Factors influencing the success of live export of South African abalone (*Haliotis midae*)

A thesis submitted in fulfilment of the requirements for the degree of

Master of Science

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Declaration

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Abstract

South African abalone (*Haliotis midae*) has been successfully farmed for 35 years, for commercial export to Asian markets. Abalone can either be exported dried, canned, or live, where live export generally fetch a higher price. However, complications arise within the transport phase during export. Abalone experience different negative effects, including a decrease in their survival rate (which ranges from 64 – 97 %) and behavioural response, including failed righting behaviour and a lower speed when locomoting, which indicates increased stress and decreased meat quality. They also experience an increased weight loss, through drip loss, of around 4 – 15% of their total weight, which is costly to farmers as exporters are paid on the landed weight.

This research, which was conducted at Aqunion (Pty) Ltd, aimed at improving current farm export practices (Chapter 4) and implementing a pre-cool method (Chapter 3) during the summer months, when increased sea temperatures prevail and further negatively influences the exporting process. This was done by comparing abalone weight, survival, behaviour, and health when subjected to eight different pre-transport conditions, and then subjected to a 42-h transport simulation. These conditions include two different acclimation temperatures, purge days, ice volumes, and for two different animal sizes, which are currently being used on the farm.

Pre-cooled tanks were able to achieve a maximum 4-degree Celsius temperature difference from the incoming ambient sea temperature while also maintaining optimal DO and pH concentrations of 8.1 mg/L, 94.5 %, and 8.4, respectively. A higher sludge accumulation was also observed within pre-cooled tanks throughout each purging process. Post transportation, animals were able to retain 170 g more with a longer 3-day purge, and survival was higher by 8 %, 9 %, and 10 % with pre-cooling, longer purge, and a more ice, respectively. Animals were also more responsive in their movement upon arrival by 9 % under a pre-cooled condition and were also able to move at a higher speed of 1.60 ± 0.17 mm s^{1}. Adhesive force was greater for both big and small animals by 1.93 N and 0.70 N when transported with more ice.

Results from this research give further insight into the current recommendations for pre-transport exporting procedures, during standard export environmental conditions and during summer conditions. This, in turn, will lead to reduced losses during export and an increase in annual revenue for abalone producers.

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Chapter 1: General Introduction

1.1 Problem statement

The high demand and premium price for abalone has made it one of the highly prized cultivation species, where the growth in this sector was due to intensive research and development to support both local and the large international market (DFFE, 2018; Wingerter *et al*., 2013). The abalone industry has contributed to the marine aquaculture sector by 94 % in total production, where values reached R 300-million per anum (Cloete, 2008). Since majority of farm output in South Africa is exported and the value of fresh abalone exports is determined by price increases and volume expansion, the optimization of space during transport while ensuring a good condition of the animals is essential for net returns (Terchunian *et al*., 1999; DFFE, 2018). The shipping of live and fresh seafood, including sea urchins, sea cucumbers, lobsters, and abalone, is dependent on species specific factors which must be considered to ensure the produce arrives alive and in good condition (Terchunian *et al*., 1999; Wilen and Reynolds, 2000; Kurni; James and Evensen, 2022). These factors include respiratory rates, ability to handle stress, excretory functions, and temperature tolerance ranges. The oxygen requirements for live seafood are also based on the body size, ambient temperature, nutritional state, and the metabolic rate of the animal. Handling animals and packing are also factors affecting export, where procedures are designed to keep metabolic rates at a low level (Terchunian *et al*., 1999; Francis, 2008). The reduction of the metabolic rate is also based on different aspects, including the use of anaesthetic, reduction of temperature, and eliminating feeding for several days prior to shipping, which is species dependent. During shipment, seafood discharge of ammonia and water is a product of the metabolic rate and is not possible to remove, which should be considered during shipment. With this, sufficient oxygen supply allows for the produce to survive for a minimum of 48-h. The volume and weight of the packaged container should always be considered, where during summer months or shipment to warmer climates, the quantity of biomass per carton should also be reduced to minimize occurrence of mass mortalities. Shipping costs can also be reduced if mortalities are low upon arrival (Terchunian *et al*., 1999; Bubner *et al*., 2009).

Currently, South African abalone seens a reduction in the weight of 4 – 15 % when landed, due to evaporation and pedal mucous production (Francis, 2008). This expected reduction, could lead to revenue loss, particularly during summer when temperatures are higher for prolonged

period. The animal's metabolic rate is impacted which contributes to a drop in water quality and negatively affects gaseous exchange, and this leads to a further reduction in landed weight (Francis, 2008; Morash and Alter, 2015; Lehto, 2023). During transportation, abalone also experience extreme stress, including a period of starvation, prolonged air exposure, handling, periods of hypoxia, and temperature fluctuations, which also affects their survival rate by between 64 – 97 %. This is made worse during the summer months due tissue hypoxia within undesirable temperatures ranges (Vosloo *et al.*, 2013; Alfaro *et al.,* 2021; James and Evensen, 2022). Therefore, a better understanding of the handling and packaging procedures, and the effect that they have on live seafood health and survival post-transport, is needed to improve efficiency and sustainability of this part of the farmed abalone value-chain.

1.2 Literature review

1.2.1 Aquaculture production

Aquaculture, which is classified as the farming of aquatic organisms (both freshwater and marine), is a very diverse industry, where different species of fish, crustaceans, aquatic plants, and molluscs are farmed for either commercial or domestic sales (Francis, 2008; Gephart *et al.*, 2020). The main aim of different aquaculture industries is to maximize profit and economic benefits by maximizing the production of certain species that are in demand but also ensuring that it is achieved sustainably (Gephart *et al*., 2020). Aquaculture is currently one of the world's fastest growing systems for food production needed to meet the growing demand for fish produce, since the rapid decline of captured fisheries in the early 1990s due to a growing increase in the global human population, which in turn led to extreme, wasteful, and unregulated fishing practices (Drammeh, 2000). Due to this, it then became the leading consequence to why over half of the world's fisheries is overexploited (Mohammed and Wahab, 2013; Mahieu, 2015; Pogue *et al.*, 2021). In consequence, great concern was raised on the sustainability of the future seafood supply, which led to conservationists, academics, and entrepreneurs to explore other alternatives sources of seafood products and in turn aquaculture was introduced as a sustainable alternative for a food source. Since the mid-1960s' the global seafood supply has increased from 9.0 kg to 20.2 kg per capita in 2015 (Gephart *et al*., 2020), where between 2015 and 2017, the global contribution equated to around 114 to 129 million tonnes, which was valued at 401-billion US dollars (Figure. 1.1) (Jayanthi *et al*., 2018; Pitcher e*t al.*, 2019; Tacon, 2020; DNV, 2021; Boyd *et al*., 2022; Naylor *et al*., 2021; Ponton, 2022; FAO, 2024). This exceeds the captured fisheries by 18.3 MT and future predictions indicate

that by 2050 the global production of cultivated aquaculture species would more than double with an increase of 30 MT every year (DNV, 2021; Pogue *et al*., 2021). Since the introduction of commercial aquaculture, it has played an important role in economic development by creating many economic opportunities and development (Shakouri & Yazdi, 2012), which assisted in poverty alleviation by contributing and supporting around 500 million people in developing countries directly including in Ghana, which is one major country that depends on fish supply as their per capita fish consumption, which is around 26 kg, exceeds the global production (20 kg) (Asiedu *et al*., 2017). Aquaculture plays an essential role in global food security as it is an important source of protein, specifically farmed fish and molluscs (Ren *et al*., 2019).

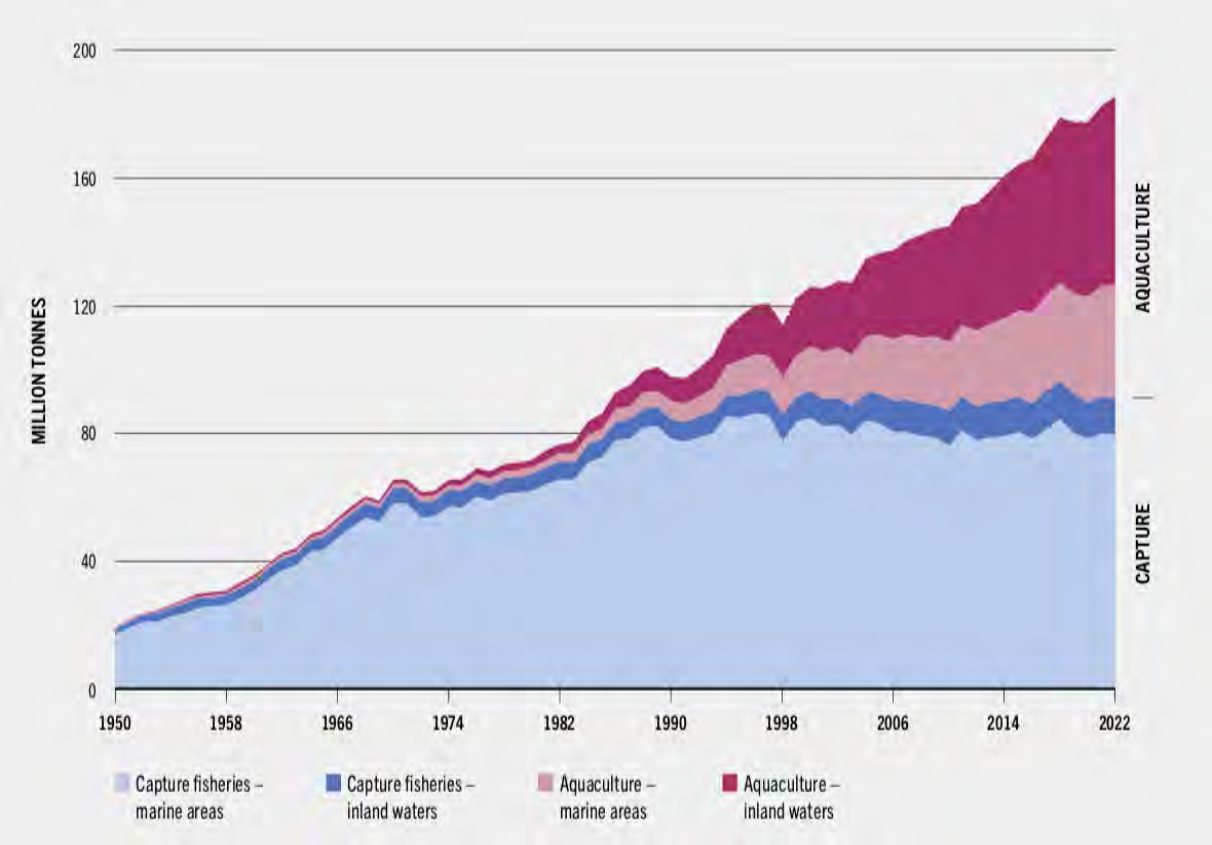


Figure 1.1: Global fisheries contribution, of both capture fisheries and aquaculture production for both marine and freshwater in Million tonnes, from 1950 until 2022. Source: FAO, 2024.

1.2.2 Aquaculture in South Africa

The initial introduction of extensive aquaculture for commercial sales into South Africa began in 1948, with the cultivation of oyster (*Crassostrea gigas*) in Knysna along the coastline (Ponton, 2022); however, the increase interest only began in the 1980s', where state-led

projects by the government aimed at supplying aquaculture products for food security and to also have another means of income generation, mainly for rural communities (Britz and Hecht, 1992; North, 2019). During this time, different species for cultivation were introduced including waterblommetjies (*Aponogeton distachyos*), and other ornamental fish, mainly goldfish (*Canassius auratus*). Production of aquaculture products gradually increased in the 1980s, as in 1982, production did not exceed 550 tonnes which was valued at around R4 million and in 1988, production of aquaculture products reached 3,094 tonnes which sold at a value of R45 million (Safriel and Bruton, 1984; North, 2019).

Although the marine sector is restricted to a few provinces with access to the ocean, including the Northern Cape, Western Cape, Eastern Cape, and Kwazulu-Natal, majority of the contribution of aquaculture production comes from this sector with 66 % of production represented as the country's total aquaculture output of around 9,800 tonnes, produced in 2020 (Cai and Zhou, 2022). Although the marine sector generates majority of aquaculture revenue for the country, this sector is still underdeveloped as other countries, including Thailand and Egypt, "with similar sized coastlines produce 1,000 times more than South Africa" (DEA, 2006; Ponton, 2022). In the marine sector, majority of production is dominated by different species including abalone (1,479.22 tons), mussels (1,758.47 tonnes), and oysters (276.85 tonnes), however finfish only contributes 77.32 tonnes since 2015. Development of the marine sector has great potential due to the country's prize species; the South African abalone (*H. midae*) (Ponto, 2022).

1.2.3 Cultivation of South African abalone (*Haliotis midae*)

Abalone (*Haliotidae*), which are also known as marine sea snails, are part of the order archaeogastropoda and the phylum Mollusca, which consists of other species, including snails, chitons, oysters, and octopuses (Dai and Cho, 2022). Abalone are slow moving, sessile creatures, that inhabit rocky reefs within the intertidal and sub-tidal zones, where they live in depths of up to 50 m. These different species are generally temperate in nature and preferred temperature ranges are between 12 to 21 °C (Vosloo & Vosloo, 2010). Abalone can be found worldwide, ranging from New Zealand in the south to Alaska in the north with distributions for species (Rousseau, 2014). In South Africa, there are five endemic species with different distributions for each species across the coastline (Figure 1.2); however, only one species is farmed for commercial export; *Haliotis midae* (Sales & Britz, 2001; Rousseau, 2014; DFFE, 2018).

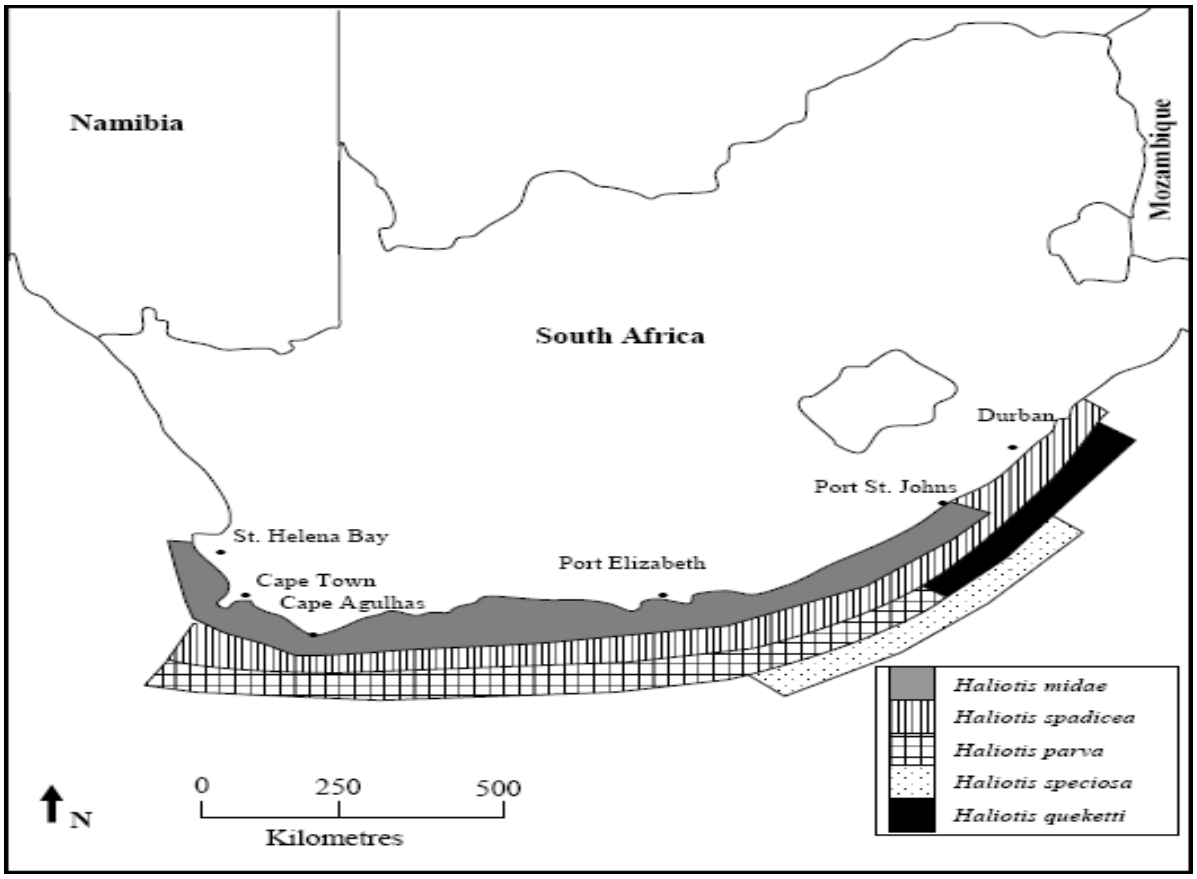


Figure 1.2: Distribution of the different 5 endemic Haliotis *species along the South African coastline, from the south west coast to the east coast (Lindberg, 1992).*

The cultivation and production of abalone worldwide is highly successful and spans many different countries, including China, Korea, South Africa, Chile, Australia, Taiwan, Japan, the US, New Zealand, Mexico, Europe, Thailand, and the Philippines (Cook, 2023). This is due to it being classified as a "premium seafood product", where an increase in popular interest due to its rich nutritional and pleasant taste has since increased (Setyaningsih, *et al*., 2020; Dai & Cho, 2022).

Even though *H. midae* can be found across the south west coast of South Africa, from St. Helena Bay towards the east coast reaching Port St. Johns, the majority of the natural abalone and farms are located on the west coast, with the exception of one farm on the east coast and a few on the north coast. This is mainly due to the colder temperate waters found within that region (Vosloo *et al*., 2013; North, 2019). Even though South Africa only contributes 1% of the total global supply, it is still classified as one of the leading countries for the global production of abalone with around 1,500 mt produced per annum since 2016 (Rousseau, 2014;

North, 2019). South Africa is also established as one of the largest suppliers, coupled with New Zealand, Australia, Japan, and the United States, however, South Africa is classified as the third largest exporter of abalone (Mau & Jha, 2018; North, 2019).

The initial cultivation of *H. midae* started in 1980 when the high demand was not met due to low stocks of wild caught individuals, which was due to over-exploitation, illegal catches, diseases, and habitat degradation (Cook, 2019; Pitcher *et al*., 2019; FAO, 2024). The illegal catches of the wild stocks, which escalated in the 1990s, was the major contributor to the rapid decline of the wild population, which led to these species being brought to the edge of commercial extinction. During this time, more abalone was impounded than legally exported, as a total of 96 million abalone units were poached from 2006 to 2016 (Steinberg, 2005; North, 2019). Since then, abalone aquaculture production has increased and was estimated at US$ 2.6-billion in 2017, where export has also increased from 246 thousand metric tonnes in 2017, to 11.1 million metric tonnes in 2023, which was valued at US $ 7.66-million and US $ 28-million (Shin, 2025). In 2015, a total number of exported abalone were valued at around 463 million Rands (38M$ US dollars) (Pitcher *et al*., 2019). Abalone farming in South Africa contributes over 90% of the total value of the marine aquaculture industry, where it supplies over half of the marine aquaculture production annually (Rousseau, 2014). Within the global market, South African exports approximately US $27-million worth of live, fresh, or chilled abalone, which in turn accounts for 19.6 % of the global export value (Trend Economy, 2024). This contributes 0.025 % to the global market, where although the contribution to the global production is low, the contribution within South Africa has generated significant economic growth, which has also produced jobs that are supported by export earnings (Antoni, 2018).

The rearing of cultivated abalone began in the 1990's where research and development was focused on understanding and developing the physical and environmental conditions of their life cycle and stages in order to ensure survival and reproduction. Research was also focused on the nutritional requirements of each life stage of abalone in order to manufacture the best feeds for each life stage so as to reach an average size of 100 mm after 5 years of growth. This is advantageous compared to the wild stock, where they reach a maximum average size of 200 mm in shell length after 30 years of growth (Ponto, 2022). More research within the abalone aquaculture industry is still of high importance, with a specific interest in increasing farm outputs while simultaneously reducing animal losses (Venter *et al*., 2018). During production, within the grow-out phase of growth, farms use high stocking densities to maximise

production and increase the economic viability (Wassnig *et al*., 2009). Abalone have an inverse relationship between growth and stocking density (Capinpin, *et al*., 1999; Harris *et al*., 1999; Harris *et al*., 1999a; Huchette *et al.*, 2003a, b), as an increased stocking density results in decreased body weight and survival rate; however, this is size dependent as this is experienced by larger animals (90 – 200 g), and on the other hand, smaller animals (20 – 50 g) experience a biomass gain with an increase in density (Mgaya and Mercer, 1995; Pang *et al*., 2022).

1.2.4 Environmental conditions for abalone cultivation

Abalone farms across the coast are reliant on the constant supply of coastal water into their land-based systems. Temperature and oxygen concentration in the incoming water fluctuations throughout the year (Morash and Alter, 2015), due to fluctuation in ocean conditions. Ocean temperatures within the Western Cape are dominated by the Benguela current, that bring cold, upwelled waters to the surface year-round (Shillington *et al.*, 2006). Ocean temperatures along the east and south-east coast of South Africa are mainly dominated by the Agulhas current, where this western boundary current provides tropical and subtropical temperatures south (Goshen *et al*., 2012). The dynamic of the Agulhas current also bring cold, deep, upwelled water to the surface, throughout the year, with exception to summer environmental conditions, due to the isotherms on the inner boundary of the water column, which curve upward (Goshen *et al.*, 2012). In recent years, since 2017, global mean temperature have risen since pre-industrial events (Mbokodo *et al.*, 2020). Different heatwave attributions, including the frequency, intensity, and duration, have also increased more with increased temperatures during summer months (Vosloo and Vosloo, 2010; Vosloo *et al*., 2013). Although South African abalone have a wide temperature range, within the Western Cape, average monthly temperatures range between 12 – 13 °C, with summer temperatures reaching 21°C and above (Vosloo and Vosloo, 2010).

Abalone are ectothermic poikilotherms and have the ability to maintain their physiological functions with fluctuating body temperatures. A higher number of mortalities occur in summer when temperatures increase beyond their thermal range (Vandepeer, 2006). This mainly effects mature reproducing animals as they have a narrower thermal tolerance compared to juveniles, as endocrine function and gonad maturation are affected by increased temperature (Portner and Farrel, 2008; Pedroso, 2017).

1.2.5 Transportation of live abalone

As a part of aquaculture production, mature abalone that are ready for commercial export can either be canned, dried or exported live. Since the pricing of live shellfish produce is dependent on its quality, it is important to ensure that the produce arrives in the best condition possible, especially since it is being shipped to distant international customers (Terchunian, *et al.*, 1999; James and Evensen, 2022). The transportation of live abalone to these international markets is a "complex operation that requires consideration of numerous conditions, including water temperature, oxygen supply during the transporting process, transportation time, and others" (Alfaro *et al*., 2023).

These different factors, during transportation, affects an abalone's metabolic activity, their stress levels, and in turn can negatively effect the quality of their meat. For example, a higher level of total volatile basic nitrogen (TVB-N) in the meat, will increase the chance of spoilage (Sikes *et al*, 2009; Siddiqui *et al*., 2024; Zhang, *et al*., 2025). When packing abalone for live transportation, two different methods can be used; dry or damp method. The damp method follows a procedure where a 10 – 30 % total volume of water is added to the storing containers, prior to transportation. The dry method, which is the most used method, follows a procedure where minimal amount of water is added, which generally consists of maintaining the moisture of the packaged individuals using sponge sheets (Yasa *et al*., 2021). The use of the dry method inhibits the growth of microorganisms, including bacteria and mould, which reduces the chances of tissue spoilage and further increasing the shelf life of seafood (Valu *et al*., 2024).

During the process of packaging live individuals for export, it is recommended by the Asia-pacific economic cooperation (APEC) guidelines to pre-cool crustaceans prior to packaging so that they become lethargic, which reduces stress during transportation (Fotedar and Evans, 2011). Although rapid cooling may result in loss of legs or claws in some animals, it is also recommended to pre-cool individuals at a slow pace to avoid a thermal shock (Vosloo and Vosloo, 2010). Depending on the species being packaged, which includes black tiger shrimp (*Penaeus monodon*), freshwater prawns (*Macrobrachium rosenbergii*), lobsters (*Homarinus capensis*), and South African abalone (*H. midae*), it is also recommended to package these individuals in sealed plastic bags, which contain oxygenated water, under cold conditions, and then package them in styrofoam containers to maintain a more constant temperature (Terchunian *et al*., 1999; Hse *et al*., 2007).

The success of live transportation of different invertebrates, including sea urchins and abalone is also dependent on different aspects, including vehicle types, installed equipment, as well as the road conditions because these different factors can affect the survival of aquaculture cultivated produce (Lekang, 2007). There are different methods of transportation, which includes transport by land (trucks or smaller vehicles) and is generally short term (<8 h). The other method of transport includes air or sea shipment, which is generally long term (>8 hours). The South African abalone is mainly transported by air and duration usually lasts up to 18-h (Yasa *et al*., 2021).

The size of the animal also plays a role in survival. Smaller specimens, with sizes one kilogram or less, have greater metabolic requirements per unit weight. Shorter transported by land is recommended for smaller animals, whereas larger sizes can be more successfully transported by sea or air (Buen-Ursua and Ludevese, 2010). Abalone, similar to sea urchins, are quite robust and with proper handling are able to survive for a significant period of time out of water (environmental hypoxia / functional hypoxia) with very low mortality after being re-immersed into seawater (James and Evensen, 2022). However, there is lack of published knowledge and information on the best pre-transport methods in terms of transportation and protocols for South African abalone that results in the most optimal health of abalone upon arrival.

1.2.6 Histology

Cultivated abalone are susceptible to immune suppression, which increases the chances of diseases outbreaks (Hooper *et al*., 2014). These occurrences can be caused by environmental stress to the host. Stress increases due to different environmental factors, including increased temperature or high stocking densities under aquaculture conditions (Dang *et al*., 2011). Investigations on the health of farmed abalone with their diagnostics are mainly based on histopathology or scanning electron microscopy (SEM), where histological results strengthen existing knowledge of normal abalone anatomical structure (Harris, *et al*., 1999).

Since abalone evolved during the Cretaceous period, their ancestral anatomy and features limits them to habitats with fast flowing, oxygenated water, with respiration occurring passively (Tissot, 1992; Figure 1.3). Although, the internal anatomy of the abalone is relatively simple, where it consists of two kidneys, a reproductive organ, and a digestive gland, they have developed and adapted different physiological strategies, which makes them well adapted to their natural environment (Morash and Alter, 2015). The anatomical structure of the abalone's

gills is described as 'feather-shaped', where it includes both gill filaments and axis, with the filaments attached to one end of the gill axis, with the outer end free (Di *et al*., 2012) (Figure 1.4). The gills also contain lateral cilia, which functions as the 'ventilatory pump' by drawing water in around the head region and the first anterior pores of the shell, where abalone relies on fast water currents for 'ram ventilation' (Morash and Alter, 2015). Within both the axis and filaments, the epithelia of the gills contain mucus cells, and there is a greater density of mucus cells within the axis. The major component of these mucus cells consists of water, which constitutes around 81.4 to 99.8% of their wet weight (W/W), which leaves 0.2 to 18.6 % of solid matter (W/W). This solid matter comprises two groups; the high molecular weight protein-polysaccharide complexes and as well as inorganic salts (Di *et al.*, 2012).

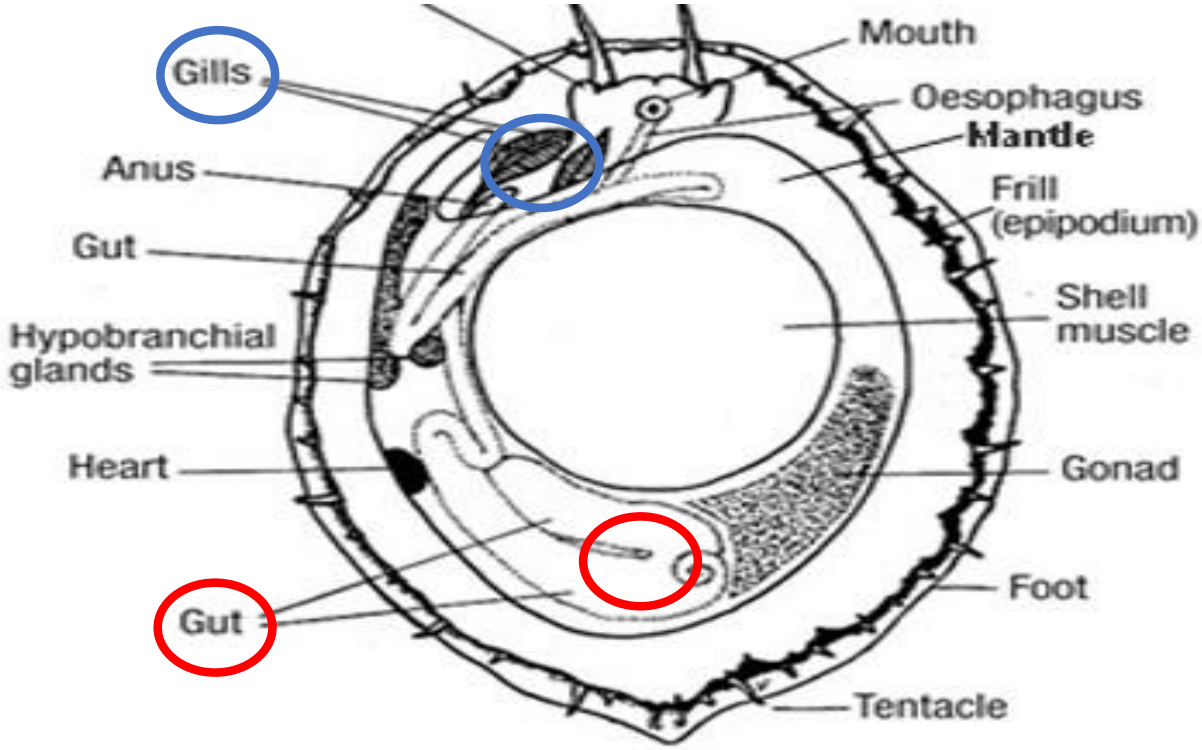


Figure 1.3: Annotated abalone internal anatomical structure with the selected tissues, including the gills and the end gut (with the exception of the right kidneys) tested for within this research. Gills are highlighted with a blue circle and the gut is highlighted in a red circle (Di, et al.*, 2012).*

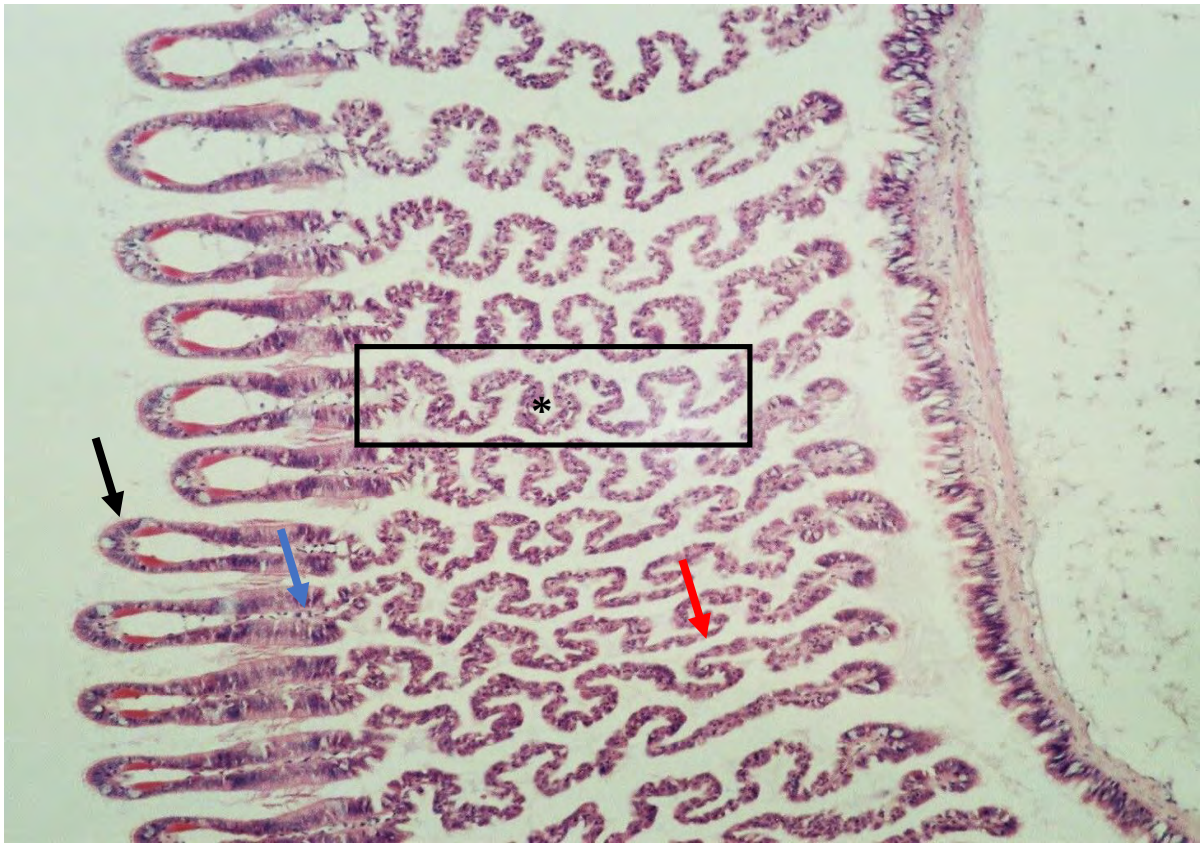


Figure 1.4: Histology of the South African abalone (H. midae) left gill under standard farming conditions. Animals were left undisturbed, prior to dissection, for a minimum of four weeks. The different characteristics, which make up the gill consists of the gill filaments (black arrow), Gill lamellae (black box with asterix), the blood vessels (blue arrow), and the epithelium (red arrow).

The digestive gland or gastrointestinal tract of an abalone contains a buccal region which consists of a radula, followed then by the oesophagus, which widens into the crop. The stomach is then followed after the crop, where it then leads to the caecum, which receives secretions from the hepatopancreas (also known as the digestive diverticulum) that contain enzymes that metabolizes lipids, carbohydrates and proteins (Bevelander, 1988). Lastly, the hepatopancreas is then followed by the intestine, where it terminates at the anus (Di *et al*., 2012). The digestive gland has multiple functions, such as the digestion of different food materials, absorption of nutrients, the storage of lipids and glycogen, and lastly it is very important as it is the main source for detoxification in different abalone species (Rizk, et al., 2014). A healthy digestive gland is characterized by having multiple oval shaped tubules, which consist of lumen which are surrounded by a single layer of epithelial cells. These lumens are connected by connective tissues and the epithelial cells. Epithelial cells are also characterized as either secretory or digestive cells. The entire digestive gland is then enclosed by an outermost membrane; the tunica propria (Figure. 1.5) (Rizk et al., 2014).

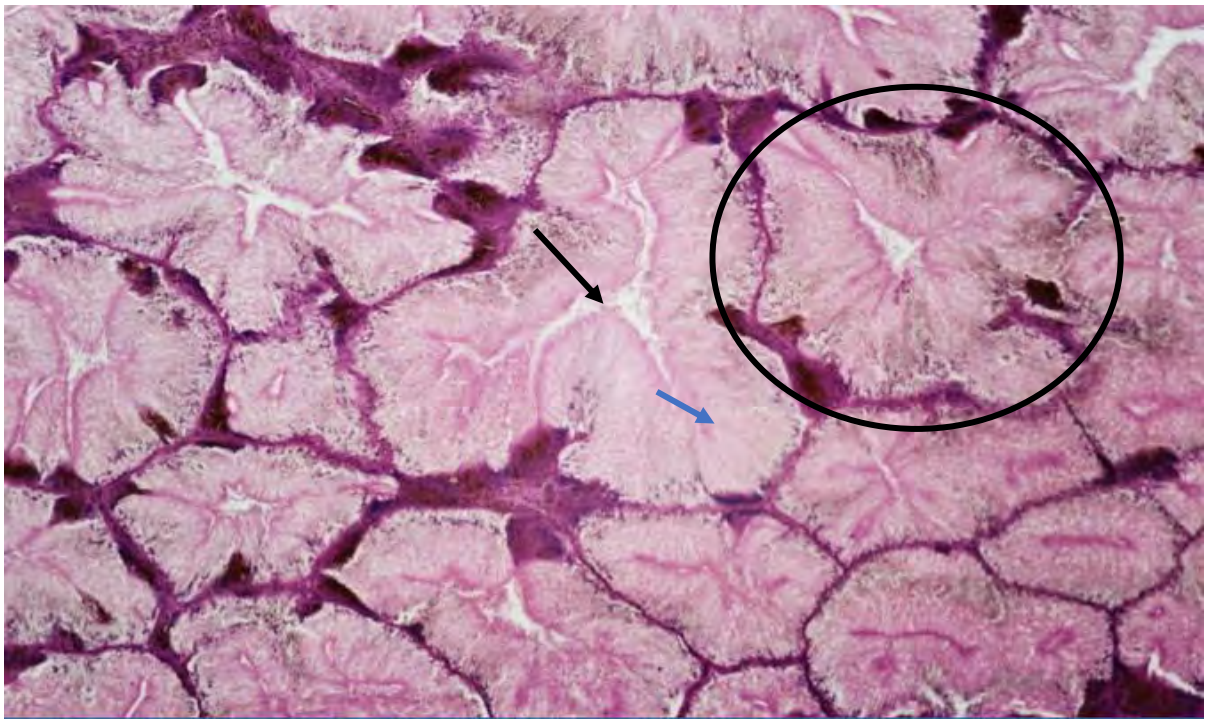


Figure 1.5: Histology section of H. midae digestive gland, depicting the different characteristics which make the digestive gland. Different characteristics include the tubule (black circle), lumen (black arrow), and the epithelium (blue arrow). The Animals collected were under routine farming conditions, and left undisturbed for a minimum of four weeks, prior to dissection.

Abalone have of two kidneys, the right and left kidneys, which are located beside the pericardium, anterior within the visceral sac, and covered by the hepatopancreas. Each kidney has a different function. The right kidney's function focuses majorly on nitrogenous excretion and resorption, whereas the left kidney's main function is the resorption of ions and to store organic solute (Harrison, 1962). Histologically, the right kidney is made up of secretory epithelial cells that lay on a basement membrane and surround a distinct lumen, which have a squamous or cuboidal shape, with centrally located nuclei. The left kidney on the other hand is relatively smaller, where it consists of densely packed epithelial cells, which have an irregular cuboidal shape, which lays on a connective tissue core. The left kidney also consists of a prominent nuclei and granular inclusions (Figure. 1.6) (Harris, 2005).

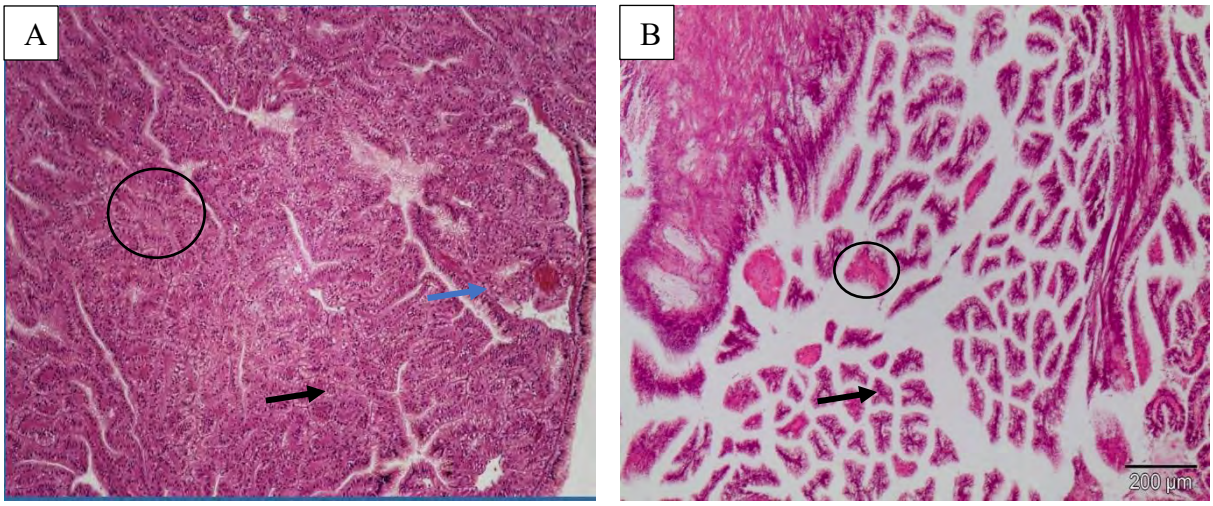


Figure 1.6: Micrograph depicting the histology of both the right (a) and left kidney (b) of Haliotis midae under 400 X magnification. Different characteristics of the right kidney consist of the renal tubules (black circle), with no lumen visible, epithelial cells (black arrow), and the duct (blue arrow). Characteristics of the left kidney consist of the nuclei (not visible), the epithelium (black arrow), and the granular inclusions (black circle).

During transportation, abalone experience histological changes. These can be biochemical modifications in response to environmental stress, and they also may experience structural changes due to hypoxic conditions and long-term anaerobic metabolism (Copedo *et al.*, 2024; Venter *et al.*, 2024). During these stressful conditions, histopathological alterations are observed within the gills, kidneys, and digestive gland of abalone. These histological structures are a good indicator of their overall health, which in turn could provide a tool to establish optimal transport and management procedures (Copedo *et al.*, 2024).

1.3 Aim and Objectives

The main aim of this thesis was to identify and assess the performance of abalone during a routine live export procedure during both favourable environmental temperature conditions and unfavourable environmental temperature conditions. The thesis then aimed to characterise the best method to mitigate the increased negative effects on abalone transport during the unfavourable summer months.

The objectives for the thesis were to:

1. Describe the main stressful events during export and to characterise the performance of animals during favourable environmental temperature conditions (Chapter 2).

2. Implement a pre-cooling purging system ahead of transport in order to steadily decrease tank temperature with minimal implications to water quality (Chapter 3).

