated with emersion temperature during handling or low oxygen immersed storage on the harvest vessel still led to an increased risk of storage mortality. Therefore, successful long-term lobster storage requires that the lobsters are in a high quality state and that have experienced little to no stress events during post-harvest handling and shipping. Minor stress events that cannot be assessed on arrival to the facility by using vigour index used in other crustacean fisheries (Stoner, 2009), must therefore rely on more sensitive measures such as what was revealed by using haemolymph lactate as a predictor in Chapter 2. The observed increased mortality risk within the high haemolymph lactate groups in the Spring season group suggests that this level of physiological stress results in irreversible damage to the lobsters.

The absence of a 48 h period of immersed recovery on arrival and before being subjected to size and quality grading and subsequent long-term storage, was identified as a significant risk factor for long-term storage mortality in the Spring season, but not in the Winter season group. The 48 h immersion would allow the lobsters to recover from the emersion event that occurs during transport from dockside to the wholesale facilities that results in haemolymph hypoxia and the accumulation of haemolymph lactate. The subsequent size and quality grading event requires that the lobsters be emersed for a further 3-4 h prior to being re-immersed in the long-term storage system and, therefore,

the 48h immersed recovery treatment on arrival and prior to this grading activity may allow the lobsters the necessary time to recover from the journey hypoxic event. As is the case with the elevated Spring haemolymph lactate levels which were due to possible exposure to high ambient environmental temperatures, the immersed recovery period may serve to decrease the haemolymph lactate concentrations and thus prevent accumulation to levels that pose a risk to long-term storage success during the subsequent quality and grading emersion period.

6.2 Emersion

Lobster core body temperature is positively correlated with metabolic rate (Forgan et al., 2014) and, therefore, during emersion events when the lobster's gills have an impaired or non-existent ion exchange capability (deFur et al., 1988; Morris and Oliver, 1999a; Chen and Huang, 2001; Bernasconi and Uglow, 2008b), the rate of nitrogen accumulation and the effects of hypoxia (e.g. the accumulation of haemolymph lactate), should be increased in warm-acclimated animals more than in cold-acclimated ones (Whiteley et al., 1990). Commercial lobster storage and emersed shipment systems are designed around this premise that it allows control the lobster's metabolic rate and reduces the factors that lead to hypoxia with its associated problems of irreversible loss of vigour (Stewart et al., 1972; Lorenzon et al., 2007). However, in the present trial this was found not to be the case. Warm-acclimated animals were observed to have significantly reduced haemolymph ammonia and lactate accumulation and loss of vigour during the emersed, simulated airfreight trials. It was also observed that lobster core body temperatures during the emersion trials adjusted to similar low levels because of the gel ice packs and insulated shipping containers which are designed to maintain a stable 3ºC ambient air temperature inside the box. Emersed lobster body temperature is significantly slower to respond to changes in ambient air temperature fluctuations. Whiteley et al. (1995) observed that it

took *Homarus gammarus* 1 h 15 min to drop 10ºC in air and 6 minutes for an identical immersed temperature change, and it was noted here that the core body temperatures of the warm-acclimated group had decreased to the 3 ºC emersion temperature by the first measurement period at 12 h. This represents a relative decrease in body temperature for the warm-acclimated lobster group, but no change for the cold-acclimated group. Thermal acclimation affects metabolic rate and temperature tolerance limits (Camacho et al., 2006) and the present study findings suggest that this is the case even with a small difference in acclimation temperatures of 2.6 ºC versus 5.6 ºC, leading to significant differences in the accumulated effects of emersion on the warm-acclimated group that had its core body temperature reduced to a sub-acclimation temperature. Based on these results, actual controlled operating environmental temperature parameter set points should be based on knowledge of the lobsters' prior acclimation temperature history and adjusting conditions to maintain a sub-ambient temperature environment to minimise the lobster's metabolic rate and extend the maximum tolerated emersion period. The sub-ambient condition may also be relevant to immersed animals but requires further trials to confirm that the controlled effect would apply in this case.