3. Identify and assess the main different pre-transport conditions that influence animal performance during transport within the summer months (Chapter 4).

Chapter 2: Transport simulation under farm conditions

2.1 Introduction

The high demand and premium price for abalone has made it a highly prized cultivation species, where the growth in this sector was due to intensive research and development to support both local and the large international markets (DFFE, 2018; Kurnaningtyas, *et al.*, 2022; Cook, 2023). Since the majority of farm output in South Africa is exported and the value of fresh abalone exports is determined by price increases and volume expansion, the optimization of space while ensuring a good condition of the animals is essential for net returns (Terchunian *et al*., 1999; DFFE, 2018). The transportation of live abalone to international customers is a "complex operation that requires consideration of numerous conditions, including water temperature, oxygen supply during the transporting process, transportation time, and others" (Sawangwong *et al*., 2019). To improve profitability, maintaining a maximum net weight and minimizing the number of mortalities during each shipment is vital, as exporters and farmers are paid based on the landed number of live and healthy abalone and they are paid on the landed weight per shipment (Bubner *et al*., 2009). The shipping of live and fresh seafood is dependent on species specific factors which must be considered to ensure the produce arrives alive and in good condition (Terchunian *et al*., 1999). These factors include respiratory rates, ability to handle stress, excretory functions, gaseous exchange and temperature tolerance ranges. The oxygen requirements for live seafood are also based on the body size, ambient temperature, nutritional state, and the metabolic rate of the animal (Volkoff and Ronnestad, 2020).

These different factors, during transportation, effect an abalone's metabolic activity, their stress levels, and in turn negatively effects the quality of their meat composition (Sikes *et al*, 2009). For abalone species, the energy budget is made up of different variables which consist of their ingested feed energy, their egested faecal energy, somatic growth energy, shell growth energy, reproduction energy, respiration energy or metabolic energy, ammonia excretion energy, and as well as mucous production energy (Peck *et al*., 1987; Barkai and Griffiths, 1988; Lopez and Tyler, 2006; Ganmanee *et al*. 2010, Duong *et al*., 2020). For South African abalone, 63 % of the energy budget is lost through food ingestion and the production of faeces, which is higher compared to other algae grazing gastropods (51.8 %) (Barkai and Griffiths, 1988. Respiration accounts for around 32 % of energy lost, which is associated with feeding and locomotion, and oxygen consumption increases by 300 % when locomotion speed increases (Barkai and

Griffiths, 1988). Locomotion has a big cost on the energy budget, and this is about five times higher in abalone compared to other running vertebrates, which is associated with mucus production (Barkai and Griffiths, 1988). Lastly an energy loss through excretion is associated with mucus production, where ammonia excretion also contributes to energy loss; however, the measurement allocation for ammonia excretion in *H. midae* has not been determined and further investigation is needed (Barkai and Griffiths, 1988).

Metabolism has a direct effect on energy expenditure for somatic growth, reproductive investment, feed utilisation, and excretion (Duong *et al.*, 2021). Since abalone are thermoconformers, their body temperature varies with the surrounding environment (Duong *et al.*, 2021). In addition to temperature, different environmental conditions, including different aerobic and anaerobic exposure episodes, can also influence a shift in energy allocation (Cardoso, *et al.*, 2006).

Abalone experience extreme stress during live transport, including a period of starvation, prolonged air exposure, handling, periods of hypoxia, and temperature fluctuations., This negatively affects their survival rate, which ranges from by 64 – 97 %, and their ability to maintain their net weight, which can drop by 4 – 15 % during transport (Alfaro *et al.,* 2021). During live aquatic animal transport, the maintenance of a low temperature, efficient purging time, and the efficient quantity of biomass per carton, is crucial for optimizing export success through low mortalities, which reduced shipping costs (Terchunian *et al*., 1999; Currie, *et al*., 2015; Kurnaningtyas, *et al*., 2022). On the other hand, the level of stress that both small and large animals experience during transport, including their weight, survival, and behavior, have not been determined. This is import as the size of the live produce also plays a role in survival, as smaller specimens, with sizes one kilogram or less, demand more oxygen per unit weight (Yasa *et al*., 2021). As such, it is recommended that smaller stock should be transported by land, whereas larger animals can be more successfully transported by sea or air (Yasa *et al*., 2021).

Cultivated abalone demonstrate different behaviours under different conditions, where an alert activity is a sign of metabolic energy expenditure. This has been investigated by Donovan *et al.* (1999), where locomotion resulted in a higher metabolic energy demand in *Haliotis kamstchatkana*. It has also been shown that locomotion is an indicator of stress in haliotids (Kaiser, *et al*., 2017). Abalone, coupled with other species such as mussels and barnacles, have

the unique ability to temporarily adhere tightly to a substrate when disturbed (Li, *et al*., 2018). The abalone's adhesion capability is similar to that of a gecko's (Zhang *et al*., 2020) as they are equipped with multiscale structures within their foot muscle that produce very high adhesive strength (Li, *et al*., 2018). This behaviour is important for haliotids as it assists them in crawling on rough and dangerous surfaces, without injury. It also assists them in rapid shell twisting and turn over and in righting themselves when dislodged to avoid predators (Baldwin *et al*., 2007; Zhang *et al*., 2020). The abalone's adhesion ability is important when they perform their self-righting behaviour as the shell side down posture or overturned posture is fatal for this species and many other animals, as it can cause asphyxia, which could lead to death (Zhang *et al.*, 2020). The abalone's adhesive capacity decreases significantly, which can also lead to death when animals are exposed to stressful conditions (Yu *et al*., 2021).

2.1.1 Aims and objectives

This chapter was carried out in order to identify the level of stress both large and small animals experience during both the purging and transport phase within a routine farm export simulation. For both large and small abalone, animal net weight, individual weight, survival, and behavior, were recorded in animals that were exposed to an export procedure, and compared to those that were left un-transported in order to quantify the amount of damage and change abalone experience during transport. A better understanding of the transport procedure, and the effect that they have on both large and small live seafood weight maintenance and survival post-transport is needed to improve efficiency and sustainability of this part of the farmed abalone value-chain.

2.2 Materials and methods

2.2.1 Experimental species

Abalone (*Haliotis midae*) of two different size classes were used. The first included abalone that were 50 – 60 g, with an average weight of 51.5 ± 0.71 g. The second were 90 – 110 g, with an average weight of 98 ± 1.41 g. They were selected from the grow-out out facilities of a commercial abalone farm (Romansbaai Abalone Farm, Aqunion Pty Ltd, South Africa; 34°36'03.7"S 19°20'04.4"E), and were randomly allocated to the treatments. Prior to the start of the trial, animals were left unhandled for a minimum of 30 days and left unfed (after having been fed a formulated diet Abfeed®; 26 – 34 % protein, 1.2 % lipid; Marifeed Pty, Hermanus) 24 hours prior to the start of the trial.

Animal size was based on economically and biologically relevant aspects within the industry. Abalone that weigh between 60 – 100 g are considered industry milestones (Sales, Britz, 2001).

Ethical clearance, from the Rhodes University animal research ethics committee (RU-AEC), was obtained prior to the start of all data collection that involved live abalone (2023-5459-7677.).

2.2.2 Routine farm practice applied to abalone before they are transported, and experimental procedures, and facilities

This trial was carried out on the same commercial abalone farm (Aqunion Pty Ltd, Romansbaai, South Africa) from which the experimental abalone originated, using the farm's routine grow-out and export procedures, facilities and equipment. The grow-out facility included concrete tanks (16 m^3) (Figure 2.1a) where abalone were suspended in oyster mesh baskets (Figure 2.1a) and a flow through production system. The export facility included fiberglass tanks (7,33 m^3; 1.2 x 8.2 x 0.745 m) (Figure 2.1c), situated within the farm's export facility, where majority of data collection took place. Under normal farm conditions, baskets, which were selected based on size ranges needed for live export, were firstly subjected to CO_2 anaesthesia in 200 L tanks for five minutes. They were then carefully removed from their respective basket with minimal external damage (Auzoux-Bordence *et al.*, 2020). Animals were then re-submerged in aerated seawater tanks for an additional five minutes before each animal was removed and transferred into basket trays (Figure 2.1b). Each basket tray was then laid out to drip dry for two minutes and the total weight of the stock in the basket was recorded (Figure 2.1b).

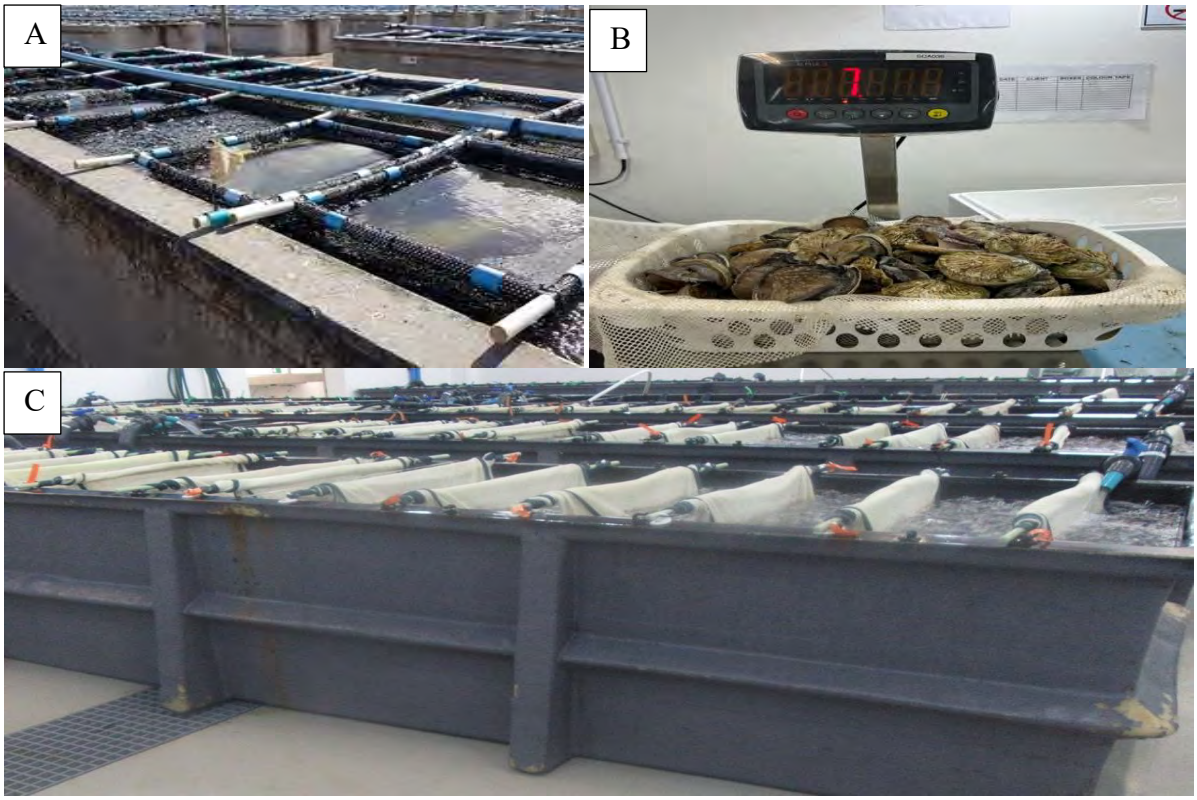


Figure 2.1: (a) Tanks selected for harvesting, with mesh baskets carrying varying selections of different size classes. (b) Basket tray used for drip drying and full basket and individual weighing. (c) Export purging tanks used for the routine export procedure, which ranged from canning export and live export purging procedures. Fiberglass tanks (7,33 m^3; 1.2 x 8.2 x 0.745 m) were cleaned before and after every export purging process.

Each animal within each different basket tray were then sorted on a large table and separated into different additional baskets depending on their respective sizes based on first observation. Individual animals were then weighed using a scale and separated into different purging bags (Figure 2.1c). Bags were then left to drip dry for an additional 15 mins before they were placed into purging tanks. Each purging bag weight was then adjusted to *ca.* 8.0 kg before being placed into purging tanks, which was the standard farm procedure ahead of packaging abalone for export (Shaw *et al.*, 2023). For each purging tank, during a standard farm export procedure, a range of 12 to 16 bags were placed in the fiberglass tank, where average stocking density was 17.57 kg/m^3. Each tank was then covered with a water proof polyamide drape (Svenmil Pty Ltd) and animals were left to purge for three days (Figure 2.1c).

Water quality parameters were recorded in the purging tanks, using an oxygen and temperature meter (Polaris C, OxyGuard, Farum Denmark) for both dissolved oxygen concentration and saturation (mg/L and %) and temperature (ºC). The pH levels were also measured for each tank (Handy pH Oxyguard; OxyGuard International, Farum Denmark). Each parameter was

recorded three times per day; first in the early morning, second at midday, and finally in the late afternoon. The water flow rate through the purging tank was maintained at 30 L/kg/h, according to currently used standing operating procedures (Shaw *et al.*, 2023), where the average flow rate for the farm routine production system was 32.13 ± 7.12 L/kg/h. Oxygen was maintained between 7.2 – 8.9 mg/L and 80 – 100% and pH was maintained between 7.2 – 8.5 during the purging period.

Abalone were then removed from their respective tanks, placed onto a dripping table for 15 min prior to being packaged. Approximately one hundred and thirty-four animals of 50 – 60 g and seventy-five animals of 90 – 110 g were then placed into an eight-compartment polystyrene box (i.e., 17 per compartment for 50 – 60 g and eight per compartment for 90 – 110 g). This was placed inside a plastic bag, and each plastic bag was then placed into a 50 L polystyrene box. Each box also included two dry absorbent pads; one placed at the bottom of the plastic bag and the other one placed on top of each wrapped up plastic bag. Two ice sheets (1085 – 1156 g) were placed on the top of the absorbent pads (Figure 2.2b). Each box of abalone was then weighed (excluding the weight of the box) and animals were either removed or added into the box in order to meet the export weight range of 7.85 – 7.90 kg of abalone per box (Figure 2.2c). After each box was weighed, pure oxygen was pumped into each plastic bag (Figure 2.2a), and after a minute of inflation, excess oxygen was released and the ends of each plastic bag were tied and taped closed. A hard board (1030 x 500 cm) was then placed on top of each plastic bag, where then two ice sheets were placed on top of each hard board. Each box lid was then placed back on top and box ends were sealed off using tape (Figure 2.3d). A number of boxes included a temperature logger (2 – 4 loggers per each size class, within each run) (Onset UA-001-64 HOBO 64K pendent, Temperature/Alarm (waterproof) Data logger) to track the change in temperature within the boxes (Figure 2.2d).

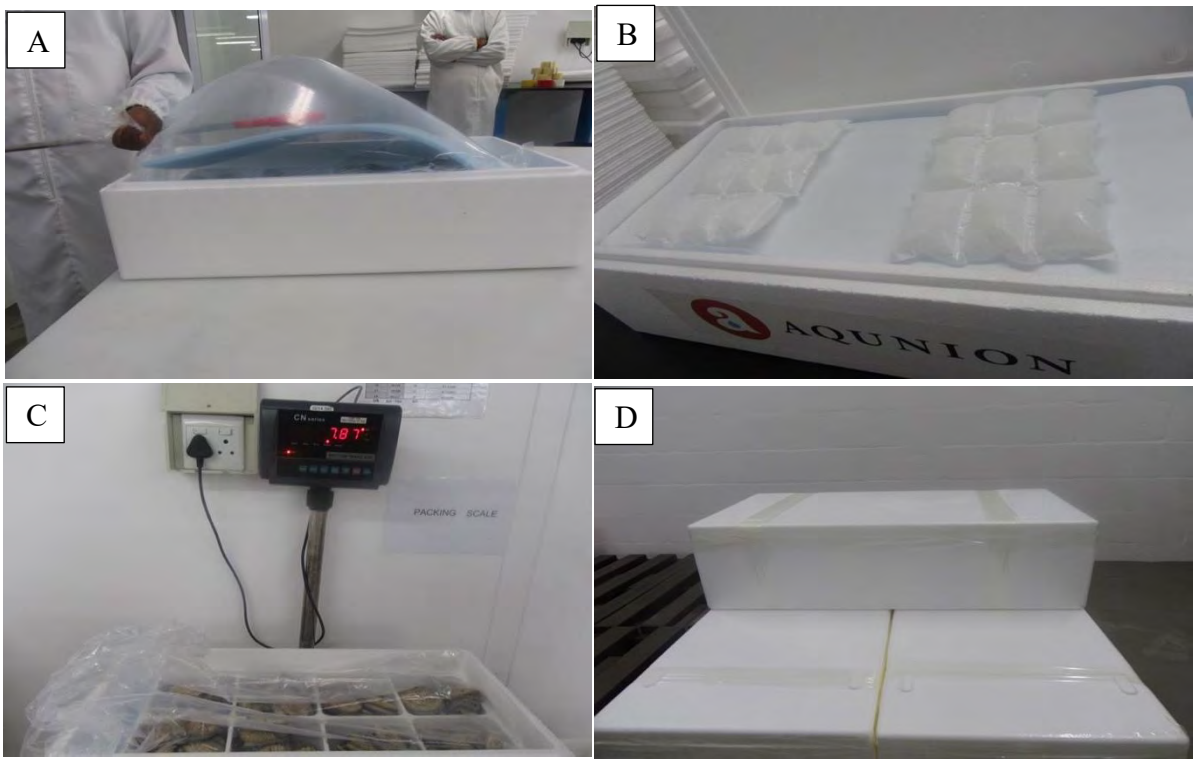


Figure 2.2: a) Pure O_2 was pumped into each box, after animals were placed *into the box compartments. b) Ice sheets placed on top of a hard board before box was sealed and stored for transport. c) Packing process of a routine export process, where boxes were weighed and excess or insufficient weight of animals were measured and weight of each box was adjusted to meet the export weight range between 7.85 - 7.90 kg. d) Boxes were stacked together and subjected to a 35-h transport simulation (procedures were based on the standard operating procedures used by the farm).*

2.2.3 Transported versus un-transported abalone

Boxes, which were the unit of replication, ready for transport were stacked and stored in a temperature-controlled room (14 – 15 °C) for 35-h, which is equivalent the duration that abalone are exposed to during export. Animals were not touched or handled during that time. These conditions were the standard operating "mock transport" farm procedures that were routinely used on a commercial abalone farm, and these farm procedures did not include a real export simulation where animals would have experienced different handling times and temperatures and being moved around, for example. Treatment ("mock transport") boxes consisted of a total of 176 replicates for the research of Chapter 2.

Animals that were subject to the same purging procedure described above, but were not subject to packing procedures, were used as a "control" group with 112 replicates, and these abalone were sent back to production after purging. In production they were subject to standard farm operating grow-out conditions for 35-h, where standard farming operations resumed and animals were not fed within the first 24-h and then fed half portions of feed thereafter.

2.2.4 Data collection

2.2.4.1 Water quality during purging

Different water quality parameters, including temperature, dissolved oxygen (mg/L), dissolved oxygen saturation (%), and pH was collected three times daily throughout the purging process within the duration of the trial. This was done using an electronic meter (OxyGuard, Polaris C) for the temperature and dissolved oxygen (mg/L and %), where pH was measured using a pH meter (OxyGuard International, Handy pH). The pH values were then converted, and hydrogen ions (M) were calculated using Equation 2.1.

$$H^+ = Log_{10}(pH) \qquad \text{Equation 2.1}$$

2.2.4.2 Post-transport simulation

A number of different data sets were collected from each box after the 35 h of transport simulation. These included the ice temperature, the temperature of the animals inside the box, using a digital laser infrared thermometer (Inspection tool specialists, GS320/ M320), the weight of the additional water in the plastic bags that accumulated due to "drip loss" from each abalone inside each plastic bag, the mortality count, the full weight of the animals combined, and the individual weight of 60 randomly selected animals from each box, using a calibrated scale (0.1 g). Survival, upon arrival and during recovery, was calculated using Equation 2.2 (Setyaningsih *et al*., 2020):

$$SL = \frac{N0}{N1} X\ 100 \qquad \text{Equation 2.2}$$

where SL represents survival rate (%), N0 was the number of deceased animals, and N1 was the initial number of abalone in the box.

Overall weight loss per box was calculated using Equation 2.3, where WL represented weight loss (%), w0 was the final weight (g), and w1 was the initial weight (g) (Bubner *et al*., 2009):

$$WL = \frac{w0-w1}{w0} X\ 100 \qquad \text{Equation 2.3}$$

From the 60 randomly selected individuals from each box, each animal's weight was recorded. If animals had a biomass lower or higher than their size class range for the two size classes,

then they were classified as “underweight” or “overweight”. The percentage of both underweight and overweight animals was then calculated using Equation 2.4 and the percentage of excess or insufficient biomass was established:

$$IEB = \frac{w0}{n0} X\ 100 \qquad \text{Equation 2.4}$$

where IEB represents Insufficient or Excess Biomass, w0 was the individual weight (g), and n0 = number of animals sampled.

The transported animals that were transferred back into baskets in the grow-out section of the farm. Additional data were collected and this included post-transport mortality, and total basket biomass. Water quality parameters, were also collected and lastly, behaviour observations were also made.

2.2.4.3 Behaviour

Righting time

Immediately after the 35-h transport simulation, or after being in the baskets for 35 h for the control group, abalone taken form the transport box (or basket in the case of the control group) and were placed upside down on their backs (shell side down) into a 20 L bucket that had been filled with ambient seawater (14 – 16 °C). The time taken for four abalone, per replicate, to return to its normal orientation with the abalone foot in full contact with bottom surface of the bucket was recorded. If animals failed to “right” themselves after five minutes, then they were recorded to have been unsuccessful and were given a total time of 300 s as a measurement.

Abalone used for this behavioural test were returned to the farms grow out facility and were not used for any further data collection.

Adhesion force

The force that abalone use when adhering to a substrate was measured. A device was designed with a flat, square, nylon board (20 x 20 cm) that was attached to a spring scale (50 kg portable scale), which was fixed to a steel frame (Figure 2.4). A total of four abalone, per replicate, selected for the behavioural trial, was attached to the bottom side of the nylon board, where it was immersed into a bucket containing ambient seawater for one minute. The board was then

removed from the water and attached to the spring scale (with the abalone on the underside), where after the scale was set to zero, and the animal was gently pulled off the board. The weight (kg) reading on the scale was recorded immediately before the abalone was removed. If the abalone was not successfully removed from the board when the reading on the scale reached 10 kg, then a reading of 10 kg was recorded and a note was made that the abalone could not be removed. If animals failed to attach themselves to the substrate, then a measurement of zero kilograms was recorded. The weights that were recorded were converted into Newtons (N) using Equation 2.4:

$$N = kg\ x\ 9.8 \qquad \text{Equation 2.4}$$

This was based on Newton's second law of motion that states that force (N) applied to an object is equivalent to the product of its mass (m) and acceleration (m s^{-2}) due to gravity, which was stated as a constant of 9.81 (Mandal, 2020).

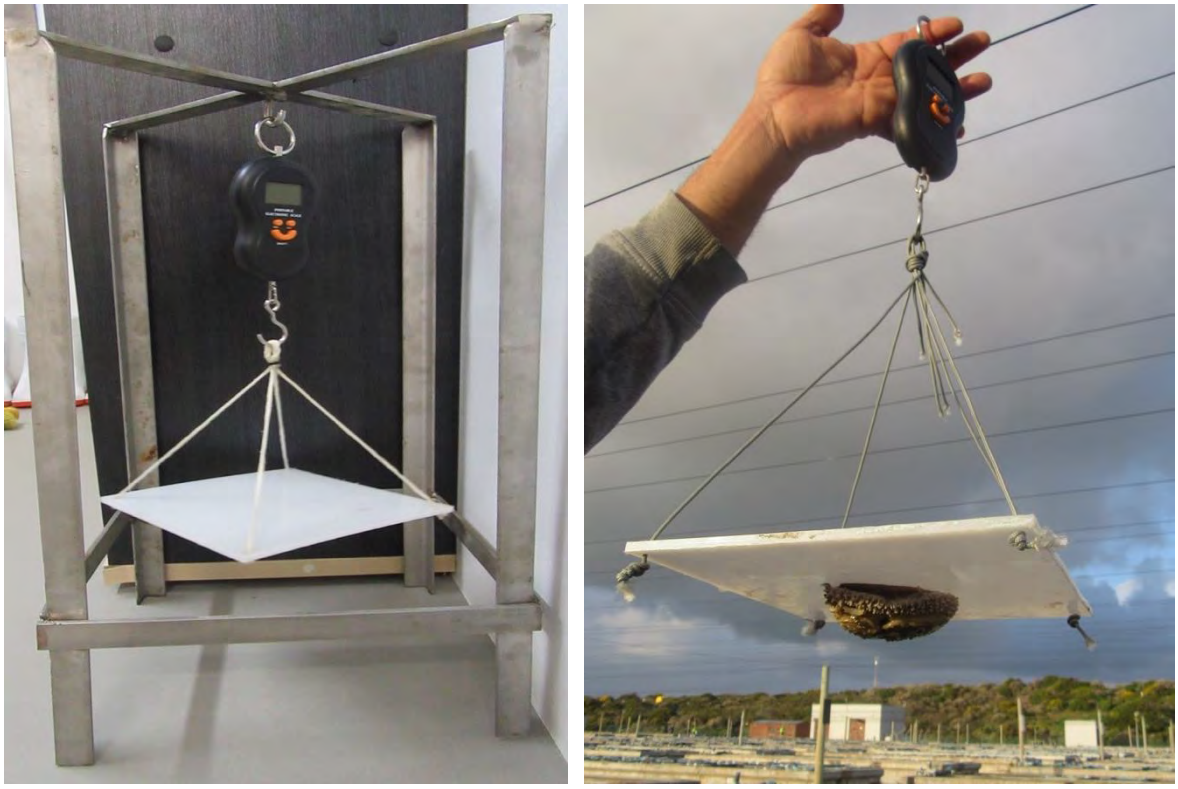


Figure 2.3: A 50 kg spring scale attached to a 20 cm x 20 cm nylon board, with a phytoplankton rope attached as a noose to the spring scale. A steel frame was attached to the spring scale for support, which was used for the adhesive force test.

Locomotion

A total of four abalone per replicate were used to determine the time taken for animals to make an initial movement of their pedal muscle, and to determine the speed with which they moved. These data were collected two hours after the abalone had been placed into the baskets after the 35-h transport simulation.

Each basket included a horizontal plate (45 x 45 cm) placed above the rack in the basket, about 10 cm below the surface of the water. The distance from the centre of the top plate to Position 1 (marked with an asterisk in Figure 2.5) was 10 cm, and the distance from the centre of the top plate to Position 2 (marked with a circle in Figure 2.6) was 19 cm. The time that it took for the first initial movement was recorded.

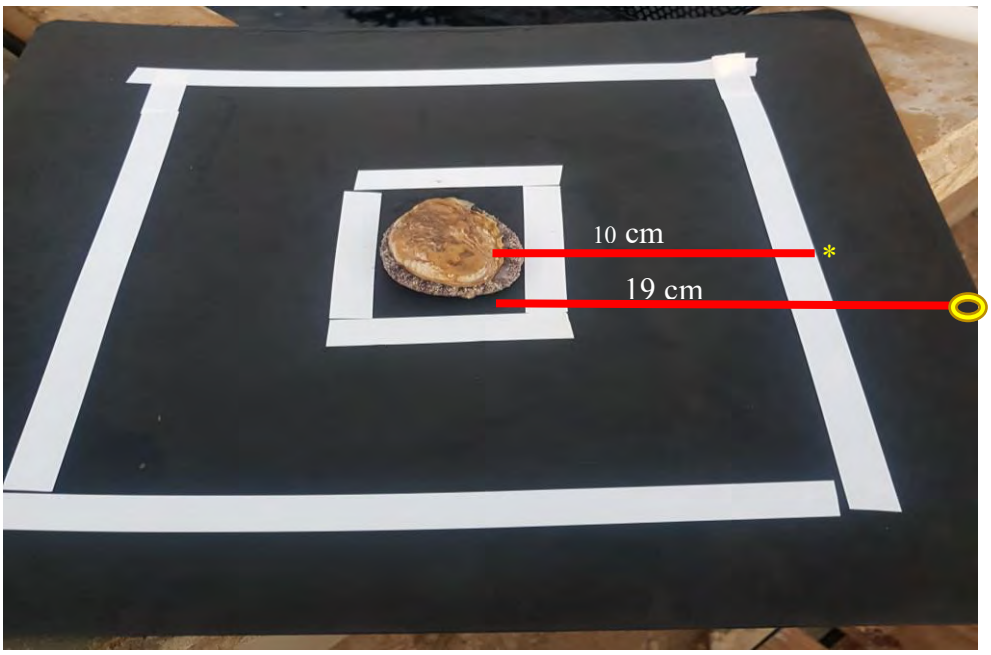


Figure 2.4: A 45 cm x 45 cm modified top plate with an abalone in the middle of the top plate, where the distance measured from the center of the middle of the top plate to position 1 was measured at 10 cm and marked with an asterisk (*). The distance from the middle of the top plate to position 2 was measured at 19 cm and was marked with circle (O).

The times taken for animals to move across the top plate, after their first initial movement was also recorded, and for it to reached Position 1, Position 2, and eventually move off the top plate were measured using a stopwatch. Their speed was then calculated, using Equation 5, where S was the speed (cm s^{-1}), t represented time (s), and D represented the distance covered (cm).

$$S = \frac{(D)}{t} \qquad \text{Equation 2.5}$$

2.2.5 Statistical analysis

Data were analysed using statical computer software (Statistica, version 14.0.0).

Majority of the post simulation data statistical analysis, including the ice temperature, temperature of animals, change in weight, weight loss (%), survival (%), and all the different behavioural parameters, were analysed using a multifactor analysis of variance (ANOVA) at $p<0.05$, with abalone size class as one factor and un-transported and simulated transported abalone as the second factor. If there was no significant interaction between factors, then means of each factor were compared.

Each of the different water quality parameters were statistically analysed using a one-way analysis of variance (ANOVA) at $p<0.05$.

All data analysed using one-way ANOVA or a multifactor ANOVA were tested for homogeneity of variance and equal distribution of residuals using the Levene's test and Welch's test (Gastwirth *et al*., 2010; Mazti *et al*., 2018). If assumptions were not met, then data were logged transformed and these data were then used in the analysis (Feng *et al*., 2014). However, if they still did not meet the assumptions of an ANOVA, then a non-parametric Kruskal Wallis test used in order to test differences between treatments with unequal samples sizes.

The change in weight of each mock transport and un-transported replicate, i.e., the weight at the time were packed compared to that when they were unboxed/re-weighed, was analysed using a repeated measures ANOVA at $p<0.05$. If these data did not meet the assumptions of an ANOVA, then a sphericity test at $p<0.05$ was used then in order to test if the assumption of sphericity is met in a repeated measures ANOVA (Grieve, 1984).

Post the transport simulation, including the percentage of insufficient and excess biomass animals, was conducted using Microsoft Excel (version 16.54), where a contingency analysis was used in order to evaluate the association and the independence between two different variables.

2.3 Results

2.3.1 Water quality during purging

Tank flow rates were kept high and obtained a mean flow rate of 32.13 ± 7.12 L/kg/h. Water quality parameters remained within the optimal range kept during purging, where dissolved oxygen obtained was 7.58 ± 0.32 mg/L and 93.83 ± 2.74 %, and H^+ ions obtained was 0.90 ± 0.001 M (Table 2.1).

Table 2.1 Mean (± 95 confidence intervals) water quality parameters, including DO (mg/L and %) and H^+ ions (Mol L^{-1}) of both un-transported and simulated transported animals of both size classes during purging (Multi-factorial ANOVA; at p<0.05)

	Simulated transport (50 – 60 g)	Simulated transport (90 – 110 g)	Un-transported (50 – 60 g)	Un-transported (90 – 110 g)	F statistics	P value
Dissolved oxygen (mg/L)	8.15 ± 0.05	8.20 ± 0.04	7.62 ± 0.05	7.59 ± 0.04	$F_{(1, 294)}$ = 0.88	p = 0.35
Dissolved oxygen (%)	94.44 ± 0.36	94.63 ± 0.27	93.91 ± 0.36	94.02 ± 0.27	$F_{(1, 294)}$ = 0.02	p = 0.90
pH (mol L^{-1})	0.90 ± 0.001	0.90 ± 0.001	0.90 ± 0.001	0.90 ± 0.001	$H_{(1, 262)}$ = 1.03	p = 0.31

2.3.2 Post transport simulation

2.3.2.1 Temperature profiles upon arrival

There was no significant difference of both the temperature of the ice and the temperature of animals upon arrival, when comparing both size classes and transported and un-transported animals that was subjected to the 35-h transport simulation (Table 2.2). Ice, from the bigger size class boxes (90 – 110 g), had a mean temperature of 10.89 ± 3.04 °C post the simulation, where temperature of animals was 11.45 ± 2.46 °C. Ice temperature profile, for the smaller size class range, obtained a mean 11.16 ± 3.16 °C, post the simulation and temperature of animals was 11.89 ± 2.30 °C post the 35-h simulation.

Table 2.2: Mean (± 95 confidence intervals) temperature (°C) of the both ice and animals of animals after being subjected to a 35-hour transport simulation (Multi-factorial ANOVA; at p<0.05).

	Simulated transported (50 – 60 g)	Simulated transported (90 – 110 g)	Un-transported (50 – 60 g)	Un-transported (90 – 110 g)	F statistics	P value
Ice temperature (ºC)	11.17 ± 0.39	10.94 ± 0.29	N/A	N/A	$F_{(1, 171)} = 0.21$	p = 0.64
External animal temperature (ºC)	11.89 ± 0.31	11.47 ± 0.23	15.61 ± 1.28	15.61 ± 1.28	$F_{(1, 171)} = 1.20$	p = 0.27

2.3.2.2 Net weight pre and post transport

There was a significant interaction of the change in net weight (g) from when animals were packed and post the 35-hour transport simulation of both the different size classes, when comparing animals subjected to a mock transport simulation and animals left un-transported (Repeated measures ANOVA, $F_{(1, 88)} = 7.41$, p = 0.008, Figure 2.6). There was a significant difference in the packed weight between the mock transport animals and un-transported animals for both size ranges (50 – 60 g and 90 – 110 g), but this was an artifact of the current standard operating procedures (SOP) used on the farm for stocking densities, where small abalone are stocked at a higher density in grow-out compared with larger abalone. As such, purged small animals sent back to baskets were stocked at a higher density compared to larger animals. Both size classes lost weight during transport; however, the interaction was a result of a difference in the stocking densities of larger stock among transported and un-transported abalone which was a result of SOP on the farm rather than an outcome of the experiment.

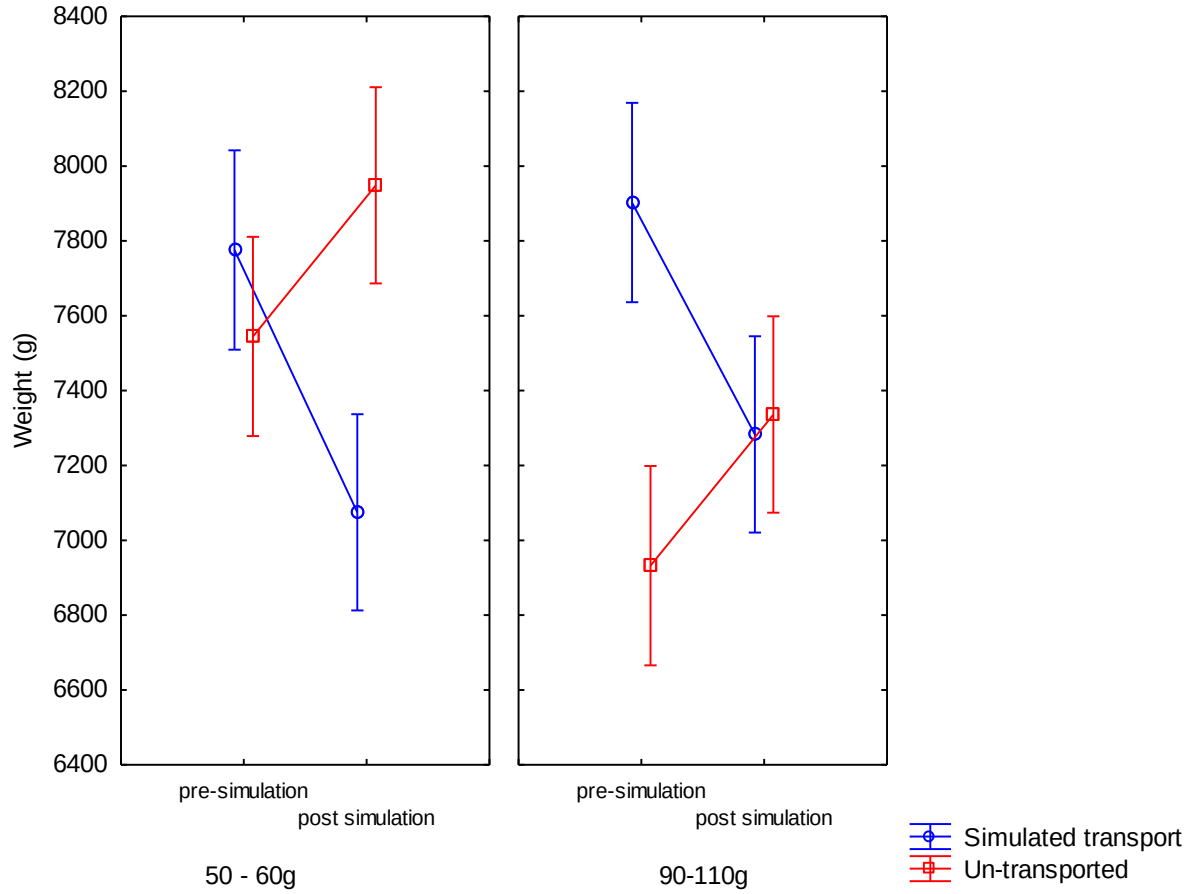


Figure 2.6: Mean (± 95 confidence intervals) weight of box (g) from pre-simulated conditions until post simulation of both size class ranges (50 - 60 g) and (90 - 110 g). red) indicates animals not subjected to the transport simulation (Un-transported n = 112) and blue) indicates individuals that were subjected to the transport simulation, n = 176, for 35-h (Mock transport simulation) (Repeated measures ANOVA, $F_{(1, 88)} = 7.41$, $p<0.008$)

2.3.2.3 Net weight upon arrival (g)

There was a significant interaction between the different size class ranges and their unboxed total weight (g) when comparing animals subjected to the transport simulation (Multifactorial ANOVA, $F_{(1, 282)} = 14.96$, $p<0.001$, Figure 2.7). Abalone lost weight during transport compared with un-transported stock and this was observed for both size classes; whereas this drop in weight was significantly greater for the smaller abalone. However, bigger animals retained a higher mean total net weight of 7274.80 ± 50.36 g compared to the smaller animals, which reached a lower mean total weight of 7071.44 ± 67.69 g.

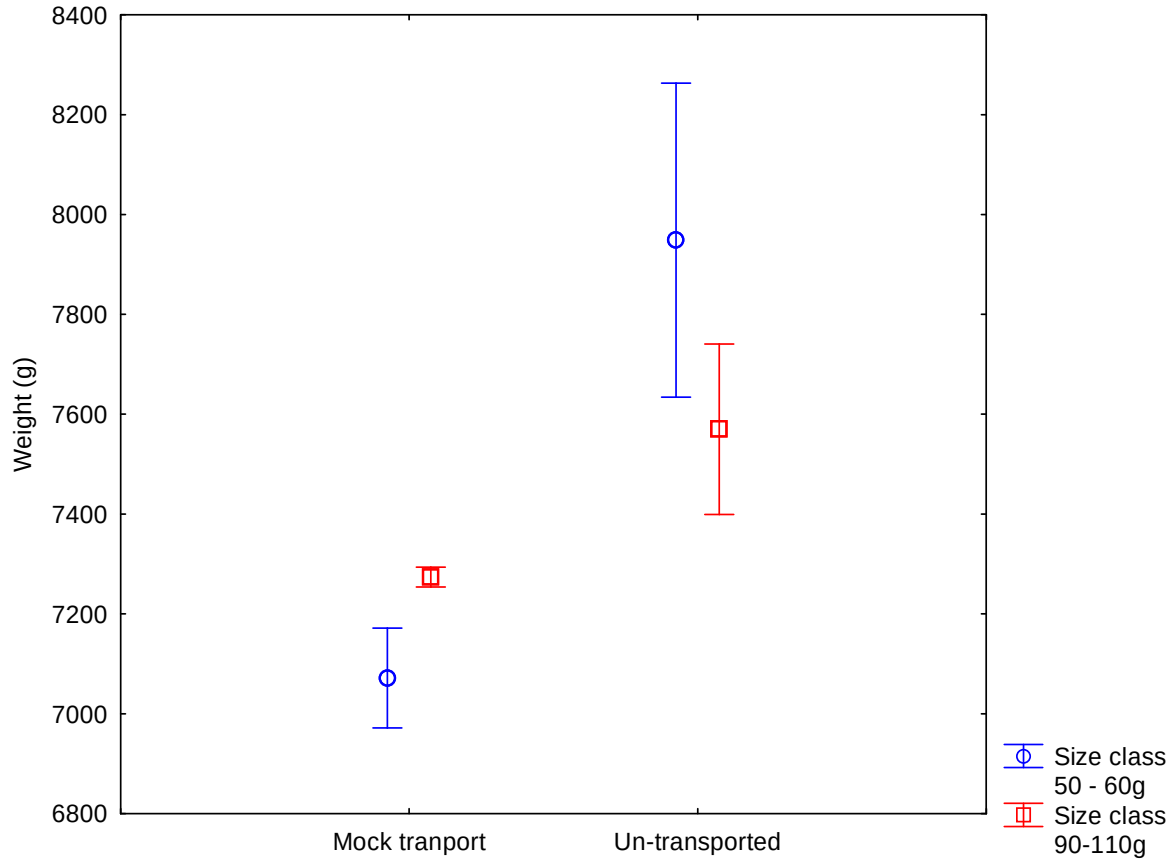


Figure 2.7: Mean (± 95 confidence intervals) weight of animals upon arrival (g) within both boxes (n = 176) and baskets (n = 112), 35-h after the transport simulation procedure for both size class ranges (Multifactorial ANOVA; $F_{(1, 282)} = 14.96$, $p<0.0001$).