Haemolymph lactate accumulation during emersion was reversed during re-immersion in all cases, with levels mostly returning to pre-emersion levels by 48h. From the risk factor results in Chapter 2, haemolymph lactate was present in high levels in some lobsters even after the pre-treatment 48h immersion period, which suggests that in these cases lobsters probably had been exposed to severe hypoxia that was still detectable after the 48h immersion period and lends additional evidence to support the contention that elevated haemolymph lactate is a strong risk factor for the prediction of long-term storage mortality. Elevated haemolymph lactate levels measured in post-harvest *Panulirus cygnus* were related to reduced survival during subsequent factory storage (Paterson et al., 1999), although the authors cautioned using a single parameter for predicting storage

success since there were other confounding factors that also caused mortality and which needed to be considered. With the present results of Chapters' 2 and 3 it is now known that lobster season is a significant factor in determining whether specific risk factors will be good predictors of storage success. Use of lactate measurement in the spring season will give valuable insight into the immediate pre-history of the lobsters prior to arrival to the storage facilities. Due to the mechanism of lactate sequestering into the shell of the lobster, as well as accumulation in the lobster's haemolymph, lactate levels persist in the haemolymph for up to 48 h post-emersion or anoxic event while the lactate reserves are flushed out. This lag time gives the quality control staff at the receiving plant the ability to use haemolymph lactate level measurements upon receipt at the facilities to determine whether the lobsters were stored in ideal conditions prior to arrival.

Haemolymph lactate analysis of incoming shipments of live lobsters, post-48 h immersion acclimation, will give insight into the immediate post-harvest history of the lobsters and indicate whether lobsters they had been exposed to hypoxic conditions and switched to anaerobic metabolism—as evidenced by the accumulation of haemolymph lactate and, given the lobsters' body temperature on receipt, one could predict how long the lobster has been using anaerobic metabolism. The lobster may have switched to anaerobic metabolism for a number of reasons during post-harvest handling including the following:

- Held immersed in low oxygen and/or high temperature water
- Held out of water at high environmental air temperatures
- Held out of water for extended periods of time

Lactate can be measured easily at the plant, either using the method described in Chapter 1, or using point of care lactate meters such as those evaluated for crustacean haemolymph by Bakke and Woll (2014). These meters offer ease of use and portability

so that they can be used by most of the commercial pound operators at any location including on the boats, at the wharves during landing, at the receiving plant and even by the end-users at the final international market wholesalers' premises.

Size had little influence over any of the emersion parameters. It may, however, play a more significant role when large loads of emersed animals are above refrigeration set points within the transport container system. In these circumstances it may take prolonged periods, if at all, to reduce core body temperatures below ambient acclimation temperatures during the consignment. However, for bulk consignments in which plastic crates are used, each crate is filled to its full weight capacity so the temperature load is likely to be the same in a consignment of small or large lobsters. Size may be a factor during emersion only, with respect to temperature shift towards the emersion environment, if the density of lobsters within the temperature-controlled environment is different due to the size of the lobsters, be it a truck load of lobsters or a box full of lobsters. This may be the case in a boxed lobster consignment in which divided partitions limit the ability to maintain constant density of packed product weight with all sizes of lobsters. Smaller lobsters within a box configured in this way will have a higher density compared with larger lobsters. Present packing parameters at the facilities of the firm used for this trial, is to allow for 36 Chix-sized lobsters to fit into a standard polystyrene shipping box for a total weight of 18 kg per box whereas only 28 Halves will fit in this same box for a total weight of 17 kg, a 6.25% lower density than the Chix box configuration and this may affect the capacity of the gel ice packs to maintain a stable, consistent temperature across all packed size classes during emersion. It may be beneficial to determine the gel ice pack requirements based on the net weight of lobsters within the packed box to adjust for the refrigerant requirements and total thermal capacity of product with each packaged size class. This may lead to a more consistent emersion temperature within the box independent of lobster size packaged for shipment, and may

help reduce the amount of ice packs used in shipments where lobster density is high enough to maintain temperature with less assistance from the gel ice. Further study into size-related packing density and gel ice pack requirements is suggested and would be relevant and useful to the live shipping industry for consignment quality maintenance, consistency and efficiency with respect to optimizing ice pack requirements.

6.3 Cardioventilatory behaviour

Heart and scaphognathite beat rates are positively correlated with temperature (Worden, 2006), specifically within the range of this study and, to a lesser degree, at temperatures above those that are typically encountered in the Canadian *H. americanus* trade. Within the present study there were no significant, size-dependent cardioventilatory differences observed in either the cooling or the warming trials, but the general trends of increasing heart and scaphognathite beat rates with increasing temperature were confirmed to occur within the temperature range of 2.5 - 7.0 ºC. After an acute temperature change, lobster core body temperatures reached the new immersion temperatures within 22 - 37 minutes. This core body temperature change rate was slower than the 10 ºC temperature change core body temperature change rates observed in *H. gammarus* (Whiteley et al., 1995) in which body temperature attained a 10 ºC difference in body temperature within 6 minutes when an acute immersion bath temperature increase or decline between 10 ºC and 20 ºC. It is likely that this difference is related to the lobster's metabolic rate differences at the different temperature ranges – the higher temperatures used for the *H. gammarus* trial causing an increased cardioventilatory rate and thus faster rate of change in body temperature compared with the present, lower temperature trials with *H. americanus*. The differences between the two suggests the cardioventilatory behaviours function as an effective heat exchange system in which the rate of branchial ventilation and cardiac output can control the rate of change of core body temperature when the animal is

subjected to acute temperature changes in their immersed environment. Future studies would be required to confirm whether similar rates of core body temperature change exist between these two species at similar starting and ending immersion temperatures.