2.3.2.4 Weight loss

There was also a significant interaction of the total weight loss (%) between un-transported and simulated transported groups with both size class ranges (Multifactorial ANOVA, $F_{(1, 282)} = 8.92$, $p = 0.003$, Figure 2.8). Smaller sized animals that went through a simulated transport scenario had a significantly higher average weight loss of 9.24 ± 0.14 % compared to larger sized animals that had an average weight loss of 7.96 ± 0.11 %. Animals that did not go through a simulated transport scenario from both size classes actually gained an average weight of 5.40 ± 0.24 % for the smaller sized animals and 5.72 ± 0.12 %.

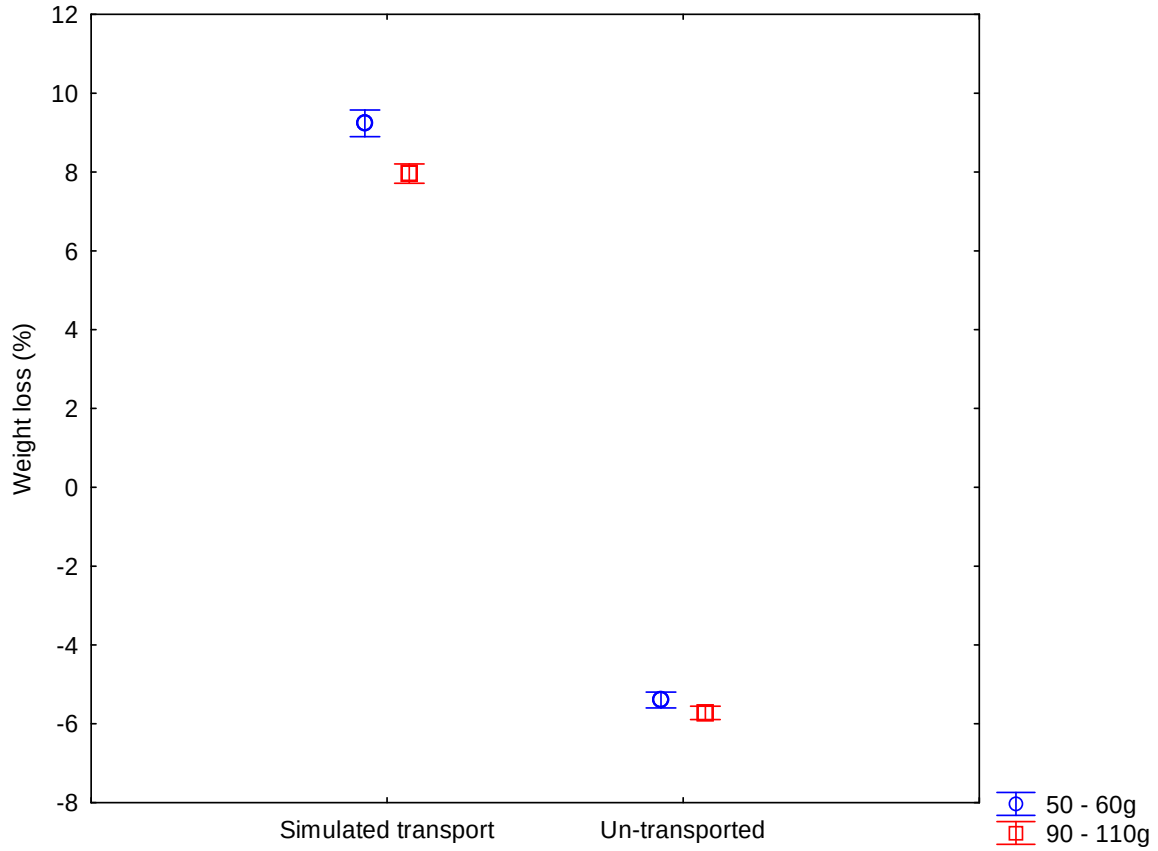


Figure 2.8: Weight loss % (± 95 confidence intervals) of both the simulated transported animals (n = 174) and Un-simulated transported animals (n = 112) of both size classes, 50 – 60 g (n = 85) and 90 – 110 g (n = 201), post the 35-h simulation (Multifactorial ANOVA, $F_{(1, 284)} = 5.71$, $p = 0.018$).

2.3.2.5 Water loss

There was a significant difference of the mean total water loss (g) through drip loss when comparing both the size class ranges from the mock transport simulation group (Factorial ANOVA, $F_{(1,172)} = 27.60$, $p<0.0001$, Figure 2.9). Bigger sized animals (90 – 110 g) reached a lower mean water loss 464.08 ± 8.36 g compared to the smaller size class range (50 – 60 g), which obtained a higher average water loss of 537.63 ± 11.23 g, 35-h post the transport simulation.

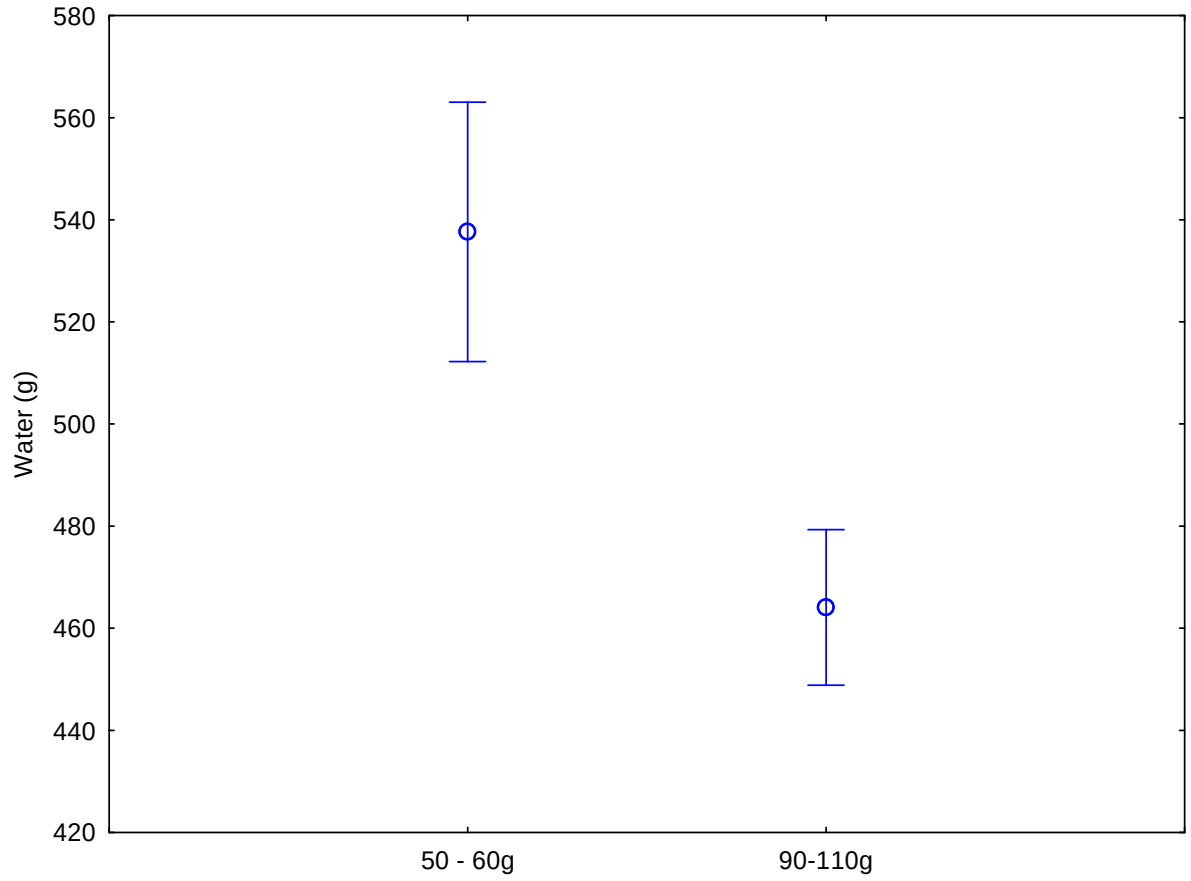


Figure 2.9: Mean (± 95 confidence intervals) water loss (g) (through drip loss) of boxes within the mock transport group, of both size classes, 50 - 60 g (n = 62) and 90 - 110 g (n = 112) after 35-h of a transport simulation. (Factorial ANOVA, $F_{(1,172)} = 27.60$, $p<0.0001$).

2.3.2.6 Individual weight pre and post transport

There was a significant interaction of the individual weight (g) with both size classes with both the simulated transported group and the un-transported group (Repeated measures ANOVA, $F_{(1, 88)} = 7.41$, $p = 0.008$, Figure 2.10). For the smaller animals, specifically the simulated transported group, animals lost an average individual weight of 2.14 ± 0.89 g, from when animals were purged until post the 35-h simulation. For the un-transported group, animals lost an average individual weight of 7.11 ± 0.78 g, from post purge until after the 35-h simulation. For the larger animals, they lost an average individual weight of 9.6 ± 0.68 g, for the simulated transported group from when animals were purged until post the 35-h transport simulation. For the un-transported group, animals lost an individual weight of 7.78 ± 0.76 g.

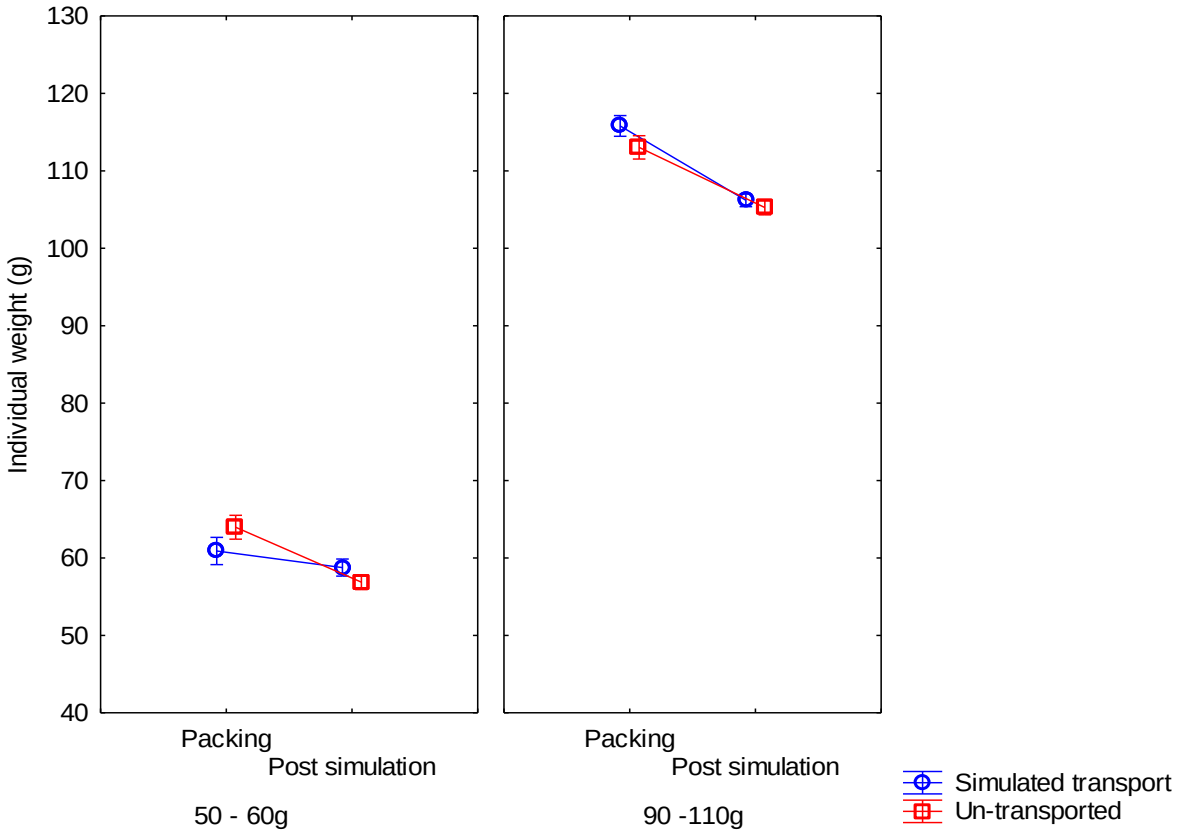


Figure 2.10: Mean (± 95 confidence intervals) change in individual average weight (g) of both size classes (50 - 60 g, n = 148) and (90 - 110 g, n = 200) and as well as the simulated transported group (n = 174) and un-transported group (n = 172). Change in individual weight ranged from when animals finished their purging process up until animals finished with the simulated transported scenario, 35 hours post the purging and packing (Repeated Measures, $F_{(1, 88)} = 7.41$, $p = 0.008$).

2.3.2.7 Excess and insufficient biomass of individual weight

There was a significant interaction of the occurrence of insufficient biomass of the individual weight, for both simulated transported and un-transported animals, for both size class ranges, post the 35h simulation (Multifactorial ANOVA; $F_{(1, 284)} = 11.25$, $p = 0.0009$, Figure 2.11). Un-transported animals, for both size class ranges, had no occurrence of insufficient biomass of individual weight, post the simulation. For the simulated transported group, smaller sized animals (50 – 60 g) had a higher percentage of occurrence of 10.60 ± 0.81 % compared to bigger sized animals, which had an occurrence of 4.51 ± 0.61 % post the 35-h simulation.

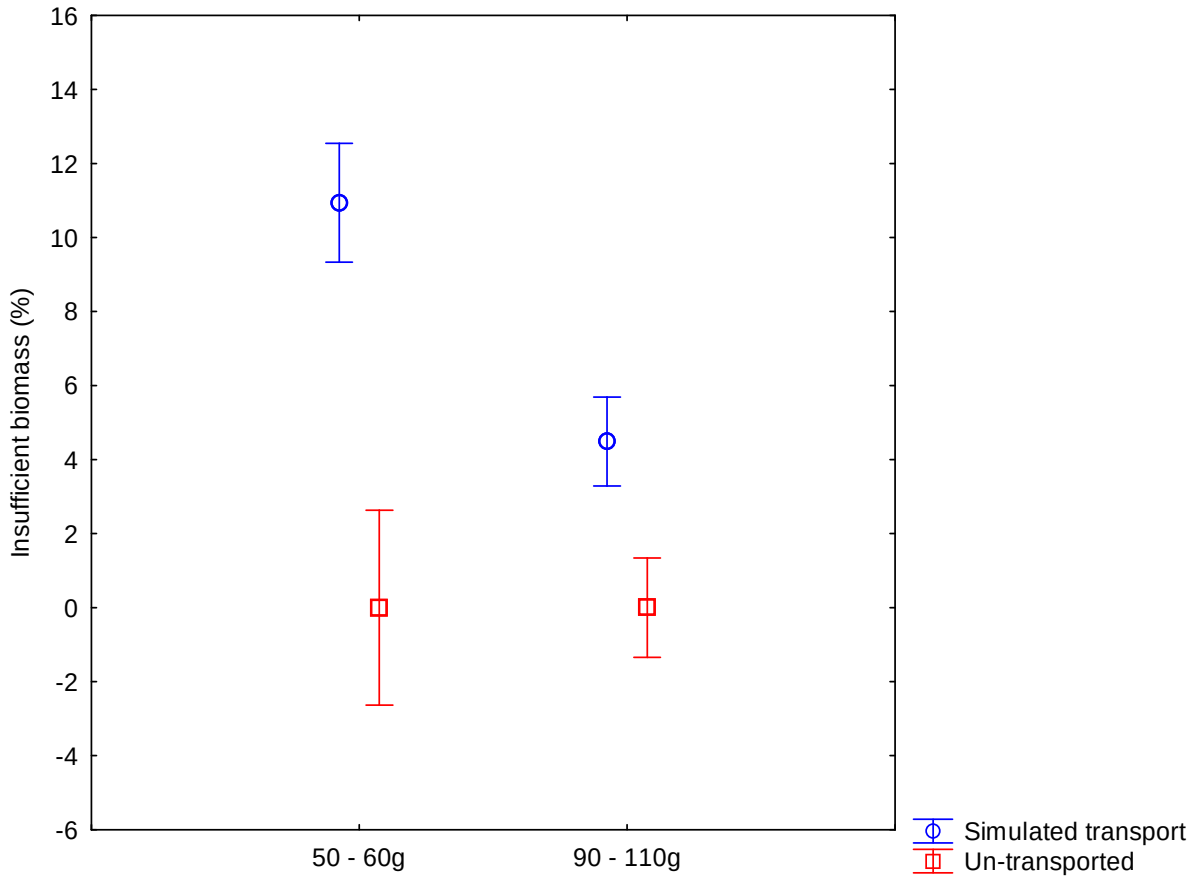


Figure 2.11: Mean (± 95 confidence intervals) insufficient biomass of animals post the 35-h transport simulation. Both size ranges, 50 – 60 g (n = 85) and 90 – 110 g (n = 200) were compared with animals subjected to a simulated transport scenario (n = 173) and animals left un-transported (n = 112) (Multifactorial ANOVA, $F_{(1,284)} = 11.25$, $p = 0.0009$).

Although there was no significant difference between the abalone sizes, there was also a significant difference of the occurrence of excess biomass of the animal's individual weight, subjected to both a mock transport simulation and animals left un-transported (Factorial ANOVA; $F_{(1,284)} = 196.53$, $p<0.0001$, Figure 2.12). Animals subjected to the mock transport simulation had a higher occurrence of the animal's excess biomass of the individual weight by 15.6 ± 0.62 % compared to animals left un-transported, which had no occurrence throughout the duration of the experimental trial.

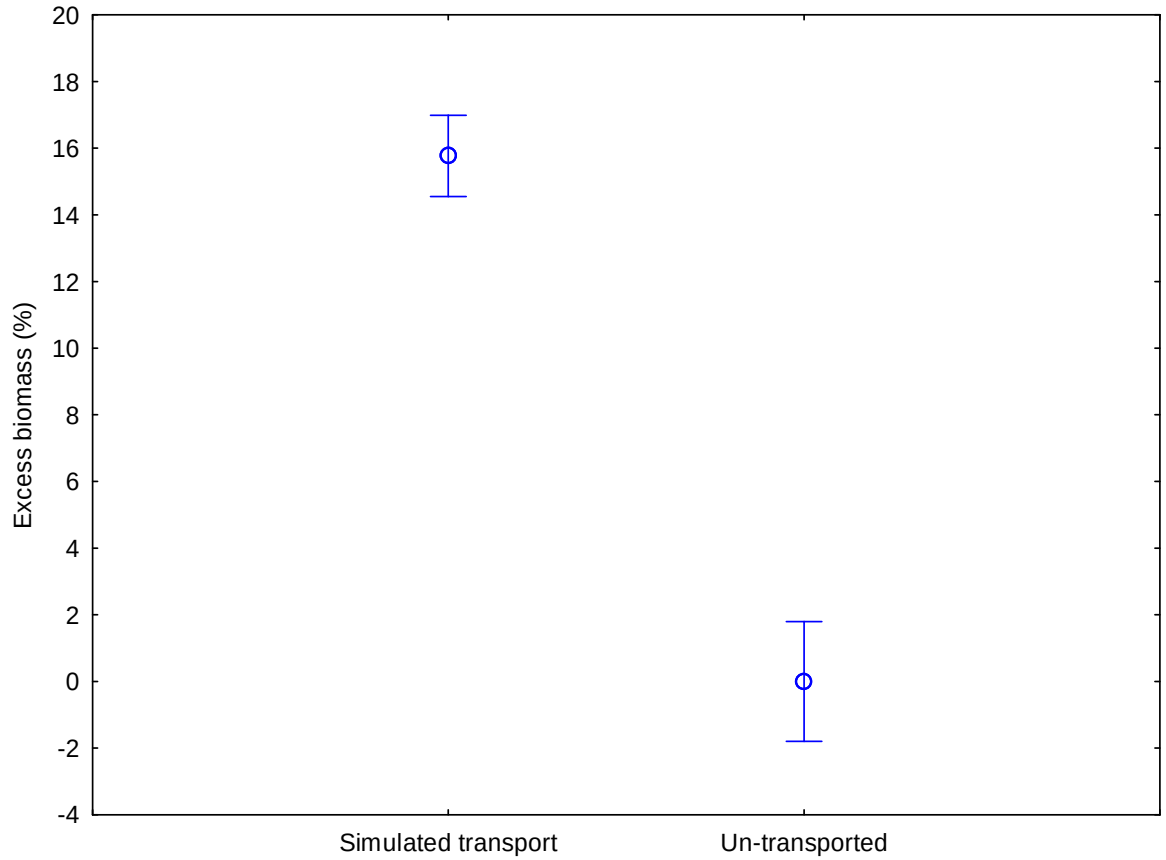


Figure 2.12: Mean (± 95 confidence intervals) excess biomass of combined size classes, post the 35-h transport simulation. Excess biomass of both mock transported animals (n = 85) and animals left un-transported (n = 200) were compared (Factorial ANOVA, $F_{(1, 284)} = 196.53$, $p<0.001$).

2.3.2.8 Mortalities upon arrival

Abalone sizes did not differ, however there was a significant difference of the initial mortality rate (%) of animals upon arrival, post the simulation when comparing the simulated group and the un-transported group (Factorial ANOVA, $F_{(1,278)} = 4.38$, $p = 0.04$, Figure 2.13). The initial mean mortality rate of animals, 35-h post the transport simulation was higher at 0.00 ± 0.04 % within the un-transported group compared to the simulated transported group, which reached a mean survival rate of 0.14 ± 0.03 %.

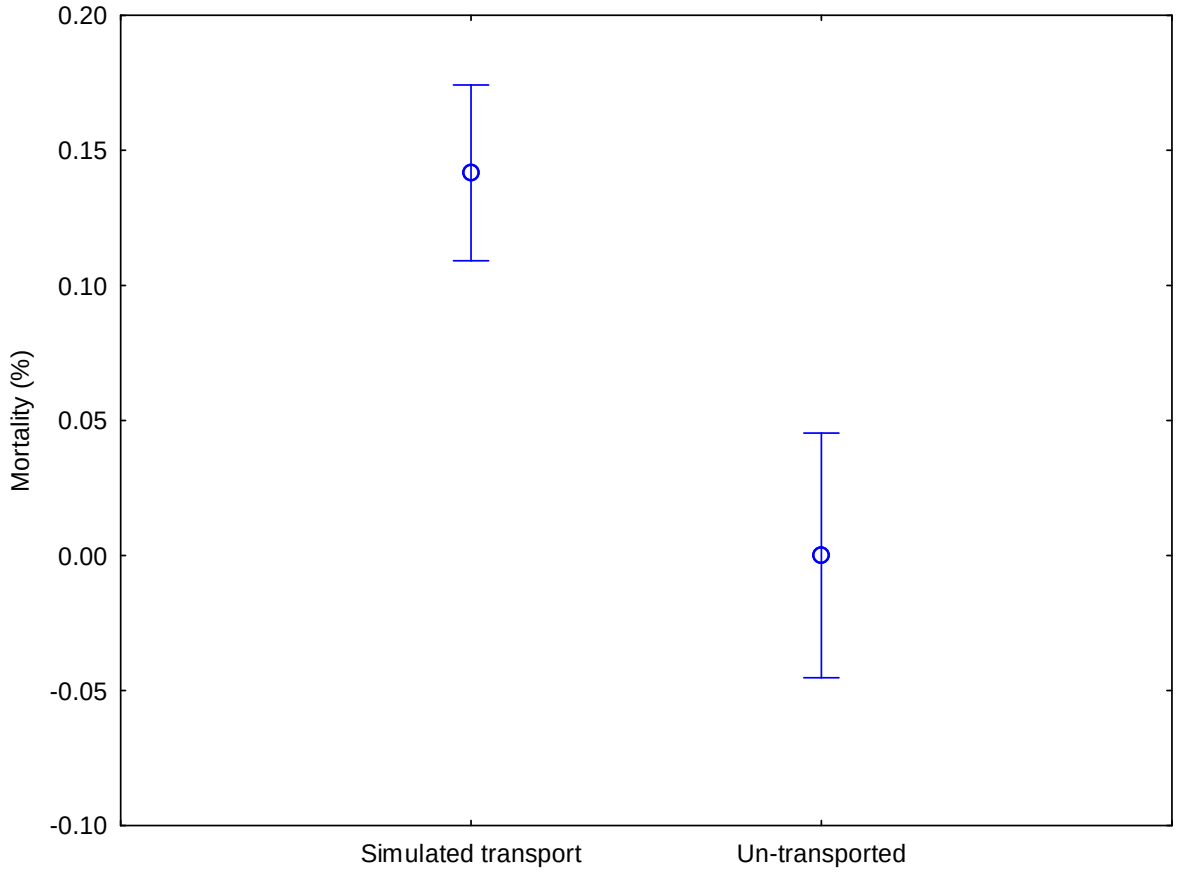


Figure 2.13: Mean (± 95 confidence intervals) mortality rate (%) of animals subjected to a mock transport scenario (n = 155) and un-transported animals (n = 111), after 35-h of a transport simulation (Factorial ANOVA, $F_{(1,278)} = 4.38$, $p = 0.04$).

2.3.3 Recovery

2.3.3.1 Survival rate

There was a significant interaction of the change in their survival rate (%) from their initial survival until the final survival rate of animals subjected to the mock transport and animals left un-transported (Repeated measures ANOVA, $F_{(1,276)} = 7.94$, $p = 0.005$, Figure 2.14). The animal's survival rate from the initial survival, post the transport simulation, compared to their final survival (%), four days after the transport simulation, was greater with the un-transported group with 100 ± 0.35 % throughout recovery, compared to the simulated transport group with a decrease in survival from 99.9 ± 0.03 % to 98.67 ± 0.25 %.

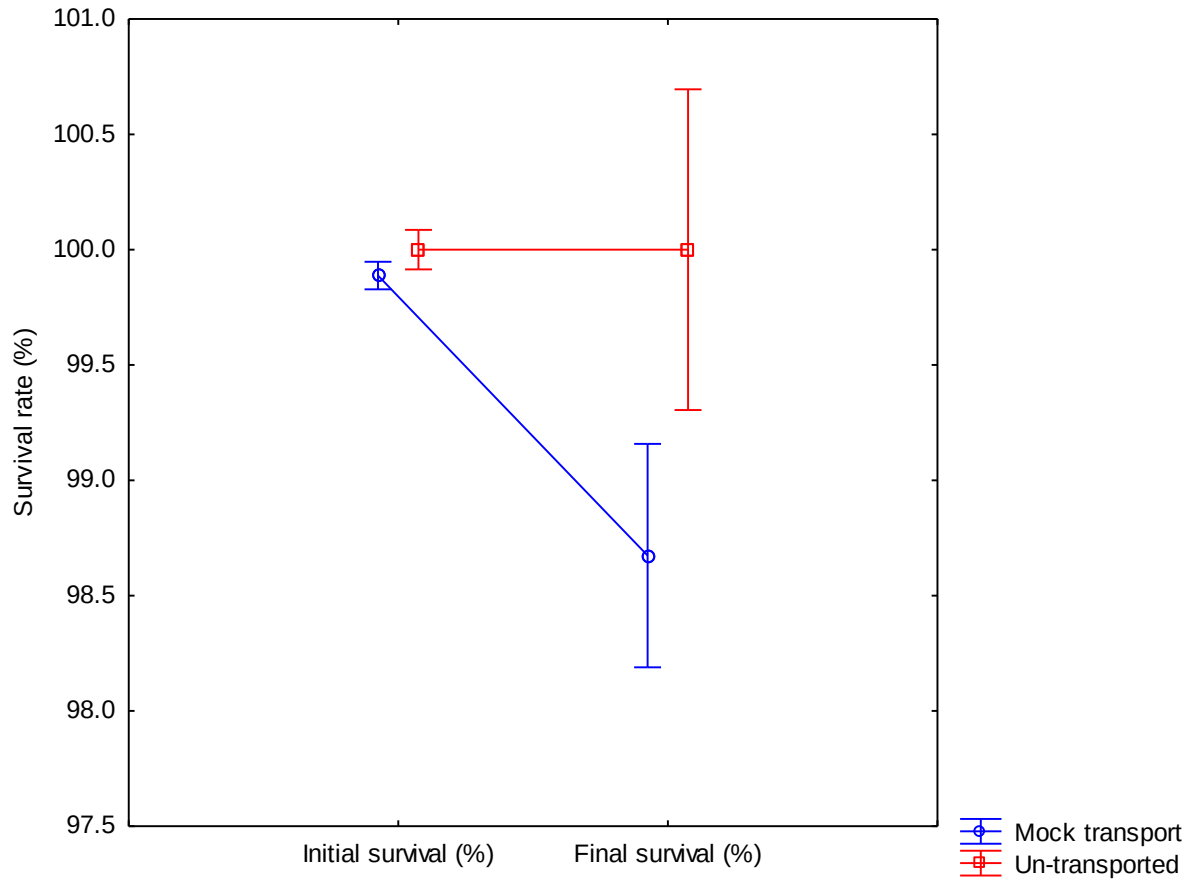


Figure 2.14: Mean (± 95 confidence intervals) survival rate (%) of combined size class animals subjected to a mock transport simulation (n = 168) and animals left un-transported (n = 112) when comparing their initial survival, from first opening them from their boxes, to their final survival (%) four days post the transport simulation (Repeated measures ANOVA, $F_{(1,276)} = 7.94$, $p = 0.005$).

2.3.4 Abalone behaviour

2.3.4.1 Righting time

There was a significant difference of the mean righting time (s) between un-transported and simulated transported animals for both size classes (Multifactorial ANOVA, $F_{(1, 100)} = 3.77$, $p<0.0001$; Figure 2.15). Both large and small animals within the un-transported group took the quickest time to right themselves, with the smaller animals having the quickest time of 40.42 ± 15.51 s compared to larger animals using an average time of 56.85 ± 17.91 s. Both large and small animals within the simulated transported group, took the longest time to right themselves with the smaller animals taking a longer average time of 229.32 ± 17.91 s.

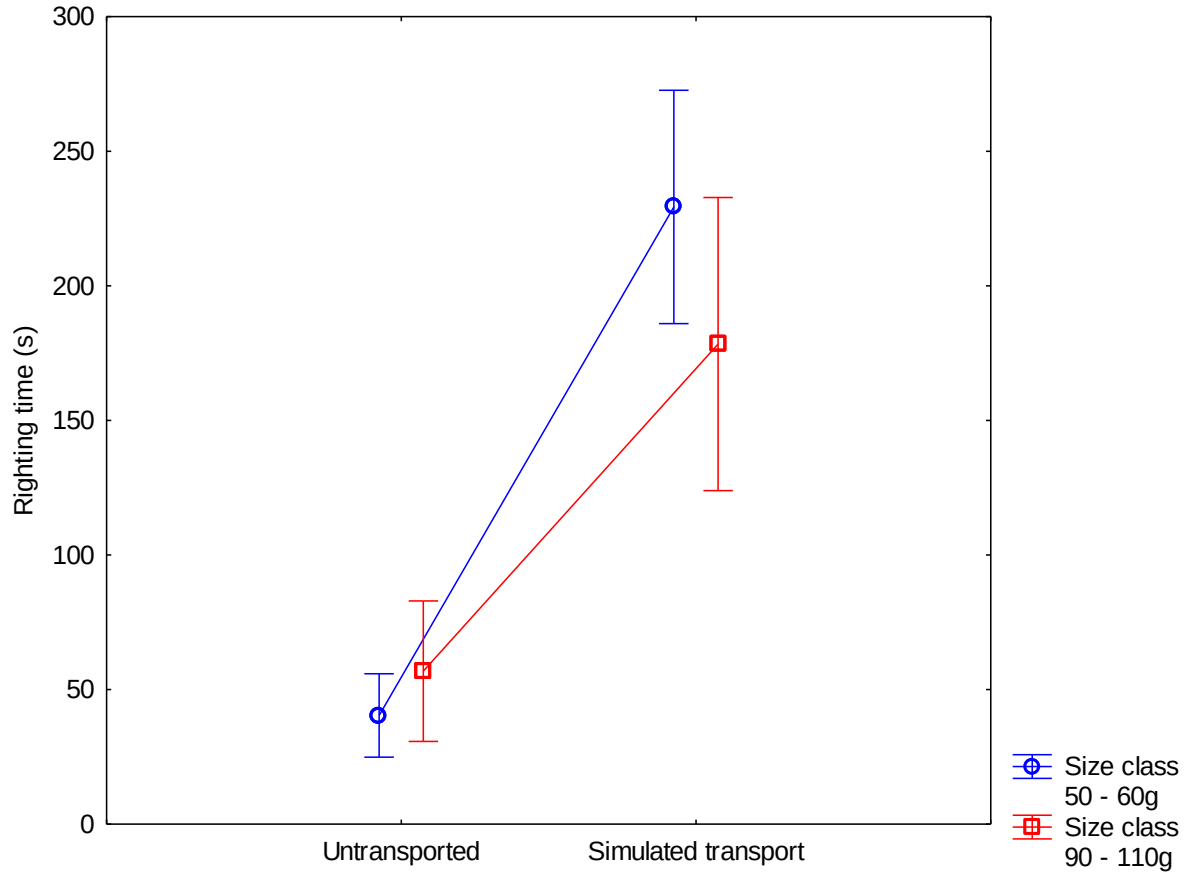


Figure 2.15: Mean (± 95 confidence intervals) righting time (s) of both size class animals, 50 – 60 g (n = 56) and 90 – 110 G (n = 48) subjected to both the mock transport simulation (n = 48) and un-transported simulation (n = 56) after 35-h (Multi-factorial ANOVA, $F_{(1,100)} = 80.11$, $p<0.0001$).

2.3.4.2 Adhesive force

There was a significant interaction between un-transported and mock transported groups compared to both size class ranges (Multi-factorial ANOVA, $F_{(1, 19)} = 6.02$, $p = 0.03$, Figure 2.16). Both size classes from the un-transported group reached a higher mean adhesive force (N) compared to the mock transport group, where bigger animals used a mean adhesive force of 68.67 ± 4.33 N, compared to the smaller animals which used a lower mean adhesive force of 42.46 ± 4.33 N. Bigger animals, within the simulated transported group, used a higher mean tension force of 7.70 ± 4.33 N compared to the smaller sized animals, which used a mean adhesive force of 3.26 ± 4.74 N after the 35-h transport simulation.

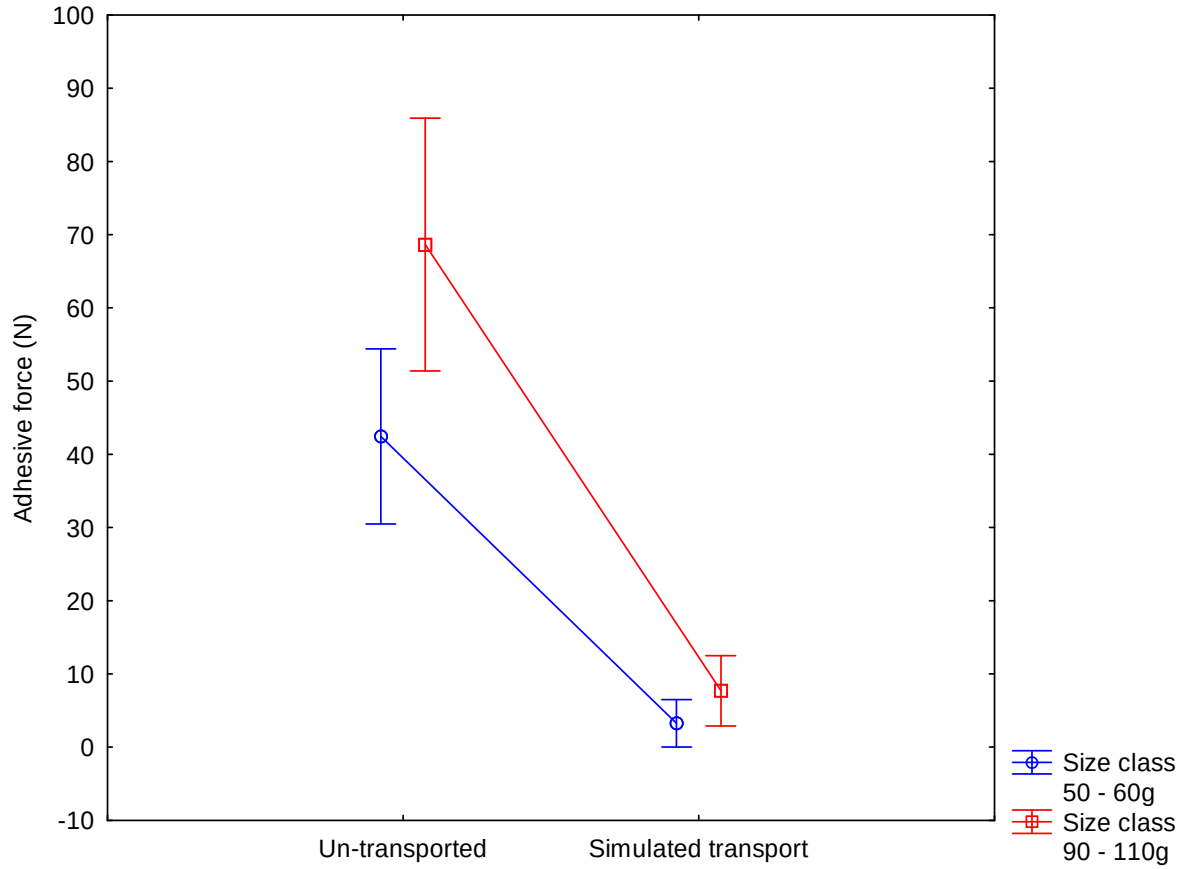


Figure 2.16: Mean (± 95 confidence intervals) adhesion force (N) of animals of both size classes, 50 - 60 g (n = 11) and 90 - 110 g (n = 12), subjected to both the mock transport (n = 11) and un-transported simulation (n = 12) of 35-h (Multi-factorial ANOVA, $F_{(1,19)} = 6.02$, $p = 0.03$).

2.3.4.3 Initial movement

Although abalone sizes did not differ, there was a significant difference of the initial movement animals took on the top plate with both the transported and un-transported animals post the 35-hour simulation (Kruskal-Wallis, $H_{(1, 24)} = 10.95$, $p = 0.0009$, Figure 2.17). Animals within the simulated transported group had a significantly lower mean initial movement of 43.83 ± 10.44 s compared to un-transported animals, which had a mean initial movement time of 10.09 ± 13.54 s.

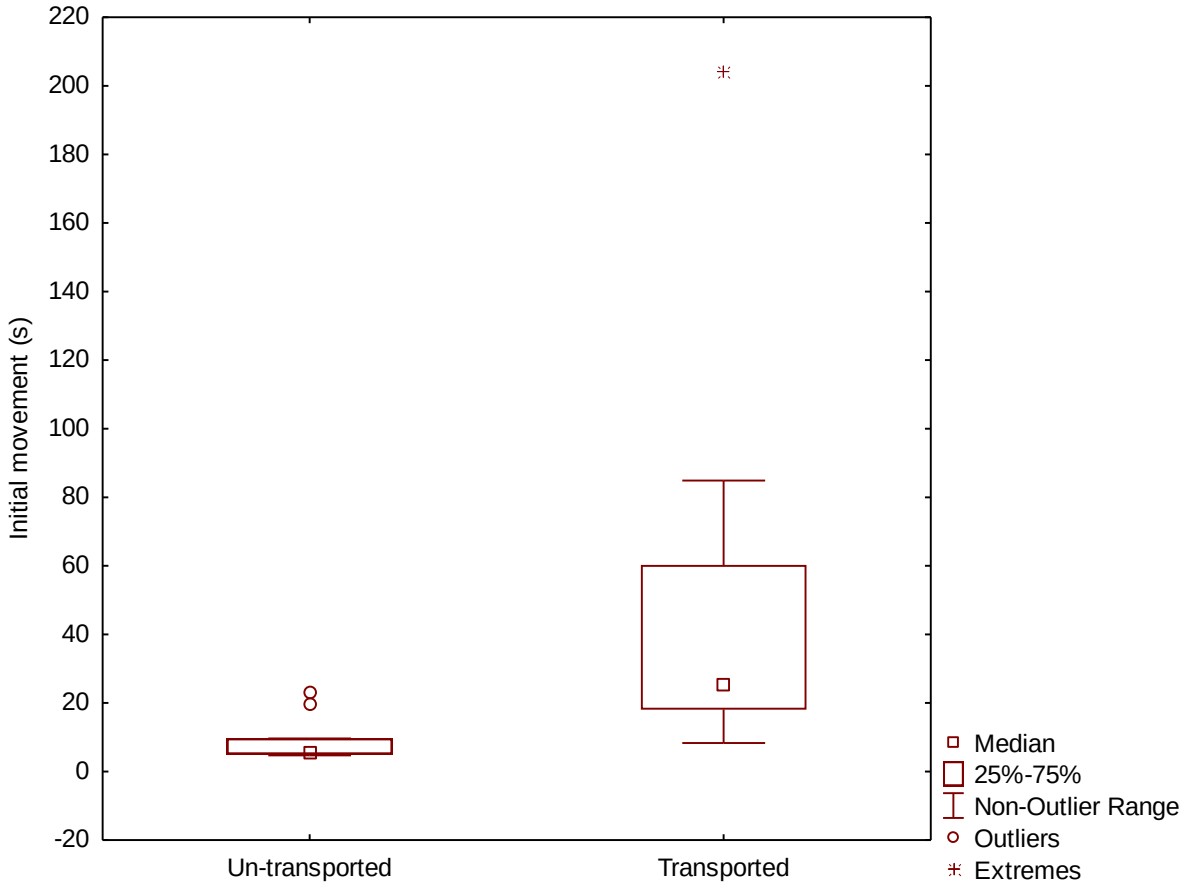


Figure 2.17: Median (± confidence intervals) initial movement of animals subjected to both a simulated transport (n = 15) scenario and animals left un-transported (n = 9), after a 35-h transport simulation (Kruskal Wallis, $_{(1, 24)}$ = 10.95, p = 0.0009).

2.3.4.4 Speed

Abalone sizes did not differ, however, there was a significant difference of both the mean and median speed (mm s^{-1}) animals took to move from the middle of the top plate to position 1 and from position 1 to position 2 when comparing the mock transported and un-transported group (Kruskal Wallis, $H_{(1, 32)} = 6.38$, $p = 0.011$, and $H_{(1, 32)} = 10.26$, $p<0.001$, Figure 2.18). Animals within the simulated transport group, had a slower mean speed of 1.99 ± 0.32 mm s^{-1} moving from the middle of the top plate to position 1, compared to un-transported animals, which used a mean speed of 3.08 ± 0.032 mm s^{-1} to move to position 1. Simulated animals also used a mean speed of 1.95 ± 0.48 mm s^{-1} to move from position 1 to position 2 compared to un-transported animals, which used a mean/median speed of 5.01 ± 0.48 mm s^{-1}.

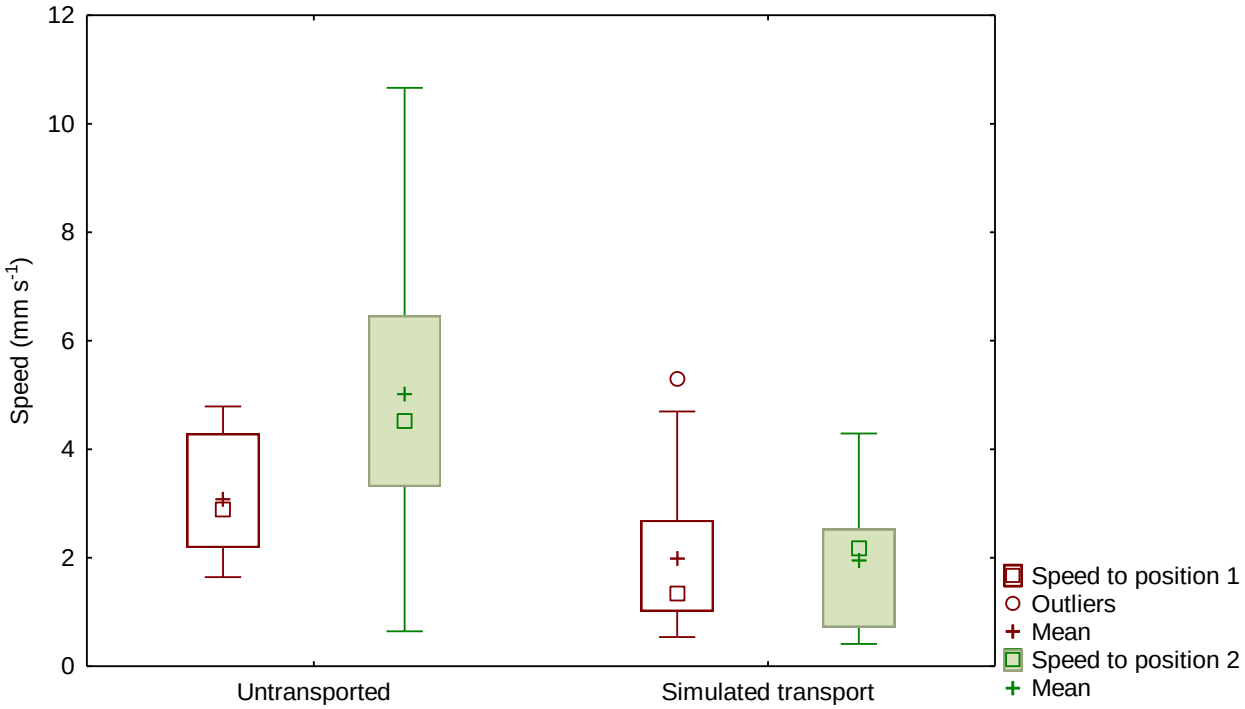


Figure 2.18: Mean/Median (± confidence interval) of both speeds to position 1 (red) and speed to position 2 (green) of combined size class animals subjected to both a simulated transported scenario (n = 16) and animals left un-transported (n = 16), after a 35-h transport simulation. (Kruskal Wallis, $H_{(1, 32)} = 6.38$, $p = 0.012$ and $H_{(1, 32)} = 10.26$, $p = 0.001$, respectively).

2.4 Discussion

2.4.1 Water quality

Within standard farming conditions, the maintenance of an optimal purging water quality conditions is one of the important factors that govern bivalve stress levels during the live export (Barrento *et al*., 2013). Bivalves, including mussels and abalone, have the capabilities to handle and recover of stressful events, including handling and grading, and poor water quality during purging. However, if a combination of all stresses would be present and intensified, then a number of mortalities and poor animal quality would be expected (Barrento *et al*., 2013). Abalone are poikilotherm animals and therefore cultivated abalone require both an optimal temperature range and high-water quality in order to mitigate the highly stressful conditions observed during export (Bullon *et al*., 2023). Temperature has a direct effect on both the diffusion ability of dissolved oxygen (mg/L and %) and a direct effect on pH (Ducharne, 2008). As temperature decreases, its ability to dissolve into the water column increases and in turn can hold higher levels of both dissolved oxygen and pH (Ducharne, 2008). This was seen within this trial, where concentration levels for both mean DO and pH observed where within the optimal ranges of 7.58 mg/L, 94 %, and 7.86 for pH, which might have been due to this trial

being conducted during the winter months. This was compared to average DO and pH during the summer months, where concentrations were 6.99 ± 0.29 mg/L and 93.72 ± 0.34 %, with pH levels reaching 7.81 ± 0.16. This was confirmed from Rabalais *et al*. (2010) and Cai *et al.* (2011) studies, which showed that DO concentration levels below 94 % and 62.5 µmol l^{1} would result in a variation of the survival rate of different fish and benthic organisms.

2.4.2 Post transport simulation

From this trial, animals that were subjected to the transport simulation process for 35 hours experienced a reduction in their total weight by 9.55 %, which is similar to Vosloo and Vosloo (2006) research where *Haliotis midae* at different air treatments, held for 36 hours, were found to have a reduction in their total body weight. As air exposure is a stressor for abalone species, they have been found to experience a reduction in their total body weight by up to 16% due to fluid loss from evaporation and as well as pedal mucus production due to the mobilization of their foot reserves due to stress (Lachambre *et al.*, 2017). Another study also showed similar weight loss results, where weight loss of 10.25 % and 8.63 % was observed after Australian abalone were subjected to a 48-h transport simulation at different pre-cooled temperatures (Kurnaningtyas *et al.* 2022).

During export, gas exchange is severely limited as the gills are unable to ventilate effectively and due to the hypoxic environmental conditions, fluid loss is even more exacerbated (Ragg and Watts, 2015). Abalone with a drip/ water loss range between 5 – 30 % have been shown to experience mortality (Hyman, 1967), which can be seen in this research, where animals subjected to an export procedure had a higher initial survival upon arrival and four days post the export, during recovery. External environmental factors, including temperature, has the ability to affect the physiological responses of shellfish, which in turn also affects their activity level and energy demand / balance (Shin and Yang, 2005). As temperature decreases to 10 °C or below, respiration and filtration rates decrease and in turn the physiological and metabolic rate decrease. In this trial, animals of both size classes, which were subjected to a 35-h simulated transport scenario were cooled down due to the ice provision, and in turn reached a lower temperature and metabolism, which resulted in a lower weight loss (g and %) upon arrival, which was also similar to Shin and Yang (2005) study, where a lower temperature, resulted in a lower overall weight loss. The relatively lower average net weight loss, compared to summer conditions with weight loss reaching 13.5 %, could have been due to temperature, which is considered a factor that affects stress during transport (Kurnaningtyas *et al*., 2022).

This trial was conducted during winter and cold storage was used during transport, both of which could account for this difference.

This excludes the added harvest weight (weight accounted for in the purging process when animals expel ammonia and their gut content) in order to mitigate weight loss abalone experience during purging but still be able to meet export weight quotas during packing (Gorfine, 2001; Barrento *et al*., 2013). When animals were harvested, for preparation of live export, a minimum excess weight of at least six grams (for smaller sizes) and seventeen grams (for bigger sizes) was calculated and graded for (Shaw *et al*., 2023). After the purging process, animals tended to retain a higher average mass prior to transport of around three to six grams, above their size range, for bigger animals, and around one to four grams extra, for the smaller sized animals. Animals lost a significant amount of body weight of 5.88 and 7.45 g for both simulated transported groups and un-transported groups, as a result of water loss from the pedal muscle, due to stress from air exposure. This was due to abalone being predominately subtidal, and not resistant to desiccation, including water loss and in turn survival (Hyman, 1967; Gorfine, 2001).

The ability of abalone to retain water is based on having a large shell aperture (Gorfine, 2001), where for this research, bigger animals were able to lose and maintain a lower weight loss through drip/water loss for both animals subjected to a simulated transport scenario and animals left un-transported for 35-h. However, during purging, animals' gut/digestive content, sand, grit, nutrition, and other organic material expelled from abalone during this process, resulted in a significant reduced weight, not only from water loss (Sales, 2001). This could be the reason un-transported animals during this trial lost significantly more individual weight loss (g) compared to the overall combined net weight after 35-h of an export simulation. The process of pre-transport fasting (purging) also had a significant impact on weight retention, as Tinsley and Bounty (2015) found that body mass can be retained during periods of starvation. Due to this process, purging was also another factor that may reduce stress during transport.

The survival rate from both un-transported and the simulated transported groups for both different animal sizes were high upon arrival (>98.5 %) as a number of aquatic organisms have a strong tolerance to hypoxic environments due to adaptation living within subtidal environments, where periods of air exposure was common (Shen *et al*., 2020). Due to this, survival rate throughout this entire trial was very high and very low levels of mortality were

observed upon arrival. Abalone species have also been shown to have delayed mortalities after an extremely stressful event or condition, which can last for several weeks (Ragg and Watts, 2015). This was similar to results from this trial where initial mortality count was low for both size ranges however as the animals were reintroduced into their baskets, their mortality count steadily increased for three days after.

2.4.3 Behaviour

The simulated transport scenario had a significant negative effect on behaviour, with the most prominent negative effect on their righting time as well as their ability to adhere to a surface. The righting behaviour of abalone is an important role in their survival rate for each individual as it is indicated that a failed righting performance results in exhaustion or mortality (Zhang *et al*., 2020). Since abalone prefer solid surfaces compared to loose surfaces, animals subjected to this experimental trial were placed in a bucket filled with ambient seawater for the full duration of this trial. Righting behaviour was negatively affected, from this trial, in animals subjected to a simulated transport. Both large and small animals, post the transport simulation, took an average of three minutes to right themselves, compared to animals that were left un-transported, which took less than a minute to right themselves. Simulated transported animals also had a number of abalone that were not successful in completing the righting behaviour. As the righting behaviour in abalone is a good indicator of the measurement of their metabolic capacity (Baldwin, *et al.,* 2007), specifically their energetic reserve, where it is also classified as a good proxy of their stress resilience, which also indicates their chances of survival (Baldwin *et al*., 2007; Lachambre *et al*., 2017). This suggests that animals during transport, had a lower chance of survival post transportation.

Abalone have a broad muscular foot, which allows them to have a strong adhesion force ability, which is three times stronger than clingfish and Guizhou gastromyzontidae, in order to adhere to slippery rocky surfaces in their natural environment (Li, *et al*., 2018). Abalone's adhesion force is even greater when in water (Li *et al*., 2018), where under high stress, abalone individuals lose their ability to adhere to a substrate as it is energetically costly (Li *et al*., 2018). This was observed in this trial where un-transported individuals had a significantly higher tension force (N) compared to individuals that were subjected to a simulated transport scenario, where animals subjected to the simulation were under high amounts of stress for a long duration of time (± 40 hours). The abalone's adhesion force also effects their ability to successfully complete the righting behaviour performance as Zhang *et al*., (2020) showed that the minimum

amount of force sufficient enough, required in the beginning of the righting behaviour to pull itself up, is 3.29 N. Results from this trial indicates that abalone subjected to the simulated transport scenario are highly stressed where their tension force ability significantly decreases.

As stress is a difficult measurement to quantify in abalone, their movement is a non-invasive method to determine their stress levels (Robinson *et al*., 2013). In this trial, abalone movement from individuals that were left undisturbed and that went through a prolonged simulated transported stress period for 35-h were compared with each other to determine if their speed varies. Within this trial, the abalone's mobility was affected by the simulated transport scenario, where due to the anatomical structure of the foot pedal of the abalone, they are capable of travelling at greater speeds compared to other crawling species (Donovan and Carefoot, 1997). Abalone increase their locomotory speed when escaping from predators (Donovan and Carefoot, 1997; Calderon-Gurrola *et al*., 2023). During this trial, animals were exposed to sunlight where animals escaped the stressful conditions by crawling off the top plate into the shaded area. Abalone that were not subjected to a prolonged extreme stressful condition and were left un-transported used an average faster speed when escaping the undesirable environment. Calderon-Gurrola (2023) also deduced that under extreme stress, abalone demonstrate significantly reduced mobility capabilities, which was seen within this trial, where speed used to escape was significantly reduced for animals that were subjected to extreme stress during the transport simulation.

2.5 Conclusion

Abalone, like other mollusc species, including mussels are impacted by transport and purging during export, where animals have the ability to recovery from purging stressful conditions, which is mitigated by water quality within the purging process. Water quality influences the purging process, by suppling animals with the most optimal environmental conditions, including dissolved oxygen, temperature, and pH. A low temperature resulted in a more optimal oxygen concentration and low pH, which were also within the "safe ranges" for export.

All animals, both transported and un-transported, showed a change in their weight parameters, including the change in net and individual weight during transport and upon arrival, their excess and insufficient biomass of their individual weight, and their weight loss and water loss. These changes were determined by the size of the animals, where smaller animals had a lower

performance during the transport simulation. Survival was mostly affected by the transport simulation alone, however, due to two of the behavioural parameters, including the righting time and adhesive force, being good indicators of their survival ability, animals were then compared. Smaller animals were also more negatively affected and took longer to right themselves, which could indicate a lowered chance of survival. Adhesive strength (force) was also more negatively affected by smaller animals; however, the size of the animal was a limitation and physiological abilities were more limited with a smaller size.

As trials were conducted within the winter months, when water quality parameters were more ideal, the effect of transport on abalone performance, during summer months, when unfavourable conditions prevail, is still unknown. The implementation of a pre-cooling method for abalone has been studied on New Zealand abalone by Kurnaningtyas *et al.,* (2022) and has yielded positive results but has not been studied on South African abalone.

Chapter 3: Development of cooling acclimation methods, prior to summer transport simulation

3.1 Introduction

Flow-through, land-based aquaculture systems are used to produce abalone in South Afirca. These systems uses a high-water volume with a single pass through the system, after which it is discharged or dispersed (Salari *et al*., 2012). On other hand, recirculating systems (RAS) are also land-based, however, the system re-use water, where the water is treated, either mechanically or biologically, to reduce water consumption and also maintain optimal water quality (Lehto, 2023). Within aquaculture systems, water flow rates provide vital information on the different water quality processes that influence production (Salari *et al*., 2012). Different water quality parameters are dependent on the environment and its surroundings, specifically within flow-through systems, where the provision of the most optimal conditions would ensure survival, feeding, optimal growth, and successful reproduction (Salari *et al*., 2012). These different water quality parameters include pH, temperature, oxidation-reduction potential (ORP/Redox), turbidity, salinity, and dissolved oxygen (DO). In RAS systems, water quality parameters are controlled and monitored closely to maintain stable conditions, where understanding the factors that change water quality is of great importance. Other biological and physiological conditions also influence water quality, where the accumulation and build-up of organic material and nutrients is influenced by the stocking densities (Lehto, 2023).

Abalone are stocked at high densities during production and harvesting, which is around 2.0 kg m^{-2} compared to the wild, where natural stocking densities are 82 – 133 g m^{-2} (Yearsly, 2007). This in turn affects the culture environment through the animal's metabolism as a higher stocking density can cause a higher accumulation of excretory products, where accumulating ammonia levels may lead to the animal's environment becoming toxic, particularly in RAS (Francis, 2008). During production, next to oxygen consumption, ammonia-nitrogen production is another limiting metabolite. This is because ammonia easily diffuses across the abalone gill membrane, and this process is linked to water pH so the pH of the water column needs to be maintained throughout production and harvesting (Lyon *et al*., 1995). With temperature being the primary environmental condition that determines the metabolic rate of abalone (Fry, 1971; Yearsly, 2007), an increase in water temperatures during summer, results in increased oxygen consumption, which in turn increases the metabolic demand of animals

and ammonia excretion. This subsequently alters the water quality during production and harvesting (Lyon *et al*., 1995; Yearsly, 2007; Vosloo and Vosloo, 2010).

Abalone, like many other species of exported seafood, are sold live to multiple international markets, where these animals are purged and experience periods of starvation for an extended time ahead of transport (Venter *et al*., 2024). This process allows food to be expelled from the digestive tract before transport, which reduces metabolic waste production during transport (Szydlowska *et al*., 2024). During this process, waste accumulates at the bottom of the tanks, which in turn affects the water quality (Flick, 2008). In the water column, ammonia exists between its unionized and ionized forms, in equilibrium with pH, temperature, and salinity, where the ionized form is more toxic to animals (Lopata *et al*., 2006). Multiple factors affecting water quality during cultivation can result in sub-optimal environmental condition for production and harvesting, making animals more susceptible to diseases and pathogens (Barrento *et al*., 2013). The constant provision of good quality water using high water exchange rates prevents this, and provides sufficient dissolved oxygen and removes ammonia (Naylor, 2012).

The environmental conditions during purging ahead of handling and transport effects the health and survival of abalone during transport since the environment influences metabolism. The most important water quality parameters include dissolved oxygen (DO), pH, temperature and ammonia, and these must be maintained at optimal levels during purging by ensuring adequate water flow rates. These environmental conditions are important because they influence the metabolism and health of aquatic animals (Boyd, 2017; Bullon *et al*., 2023). Abalone exposed to warmer water have a higher metabolic rate compared to those kept under colder conditions, which in turn affects the water quality during harvesting and purging (Luo *et al*., 2010; Bullon *et al*., 2023).

3.1.1 Aims and objectives

The aim of this trial was to establish the effect of temperature change during a purging period using a semi-recirculating flow through system. The aim of this trial was also to assess a method of pre-cooling tanks by establishing the effectiveness of the cooling ability of a recirculating chilling unit. This was done by reducing the incoming ambient flow rates significantly over 24 h and comparing the temperature difference of both the incoming ambient temperature and temperature of the tank. This was conducted in order to develop a method to

pre-cool abalone, prior to transport, so as to prevent animals from experiencing thermal shock during purging, and that could subsequently be used as a method in Chapter 4.

3.2 Materials and methods

3.2.1 Routine farm practice applied to abalone before they are transported, and experimental procedures, and facilities

The same routine farm practice that was used ahead of abalone transport and the same experimental procedures and facilities, and that were presented in Chapter 2 (Section 2.2.2), were also applicable to the work carried out here in Chapter 3.

3.2.2 Experimental system and design

It is standard farm practices during harvest for each purging tank to be supplied with seawater at ambient temperature (Figure 3.1), where flow rates were kept and maintained at a minimum of 30 L/h/kg, in order to supply animals with a flowthrough system with consistently good water quality (Shaw et al., 2020). The fiberglass tanks were also equipped with three airlines at the bottom. For this experiment, a cooling system was set up using a heat pump with both a heating and cooling feature (Standard TFS090/3 Heat Pump, Aqua-heat, Cape town), where it was connected to two tanks (Figure 3.1) using a PVC pipe, which supplied water from the cooling system (CS) into the two tanks (Figure 3.1). One additional PVC pipe was also connected to each tank, which supplied water to the chiller system in order to decrease the temperature of the water that was used to top up both tanks (Figure 3.1). Three fiberglass purge tanks were used in this trial. One tank operated as a flow through tank (which is normal farm procedure, and which acted as a control). This tank received ambient seawater from the farm's water supply at a rate equivalent to 30 L/kg/h (had the tanks been stocked with abalone), and water flowed via an inflow pipe at one end and an outflow pipe at the other. The other two tanks were run as a recirculating system (Figure 3.1). They also had an ambient inflow and outflow pipe, which were used to fill tanks ahead of data collection. These two tanks were connected to a heat pump (GTP28 – ES series variable speed water pump) with a heating and cooling mechanism (Figure 3.1). Ambient water was turned off during data collection and water flowed through the heat pump at a rate similar to the ambient water flow.

Six temperature loggers (Onset UA-001-64 HOBO 64K pendent, Temperature/Alarm (waterproof) Data logger) were placed into the tanks. One at the inlet and one at the outlet of each tank. The DO (mg L^{-1} and percent saturation) and pH were recorded in each tank.

Tanks were not stocked with abalone in this trial in order to finalize the different flow rates without causing unnecessary physiological and physical stress.

Tanks were cooled and tested during peak summer months (Nov – Feb), when westerly winds prevailed for a prolonged period and ambient sea surface temperatures reached and exceeded 18 °C. Methods tested during this trial was used in order to implement a pre-cooling acclimation environment for a more optimal export procedure.

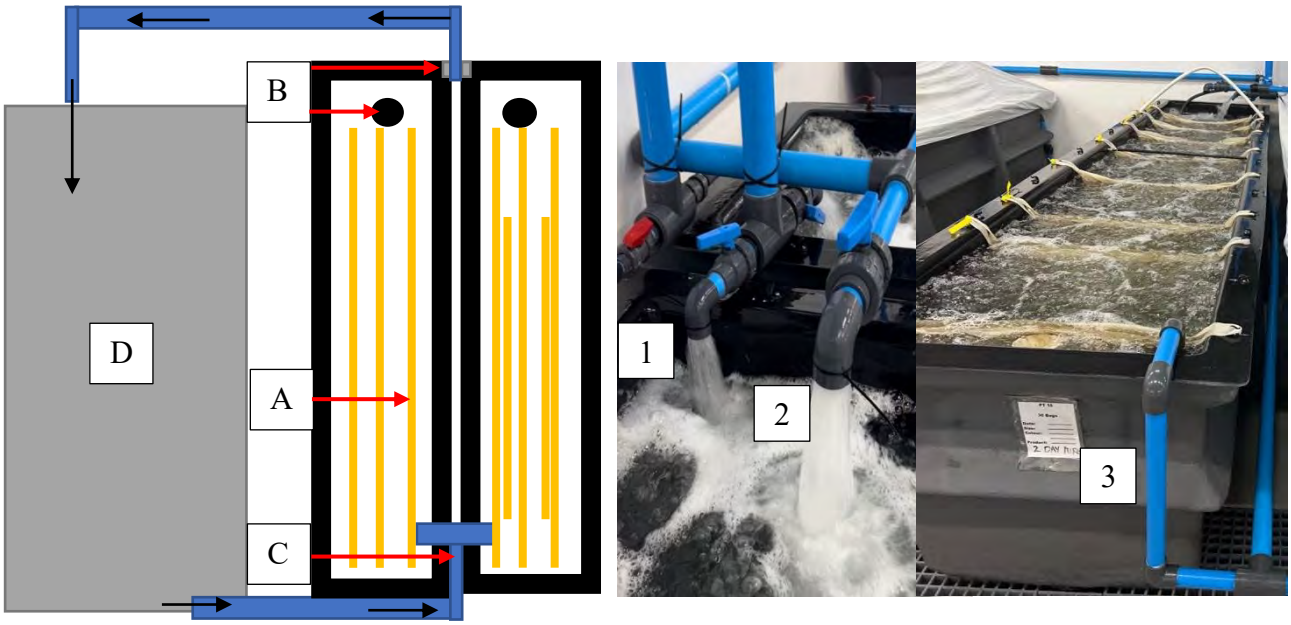


Figure 3.1: Experimental system, which was used in order to establish the effectiveness of cooling tanks for the purging process of exportation. Black arrows indicate the flow of water throughout the entire system (from the cooling system, to the tanks, and back to the cooling system. A) indicates the air-line placed on the bottom of each tank. B) indicates the outlet from each tank, that takes water from the tank to the cooling system (upper arrow) and also discards old water (lower arrow). C) indicates the outlet pipe that takes cooled down water from the cooling system back to the tanks. D) indicates the cooling system within both tank pipes. pipe 1) indicates the incoming ambient seawater, pipe 2) indicates the recirculated, chilled water from the cooling unit, and pipe 3) indicates the outflow pipe that takes water from both tanks and chills it down from the cooling unit before giving it back to both tanks.

3.2.3 Experimental cooling system

There was a total of three different flow rates used when using the CS which was used to determine the best method to maintain a minimum of a 2 °C difference or a lower temperature difference from the incoming ambient temperature. In order to limit the occurrence of a thermal

shock, the tank temperature was decreased by a 1 °C difference for a total of 24 h before temperatures were further decreased. Tank temperature was decreased every 24 hours, until a 3 or 4 °C temperature difference was achieved. Within each 24-h period, flow rates were taken every 3-4 h per day using a 5L bucket and stopwatch, where flow rates from each tank were calculated, using Equation 3.1. FL = flow rate L/kg/h, s = mean time (s) taken to fill a 5 L bucket throughout the purging period, and g = total weight (g) of animals inside each tank.

$$FL = \frac{(\frac{5}{\Delta s})*3600}{(g)} \qquad \text{Equation 3.1}$$

As animals were not used during this trial, average total weight of animals in tanks during a routine export was used to calculate flow rates. Flow rates calculated were adjusted for in order to use during a standard export procedure. Average weight of animals in tanks during a routine export ranged from 112 to 128 kg.

The cooling ability of the unit itself was based on four different factors. The surface area of the corrugated panels within the chilling unit, the flow rate (L/kg/h) of the CS pipe, which remained at a consistently high mean flow rate of 122.80 L/kg/h. The third factor consisted of the flow rate of the incoming seawater pipe (L/kg/h), which varied throughout the day due to the farm's daily fluctuations in their reservoir. The fourth and final factor was the temperature of the incoming seawater temperature, which also fluctuated daily throughout the entire duration of the trial (Lloyd, 2022).

Flow rate 1: (-1-degree temperature difference)

Flow rates for the first decrease in temperature by one degree was set at 37.51 L/Kg/H, where temperature difference ranges were set between 0.3 – 0.6. Temperature difference ranges was based between the incoming seawater temperature and the temperature towards the outlet of each different tank throughout the trial.

Flow rate 2: (-2-degree temperature difference)

Flow rates in order to achieve a temperature difference of two degrees was decreased and set at 19.87 L/kg/h. The temperature difference ranges were set between 0.4 – 0.6°C for a total duration of 24 hours.

Flow rate 3: (-3 and -4-degree temperature difference)

Flow rates, which were used in order to achieve the final temperature decrease of three to four degrees, was set between 8.57 – 5.91 L/kg/h due to the reduced supply of the incoming seawater temperature, which allows for a quicker cooling period between the three-temperature difference and the four-degree temperature drop from the incoming seawater and area within each tank towards the outlet. Temperature difference ranges were set at a higher range between 0.4 until 1.5, compared to the other phases due to the faster cooling ability of the chilling system when flow rates are adjusted at a low flow rate.

The maintenance of the temperature decreases by a degree a day is used in order to gradually acclimate animals into a cooler environmental temperature and eliminate the potential of animals experiencing thermal shock during acclimatization during the entire purging period. Water quality parameters were also collected at the end of the cooling experimental trial, using the same methods mentioned in Experiment 1 (Section 2.2.4; Chapter 2).

3.2.4 Data collection

Temperature loggers (Onset UA-001-64 HOBO 64K pendent, Temperature/Alarm (waterproof) Data logger) were placed into each respective tanks, with loggers placed at the outlet of each pipe, were set to record the sea temperature, of each flow rate, from each pipe, in 10 min intervals for the duration of the entire trial. Temperature readings from when HOBOs were placed into the tank and an hour after being placed into the tanks were excluded, in order to calculate the temperature difference from when tanks were cooled compared to the incoming ambient sea temperature. Temperature recordings that showed had errors, from malfunctions from the chilling unit system, due to load shedding, were also excluded.

The same water quality parameters collected from Experiment 1 (Chapter 2: Farm condition (mock pack) transport simulation) were also monitored and collected using both the Polaris C Oxyguard and the Handy pH OxyGuard. Polaris C OxyGuard was used for collecting both dissolved oxygen concentration (mg/L) and dissolved oxygen saturation (%). Handy pH OxyGuard was used to collect pH levels, where all water quality parameters were only collected at the end of the trial.

3.2.5 Statistical analysis

A multiple regression analysis was used in order to determine if there was a significant relationship between time and the change in temperature. Microsoft Excel (version 16.54) was also used simultaneously in order to generate the models, R^2 value, and as well as p-value of each flow rate slope. Incoming water temperature was then tested against the end of both tanks for each degree difference tested (-1-degree difference, -2-degree difference, and -3-degree difference), using a repeated measures ANOVA at $p<0.05$. If the dataset also did not meet the assumptions of an ANOVA, then a sphericity test was also used.

The water quality parameters for both chilled tanks and ambient tanks were recorded throughout the cooling experimental tests, where currently used water quality parameters, including dissolved oxygen (mg/L) and dissolved oxygen saturation (%), and pH, were compared with each other after the entire experiment using Factorial ANOVA at $p<0.05$. If the assumption of variance were not met using the Levene's test, then the Kruskal-Wallis non-parametric test was then used in order to compare the distribution of the dataset comparing the different treatments or different size classes (Bewick *et al*., 2004).

3.3 Results

Throughout the entire cooling experimental trial, incoming ambient temperature ranged from low of 13 °C to the highest of 20 °C. Mean temperature reached throughout the entire trial, for all tested tanks, reached 14.85 ± 1.18 °C (Table 3.1).

Table 3.1: Mean (± 95 confidence intervals) incoming ambient temperature of water into each tested tank, throughout the duration of the trial (Factorial ANOVA; at p<0.05)

	Chilled tanks	Ambient tanks	F-statistics	P value
Incoming ambient sea temperature (°C)	14.85 ± 1.18	14.85 ± 1.18	$F_{(5, 138)} = 57.213$	$p = 0.79$

3.3.1 Degree temperature drop during purging

There was a significant difference of the temperature drop of both tanks when comparing the temperature difference, the incoming ambient water, and the chilled tanks over a period of 48 h for each degree difference (Repeated measures ANOVA, $F_{(4,234)} = 851.61$, $p<0.001$, Figure 3.2 and 3.3). With the mean incoming temperature reaching 15.01 ± 0.28 °C, the temperature

difference for the first-degree difference was maintained at 13.95 ± 0.37 °C for tank 1 and at 14.10 ± 0.37 °C for tank 2. Temperature difference for the second-degree difference was maintained at 13.27 ± 0.37 °C for tank 1 and at 13.36 ± 0.37 °C for tank 2. The final third-degree temperature difference was maintained at a mean 11.47 ± 0.38 °C for tank 1 and at 11.60 ± 0.38 °C for tank 2.

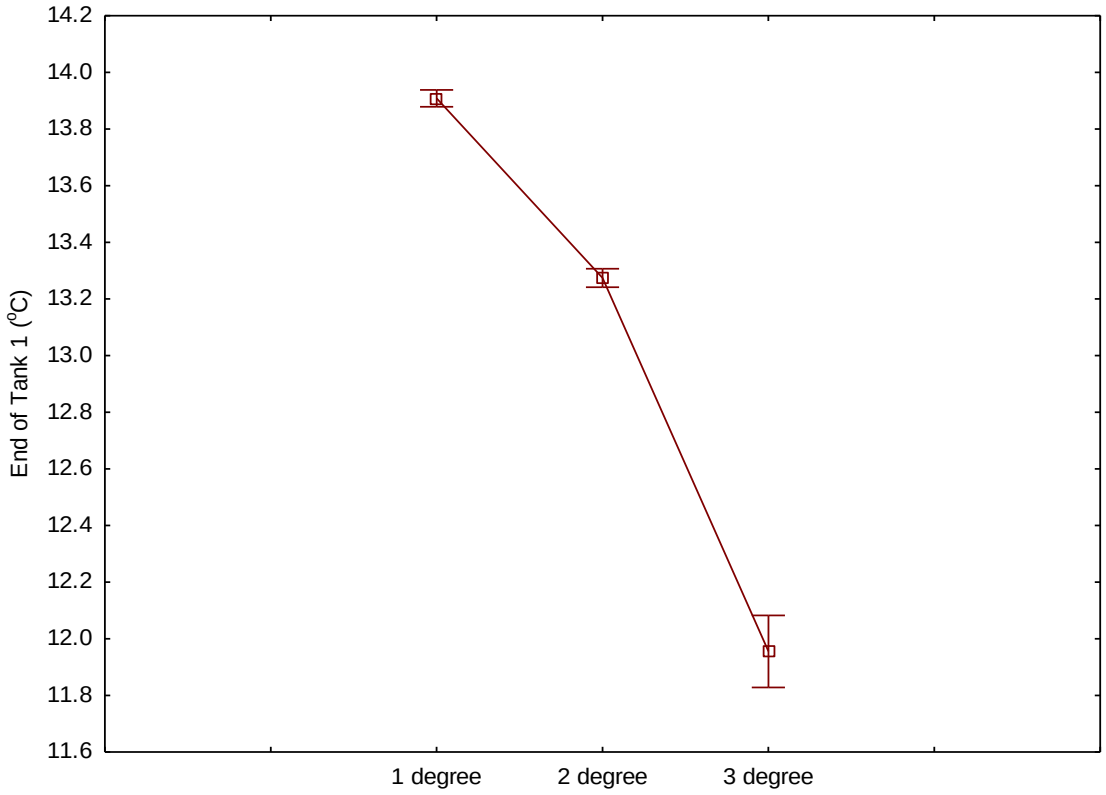


Figure 3.2: Mean (± 95 confidence intervals) temperature difference of tank 1 (n = 6) when comparing the incoming ambient temperature to the end of tank after tanks have been chilled for 48-h per each degree difference (Repeated measures ANOVA, $F_{(4, 234)} = 851.61$, $p<0.001$).

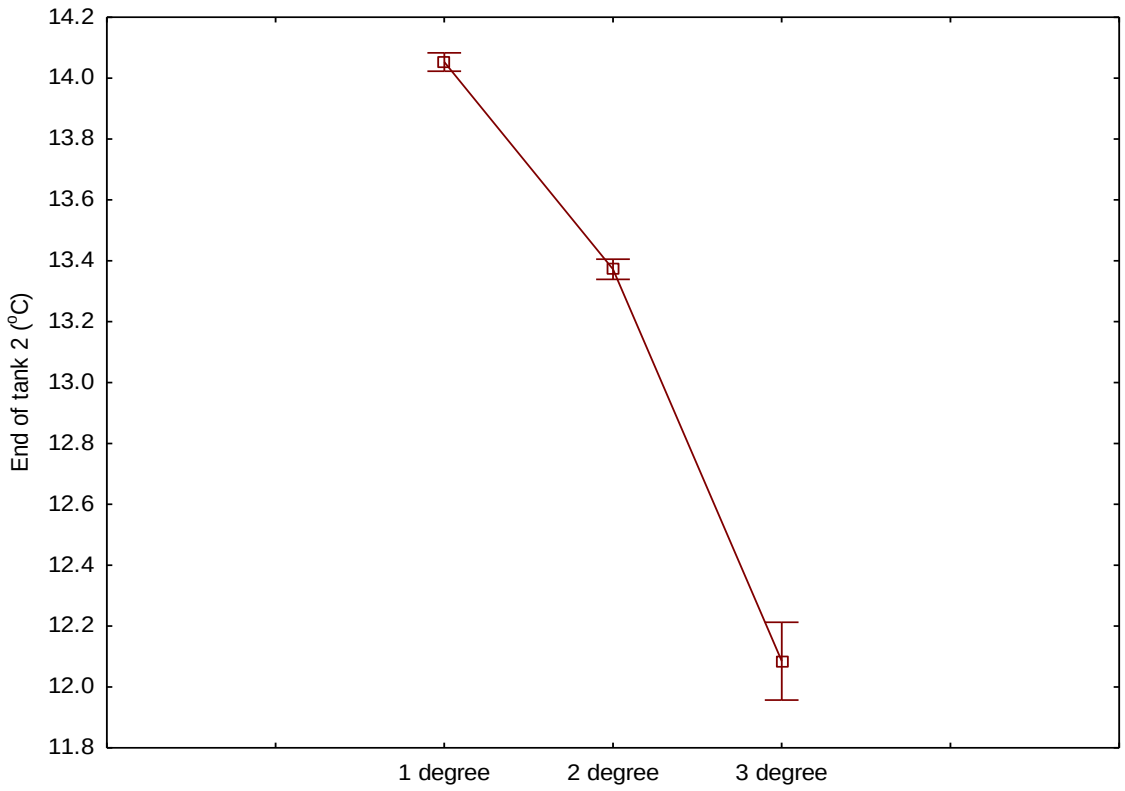


Figure 3.3: Mean (± 95 confidence intervals) temperature difference of tank 2 (n = 6) when comparing the incoming ambient temperature to the end of tank after tanks have been chilled for 48-h per each degree difference (Repeated measures ANOVA, $F_{(4, 234)} = 851.61$, $p<0.001$).

Flow rate 1: 37. 51 L/kg/h

Once the chilling system was switched on, both tank unit temperatures and outlet temperatures took 2.5 h in order to achieve the first temperature drop of the entire system. Flow rate (L/kg/h) for each treatment tank fluctuated throughout the entire trial duration, however, for tank 1, mean flow rates for the incoming ambient flow rate achieved reached 38.01 ± 1.19 L/kg/h and flow rates for Tank 2 reached 30.61 ± 1.17 L/kg/h. Mean flow rates for the control tank reached a mean 33.64 ± 0.41 L/kg/h. The highest temperature difference between the incoming ambient temperature and the end of tank 1 recorded was 1.05 °C, the lowest recorded was 0.67 °C, and the average temperature recorded was 0.82 °C. For tank 2, the highest temperature difference recorded was at 0.86 °C, the lowest temperature difference recorded was 0.67 °C, and the average temperature difference recorded was 0.68 °C.

Flow rate 2: 19.87 L/kg/h

Mean flow rate (L/kg/h) for each tank fluctuated throughout the entire trial duration, where for tank 1, mean flow rates achieved reached 19.47 ± 0.84 L/kg/h and flow rates for tank 2 reached 20.08 ± 0.83 L/H/Kg. The highest temperature difference recorded for tank 1 was 1.83 °C, the

lowest recorded was 1.44 °C, and the average temperature difference recorded was 1.67 °C. For tank 2, the highest temperature difference recorded was 1.73 °C, the lowest temperature difference recorded was 1.44 °C, and the average temperature difference recorded was 1.58 °C.

Flow rate 3: 8.57 L/kg/h

Mean flow rate (L/h/kg) for each tank fluctuated throughout the entire trial duration, where for tank 1, mean flow rates achieved reached 8.57 ± 1.17 L/kg/h and flow rates for tank 2 reached 5.91 ± 1.17 L/kg/h. The highest temperature difference recorded for tank 1 was 3.64 °C, the lowest recorded was 2.67 °C, and the average temperature recorded was 3.03 °C. For tank 2, the highest temperature difference recorded was 3.55 °C, the lowest temperature difference recorded was 2.58 °C, and the average temperature difference recorded was 3.00 °C.

3.3.2 Water quality parameters

There was a significant difference of the mean/median of the hydrogen ions (H^+) (M) of both the ambient and chilled tanks (Kruskal-Wallis ANOVA, $H(1, 298) = 51.71$, $p<0.0001$, Figure 3.4). Chilled tanks obtained a higher median H^+ ions of 0.91 ± 0.01 M, compared to ambient tanks, which obtained a mean H^+ ions of 0.90 ± 0.01 M, under normal farm conditions.

There was also a significant difference of the median/mean for both dissolved oxygen parameters (mg/L and %) when comparing both ambient and chilled tanks (Kruskal – Wallis; $H_{(1, 298)} = 116.36$, $p<0.0001$ and $H_{(1, 298)} = 4.14$, $p = 0.042$, Figure 3.5 and Figure 3.6, respectively). Chilled tanks for both dissolved oxygen parameters, reached a higher median/mean of 8.2 mg/L when compared to the ambient tanks, which reached a median/mean of 7.6 mg/L after 96 hours of purging. Dissolved oxygen saturation means did not differ, however DO saturation of chilled tanks reached a higher median of 94.5 %, compared to ambient tanks, which reached a saturation of 94.0 % after 96 hours of purging.

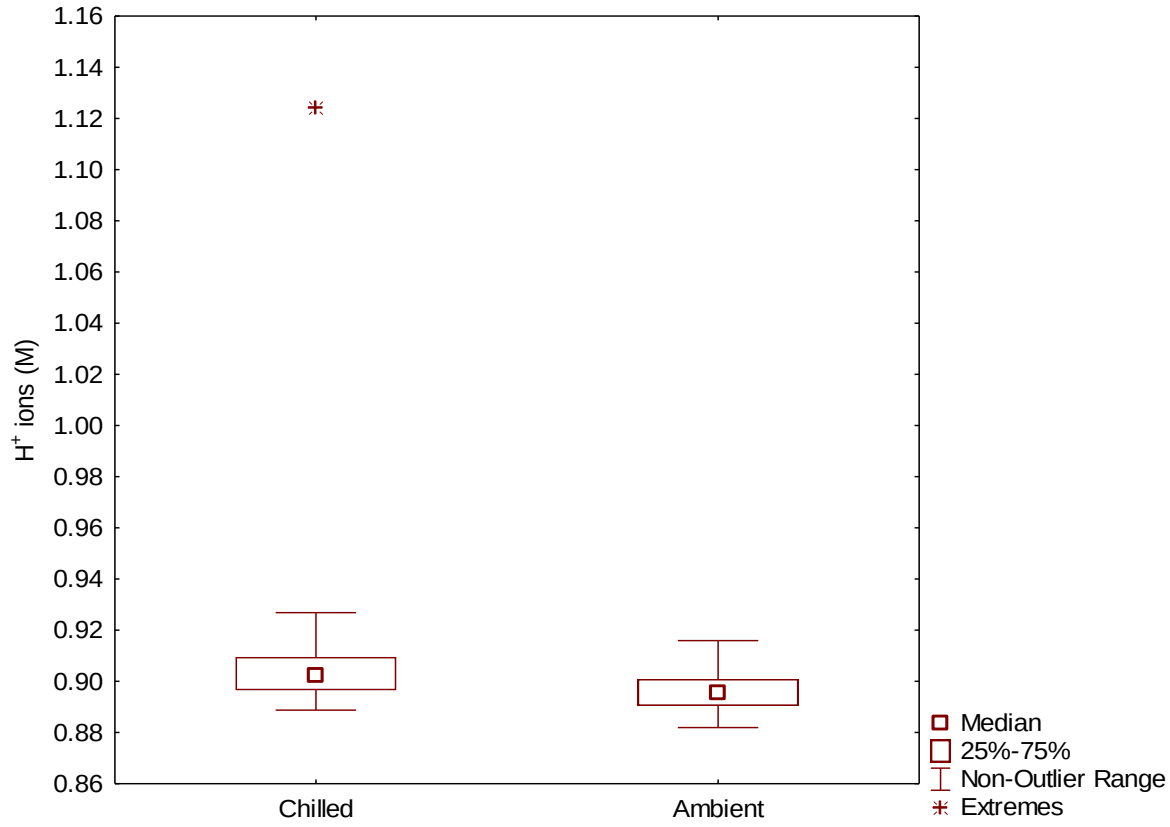


Figure 3.4: Median (± 95 confidence intervals) H^+ ions (mol. L^{-1}) of both chilled tanks (n =149) and ambient tanks (n =149) after 96-h purge (Factorial ANOVA, $F_{(1,294)} = 230.32$, $p<0.0001$).