H. americanus has been shown to be able to detect temperature changes as small as 0.15 ºC (Jury and Watson, 2000) using cardiac assays, and thermoregulatory behaviours exist with respect to temperature avoidance and temperature preference responses through movement away from or towards specific habitat temperatures (Crossin et al., 1998). The non-lethal methods of IR plethysmography for the determination of cardioventilatory activity during acute temperature change events offers a sensitive, relatively simple method to assess the various temperature change scenarios that exist in the live *H. americanus* distribution trade and to help determine which practices are within the tolerance limits of the species.

6.4 Model HACCP plan for the live lobster supply chain

Homarus americanus is an aquatic species that is relatively tolerant of a wide range of emersion and immersion environmental parameters and they are capable of withstanding the rigours of the commercial supply chain transport and storage activities (Danford et al., 1999; Lavallée et al., 2000; Lorenzon et al., 2007). However, there still exists repeated examples in which lobsters are exposed to extreme conditions that cause excessive mortality events (Harris, 2004; Giomi et al., 2008; Stoner, 2009). These events can be expensive economically, have a negative impact on the marketability of the species as a premium product, and suggest that there is a need to control the execution of commercial activities to provide conditions that are kept within the tolerance ranges of the species. A model HACCP plan application to the entire industry has challenges both with respect to the availability of defined critical controls and critical limits that can be monitored realistically and the ability of a fragmented industry like that of the *H. americanus* live

trade to be committed to maintain quality chain control measures. Successful implementation of HACCP - based programs require the identification of formal management responsibilities. In the *H. americanus* industry model, a management body that oversees the entire supply chain logistics does not exist currently, and it is beyond the scope of any current company or stakeholder in the industry to take on this responsibility. A proposed solution would be to form an industry coalition made up of stakeholders from all sectors of the live lobster distribution trade with aligned values of supplying a premium quality live lobster to the market. This group could create and enact their own set of operating standards and use a system such as the proposed HACCP plan model to control the activities and ensure the harvested lobsters are handled, transported and stored using proven methods of maintaining intrinsic, freshly-harvested commercial quality.

6.5 Final summary and conclusions

The work in this thesis was designed specifically to investigate the implication of commercial practices on the maintenance of quality throughout the post-harvest distribution chain of live *Homarus americanus*. The study identified risk factors that were related to long-term storage success, with seasonally-dependent effectiveness as quality maintenance predictors. When properly applied based on these studies, the factors can be used to determine the capacity of the lobster to withstand further handling upon arrival to the storage facility. In the Winter season, measures of haemolymph total protein and its analogues including haemolymph refractive index (% Brix) and haemocyanin, as well as exoskeleton hardness and pleopod staging, were most effective in predicting storage success. These factors are affected by, and are related to, the lobster moult stages following ecdysis and progression into intermoult that occurs in the months leading up to the opening of the Winter harvest season. In the Spring harvest season, haemolymph

lactate and arrival immersion recovery treatment prior to subsequent handling were relevant factors in predicting long term storage success. These two identified risk factors are associated with post-harvest environmental exposure history and speak to the need to ensure proper on-boat and dockside environmental conditions are maintained during the warmer spring weather months to protect against loss of quality further along the distribution chain. The moult-related risk factors that are important in the winter fishery are not effective for predicting storage outcome in the spring, and this is probably due to most of the environmental condition requirements being met in the winter due to favourable seasonal weather conditions on board the boats and at dockside prior to shipment to the storage facility.

Post-harvest emersion is unavoidable when transporting lobsters from the harvest region to the far-off international markets where their value as a live seafood product is realised. During emersion, the lobster's gill function is impaired and eventually their haemolymph oxygen reserves become depleted and they switch to anaerobic metabolism that leads to an accumulation of haemolymph lactate and ammonia as well as a haemolymph acidosis. Pre-shipment differences in lobster acclimation temperature, even less than a 5 ºC difference, can lead to significant differences in final vigour at the end of the emersion event. Data from these studies revealed that environmental temperatures required to maintain vigour during typical maximum commercial shipment emersion times of 60 to 72 h were dependent on the acclimation history of the lobster. Lobster size also affects the outcome of emersion tolerance in a commercially-packaged lobster for shipment due to the differences in packing density in the shipment that influences the internal box air temperature. This identifies the problem that a simple standardised set of temperature controls or packing conditions will always provide the optimum emersion environment to maintain quality during a commercial consignment.