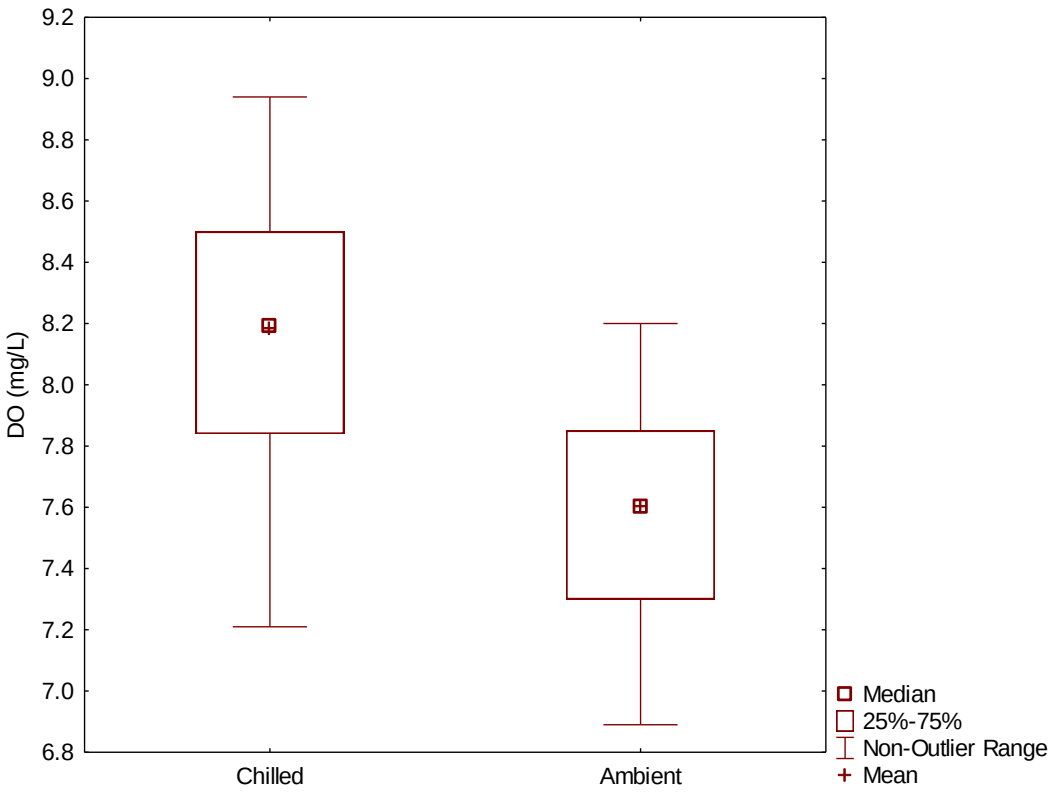


Figure 3.5: Mean (± 95 confidence intervals) dissolved oxygen (DO, mg/L) of both tanks that were chilled (n = 149) and left at ambient temperature (n = 149) after the full purging process (Kruskal Wallis, $H_{(1, 298)} = 116.36$, $p = 0.001$).

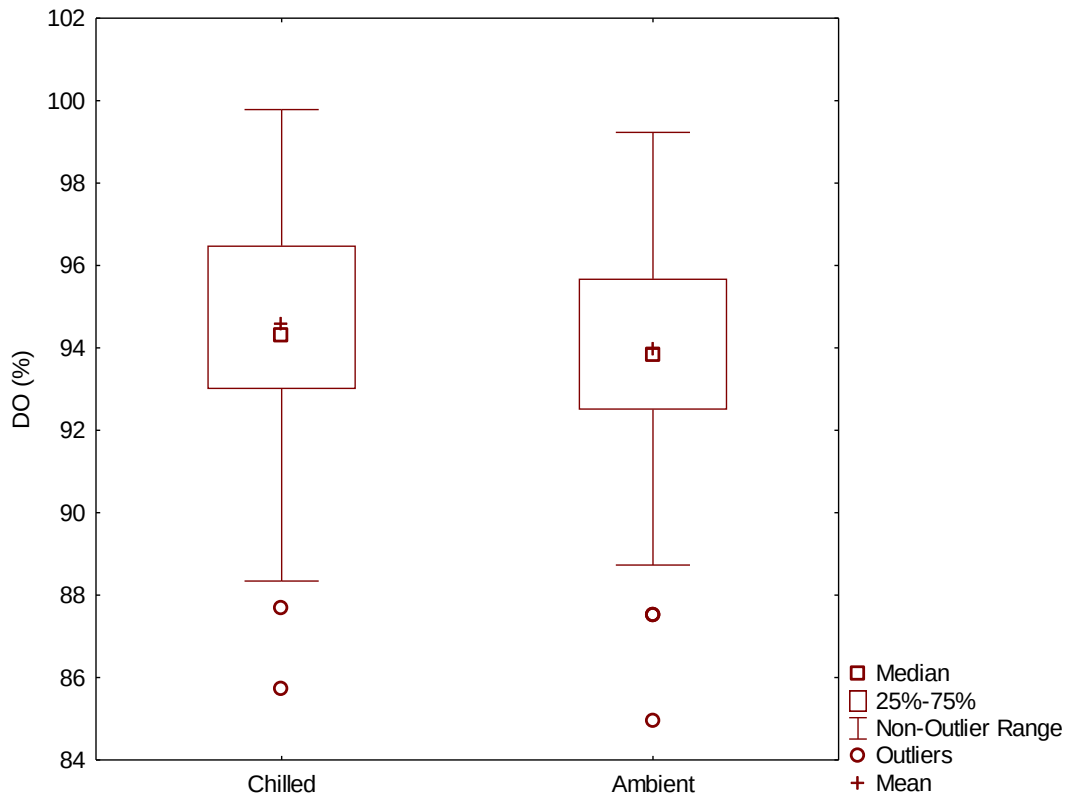


Figure 3.6: Median/Mean (± 95 confidence intervals) dissolved oxygen saturation (%) of both ambient tanks (n = 149) and chilled tanks (n =149) after the purging process of the experimental cooling system (Kruskal Wallis, $H_{(1, 298)} = 4.14$, $p = 0.042$).

3.4 Discussion

3.4.1 Cooling effectiveness of chilling system

The cooling capabilities of the chilling system was based on both structural features within the system itself and as well as the total velocity of the waterflow rate from the tanks to the system (Karaca *et al., 2016*). Within this trial, the cooling ability or rate of temperature decrease of each tank was the lowest with the lowest flow rate at 8.57 L/kg/h. This was similar to results by Karaca *et al.* (2016) that found that the cooling effectiveness values were lowest with the lowest water flow rate of 6 L min^{-1} m^{-2}.

The importance of pre-cooling crustaceans at a slow rate has been studied in multiple crustacean species, as it has been shown and demonstrated that a high fluctuation in sea temperatures during the purging process could result in variation of the survival rate and quality of meat (Kang *et al*., 2019; Bi *et al.,* 2023). This could also be applied to ectothermic molluscs, including abalone (Seuront *et al*., 2018). Implementation of a slow cooling method was of importance as rapid cooling has been demonstrated by Schoenfuss *et al.,* (2023) to result in thermal shock and increased mortalities of different fish species.

3.4.2 Water quality during the cooling process

Since abalone require both an optimal temperature range and a high-water quality (Bullon *et al.*, 2023), of dissolved oxygen (mg/L and %) and as well as pH (Ducharne, 2008), the provision and availability of these parameters during purging is of vital importance to increase chances of survival during transport (Barrento *et al.*, 2013).

As temperature decreases, its ability to dissolve into the water column increases and in turn can hold higher levels of dissolved oxygen (Ducharne, 2008), which is seen within this trial, where both chilled tanks reached a higher mean dissolved oxygen, for both mg/L and 100% saturation, at the end of each 48-h trial. The same was observed for pH, where chilled tanks reached a higher mean pH at the end of each 48-h trial, with each different flow rate. Temperature has a direct effect on the pH, where a decrease in temperature results in fewer H^+ ions being formed (Bandura and Lvov, 2006). Water quality parameters tested for were in the "safe" ranges of 8.4 (Shaw *et al.*, 2023) during production and harvesting, where pH values ranged between 7.30 to 8.50 in terms of optimal purging environment.

Additional oxygen was also provided in each tank in order for the minimum dissolved oxygen concentration levels to be higher than 6 mg/L (Gao *et al.*, 2022), where for this trial the lowest DO recorded reached 7.6 mg/L and highest reached 8.1 mg/L.

Pre-cooled tanks were also able to hold a higher mean H+ ion within the tanks, which could be due to the semi-recirculating system accumulating higher levels of sludge. As more time proceeds during the purging process, and as each degree difference gets higher and flow rates decrease, more discharged water is being recirculated, with no filtration system, by the chilling system and brought back to the purging tanks once water temperatures reached a desired minimum three-degree difference from the incoming ambient temperature from each tank. Due to this, more suspended solids are likely to accumulate, which can also affect ammonia (pH) concentrations in the water column. (Gao *et al.*, 2022).

Metabolomics could be used to measure stress associated with transport in future studies. Metabolomics is an indicator of the metabolic pathways and accumulation of different metabolic end products during periods of stress in invertebrates (Alfaro *et al.*, 2021). The production and distribution of different metabolic end products (pyruvates), including

tauropine and D-lactate, is species specific. Therefore, it has been said that the accumulation of both the D-lactate and tauropine in the muscles of abalone can provide a useful index of the level of metabolic stress, which can influence the quality and in turn effect the food value for export (Baldwin et al., 1996). This approach could be used in future studies.

3.5 Conclusion

Despite the innovative technology within aquaculture, the implementation of a cooling system within harvest and purging prior to export has still been limited. The implementation of a pre-cooling system within a slow pace, in order to decrease the likelihoods of animals experiencing a thermal shock, is possible dependent on the flow rate of each tank, using a semi-recirculating cooling system. Based on the results from this trial, the cooling effectiveness of the system was based on the maintenance of a specific flow rate in order to a achieve a degree difference of the overall temperature of the tank against the incoming ambient sea water during purging.

As animal production and harvest is dependent on good water quality, the implementation of a cooling system, without negatively influencing the water quality was of vital importance, where it can be concluded that the implementation of a cooling system would positively influence the oxygen concentration levels within the tanks during purging, which could improve purging conditions during summer export when unfavourable temperature conditions persist. As sludge build-up within the water column is a common attribute within the purging process, a higher accumulation of sludge, and in turn pH, within the chilled-treatment tanks is to be expected as a semi-recirculating system accumulates more sludge build up and in turn increases the pH values. This in turn could negatively affect the purging process, however since experiments were conducted on empty tanks, the effect it could potentially have on animals was not tested.

Results from this chapter will also be used to inform subsequent experimental chapters.

Chapter 4: Summer live export transport simulation

4.1 Introduction

In abalone aquaculture, a surplus production of 60% from total production is used for live export where prices may range between US$ 60 to 80 per kilogram (Venter *et al*., 2024). South African abalone (*Haliotis midae)* and Mexico's abalone (*Haliotis fulgens*) are among the top selling imported products in China's live produce markets, and fetch a higher price compared to other competitive countries such as New Zealand or Australia, due to their high quality and high demand (Oakes and Ponte, 1996; Vosloo and Vosloo, 2006).

Abalone, coupled with other crustacean species such as lobsters, are prone to experience different stress responses during transportation, which include, air exposure, salinity shock, physical injuries, overcrowding, temperature shifts, and low humidity and oxygen (Alfaro *et al.,* 2021). During the summer months, water temperatures in South Africa increase for a prolonged period of time with increased westerly winds along the coastline (Goschen et al., 2012), which in turn impacts production, harvesting, and purging prior to export as increased temperature increases abalone metabolic rate (Frederick et al., 2022; Bullen *et al*., 2023). Aerobic energy is used during periods of hypoxia, and this can cause cell and tissue damage due to changes in osmotic pressure (Venter *et al*., 2024). Different invertebrates have the capability of handling stressful environmental conditions, but the scope of survival is species specific. When exposed to air, abalone such as the *H. asinina*, reduce their oxygen intake (Baldwin et al., 2007) and in the case of prolonged increased temperatures, different species, including the pacific abalone (*H. discuss* hannai) shift their metabolic state and switch from aerobic to anaerobic metabolism (Kyeong, et al. 2020). During the transportation of live abalone and as well as increased temperature, aerobic respiration diminishes and in turn leads to anaerobic respiration, which then leads to the production of multiple different anaerobic glycose end products (O'Omolo et al., 2003).

Due to these stress responses, it is recommended to purge these species, coupled with pre-cooling, prior to packaging in order to reduce the amount of nitrogenous waste that is produced. This further reduces the level of stress that these species may encounter during packaging and transportation associated with exportation (Currie, *et al*., 2015). Pre-cooling is also recommended as a lowered body temperature reduces crustacean's and mollusc's standard

metabolism and in turn optimizes their survival rate (Karanova and Gakhova, 2007). Within South Africa, cultivated abalone currently follow these recommended procedures of purging abalone prior to packaging, however live abalone are still experience different complications due to transportation. This includes an increase in their weight loss by 4 – 15 % and a decrease in their survival rate, meat quality, and health after 30 to 42-h of transport (Francis, 2008). Currently there are no recommended standard operating procedures in place to reduce mortalities and weight loss, and that ensures a healthier looking animal upon arrival.

4.1.1 Aims and Objectives

The aim was to investigate the effect that different pre-transport conditions including different temperature treatments, through pre-cooling, different purge days, different abalone sizes, and different ice volumes, have on the change in weight of abalone, and as well as their survival, their appearance upon arrival (through behavioural analysis), and as well as their health, through histology analysis. The main objectives for this research were to compare abalone survival, weight loss, behaviour, and their health to the different pre-transport conditions; temperature, purge days, ice volumes, and size class.

4.2 Materials and methods

Abalone were cultivated and farmed at Aqunion (Pty) Ltd, Romansbaai Farm. Prior to abalone being chosen for the experimental trials and pre-transport conditions, they were kept in aerated flowthrough concrete tanks (16 m^3) with an average incoming ambient sea temperature ranging between 18 to 22 °C. Tank water quality, including the water temperature, dissolved oxygen (DO; mg/L and %), and pH were checked twice daily. Prior to the start of the trial, they were left unhandled for a minimum of 30-d and left unfed for 24-h ahead of the experiment.

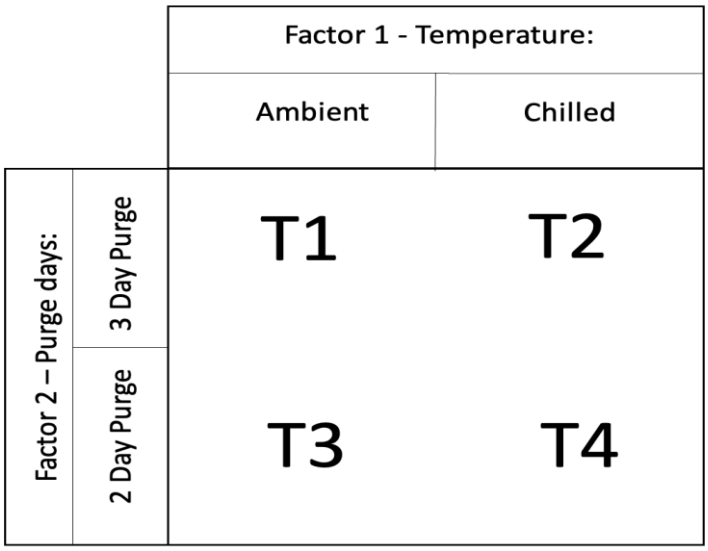
4.2.1 Experimental species

Roughly 9,600 randomly selected mature South African abalone (*H. midae*) were collected with two different size classes range, one with an average weight of 51.5 ± 0.71 g (i.e., 50 – 60 g) with a total of 6,600 animals, and the other with an average weight of 98 ± 1.4 g (i.e., 90 – 110 g) with a total of 3,000 animals.

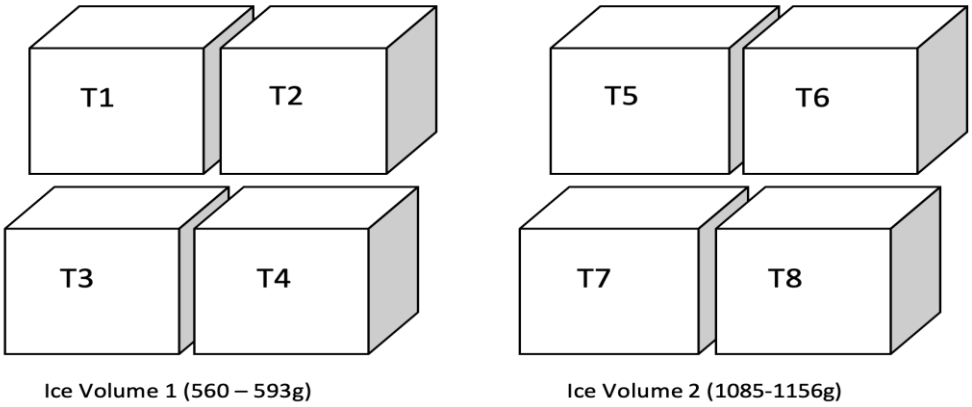
4.2.2 Experimental treatment

There were 16 treatments in this experiment, which made a 2 x 2 x 2 x 2 multifactor experimental design (Figure 4.1). Prior to packaging and transport, the abalone were held at one of two temperatures: (a) chilled water or (b) ambient seawater temperature (i.e., a control of 18 to 22 °C; Figure 4.1). The water in the chilled treatment was cooled by approximately 2 to 4 ºC using a semi-recirculating flowthrough system (Chapter 3, Section 3.2.2). The abalone in each of these treatments were purged for either (a) two days or (b) three days prior to handling and transport (Factor 2; Figure 4.1). When the abalone were packed, two different volumes of ice-packs were included in each box of abalone: (a) 504 – 593 g of ice per box or (b) 1085 – 1156 g of ice per box (Figure 4.1). The eight treatments described above were carried out using abalone of two size classes, either (a) 50 – 60 g abalone or (b) 90 – 110 g abalone (Figure 4.1). These four factors made the total of 16 treatments. Each of these treatments were represented by three replicates per treatment, where one box of abalone represented one independent replicate. The experiment included a total of 96 boxes of abalone.

(A) Factor 1 – Temperature; and Factor 2 – Purge days:



(B) Factor 3 – Ice volume:



(C) Factor 4 – Abalone size:

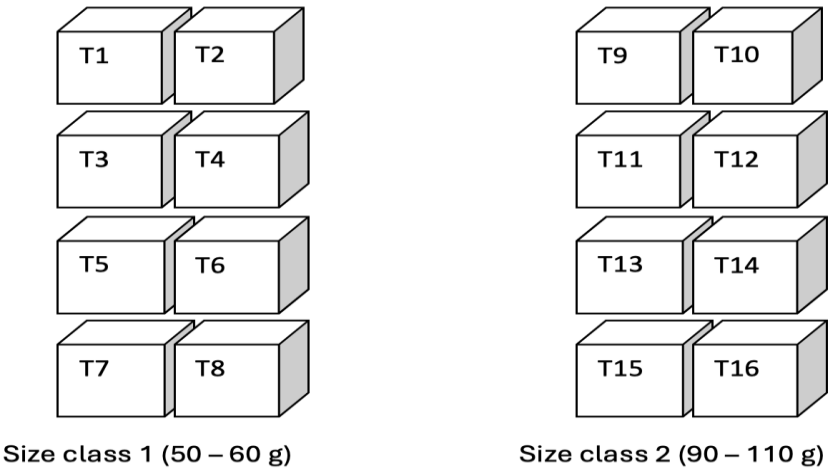


Figure 4.1: There were 16 treatments that made up the 2 x 2 x 2 multifactor experiment. The design included (A) Factor 1 which was one of two pre-transport temperatures (chilled and ambient), each of which was represented by Factor 2 which was one of two pre-transport purge days (two- and three-days purge), and these made up four treatments (T1-T4). (B) Each of the treatments in "A" were subject to Factor 3, which was one of two ice volumes: Ice volume 1 (T1-T4) or ice volume 2 (T5 - T8). Factor 4 was abalone size. The first eight treatments were represented by abalone size class 1 (T1-T8) and size class 2 (T9-T16). Each of the 16 treatments were represented by three replicates, where a box of abalone was considered one independent replicate, which resulted in a total of 96 boxes of abalone.

4.2.3 Experimental system: Live transport simulation

The same packaging facilities and packaging routine that was presented in Section 2.2.2 (Chapter 2) were used here, with the exceptions presented in Section 4.2.2. One temperature data logger (Logtag USB temperature recorder or Logtag coupler temperature recorder) was then added to each box to monitor the temperature fluctuations during transport simulation.

The transport simulation data collected in this experiment were collected from actual farm transport simulations, which form part of routine farm practice. Therefore, the method used in collecting these data were prescribed by the transport simulation procedures that were used on the abalone farm. After 42-h, the boxes were opened and various data were collected from each box.

The boxes of abalone were subject to five phases for a routine "mock export" conditions, while in the temperature-controlled room (Figure 4.2):

- Phase 1 - Transit to the airport: animals were stored at temperatures set between 10 - 11°C for two and half hours;

- Phase 2 - Offloaded and then stored at the airport ahead of boarding the aeroplane: boxes were exposed to ambient temperature (6.3 – 15.5 ºC) for six to eight hours;

- Phase 3 - Boxes loaded onto the plane: stored at a set temperature between 5 – 10°C.

- Phase 4 - Airborne transportation: stored between 5 – 8 °C for 18 h;

- Phase 5 - Arrival at desired destination: boxes stood for an average 10 – 15 h at ambient temperature (30 °C).

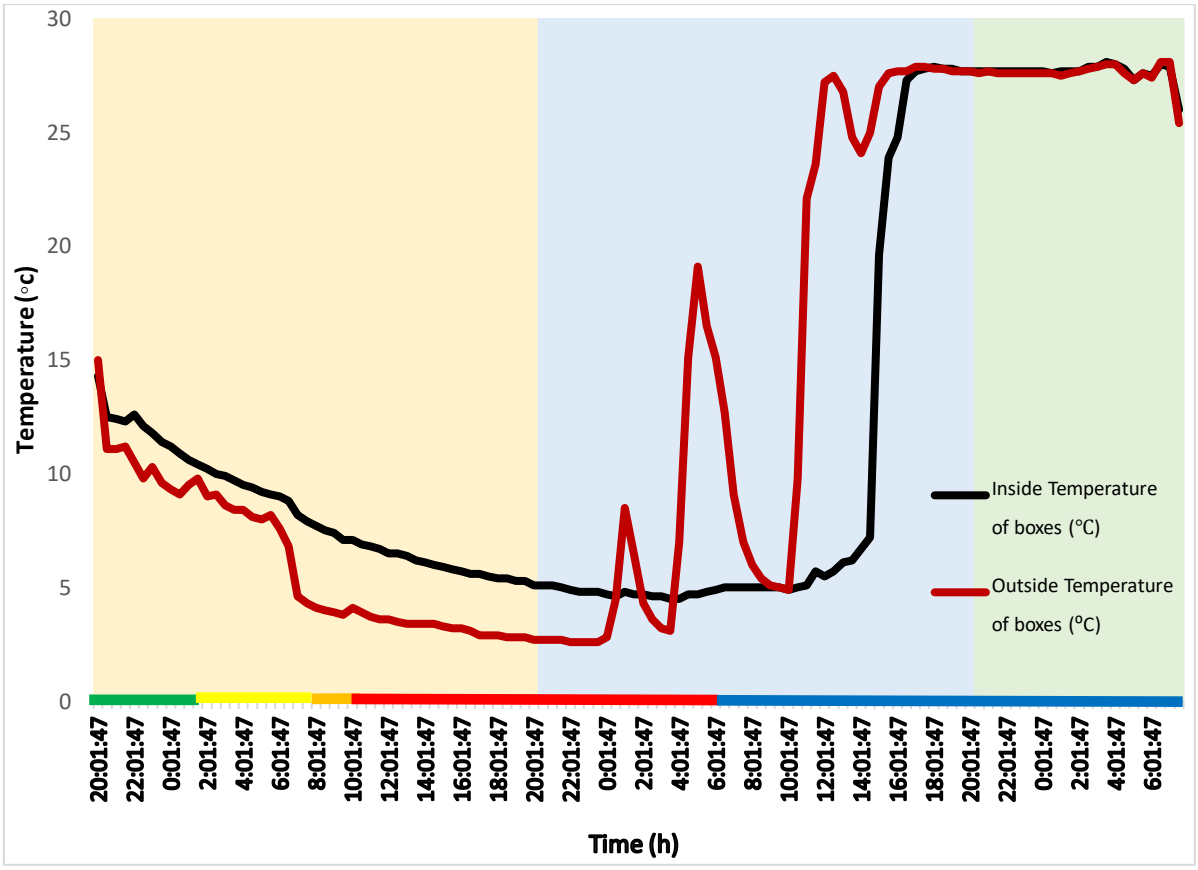


Figure 4.2: Temperature fluctuations from both the internal and external areas of one box throughout the entire export procedure. Green duration reflects the time when boxes were within the truck transit phase. Yellow indicates when boxes were within the check-in phase (waiting to be loaded). Orange indicates when boxes were loaded onto the plane, red indicates aerial transit, and blue indicates when boxes arrived at their intended destination until boxes were opened.

4.2.4 Data collection

4.2.4.1 Water quality during purging

Water quality parameters, including both the dissolved oxygen (DO) levels (mg/L and %), and pH, of all four tanks used during purging, were collected with the same methods and equipment as described in Chapter 2 (Section 2.2.3). The same water quality ranges, for an optimal purging environment (Shaw *et al.*, 2023), remained the same for duration of this experimental trial.

4.2.4.2 Post transport simulation

All data collected, including the temperature of both the ice and animals (°C), the change in both net weight and individual weight (g) from when animals were packed to when they were unboxed 42 hours post the transport simulation, excess and insufficient biomass (%), weight loss (%), water loss (g), and the initial survival (%) upon arrival, followed the same methods and data collection procedures from Chapter 2 (Section 2.2.3).

4.2.4.3 Behaviour

All behaviour parameters tested, including the righting time (with the exception of the side-to-side movement behavioural test), adhesive force (N), initial movement (s), and change in speed from the middle of the top plate to position 1, and from position 1 to position 2 (mm s^{-1}), were all measured in the same as Chapter 2 (Section 2.2.3).

Side to side movement

During the righting behavioural test, the four random selected individuals placed shell side up, were also used to record the number of times individuals move in a "side to side" behaviour within five minutes. Individuals which were placed in a stationary position (Figure 4.3a) consisted of the head of the abalone being in the same position with the foot of the animal, either relaxed or extended. Animals in a side position (Figure 4.3b) consisted of the foot of the animal being placed either on the left or the right from the centre of the shell or perpendicular to the head of the animal. The number of times each animal moved from a stationary position to a side position and back was recorded for the total five minutes.

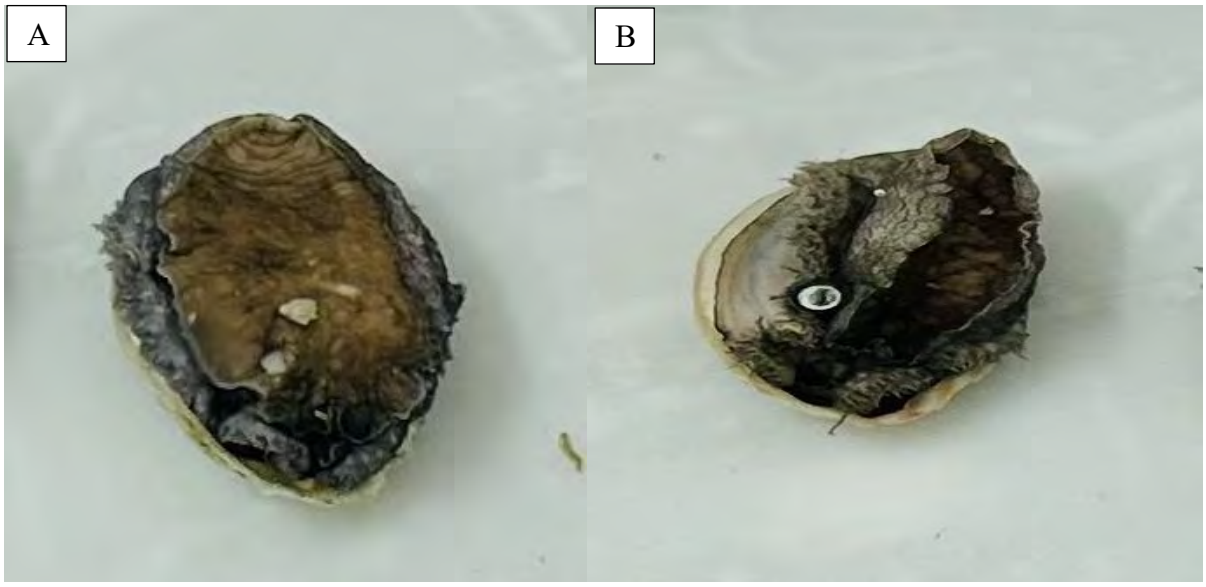


*Figure 4.3: Side to side movement of South African abalone (*H. midae*) during the righting behavioral test. A) depicts the abalone foot during the stationary position and B) depicts the abalone foot within a "side" position.*

4.2.4.4 Histology

The final dataset that was collected consisted of the abalone's gill, digestive gland, and kidney tissue samples, where they were analysed at Amanzi biosecurity following standard operating procedures in order to dissect and remove each tissue sample. After two randomly selected

individuals, from each box from both size classes, were weighed and their shells shucked. Their organs were then located and made visible in order to dissect a small portion of their tissue. Once the gill, digestive gland, and kidney tissues were located, they were then dissected even further using a scalpel (LASEC dissection kit, 9-piece set) and a piece, no bigger than 5 mL, was removed and placed into the same cassette (41.5 x 28.5 x 5 mm), per animal. Once each cassette contained each tissue sample, they were then closed shut and placed into 1L plastic jar containers with a maximum of 16 different cassettes within each eight containers. A preserving agent (Davidson's fixative), which was also supplied by Amanzi Biosecurity, was then poured into each container, just enough to submerge all of the cassettes. Each dissected animals' shell with its remaining tissue was then discarded within mortality bins, and placed within a deep freezer. The samples were then sent to Amanzi biosecurity in Hermanus, within 24-h from collection, where they were processed and made into histological slides for a light microscope.

Histology observation for abalone gills, digestive gland, and right kidney were conducted using a light microscope, specifically the Olympus IX50 microscope, with a 4x lens objective provided by the physics department of Rhodes University, South Africa. Images of abalone gill lamellae, filaments, epithelial cells, digestive gland tubules and epithelial cells, and right kidney tubules with epithelial cells, were all then photographed using an attached AxioCam ERc5s camera. Data collection and analysis was done by both a light microscope analysis computer package at the physics department or by Image_J, which is a free downloadable software package for image analysis, complete with measurement and counting tools (Schneider *et al*., 2012). In this research abalone tissue from each slide had a total of 5 pictures taken randomly, where in each image taken, a set of variables was collected per tissue. This data was taken using Image_J, where 4 quadrates were chosen from each picture (1^{st} quadrate = top left area, 2^{nd} quadrate = bottom left area, 3^{rd} quadrate = bottom right area, and 4^{th} quadrate = top right area) and two random measurements of the shortest and longest observed lengths between two cells were taken and then averaged. After all measurements were collected from each quadrate, averaged measurements were again averaged combined with the other quadrates to use as the data for the average distance between cells for each picture.

The average damage observed for each treatment were then established and compared with the rest of the treatments to compare the severity of the damage when subjected to different pre-transport conditions.

Gills

Two different changes in gill tissue (stress indicators) parameters, specifically the occurrence or percentage of haemolymph infiltration (%) and the size of the gill epithelial lamellae (µm), were measured and collected through histological slides, in order to determine if there is a significant difference between the amount of damage abalone experience within the different treatment scenarios during live export. Each gill lamellae, with both the skeletal rods and goblet cells, were counted within each slide, where the total number of goblets cells were counted as well as the number of goblets cells which had haemolymph filtration within the goblet cells. The percentage of haemolymph infiltration was then calculated using formula HI (%), where H0 = number of goblet cells with haemolymph infiltration, and H1 = the total number of goblet cells within each slide.

$$HI = \left(\frac{H0}{H1}\right) * 100 \qquad \text{Equation 4.1}$$

Within each slide, the gill epithelial lamellae thickness (µm) was measured randomly, specifically the middle area of each randomly selected gill lamellae. The end of each lamella, from one end to the other (width) was measured and the average size was established for both size classes and also to determine the difference in size between the different treatments.

The grading damage score of the gills of abalone was based on the number of different stress indicators observed and as well as the frequency of occurrence of each specific stress indicator (Hooper *et al*., 2014). A grading score from 0 – 3 was selected for the Gleason's damage grading pattern to assess the overall damage level of each tissue from each different treatment (Copedo *et al*., 2023). A "0" (least severe) grading score indicates no or very minimal stress indicators and frequency of occurrence observed, and tissue closely resembled a healthy and undisturbed abalone gill tissue. A "1" grading score represents an observation of a slightly higher frequency of any one or two stress indicators, which can also be accompanied with a change in overall colour H&E staining within the slide, which is an indication of a burst tissue and free fluid within the slide (Hooper *et al*., 2014; Copedo *et al*., 2024). A "2" grading score was classified with a higher count of stress indicators of either two or more, and as well as a higher frequency of occurrence across the tissue. observation of these different stress indicators consisted of at least two or more, where it is normally accompanied with more deterioration of the tissue. The third and final damage grading score was a combination of all the stress

indicators with the highest level of tissue damage and deterioration observed, where little to no tissue can be separated and distinguished between each other.

Digestive gland

The digestive gland damage grading score was also based on 0 – 3 criteria to assess the overall health of the tissue. "0" score was similar to the gills grading score, where no change or very minimal change is observed with the tissue and any one of the stress indicators is barely identified within the slide. "1" score was observed where one or two different stress indicators were observed, where the degradation of the epithelial cells was low and highest seen was 0-10% degraded. "2" score had more different stress indicators observed and a higher level of epithelial cell degradation ranging from 0-10 % and 10-20 % degraded. The "3" final score consisted of all the stress indicators observed, with the exception of the presence of the brown pigmentation, where the level of degradation of the epithelial cells of the tubules ranged between 20-40 %. Distinction of the different characteristics of the digestive gland was also difficult and not possible for a few slides.

Right kidney

Damage grading score was based on the overall health of the right kidney tubules, where each grading score compensated the observation of different stress indicators and as well as the frequency of observed stress indicators across each image.

Within the damage grading score, "1" score consisted of majority of the tubules having one or two stress indicators, mainly the observation of enlarged tubules and a subdued degradation of the epithelial cells. "2" grading score consists of an increased observation of both stress indicators mentioned and as well as another stress indicator observation, including the variation in lumen distancing of majority of tubules. The final "3" grading score consisted of the observation of all the stress indicators including a higher frequency and deterioration of majority of the epithelial cells of the tubules.

Each observed stress indicator, within the gills, digestive gland, and right kidney were also measured and compared to the different treatments and as well compared to healthy histological slides of abalone left undisturbed or handled for at least two weeks prior to dissection (Section 1.2.8).

4.2.5 Statistical analysis

All Data was analysed using statical computer software (Statistica, version 14.0.0) or Microsoft Excel (16.54).

Statistical analysis on the water quality, including both dissolved oxygen parameters (mg/L and %) and the pH were compared using a multifactorial analysis of variance (ANOVA), at $p<0.05$. Different purging temperatures were considered as one factor (Factor 1), with different purge days as another (Factor 2), with abalone size as the final factor (Factor 4). If there was no significant interaction between the factors, then means of each factor were compared.

For the farm export temperatures within boxes(°C), the transport simulation temperature of boxes (°C), the change in both net weight (g) and individual weight (g), the change in survival rate during recovery (%), and the speed animals used to move off a top plate (mm s^{-1}) were compared using the repeated measures analysis of variance (ANOVA) at $p<0.05$. For all temperature profiles, the change in temperature was analysed across the five different export phases; boxes in truck transit, boxes checked in, boxes loaded, boxes during aerial transit, and boxes upon arrival. The change in net weight and individual weight was compared from when animals were packed, post purging, and then 42-h post the transport simulation. Survival during recovery was compare over a span of twelve days. Speed was lastly compared from when animals had their first initial movement until animals moved across both positions on the top plate. All statistical analysis was compared with different purging temperatures as one factor (Factor 1), different purge days, as another factor (Factor 2), different ice inclusion as the third factor (Factor 3), and abalone size as the final factor (Factor 4).

Majority of the post simulation, including the ice temperature, the temperature of the animals, the excess and insufficient biomass of animals, the net weight upon arrival, weight loss (%), water loss (g), initial survival (%), all behaviour parameters, excluding righting behaviour, and as well as all health parameters were analysed using a multifactorial analysis of variance (ANOVA) at $p<0.05$. Different behaviour parameters consisted of their adhesive force, initial movement, and their side-to-side movement. The different health parameters consisted of different analysis of the gills, digestive gland, and right kidney, where comparison was done with different acclimation temperatures used as the first factor (Factor 1), different purging duration as the second factor (Factor 2), different ice volume inclusions as the third factor (Factor 3), and abalone size as the final factor (Factor 4).

If assumptions for all statistical analysis did not meet the levene's test of homogeneity of variance, then the data was logged transformed and then used for analysis. If assumptions were still not met, then a non-parametric Kruskal Wallis test was then used in order to test the differences of the dataset between the different treatment factors with unequal samples sizes.

For all the repeated measures analysis of variance (ANOVA) that did not meet their assumptions, then the mauchly's sphericity test was then used to test if the assumption of sphericity was met.

4.3 Results

4.3.1 Purging

4.3.1.1 Water quality

There was a significant difference of the median/mean of both dissolved oxygen parameters, (mg/L and %), when comparing different acclimation temperatures (Factor 1) after purging (Kruskal Wallis, $H_{(1, 84)} = 33.08$, $p<0.001$, Figure 4.4 and $H_{(1, 84)} = 20.59$, $p<0.001$, and Figure 4.5). Tanks subjected to a chilled purging temperature were able to reach and maintain a higher median/mean dissolved oxygen of 7.57 ± 0.06 mg/L during purging, compared to ambient tanks, which obtained a median/mean dissolved oxygen of 7.00 ± 0.06 mg/L. Chilled tanks also obtained a higher median/mean DO saturation of 96.24 ± 0.34 % compared to ambient tanks, which had a mean saturation of 93.72 ± 0.34 %.

There was also a significant difference of the median H^+ ions with different acclimation temperatures (Factor 1) (Kruskal Wallis, $H_{(1, 84)} = 04.67$, $p = 0.031$, Figure 4.6). Ambient tanks obtained a higher concentration of H^+ ions and median values were 36.76 mol L^{-1} compared to chilled tanks, which obtained a median H^+ ion value of 48.24 mol L^{-1}, during the purging process.

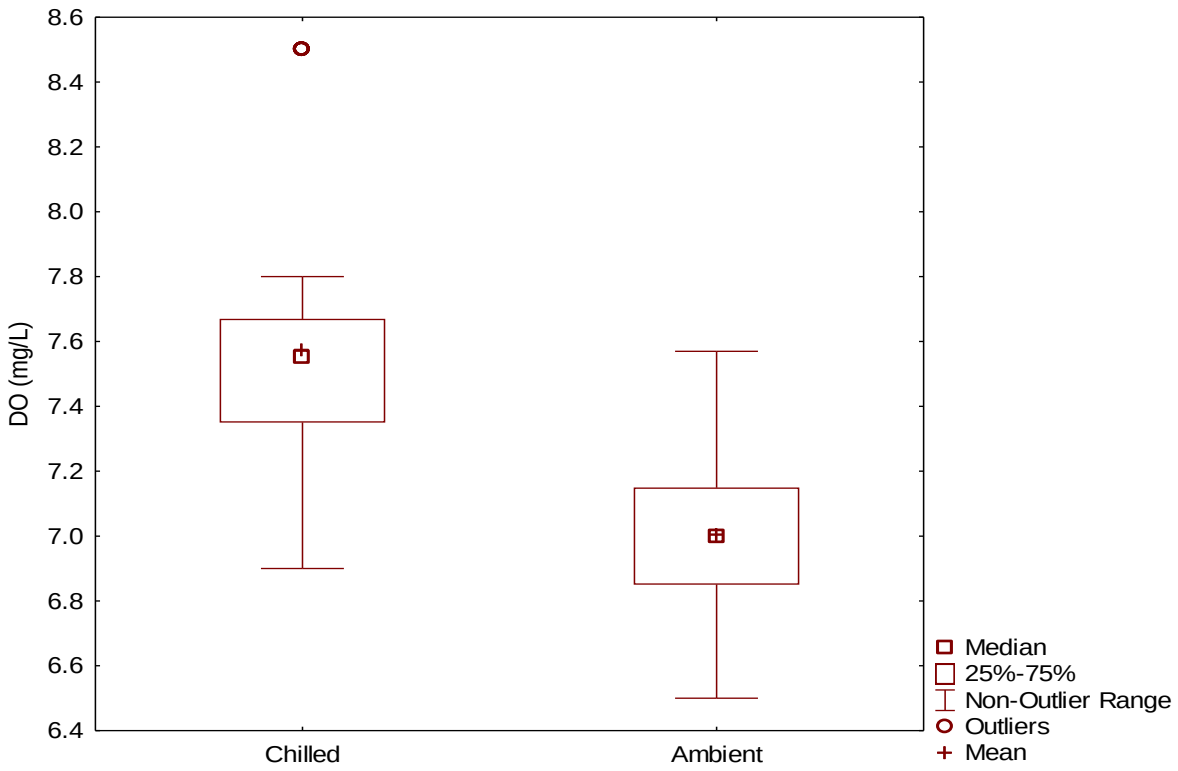


Figure 4.4: Mean/Median distribution of animals subjected to both a chilled purging environment (n = 42) and animals subjected to a high temperature purging environment (n = 42) (Kruskal Wallis, $H_{(1, 84)} = 33.08$, $p<0.001$).

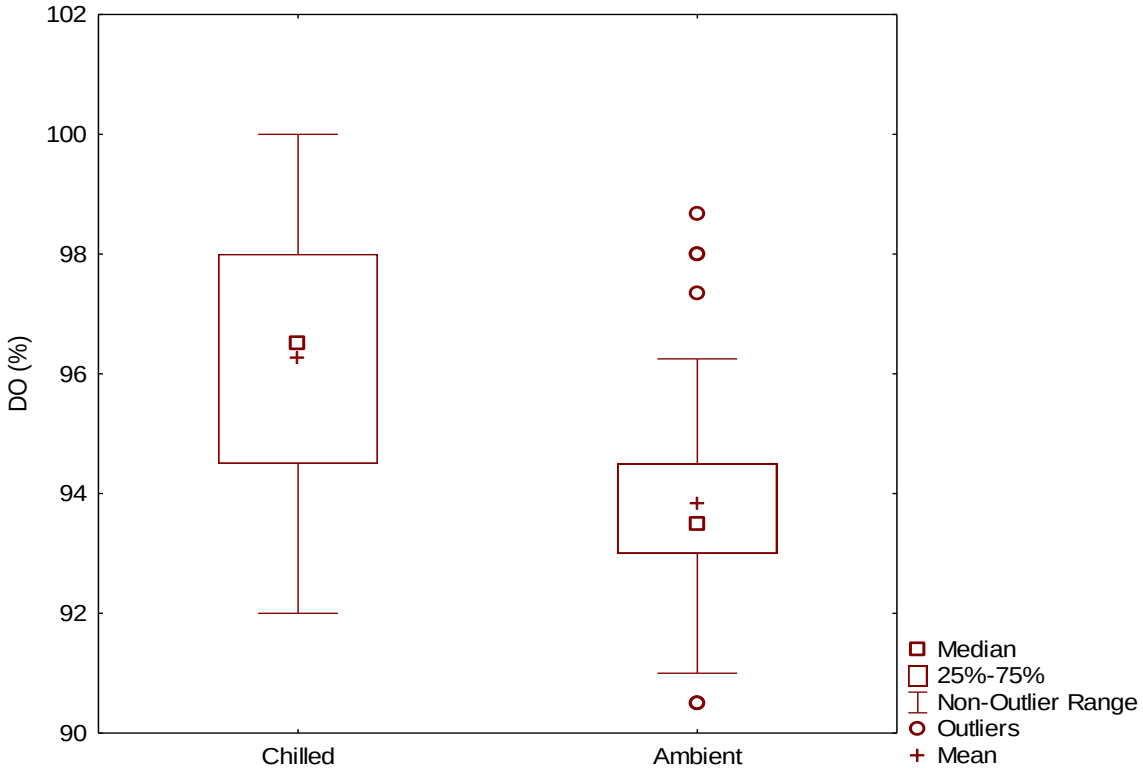


Figure 4.5: Mean/ Median distribution of animals subjected of animals subjected to both a chilled purging environment (n = 42) and animals subjected to a high ambient purging temperature (n = 42) (Kruskal Wallis, $H_{(1, 84)} = 22.59$, $p<0.001$).

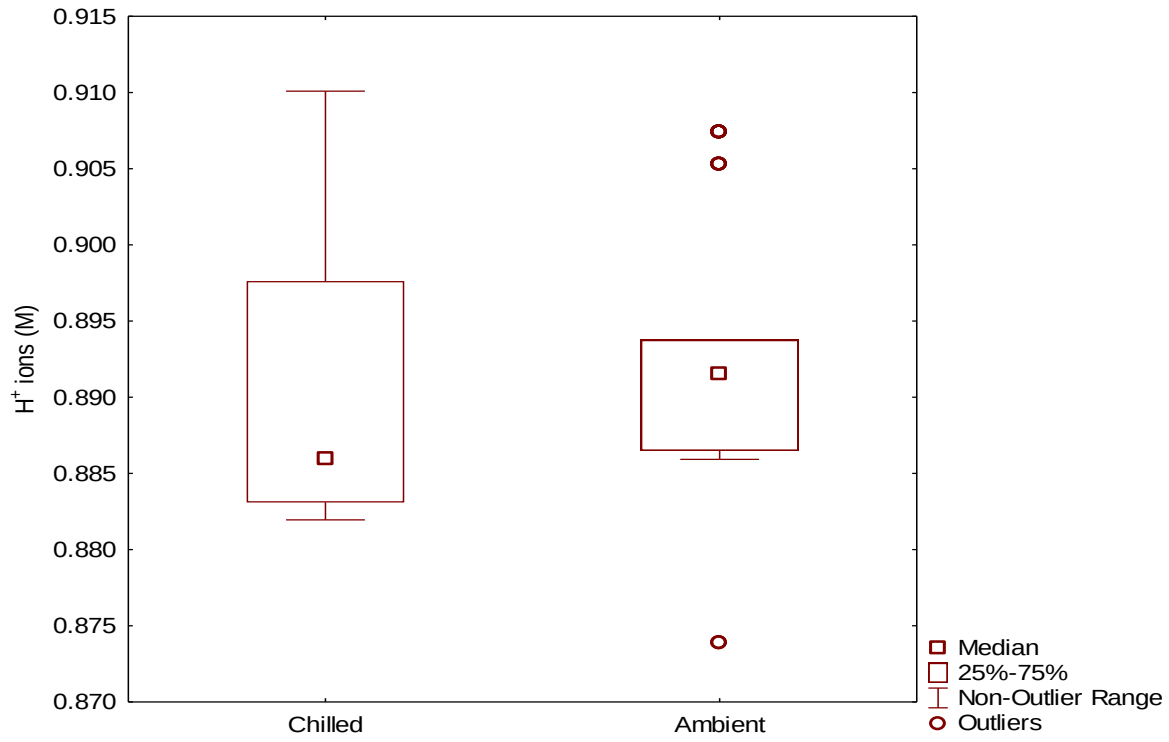


Figure 4.6: Mean/ Median distribution of animals subjected to a chilled purging environment (n = 42) and animals subjected to a high ambient purging temperature (n = 42) prior to the 42-h transport simulation. (Kruskal Wallis, $H_{(1, 84)} = 4.67.$, $p = 0.031$).

4.3.2 Export temperature profiles

4.3.2.1 Standard farm export temperature outside of boxes

Mean air temperature obtained was 13.43 ± 1.37 °C during phase 1 of export. Mean temperature during phase 2 of export, when boxes were checked in, stored and waited to be loaded onto the plane, was 10.98 ± 1.76 °C. During phase 3, when boxes were then loaded onto the plane, mean temperature obtained was 10.43 ± 1.85 °C. The exterior temperature of boxes during the 18-h flight duration was of 9.55 ± 3.83 °C. Upon arrival, at the final export phase, boxes were stored at a mean ambient air temperature of 23.03 ± 4.32 °C, from when boxes arrived at their destination until boxes were opened.

4.3.2.2 Standard farm export temperature inside of boxes

There was a significant difference of the change in temperature of boxes were in all five different phases of export (Repeated measures ANOVA, $F_{(4, 12)} = 37.85$, $p<0.0001$, Figure 4.7). During phase 1, internal box mean temperature obtained was 15.23 ± 0.13 °C during the 2-h truck transit. During phase 2, when boxes were checked in and left at ambient air temperature, mean temperature of boxes obtained was 13.18 ± 0.43 °C. During phase 3, when boxes were loaded onto the plane, mean temperatures obtained was 12.05 ± 0.63 °C. During phase 4, when

boxes were in aerial transit for 18-h, mean temperatures obtained was 10.44 ± 0.81 °C. Lastly, during phase 5, upon arrival, temperature of boxes was higher and was 15.40 ± 0.06 °C.

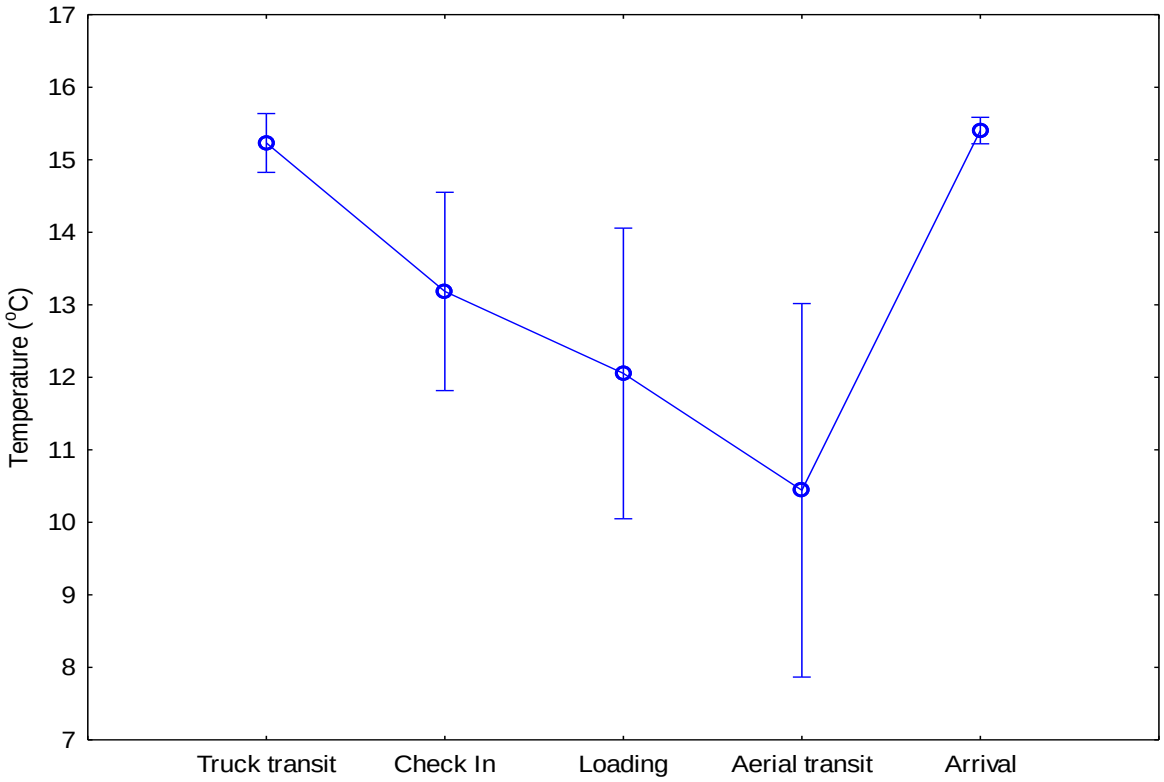


Figure 4.7: Mean (± 95 confidence intervals) temperature profile inside the boxes during each different transportation phase of a real-time routine export process (n = 14) (Repeated measures, $F_{(4, 52)} = 459.04$, $p<0.0001$).

4.3.2.3 Transport simulation temperature outside of boxes

Outside temperatures for the first phase of export, when boxes were transported by a truck in a temperature-controlled room, obtained a mean temperature of 14.30 ± 4.28 °C. Temperatures for the second phase of export, when boxes were checked in and stored at ambient temperatures for a duration of 6 h, obtained a mean temperature of 18.37 ± 0.80 °C. During the third phase of export, when boxes were loaded onto the plane and were stored at a mean ambient temperature of 11.54 ± 0.03 °C, where under normal export conditions, the loading duration lasts a minimum of an hour, however for this trial, loading lasted a total duration of 10 minutes. For the fourth export phase, where boxes were stored within the temperature-controlled air craft for a total duration of eighteen hours, mean temperatures obtained was 8.60 ± 0.23 °C. For the final fifth phase of export, when boxes reached their desired destination (which is export dependent) and were left at an ambient temperature of 19.35 ± 4.90 °C until boxes were opened.

4.3.2.4 Transport simulation temperature inside of boxes

The internal temperatures in the boxes differed significantly between for treatments with different acclimation temperatures (Figure 4.8), different ice volumes (Figure 4.9) and different purge days (Figure 4.10; Repeated Measures ANOVA, $p < 0.05$). Internal box temperature dropped progressively over the duration of the simulated transport, from about 14.75 ± 0.29 °C at the start to 10.53 ± 0.29 °C by the end of the aeroplane journey, and it subsequently increased to around 15.17 ± 0.56 °C on arrival at the destination. This trend was the same for boxes of abalone that were subjected to ambient and chilled preparation ahead of transport (Figure 4.8), for boxes with different volumes of ice (Figure 4.9) and for boxes of abalone subjected to different number of purge days (Figure 4.10). By the end of the aerial transit the temperature in boxes with more ice was significantly cooler than those with less ice (Figure 4.9). With the exception of the arrival phase of simulated transport, the internal temperature of abalone subject to longer 3-day purge were significantly cooler than those subject to a 2-day purge ahead of transport (Repeated measures, $F(8, 40) = 3.69$, $p = 0.003$, Figure 4.10).

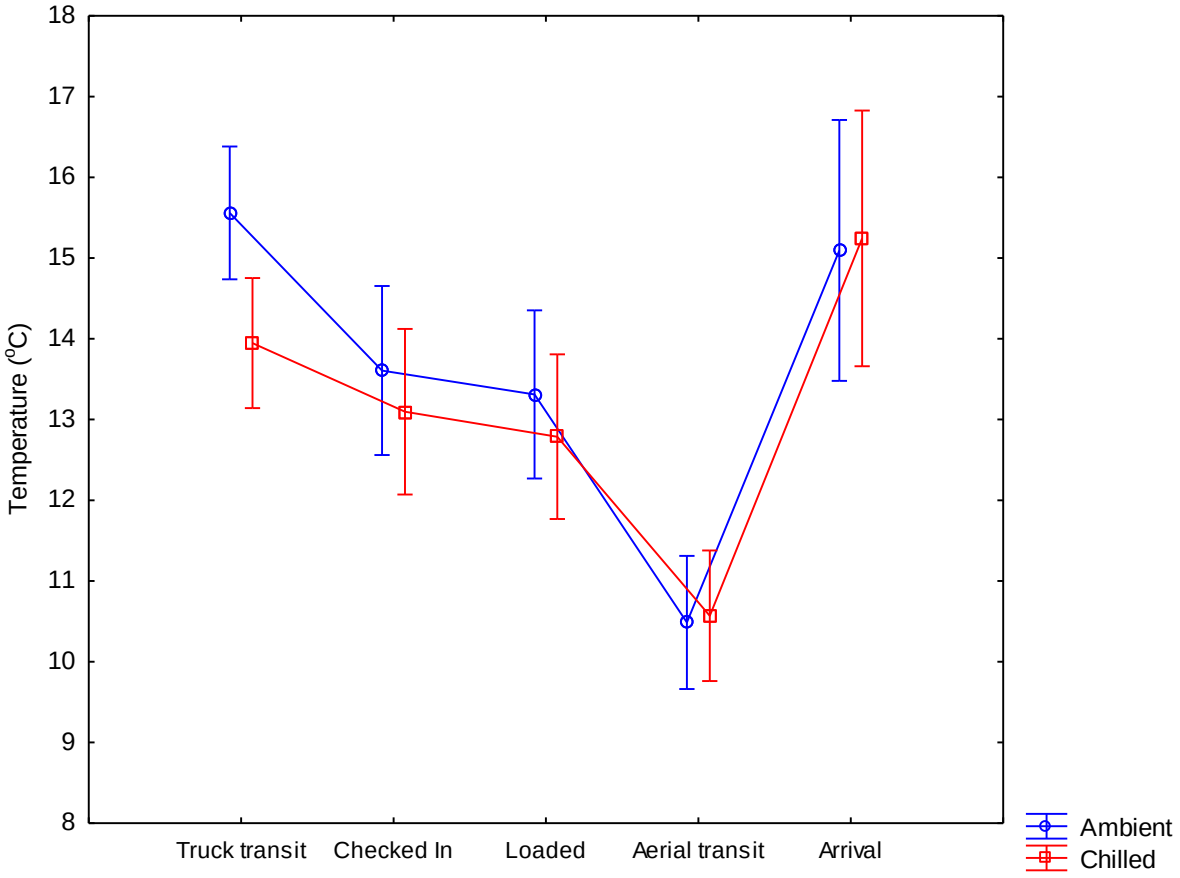


Figure 4.8: Mean (± 95 confidence intervals) temperature of boxes, with animals subjected to both a chilled purging environment (n = 32) and high ambient purging temperature (n = 31), within each of the five different export phases (Repeated measures ANOVA, $F_{(8, 40)} = 5.62$, $p<0.001$).

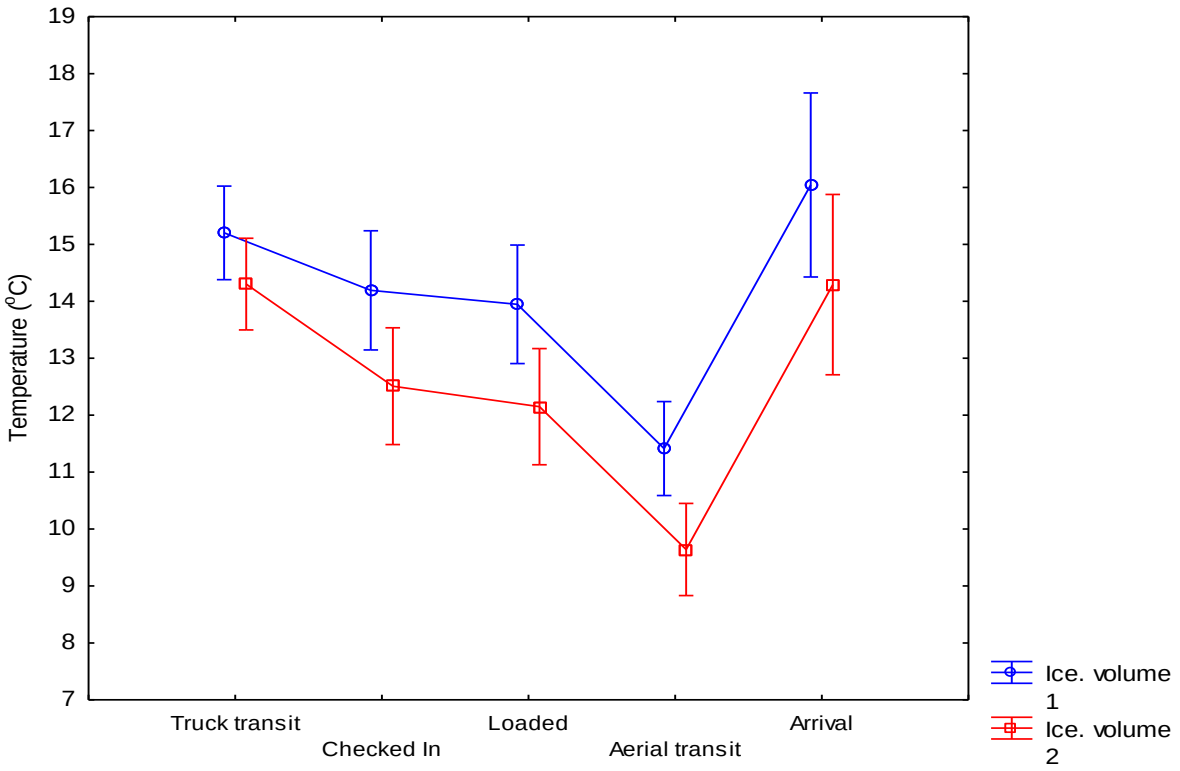


Figure 4.9: Mean (± 95 confidence intervals) internal temperature of boxes, where animals subjected to both a lower ice volume provision (vol. 1; 548.5 g, n = 31) and a higher ice volume provision (vol. 2; 1090.88 , n = 32) during packing, across each of the five different export phases (Repeated measures ANOVA, $F_{(8, 40)} = 2.54$, $p = 0.02$).

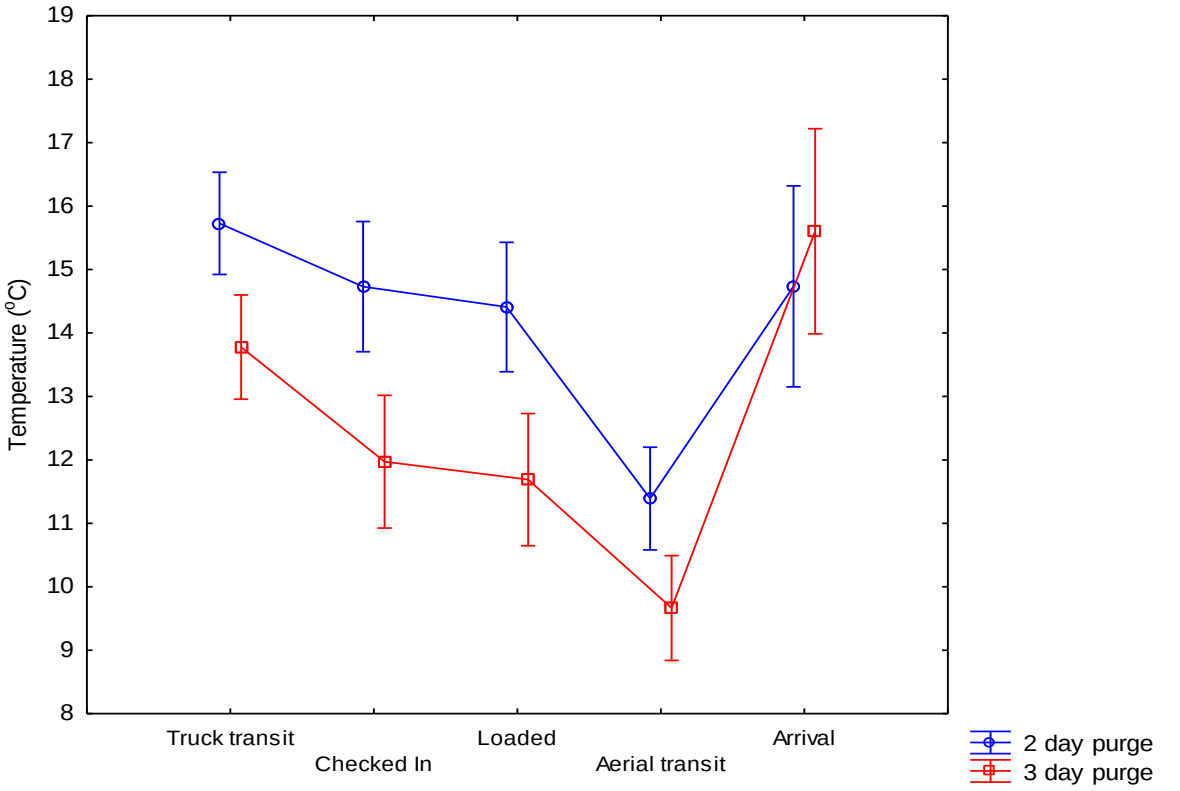


Figure 4.10: Mean (± 95 confidence intervals) internal temperature of boxes, with animals subjected to both a shorter purging period (two days, n = 32) and longer purging period (three days, n = 31), within each of the five different export phases (Repeated measures ANOVA, $F_{(8, 40)} = 3.69$, $p = 0.003$).

4.3.3 Post transport simulation

4.3.3.1 Ice temperature

There was a significant difference of the temperature of the ice upon arrival, post the 42-hour transport simulation, with different ice volume inclusions (Factor 3) (Factorial ANOVA, $F_{(1, 64)} = 10.93$, $p = 0.002$, Figure 4.11). Boxes which had a higher volume of ice prior to transport obtained a lower temperature of 13.03 ± 0.39 °C compared to boxes with one ice sheet, which had a mean temperature of 14.89 ± 0.39 °C upon arrival.

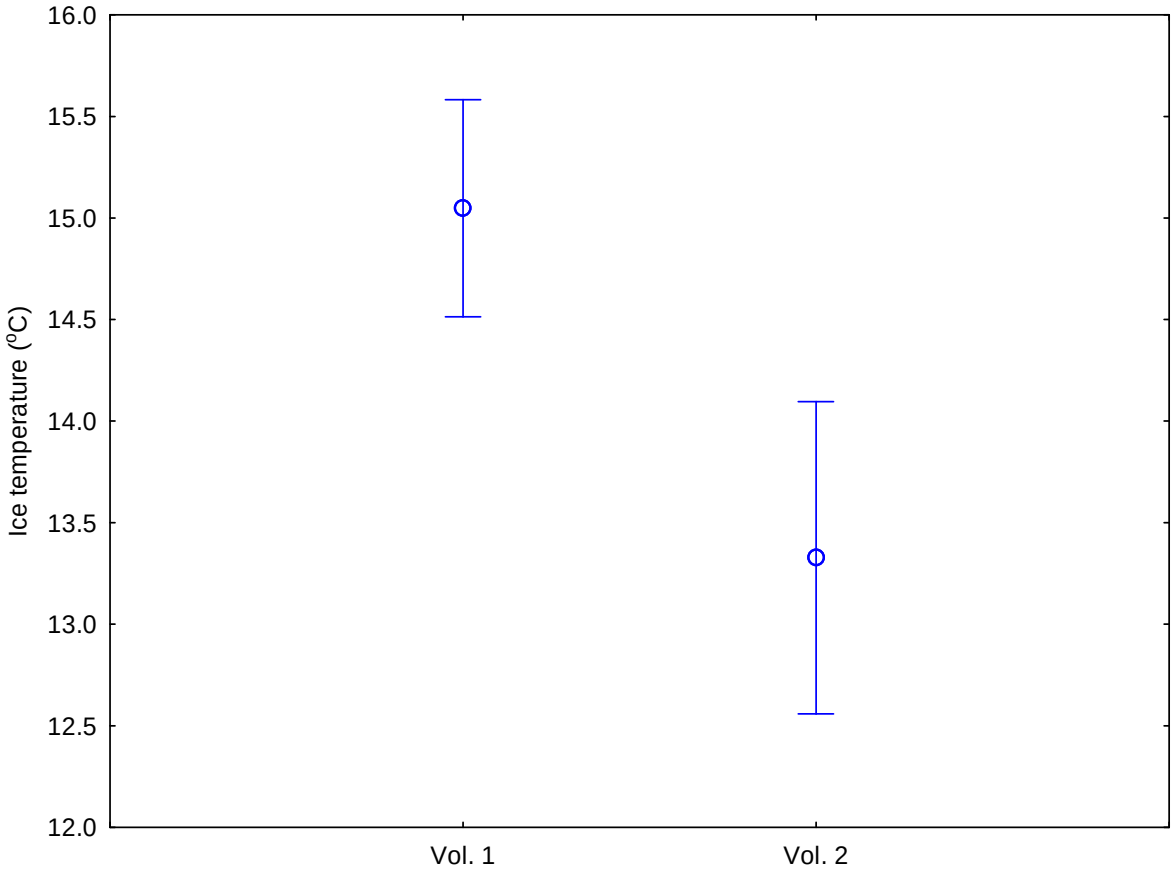


Figure 4.11: Mean (± 95 confidence intervals) temperature of ice within boxes subjected to ice vol. 1 (548.5 g, n = 48) and ice volume 2 (1090.88 g, n = 48), post the 42-h transport simulation. (Factorial ANOVA, $F_{(1, 64)} = 10.93$, $p = 0.002$).

4.3.3.2 External animal temperature

There was a significant difference of both the mean temperature of animals post the transport simulation and the distribution of the dataset, with different ice volume inclusions (Factor 3) (Kruskal Wallis, $H_{(1, 96)} = 7.96$, $p = 0.005$, Figure 4.12). Animals that had a higher volume of ice within the boxes during transport, obtained a lower mean temperature of 13.89 ± 0.30 °C compared to animals that had less which obtained a mean temperature of 15.05 ± 0.30 °C.

Animals subjected to a higher volume of ice had a lower median of 40.48 compared to animals with a lower volume of ice, which had a higher mean/median 56.52 post the transport simulation, where the range of the dataset was higher in values compared to animals subjected to a higher ice volume.

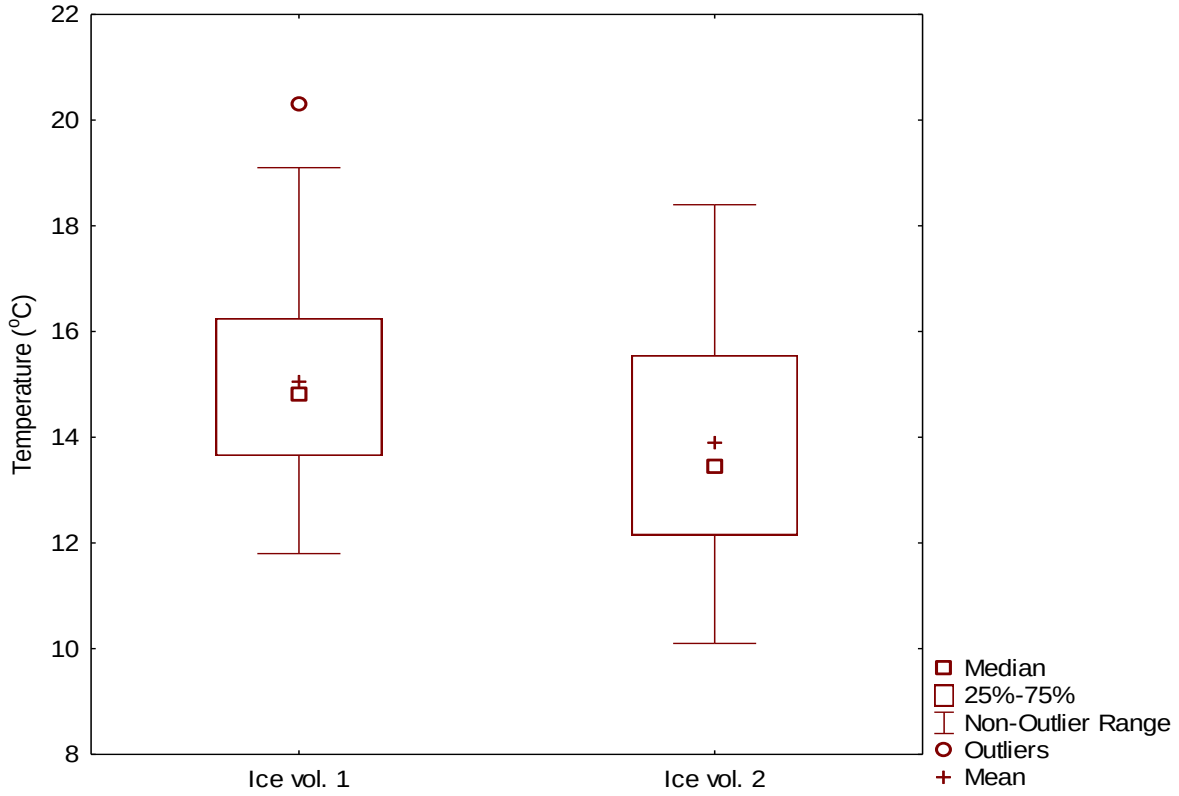


Figure 4.12: Mean/ Median distribution of animals subjected to ice volume 1 (548.5 g, n = 48) and ice volume 2 (1090.88 g, n = 48) during packing, post the 42-h transport simulation, upon arrival. (Kruskal Wallis, $H_{(1, 96)} = 7.96$, $p = 0.005$).

4.3.3.3 Net weight of boxes prior and post the transport simulation

There was a significant interaction of the net weight (g) of animals from when they were packed, prior to the transport simulation, to when they were unboxed 42-h after the transportation simulation, with different purging durations (Factor 2) (Repeated measures ANOVA, $F_{(1, 80)} = 7.96$, $p = 0.006$, Figure 4.13). Animals that had a longer purging period of three days lost a significantly lower amount of weight compared to animals that had a shorter purging period of two days. Animals that were subjected to a longer purging period had a significantly lower change in weight of 802.4 g from 7891.88 ± 4.28 g to 7089.48 ± 41.66 g after the 42-h simulation. This loss was substantially less than that of animals with a shorter

purging period, which lost a net weight of 972.54 g from 7891.67 ± 4.28 g to 6919.13 ± 41.66 g (Figure 4.13).

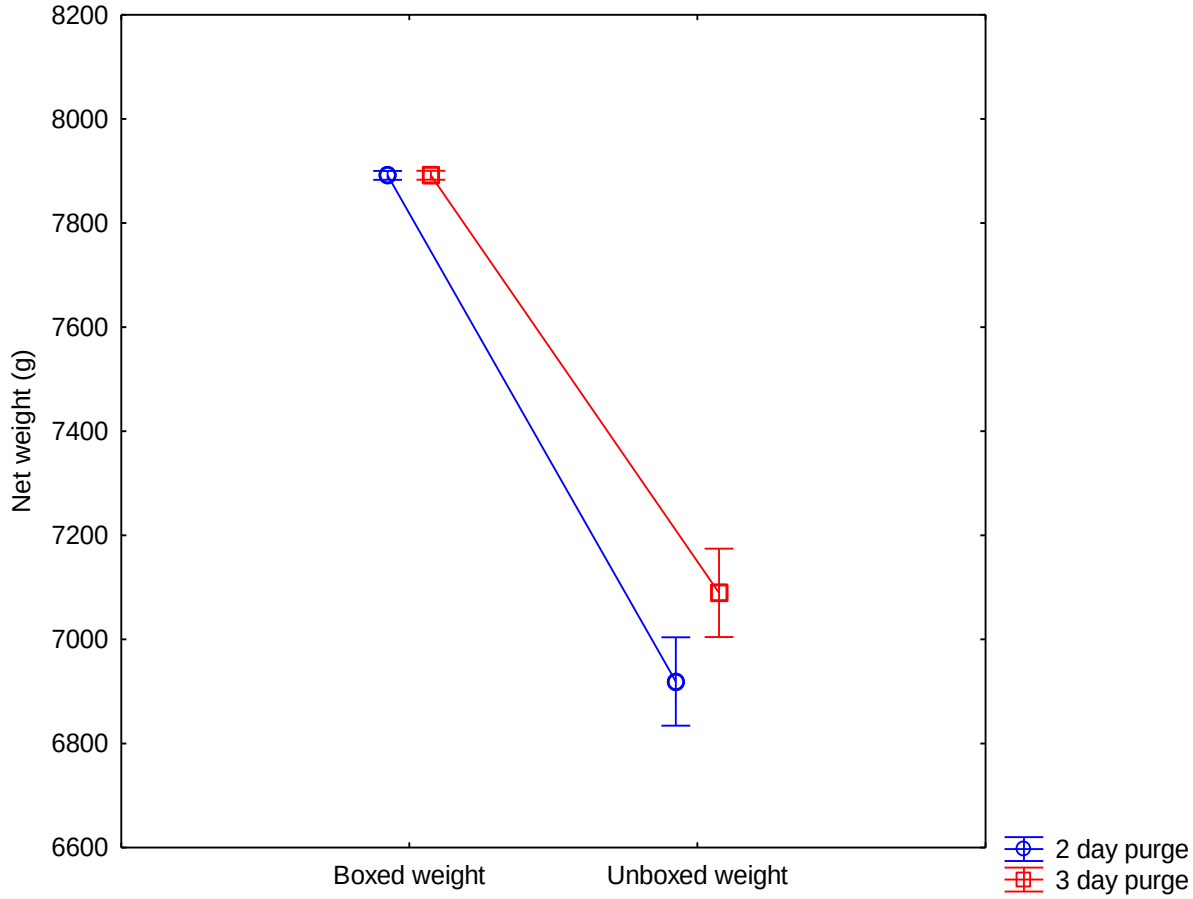


Figure 4.13: Mean (± standard error) net weight (g) of animals subejcted to both a shorter purge of two days (n = 48) and a longer purge of three days (n = 48) within boxes, from when they were packed until they were unboxed, after the 42-h transport simulation (Repeated Measures, $F_{(1, 80)} = 7.96$, $p = 0.006$).

4.3.3.4 Net weight upon arrival

There was a significant difference with different purge days (Factor 2) on the weight (g) of boxes upon arrival (Factorial ANOVA, $F_{(1, 80)} = 7.83$, $p = 0.006$, Figure 4.14). Animals subjected to a longer purging period of three days prior to transport resulted in a higher net weight upon arrival of 7089.48 ± 42.66 g, compared to animals subjected to a shorter purging period of two days, which obtained a lower net weight of 6919.13 ± 42.66 g upon arrival.

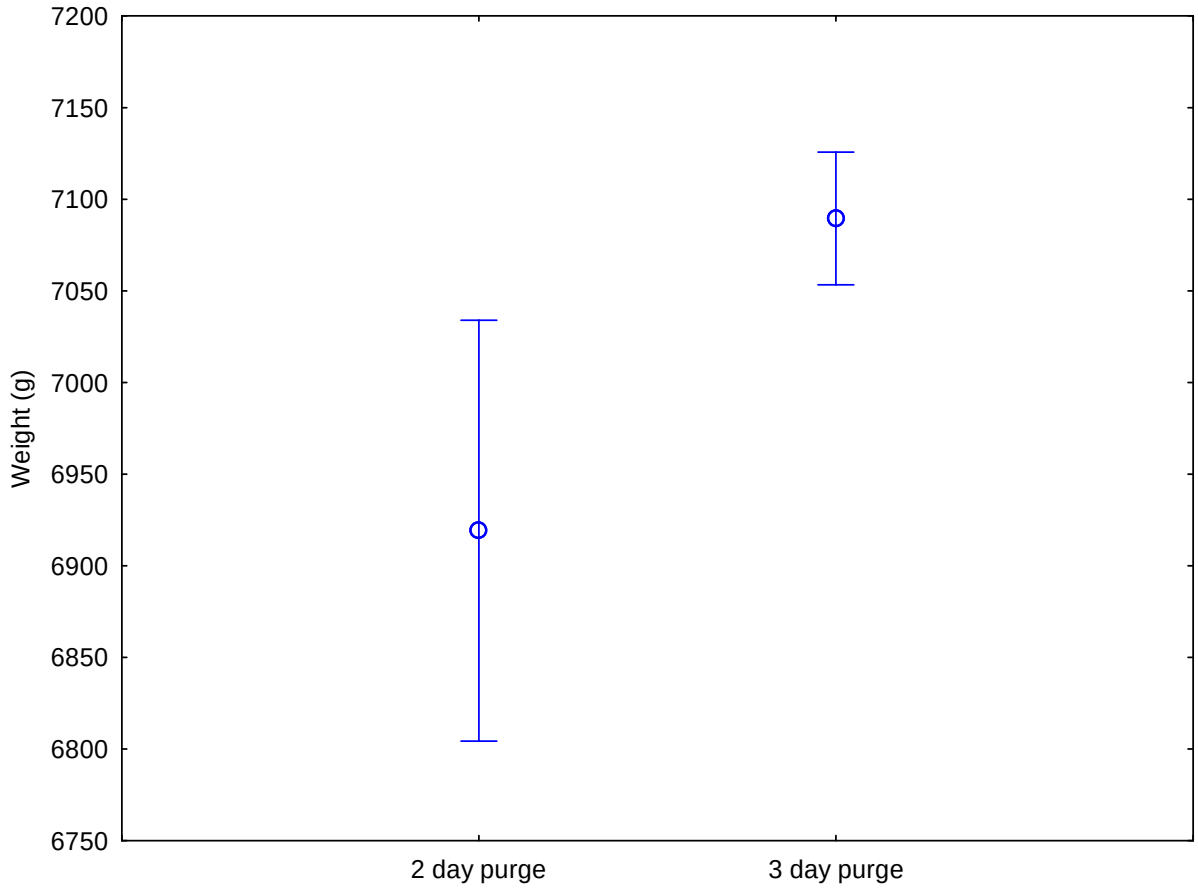


Figure 4.14: Mean (± 95 confidence intervals) weight of animals subjected to both a shorter purging period (two days, n = 48) and longer purging period (three days, n = 48), upon arrival, post the 42-h transport simulation. (Factorial ANOVA, $F_{(1, 80)} = 7.97$, $p = 0.006$).

4.3.3.5 Weight loss (%)

There was also a significant difference of the percentage of weight loss (%) with different purging durations (Factor 2) post animals subjected to a 42-h transport simulation (Factorial ANOVA, $F_{(1, 80)} = 7.97$, $p = 0.006$, Figure 4.15). Animals with a longer purging period of three days had a lower percentage weight loss of 10.17 ± 0.54 % compared to animals subjected to a shorter purging period of two days, which obtained a higher weight loss of 12.33 ± 0.54 % 42-h post the transport simulation.