H. americanus is an ectotherm, and therefore, there is a direct influence by the external environment on their body temperature and metabolic processes, including respiration, ventilation and cardiac performance. Within the live lobster distribution chain there are instances where the lobsters may be subjected to different immersed temperature environments depending on whether the particular facility utilises chilling systems for temperature control or a simple flow-through, ambient seawater reservoir. Although the range of temperature differences is usually within 4.5 ºC, the measurement of cardio-ventilatory activities were shown to be sensitive enough to detect small differences in the lobster's response to temperature changes. This technique may be useful in resolving whether temperature change events have a negative impact on the lobster before a difference is detected using haemolymph parameters (e.g. glucose or lactate) from acute environmental change stress.

Through the identification of measureable risk factors and environmental condition effects on lobster quality preservation during commercial handling and storage practices, and the development of the tools that allow for refinement of these factors and parameters, the foundation to begin effective control of conditions throughout the distribution chain can be realised. Controlling the risk factors and conditions through the use of a common quality control system such as HACCP, commonly used in the food industry worldwide, provides a platform within which standard codes of practice can be documented, implemented and monitored for effectiveness. The HACCP quality control system relies on the participants within the targeted process that requires control to be responsible for the maintenance of written procedures that specify proper conditions to ensure quality is maintained. Implementation of HACCP principals are already a license requirement in the Canadian live lobster export industry so the concepts and terminology are familiar to most of the *H. americanus* distribution chain participants. Therefore, the use of HACCP principals in this study, which incorporate factors investigated in earlier Chapters of this

thesis, as well as previously published articles in the literature, should be an effective platform that encourages structure, sound advice based on relevant research and rigorous control and oversight of the many processes that are used to handle, store and ship live lobsters to premium markets.

6.6 Further work

The findings in the present studies have contributed to our knowledge of the effects of temperature on the behaviour and physiology of *Homarus americanus* and have identified risk factors that affect the successful long-term commercial storage of the species. These results suggest that there is more work to be done with respect to collecting and analysing data on other events observed within the lobster industry to further refine the conclusions and recommendations to improve handling, storage and transport conditions in an attempt to provide a consistent supply of premium live lobster product to global seafood markets. Some suggested areas for further investigation include the following:

- Risk factors identified for long term storage mortality should be trialed within the commercial environment in a pilot project to confirm the validity of the findings of this study and to determine whether the risk factor categories defined in Chapter 2 require further refinement to properly classify risk groups of short-, medium- and long-term stored lobsters.
- Risk factors that were associated with long-term storage mortality are able to be sampled only from a representative group of lobsters from each shipment. There exists a within-shipment variability of measurement levels, and it would be desirable to be able to identify specific animals that are outside of the acceptable, low-risk categories using a low impact, preferably non-invasive method. It is impractical to consider sampling haemolymph from every single animal and

therefore an automated sensor that could estimate or sort individual animals by risk factor categories would be an attractive option for many stakeholders within the live lobster trade.

- There still exists a need to conduct more trials to determine the acceptable parameters for improving emersion tolerance and extending emersion time based on the results of this current work. Specific attention to acclimation pre-history, refining emersion box air temperatures and validating current industry practices which incorporate ice packs in every shipment to bring the emersion air temperature as low as possible without causing freezing conditions. There may exist a specific temperature range for specific acclimation temperature conditions, and investigation of these range limits would provide further refinement of recommended operating procedures for the live lobster trade.
- Cardioventilatory assay trials have highlighted the usefulness of using such methods for sensitive, non-invasive methods to determine thermal sensitivity limits of acute environmental changes. Further work to observe the cardioventilatory effects of the full spectrum of commercial practices from post-harvest emersion to long-term, low-temperature immersed storage would could potentially provide useful data to suggest further improvements to the current commercial practices used in the trade of live *H. americanus*.

Investigations directed to the suggested areas of further research, outlined above, could provide the knowledge necessary to make effective improvements to current commercial practices within the live lobster industry. The trials were selected either to simulate commercial practices to make observations of their effects, or to observe the impacts of actual commercial activities. This type of work is best undertaken with the support and involvement of industry partners who can provide effective guidance on the relevance of the research with respect to it applicability to the industry being observed. It was the focus

of this research to use an industry-experienced perspective in the designing of the trials in order to obtain conclusions that are relevant to industry.

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