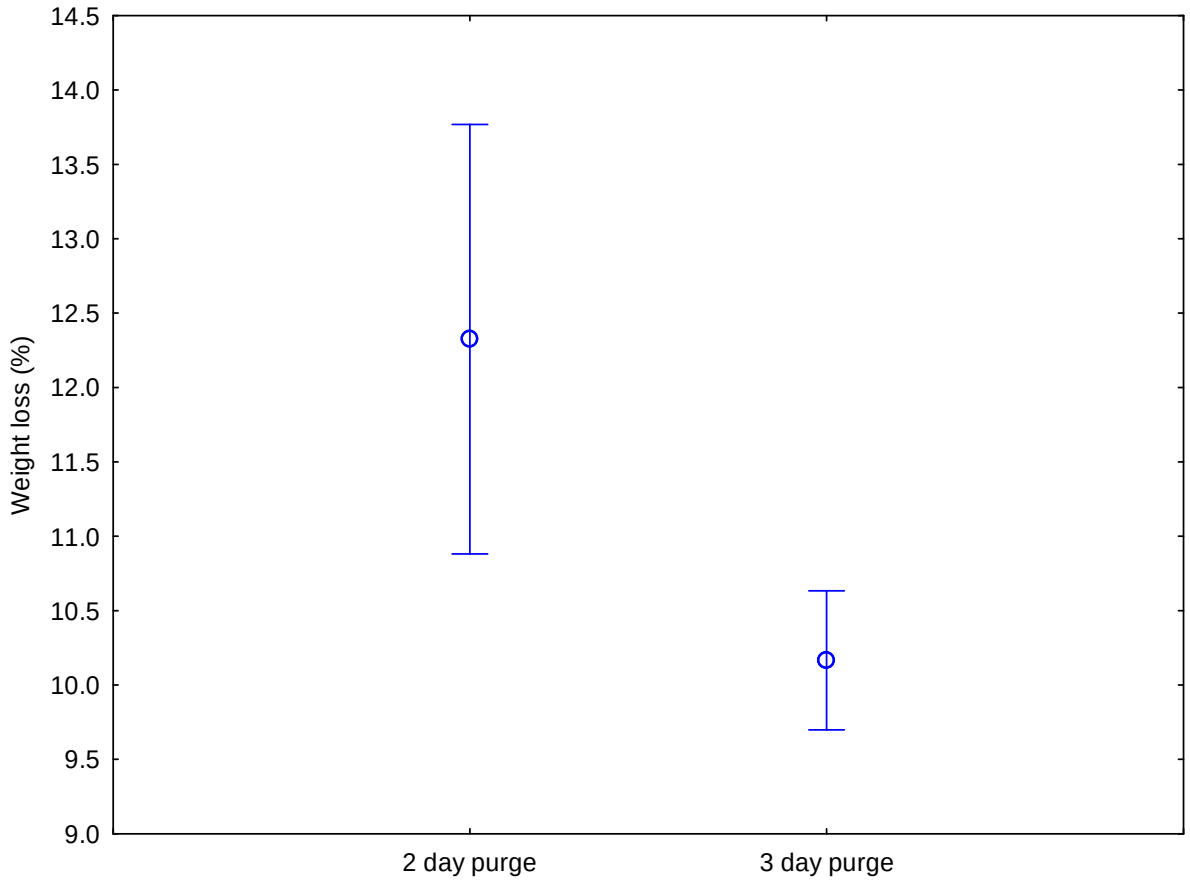


Figure 4.15: Mean (± 95 confidence intervals) weight loss (%) of animals subjected to both a shorter purging period (two days, n = 48) and a longer purging period (three days, n = 48), post the 42-h transport simulation. (Factorial ANOVA, $F_{(1, 80)} = 7.97$, p = 0.006).

4.3.3.6 Water loss (g)

There was a significant difference of the water loss (g) through drip loss, post the transport simulation with different size class ranges (Factor 4) (Factorial ANOVA, $F_{(1, 80)} = 8.17$, p = 0.006, Figure 4.16). Bigger sized animals (90 – 110 g) lost less average water weight of 534.27 ± 18.73 g, 42-h post the transport simulation, compared to smaller sized animals (50 – 60 g) which lost a higher average water weight of 609.98 ± 18.73 g.

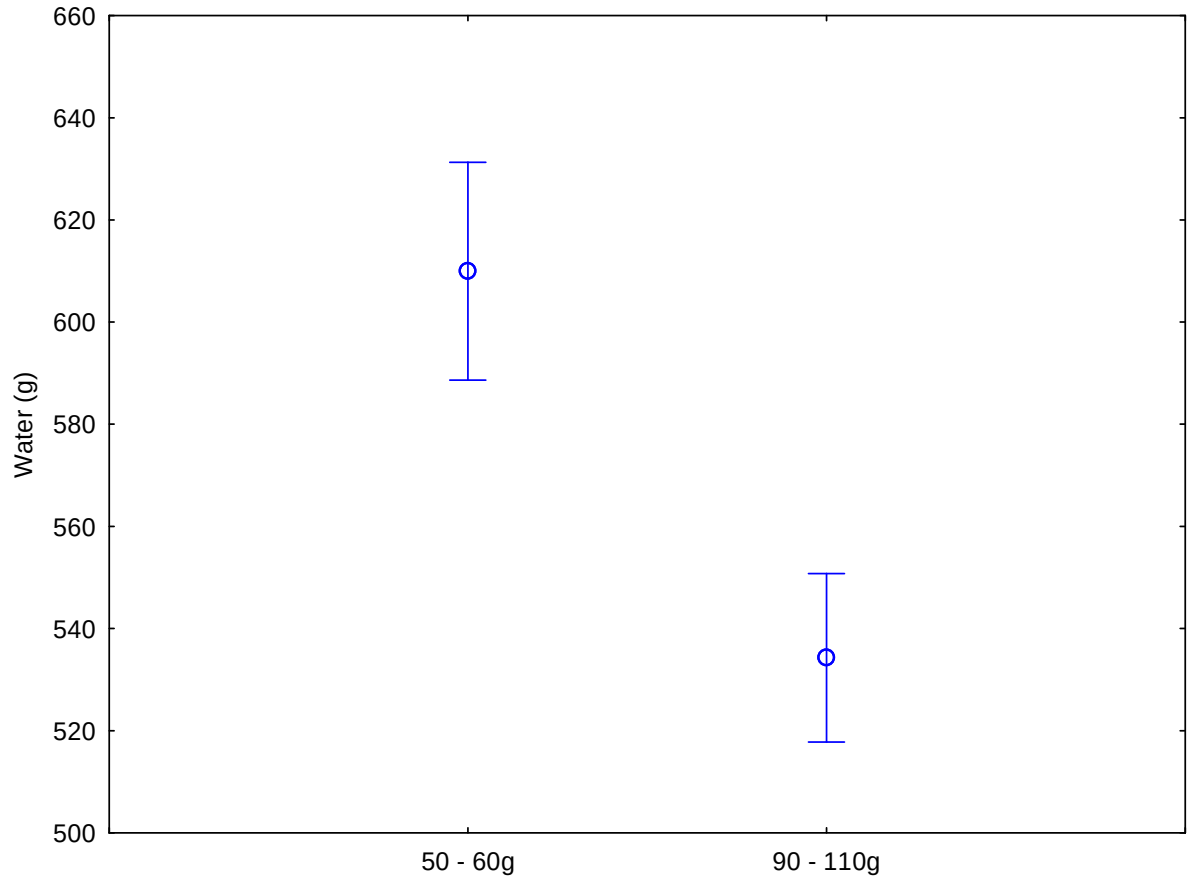


Figure 4.16: Mean (± standard error) water weight (g) of both abalone sizes, 50 - 60 g (n = 48) and 90 - 110 g (n = 48), post the 42-h transport simulation (Factorial ANOVA, $F_{(1, 80)} = 8.17$, $p = 0.006$).

4.3.3.7 Individual weight prior and post the transport simulation

There was a significant interaction in individual abalone weight over the duration of the simulated transport, for different size abalone packed with different volumes of ice (Repeated Measures, multifactor ANOVA, $F_{(1, 16)} = 29.52$, $p<0.001$; Figure 4.17). There was a reduction in weight from post purging to post transport, and this trend was evident for both size classes of abalone; however, the drop in weight was less for larger abalone when they were packed with more ice, whereas additional ice did not influence the weight of smaller abalone over the transport period (Figure 4.17).

Animals, in general, lost an average weight of 8.88 g from when they were purged at an average weight of 84.75 ± 0.39 g to when they were unboxed 42-h after the transport simulation started, where they obtained an average weight of 75.87 ± 0.36 g.

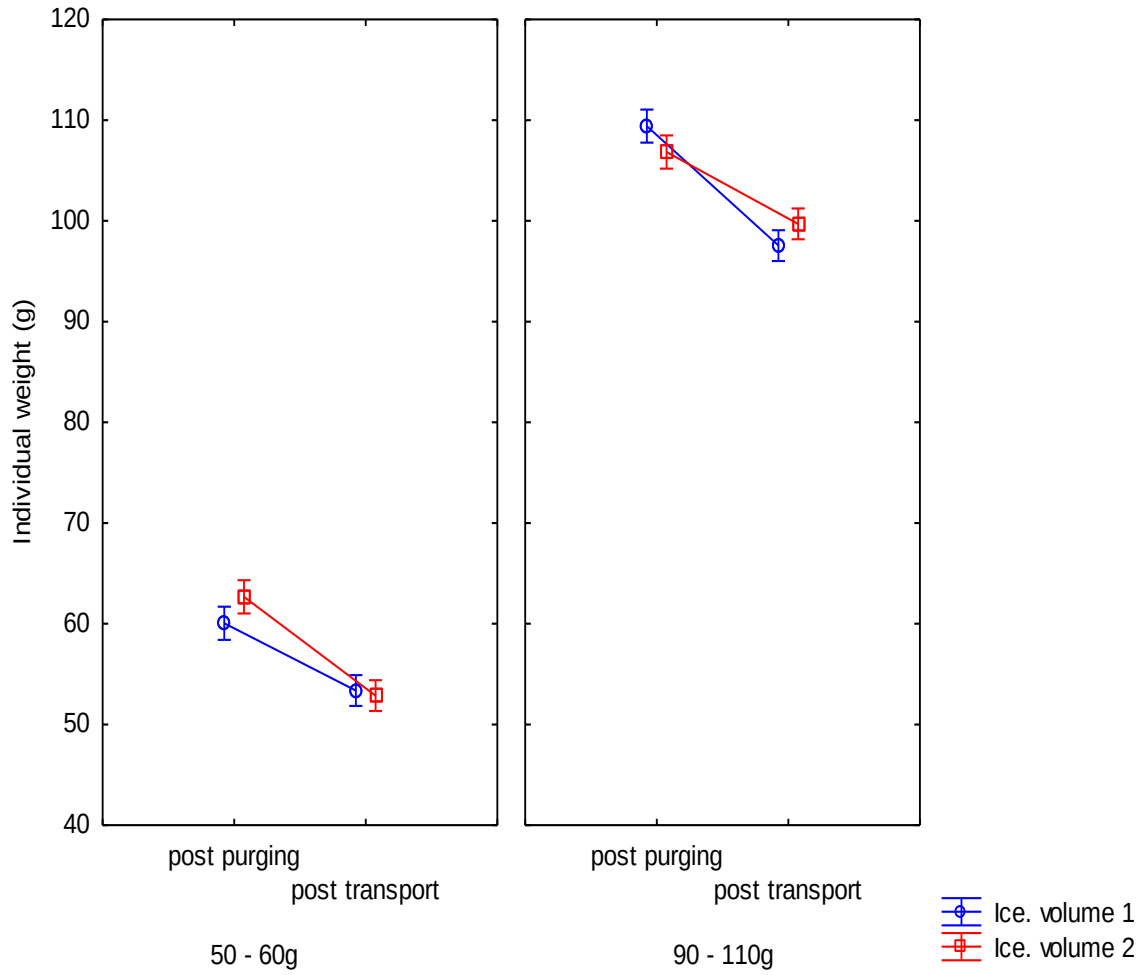


Figure 4.17: Mean (± 95 confidence intervals) individual weight of both size classes, with different abalone sizes (50 – 60 g, n = 16, and 90 – 110 g, n = 8) subjected to both ice volume 1 (548.5 g, n = 16) and ice volume 2 (1090.88 g, n = 16) (Factor 3), from animals which were checked post the purging process (packing), to when they were opened 42-h post the transport simulation (Repeated Measures ANOVA, $F_{(1, 16)} = 29.52$, $p<0.001$).

4.3.3.8 Individual weight upon arrival

There was a significant difference of the average individual weight upon arrival for abalone of different sizes (Factor 4), after a 42-h simulated transport (Multifactorial ANOVA, $F_{(1, 2384)} = 6.69$, $p = 0.009$, Table 4.1). Although there was significant interaction or difference with the other three factors, both abalone sizes lost individual weight upon arrival, with smaller sizes significantly lower. However, the difference of individual weight was a result of the chosen sizes and not an outcome of the experiment.

Table 4.1: Mean (± 95 confidence intervals) individual weight upon arrival, post the 42-h transport simulation, of both size classes (Factor 4), different volumes of ice within the boxes (Factor 3), different purge days (Factor 2), and different acclimation temperatures (Factor 1). Different alphabetical letters indicate a significant difference (Multi-factorial ANOVA, at p<0.05).

Factor 1 – Temperature	Chilled	Ambient	F statistics	P value
	76.12 ± 0.48	76.28 ± 0.48	$F_{(1, 24)} = 0.05$	= 0.82
Factor 2 – Purge Days	2-day purge	3-day purge	F statistics	P value
	75.53 ± 0.53	76.87 ± 0.49	$F_{(1, 24)} = 3.91$	p = 0.06
Factor 3 – Ice Volume	1 ice sheet	2 ice sheets	F statistics	P value
	75.82 ± 0.48	76.58 ± 0.48	$F_{(1, 24)} = 1.24$	p = 0.28
Factor 4 – Size class	50 – 60 g	90 – 110 g	F statistics	P value
	53.13 ± 0.48[a]	99.28 ± 0.48[b]	$F_{(1, 24)} = 4612.2$	p<0.001

4.3.3.9 Excess biomass of individual weight

The volume of ice significantly influences the interaction of the volume of ice and abalone size on excess biomass (Multifactorial ANOVA, $F_{(1, 80)} = 4.56$, p = 0.036; Figure 4.18). There was no difference in excess biomass between big and small abalone when both were packed with a low volume of ice; whereas, the big abalone had a significantly greater excess biomass than the small abalone, when the larger animals were packed with more ice (Figure 4.19).

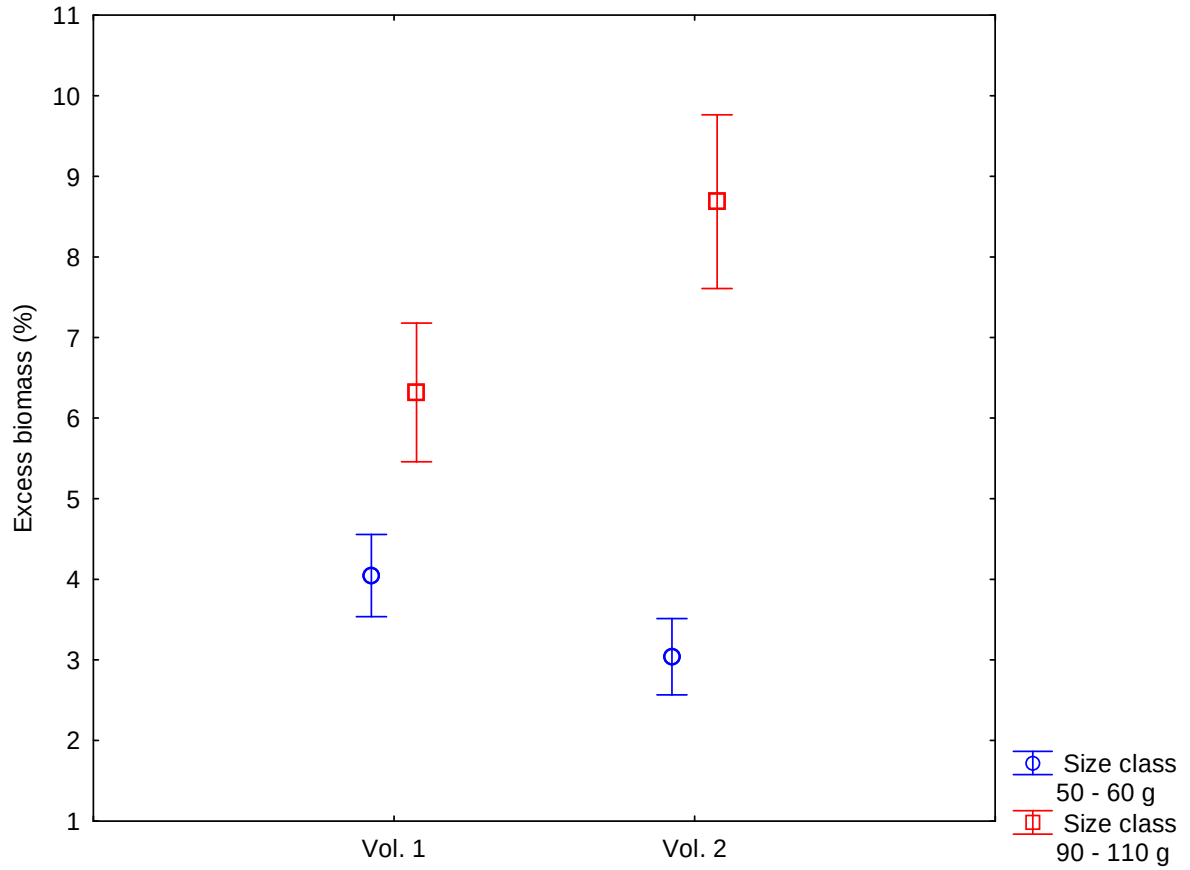


Figure 4.18: Mean (± 95 confidence intervals) excess biomass of both abalone sizes, 50 - 60 g (n = 48) and 90 - 110 g (n = 48), subjected to ice volume 1 (548.5 g) and ice volume 2 (1090.88 g), packed within boxes, post the 42-h transport simulation, upon arrival. (Multifactorial ANOVA, $F_{(1, 80)} = 4.56$, $p = 0.036$).

4.3.3.10 Insufficient Biomass of individual weight

There was no significant interaction or significant difference of the insufficient biomass (%) of animals with any of the four factors, temperature, purge days, ice volume, and size class, post the 42-h transport simulation (Multifactorial ANOVA, $F_{(1, 80)} = 0.05$, $p = 0.82$; Table 4.2). Animals which reached an insufficient biomass of 49 g and under and as well as 89 g and under occurred randomly throughout each experimental event, where the highest insufficient biomass of 13.71 ± 5.43 % occurred with the smaller sized animals, that were chilled, subjected to two ice sheets and also subjected to a shorter purging period. The lowest insufficient biomass of 6.29 ± 4.80 % occurred with the bigger sized animals that were subjected to an ambient purging environment for two days and as well as higher volume of ice during the transport phase of export.

Table 4.2: Mean/Median (± 95 confidence intervals) of the insufficient biomass (%) of animals, subjected to the four different pre-transport factorial conditions and subjected to a 42-h transport simulation (Kruskal Wallis, at p<0.05).

Factor 1 – Temperature	Chilled	Ambient	F statistics	P value
	10.44/51.31 ± 0.72	9.17/45.69 ± 0.72	$H_{(1, 96)} = 0.98$	p = 0.32
Factor 2 – Purge Days	2-day purge	3-day purge	F statistics	P value
	10.24/49.74 ± 0.72	9.37/47.26 ± 0.72	$H_{(1, 96)} = 0.19$	p = 0. 66
Factor 3 – Ice Volume	1 ice sheet	2 ice sheets	F statistics	P value
	10.58/53.56 ± 0.72	9.02/43.44 ± 0.72	$H_{(1, 96)} = 3.17$	p = 0.08
Factor 4 – Size class	50 – 60 g	90 – 110 g	F statistics	P value
	10.24/51.29 ± 0.72	9.36/45.71 ± 0.72	$H_{(1, 96)} = 0.96$	p = 0.33

4.3.3.11 Mortalities

There was a significant difference of the mortality rate of abalone on arrival after 42 hours of transport with different purging durations (Factor 2) (Factorial ANOVA, $F_{(1, 80)} = 4.51$, p = 0.037, Figure 4.19). Animals with a longer purging period of three days had a lower initial mortality rate of 0.11 ± 0.15 % after boxes were opened 42-h post the transport simulation, compared to animals subjected to a shorter purging period of two days, which reached an initial survival rate of 0.57 ± 0.15 % post the transport simulation.

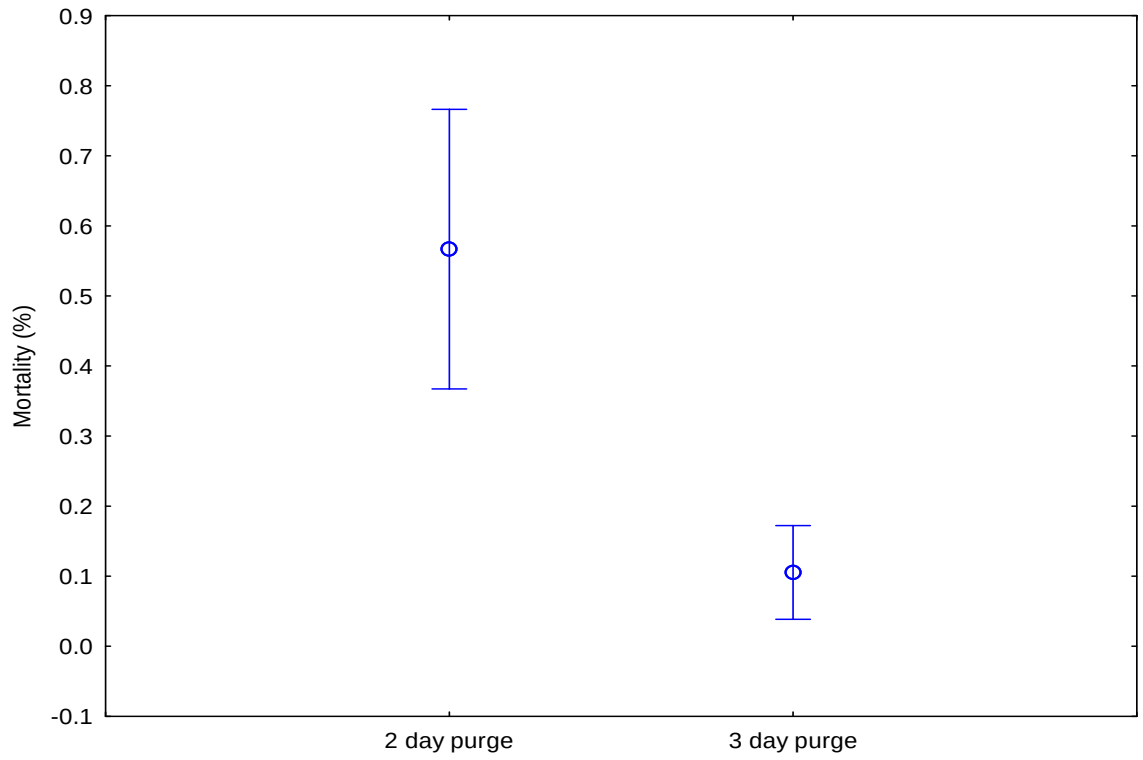


Figure 4.19: Mean (± 95 confidence intervals) initial mortality rate (%) upon arrival of animals subjected to both a shorter purging period (two days, n = 48) and a longer purging period (three days, n = 48), 42-h post the transport simulation. (Factorial ANOVA, $F_{(1, 80)} = 4.51$, $p = 0.0037$).

4.3.4 Recovery

4.3.4.1 Survival rate

The survival rate of animals, during the recovery period, differed significantly between the treatments with different acclimation temperatures (Factor 1), where there was also a significant interaction of the survival rate between different purge days (Factor 2) and different ice volume inclusions (Factor 3) (Repeated measures ANOVA, $p<0.05$, Figure 4.20, Figure 4.21, and Figure 4.22). Survival dropped significantly from the start of recovery on day one, upon arrival, from 99.66 ± 0.12 % until 85.20 ± 1.78 % on the third day of recovery, where survival remained relatively similar during the rest of recovery from Day 6 until Day 12. During the rest of recovery, survival reached 83.16 ± 1.71 % on day six until 81.86 ± 1.81 % on day twelve. This trend was identical to animals that were subjected to different acclimation purging temperatures, prior to transport (Figure 4.20), for animals which were subjected to different purge days (Figure 4.21), and animals which were subjected to different ice volume inclusions during packing (Figure 4.22). By the third day, survival was significantly higher for animals which were pre-cooled prior to transport (Figure 4.20), animals which had a longer purge (Figure 4.21), and animals which had more ice within the boxes during transport (Figure 4.22).

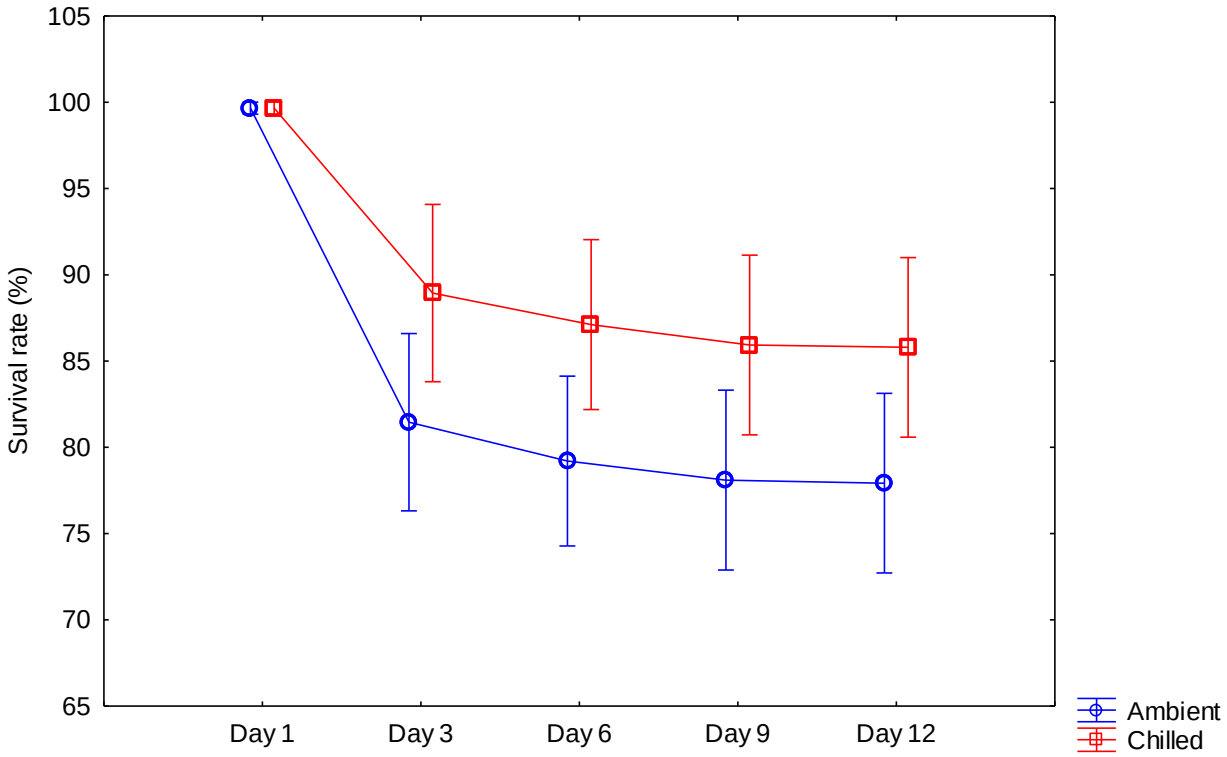


Figure 4.20: 4.20: Mean (± 95 confidence intervals) survival rate (%) of animals subjected to both a chilled purging environment (n = 24) and a high ambient purging temperature (n = 24), at different days during recovery when animals were sent back to their respective tanks post the 42-h transport simulation. (Repeated measures ANOVA, $F_{(4, 128)} = 4.98$, $p<0.001$).

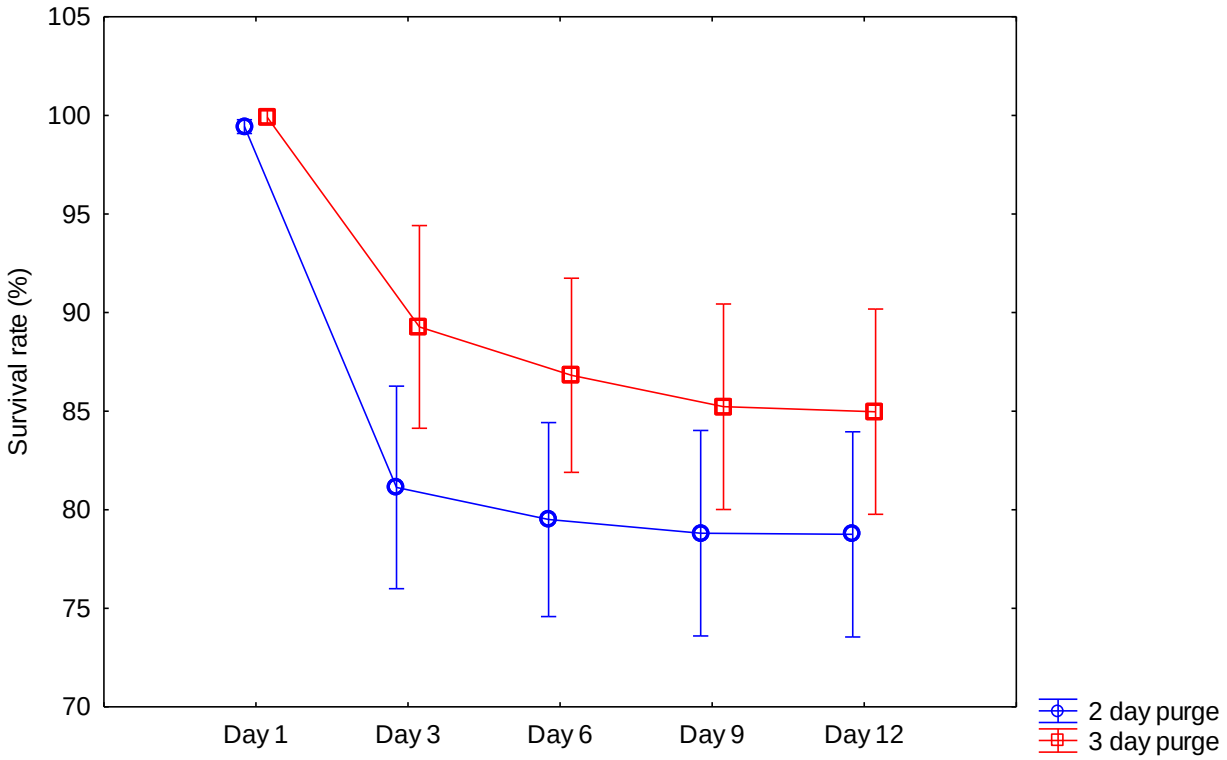


Figure 4.21: Mean (± 95 confidence intervals) survival rate (%) of animals subjected to both a shorter (two days, n = 24) purging period and a longer purging (three days, n = 24) period, across the recovery days when animals were sent back to their respective tanks, post the 42-h transport simulation. (Repeated measures ANOVA, $F_{(4, 128)} = 3.79$, $p = 0.006$).

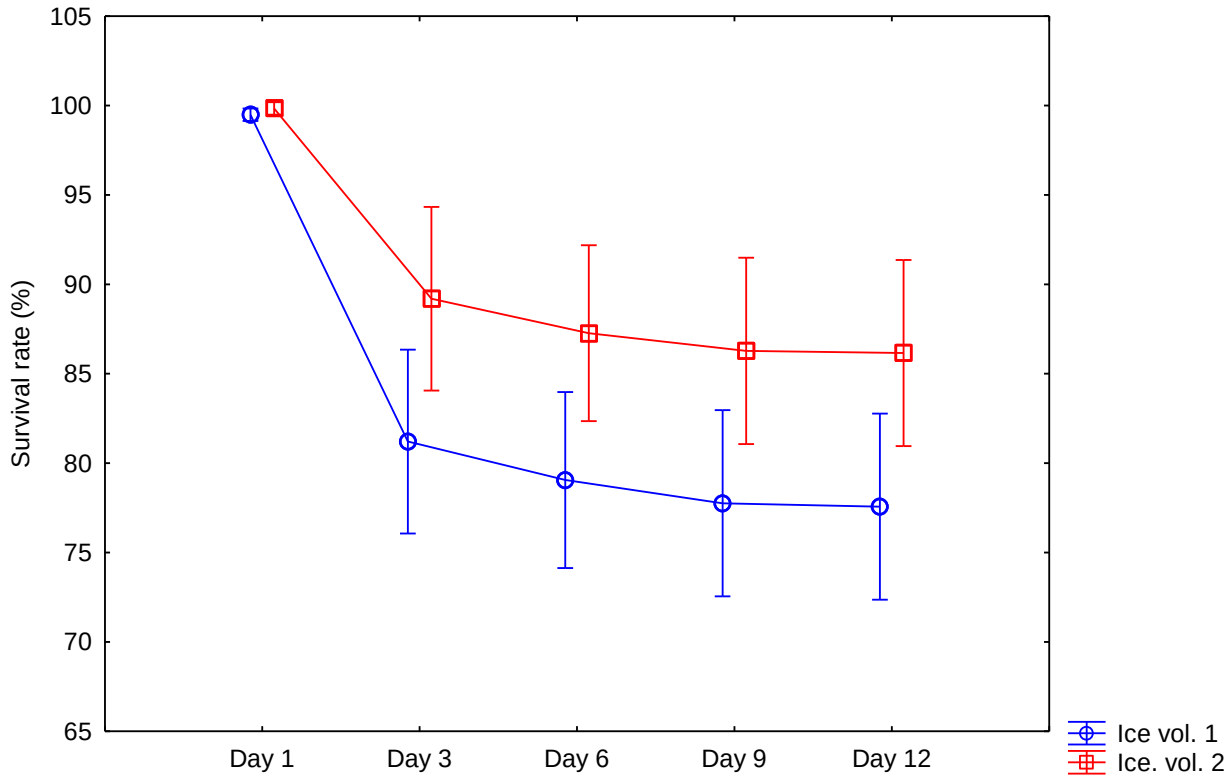


Figure 4.22: Mean (± 95 confidence intervals) survival rate (%) of animals subjected to both ice volume 1 (548.5 g, n = 24) and ice volume 2 (1090.88 g, n = 24) provisions during packing prior to the transport simulation, across the different recovery days, after animals were transferred to their respective recovery tanks, post the 42-hour transport simulation. (Repeated measures ANOVA, $F_{(4, 128)} = 5.27$, $p<0.001$).

4.3.5 Behaviour

4.3.5.1 Righting time

All animals randomly selected for this behavioural test, from each box within every replication, failed to right themselves after five minutes (mins), after the 42-h transport simulation.

4.3.5.2 Side to side movement

There was a significant difference of the side-to-side movement of animals with different acclimation temperatures (Factor 1), post the 42-h transport simulation. (Factorial ANOVA, $F_{(1, 48)} = 9.49$, $p = 0.003$, Figure 4.23). Animals subjected to a chilled purging environment had a higher side to side movement (11.46 ± 1.95) when attempting to right themselves, when comparing animals subjected to an ambient purging environment, which attempted to right themselves with a fewer mean side to side movement (2.99 ± 1.95).

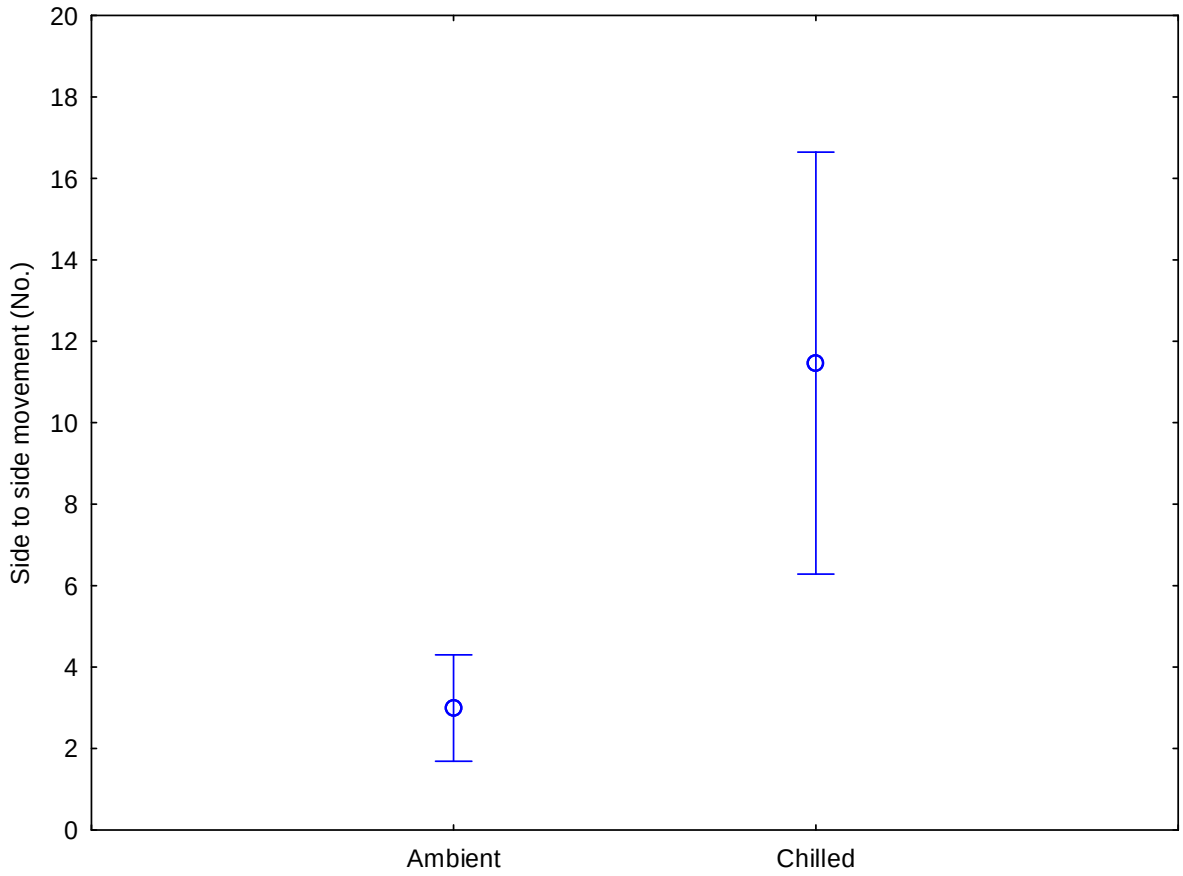


Figure 4.23: Mean (± 95 confidence intervals) side to side movement (no.) of animals subjected to both a chilled purging environment (n = 32) and a high ambient purging temperature (n = 32), post the 42-h transport simulation, upon arrival. (Factorial ANOVA, $F_{(1, 48)} = 9.49$, $p = 0.003$).

4.3.5.3 Adhesive force

There was a significant interaction with the adhesive force (N) animals use to stick to a substrate with different purging durations (Factor 2), different ice volumes (Factor 3), and different size classes (Factor 4), 42-h post the transport simulation (Multifactorial ANOVA, $F_{(1, 80)} = 6.63$, $p = 0.012$, Figure 4.24). Larger animals had the highest overall adhesive force of 2.17 ± 0.52 N, which was greater with a higher inclusion of ice and a longer purge, which was a similar trend for smaller animals, however adhesive force was greater at 0.75 ± 0.52 N with a shorter purge. Both abalone sizes had a relatively lower adhesive force with less ice, however, adhesive force for larger animals was greater at 1.07 ± 0.52 N with a shorter purge and greater for smaller animals at 0.19 ± 0.52 N with a longer purge.

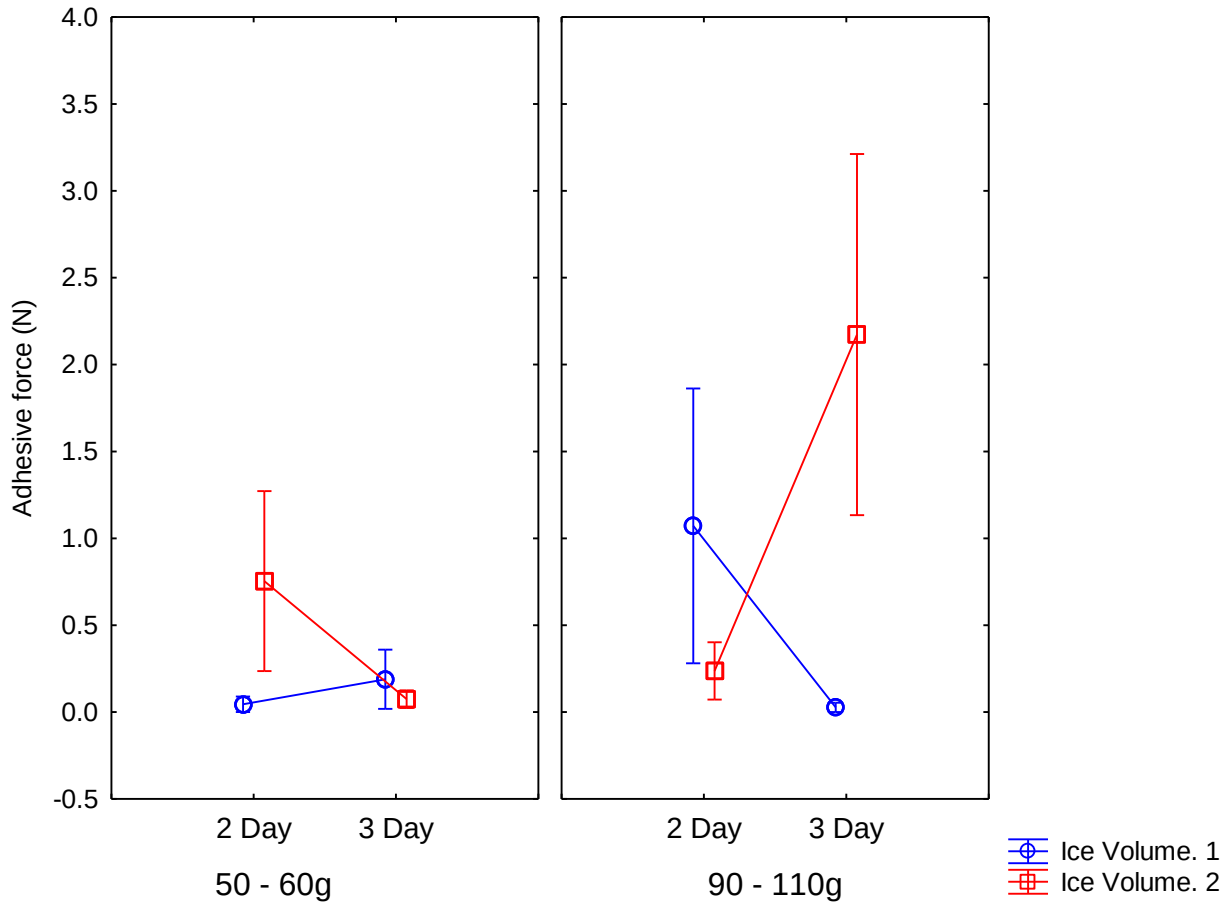


Figure 4.24: Mean (± 95 confidence intervals) of both size class ranges (Factor 4) subjected to both different purging periods (Factor 2) and different ice volume provision (Factor 3), during packing (n = 12) and post the 42-h transport simulation (n = 12). (Multifactorial ANOVA, $F_{(1, 80)} = 6.63$, $p = 0.012$).

4.3.5.4 Initial movement

There was no significant interaction or significant difference of the initial movement (s) animals take when placed onto a modified top plate with any of the four factors, temperature, purge days, ice volume, and size class (Table 4.3). Animal initial movement varied from 28.97 s until 85.10 s post the transport simulation, across each of the 16 treatments.

Table 4.3: Means/Median (± 95 confidence intervals) of the initial movement of animals after being subjected to a 42-h transport simulation (Kruskal Wallis; at p<0.05).

Factor 1 - Temperature	Chilled	Ambient	F – statistics	P value
	64.88/28.97 ± 6.15	54.21/36.03 ± 6.15	$H_{(1, 64)} = 2.30$	$p = 0.13$

Factor 2 – Purge days	2-day purge	3-day purge	F statistics	P value
	63.68/35.06 ± 6.15	55.41/29.94 ± 6.15	$H_{(1, 64)} = 1.22$	p = 0. 27
Factor 3 – Ice volume	1 ice sheet	2 ice sheets	F statistics	P value
	64.47/35.59 ± 6.15	54.62/29.41 ± 6.15	$H_{(1, 64)} = 1.77$	p = 0.18
Factor 4 – Size class	50 – 60 g	90 – 110 g	F statistics	P value
	55.69/30.34 ± 6.15	63.40/34.66 ± 6.15	$H_{(1, 64)} = 0.86$	p = 0.35

4.3.5.5 Speed

There was a significant interaction of the speed abalone use when moving from the middle of the top plate to position 1, and from position one to position two, when comparing different purge days (Factor 2), two hours post the 42-h transport simulation (Repeated measures ANOVA, $F_{(1, 48)} = 7.71$, p = 0.008, Figure 4.25). Animals that were subjected to a longer purging period of three days used an initial maximum average speed of 1.60 ± 0.17 mm s^{-1} from the middle of the top plate to position 1, where animals moving from position 1 to position 2, used a mean speed of 1.18 ± 0.11 mm s^{-1}. This was compared to animals which were subjected to a shorter purging period of two days, which used relatively a similar speed moving from the middle of the top plate to position 1 with a speed of 1.20 ± 0.17 mm s^{-1}, where animals moving from position 1 to position 2, used a mean speed of 1.15 ± 0.11 mm s^{-1} to escape completely off the top plate, two hours post the 42-h transport simulation. However, animals overall used a higher speed to move from the middle of the top plate to position one and gradually reduced their speed to move off the top plate.

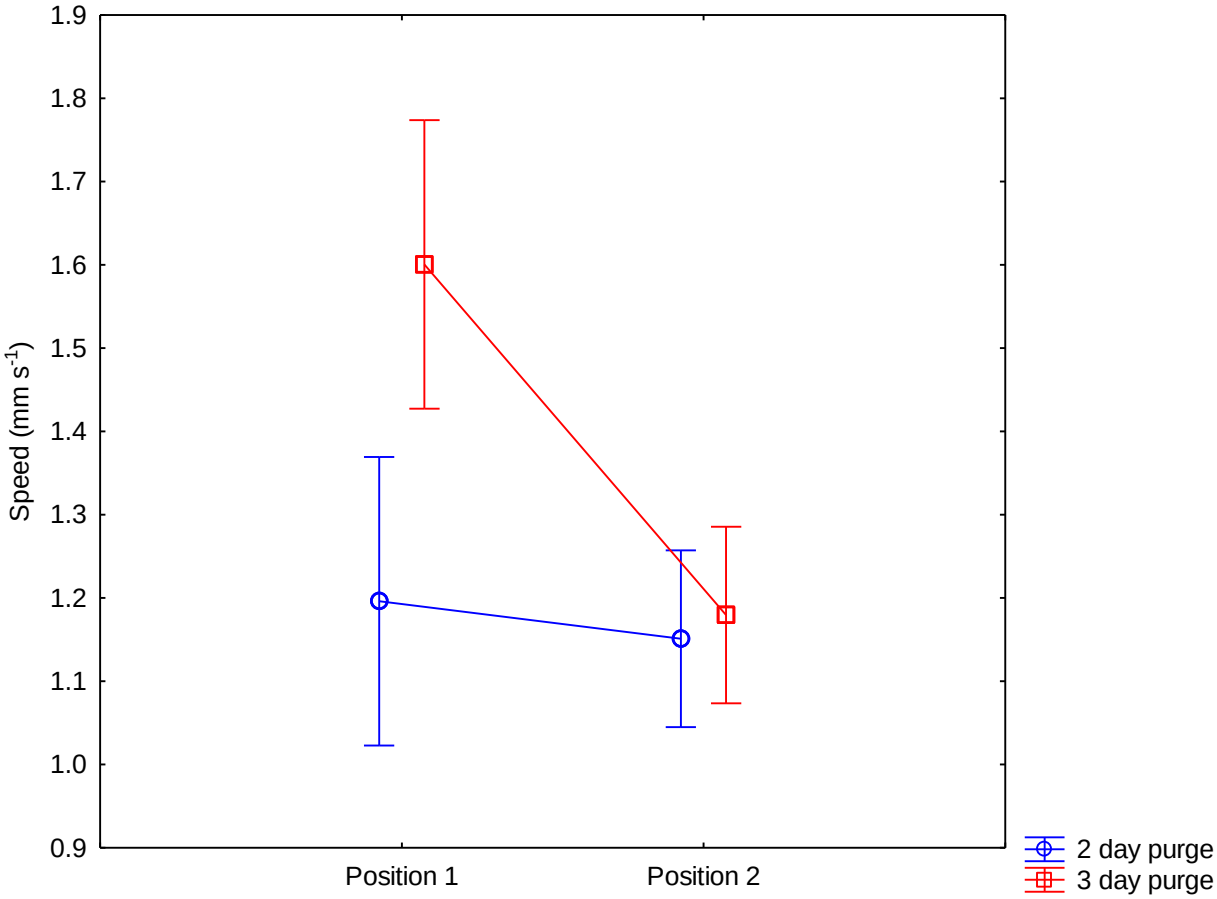


Figure 4.25: Mean (± 95 confidence intervals) change in speed (mm s^{-1}) of animals moving from the middle of the top plate to position 1 and from position 1 to position 2, when comparing a shorter purge (two days, n = 32) and a longer purge (three days, n = 32), two hours post the 42-h transport simulation. (Repeated measures ANOVA, $F_{(1, 48)} = 7.71$, $p = 0.008$.).

4.3.6 Histology

4.3.6.1 Gills

Different stress indicators within the gills of abalone were observed across all 16 treatments, including the enlargement of the gill lamellae of different leaflets (Figure 4.26). Haemolymph infiltration or eosinophilia in the sinus fluid of different leaflets and goblets cells was also observed (Figure 4.27). Lastly, the observation of unusual and deformed shaped gill lamellae was also found in the abalone's gill, 42-h post the transport simulation (Figure 4.28).

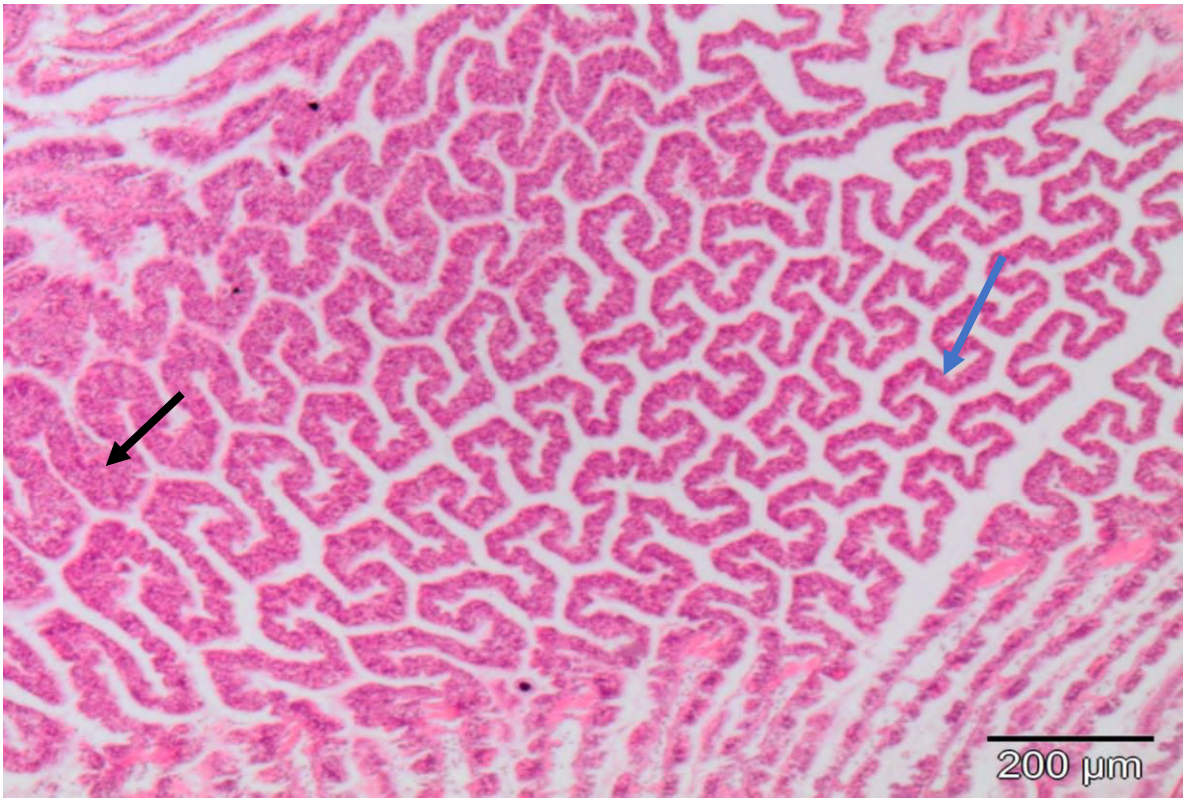


Figure 2.26: Histology of the left gill of South African abalone (H. midae), post a 42-h transport simulation using a light microscope under x400 magnification. Enlargement of the gill lamellae (Black arrow) compared to a uniform gill lamellae size (blue arrow).

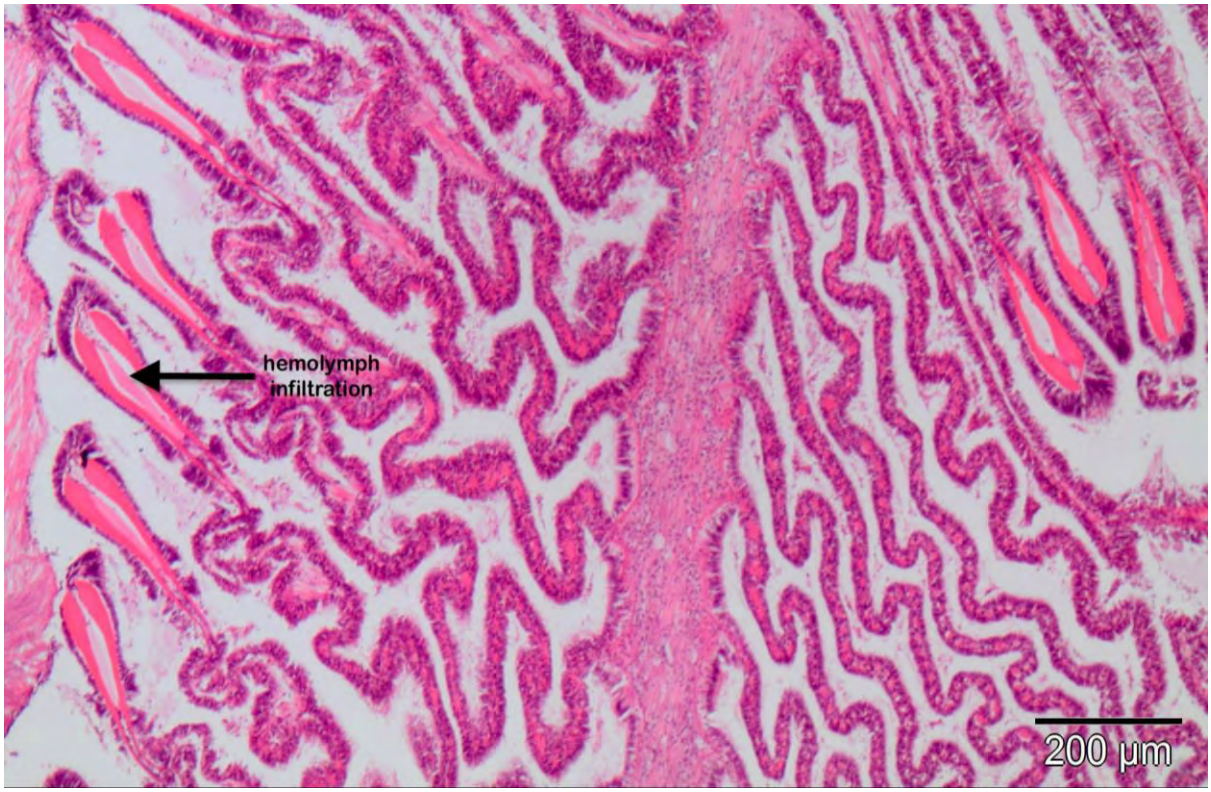


*Figure 4.27: Hemolymph infiltration of the gill lamellae (black arrow) of South African abalone (*H. midae*), after a 42-h transport simulation, under a light microscope with x400 magnification.*

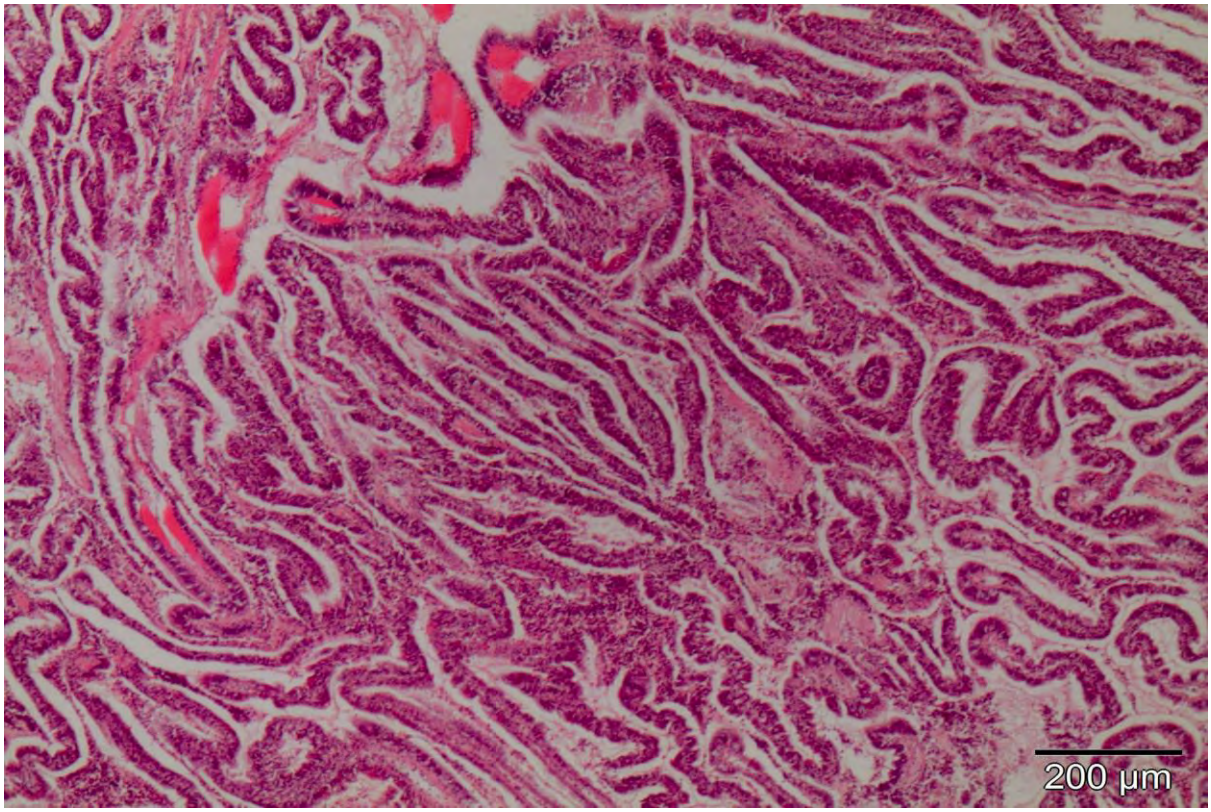


*Figure 4.28: Irregular or deformed gill lamellae histology of the South African abalone (*H. midae*), post a 42-h transport simulation, under x400 magnification.*

4.3.6.2 Gill grading score

There was no significant difference of the gill damage grading score of animals subjected to a 42-h simulated transport simulation. Animal damage grading score ranged from 1.57 ± 0.10 to 1.77 ± 0.10 within the 16 different treatments (Table 4.4).

Table 4.4: Histopathology gill grading score of live abalone between four different treatments, post a 42-h transport simulation (Multifactorial ANOVA, at p<0.05).

Factor 1 - Temperature	Chilled	Ambient	F - statistics	P value
	1.63 ± 0.10	1.71 ± 0.10	$F_{(1, 16)} = 0.28$	p = 0.60
Factor 2 – Purge days	2 days	3 days	F - statistics	P value
	1.68 ± 0.10	1.66 ± 0.10	$F_{(1, 16)} = 0.01$	p = 0.91
Factor 3 – Ice volume	1 ice sheet	2 ice sheets	F - statistics	P value
	1.71 ± 0.10	1.63 ± 0.10	$F_{(1, 16)} = 0.24$	p = 0.63
Factor 4 – Size class	50 – 60 g	90 – 110 g	F - statistics	P value
	1.77 ± 0.10	1.57 ± 0.10	$F_{(1, 16)} = 1.92$	p = 0.19

4.3.6.3 Haemolymph infiltration

There was no significant difference of the haemolymph infiltration within the goblet cells of abalone gills, post the 42-h transport simulation. Haemolymph infiltration ranged between 8.09 ± 4.10 % to 17.34 ± 4.10 % between the 16 different treatments. (Table 4.5).

Table 4.5: Percentage of hemolymph infiltration within the goblet cells of abalone gills, post the 42-h transport simulation (Multifactorial ANOVA, at p<0.05).

Factor 1 – Temperature	Chilled	Ambient	F statistics	P value
	12.30 ± 4.10	13.14 ± 4.10	$F_{(1, 16)} = 0.02$	p = 0.89
Factor 2 – Purge days	2 Day Purge	3 Day Purge	F statistics	P value
	8.09 ± 4.10	17.34 ± 4.10	$F_{(1, 16)} = 2.54$	p = 0.13
Factor 3 – Ice volume	1 ice sheet	2 ice sheets	F statistics	P value
	10.72 ± 4.10	14.72 ± 4.10	$F_{(1, 16)} = 0.47$	p = 0.50
Factor 4 – Size class	50 – 60g	90 – 110g	F statistics	P value
	14.19 ± 4.10	11.25 ± 4.10	$F_{(1, 16)} = 0.26$	p = 0.62

4.3.6.4 Gill epithelial lamellae thickness

There was a significant interaction of the thickness of the gill epithelial cells (µm) with different ice volumes (Factor 3), and different size classes (Factor 4) (Multifactorial ANOVA, $F_{(1, 16)} = 6.66$, p = 0.02, Figure 4.29). Bigger sized animals (90 – 110 g) with one ice sheet during export, had the highest thickness width at 40.80 ± 2.38 µm, compared to animals with two ice sheets during transport, which obtained a mean thickness width of 35.36 ± 2.38 µm. Smaller sized animals (50 – 60 g), which were subjected to one ice sheet during transport, reached the lowest gill epithelial thickness width of 28.50 ± 2.38 µm, compared to animals which were subjected to two ice sheets during transport and obtained a thickness width of 35.33 ± 2.38 µm.

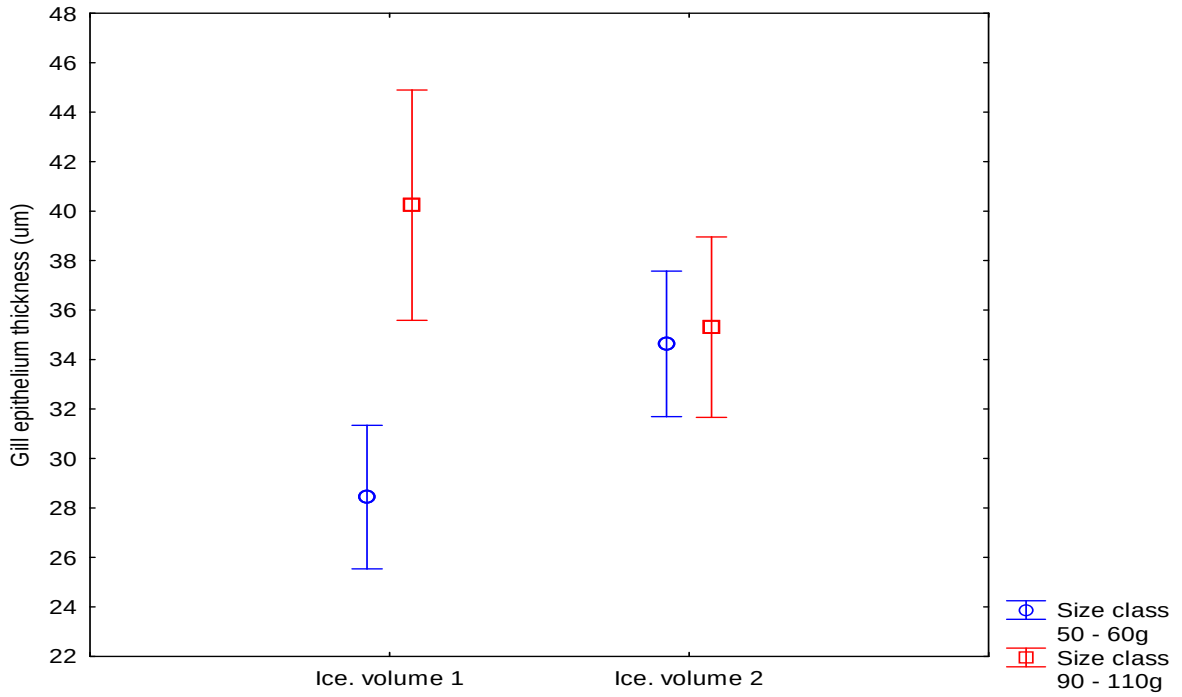


Figure 4.29: Mean (± 95 confidence intervals) gill epithelial lamellae thickness (um) of both 50 – 60 g (n = 143) and 90 – 110 g (n = 142) (Factor 4), subjected to ice volume 1 (548.5 g, with 144 replicates) and ice volume 2 (1090.88 g, n = 141) during packing (Factor 3), post the 42-h transport simulation. (Multifactorial ANOVA, $F_{(1, 16)} = 6.66$, $p = 0.02$).

4.3.7 Digestive Gland

The digestive gland damage consisted of a selection of stress indicators observed, including an observation of enlarged tubules (Figure 4.30) and increased distance or dilation of the lumen of each tubule (Figure 4.30).

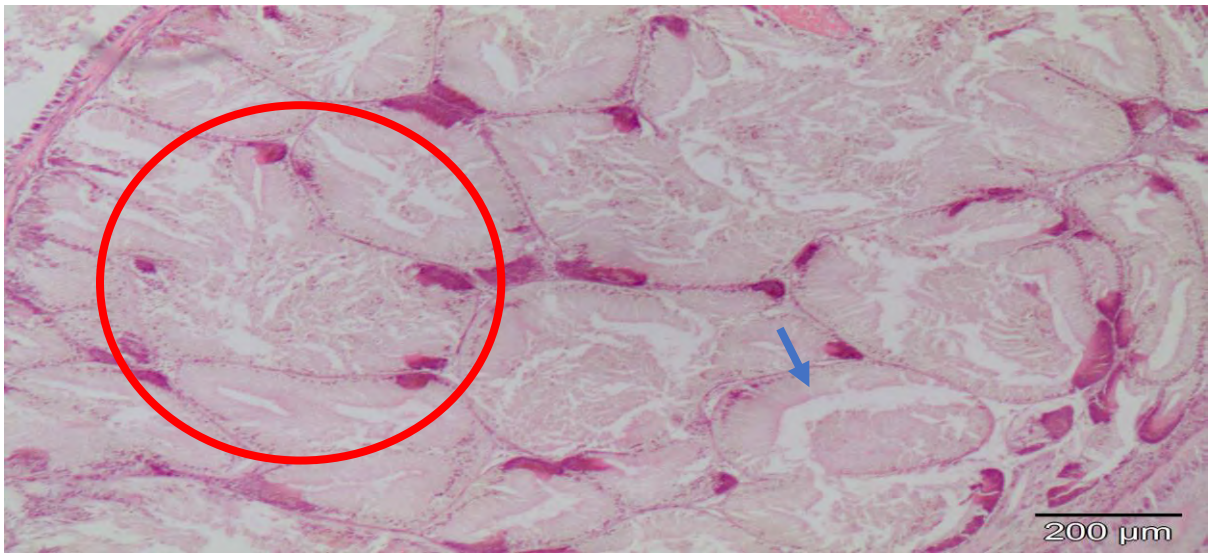


Figure 4.30: Abalone digestive gland histology, of animals post the 42-h transport simulation, under x400 magnification. Enlargement of the digestive gland tubule (red circle) was observed and as well as an increased lumen dilation from the center of specific tubules (blue arrow). Observation of digestive gland aletrations was from treatment 1, where small animals (50 – 60 g) were subjected to ambient temperature conditions, with a shorter purging period (two days), and ice volume 1 (548.5 g).

Detachment of the tubules from the base was also observed (Figure 4.31), where the presence of a brown pigmentation or inorganic granule accumulation in the cytoplasm of the digestive gland tubules was also randomly observed between the different treatments (Figure 4.32). The last stress indicator observed was the presence of the degradation and deterioration of the epithelial cells from either the basal lamina or the lumen of different tubules (Figure 4.33).

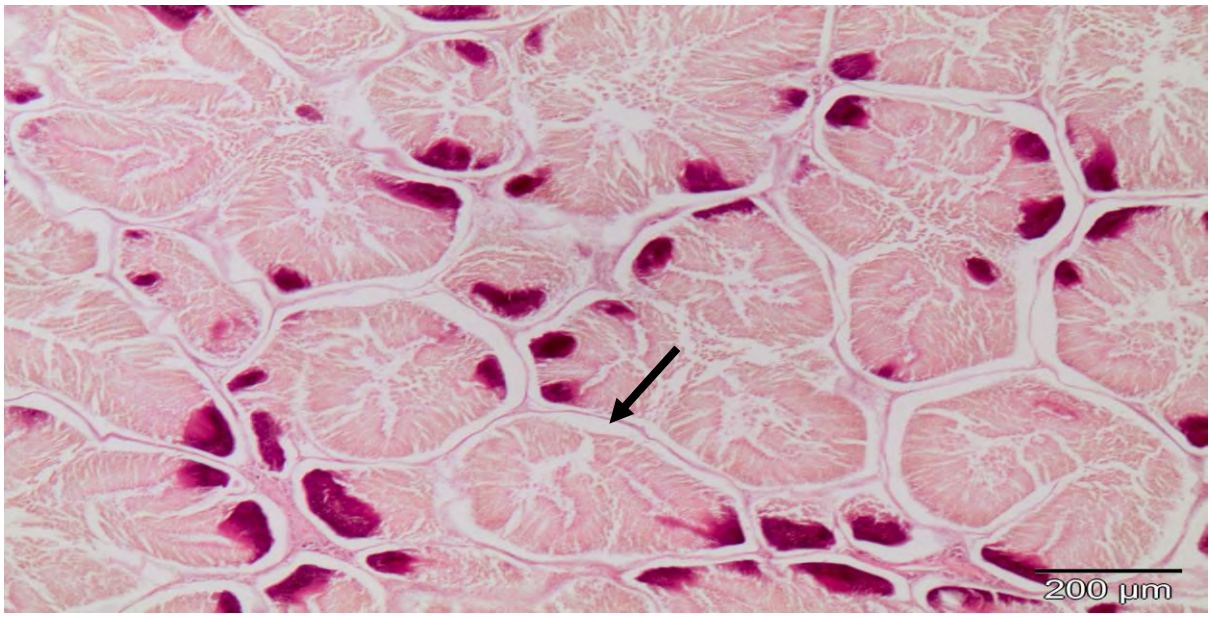


Figure 4.31: Abalone digestive gland histology of animals post a 42-h transport simulation, under x400 magnification. Separation of the tubule from the base of the digestive gland (black arrow). Alterations observed was from treatment 5, where small animals (50 – 60 g) were subejcted to a chilled acclimation temperature, a shorter purge (two days), and ice volume 1 (548.5 g).

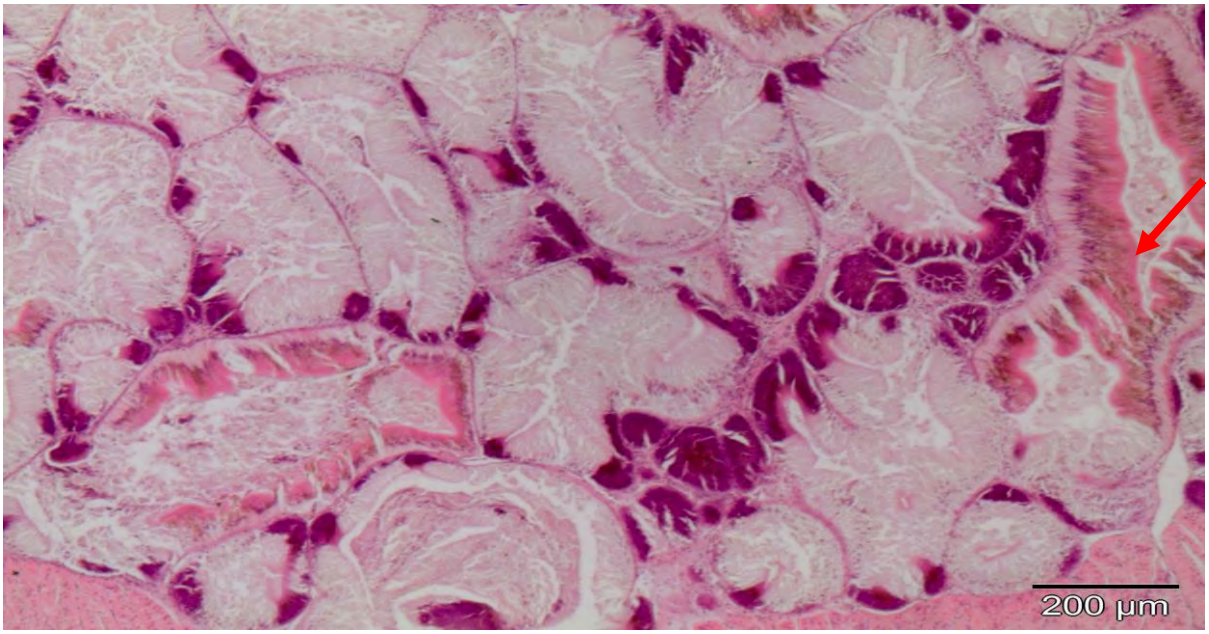


Figure 4.32: Abalone digestive gland histology of animals post a 42-h transport simulation, under x400 magnification. Brown pigmentation of inorganic granule accumulation (red arrow). Alterations observed was from treatment 14, where large animals (90 – 110 g) were subejcted to a chilled acclimation temperature, a longer purge of three days, and ice volume 1 (548.5 g).

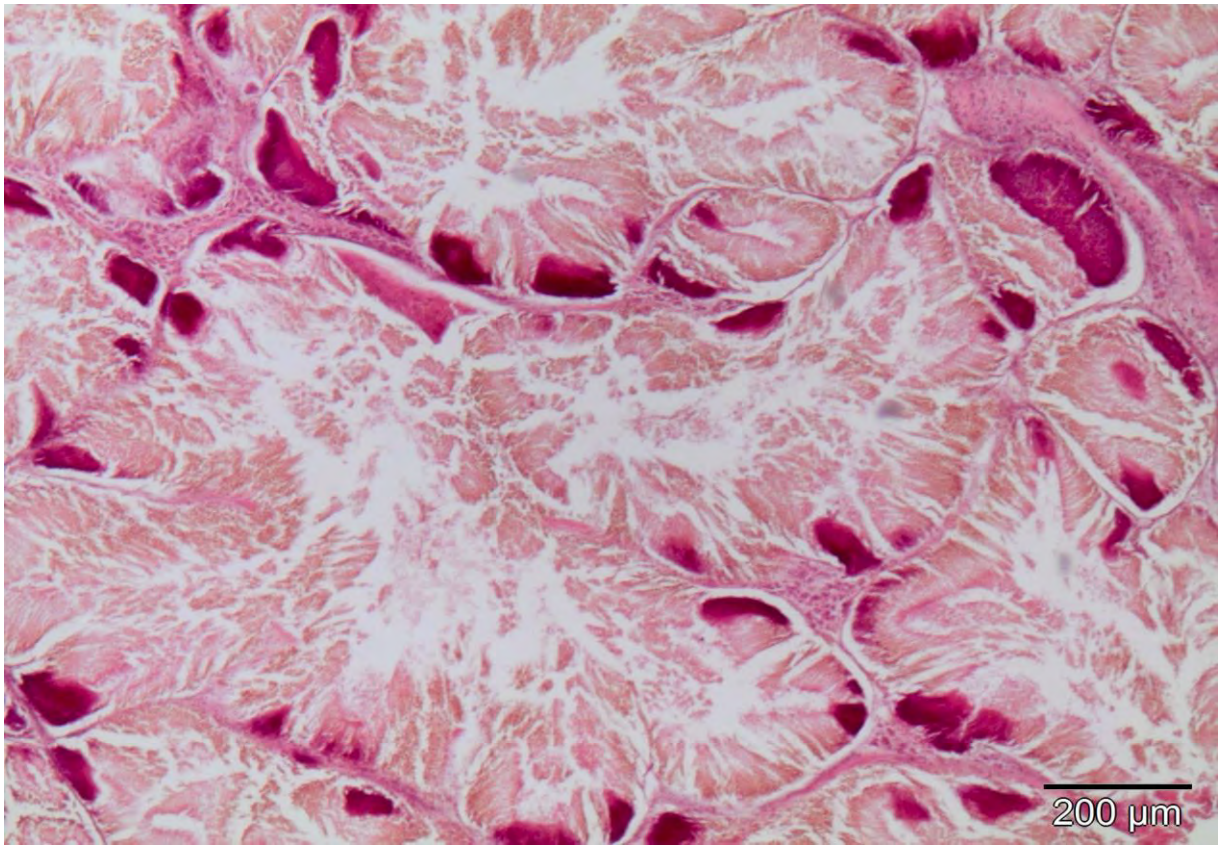


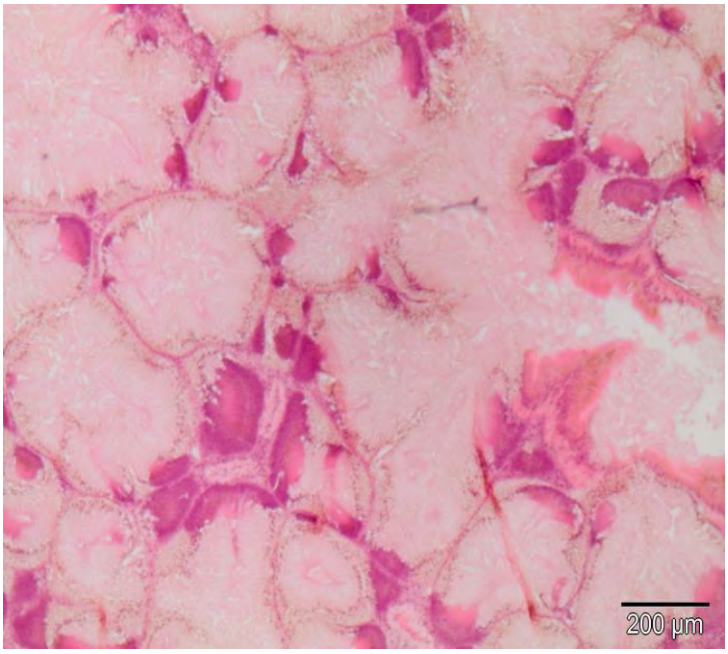
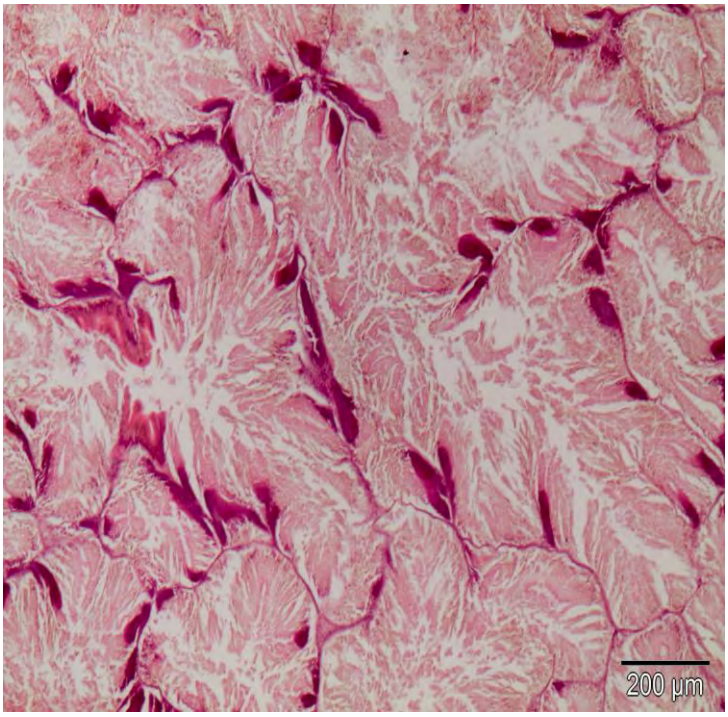
Figure 4.33: Abalone digestive gland histology, post a 42-h transport simulation, under x400 magnification. Observation of deteriorated epithelial cells of each tubule. Digestive gland aletrations was from treatment 1, where small animals (50 – 60 g) were subjected to ambient temperature conditions, with a shorter purging period (two days), and ice volume 1 (548.5 g).

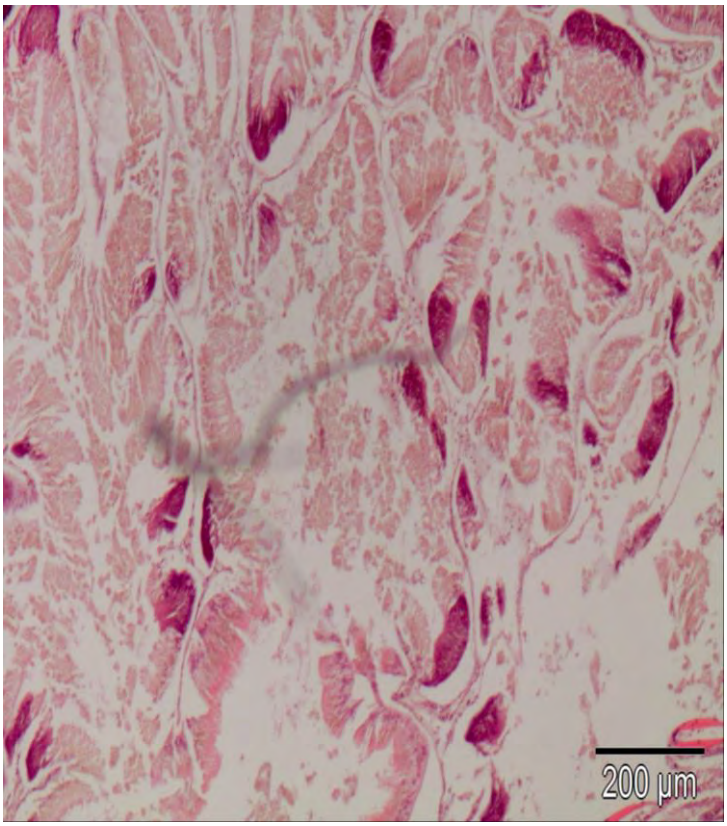
4.3.7.1 Qualitative histological results of the digestive gland

The degradation of the epithelial cells of the digestive gland tubules was determined based on the level of deterioration of the epithelial cells of each tubule and as well as the frequency of observed deterioration epithelial cells throughout each different slide.

There was a total of three different epithelial cell deterioration and degradation criteria observed, where for the first criteria, epithelial cell deterioration ranged from 0 – 5 % (Table 4.6), where epithelial cells remained mostly intact, for the best-case scenarios, and if cell deterioration was observed then cells had very minimal deterioration across each tubule. For the second criteria, epithelial cell deterioration ranged from 6 – 15%, across majority of the tubules. Epithelial cell deterioration was highest nearest the lumen of each tubule and gradually decreased towards the edge, dependent on the image. The last 3$^{rd.}$ criteria, consisted of epithelial cells deterioration ranging from 20 – 40 %, where deterioration occurred highly towards the centre of the lumen and as well as the edges of each tubule.

*Table 4.6 Epithelial cell degradation levels observed of the digestive gland tubules of South African abalone (*H. midae*) after subjected to 42-h transport simulation.*

	Digestive gland tubules were closely compacted together and easily distinguishable between each other. Very minimal or no distance of the lumen of each tubule was observed. Epithelial cell deterioration ranged from 0 – 10 % across majority of the different tubule.
	Majority of the digestive gland tubules were still easily distinguishable between each other. The occurrence of distance of lumen in each tubule was observed and frequency of observation increased. Epithelial cell deterioration increased and ranged from 11 – 20 % for majority of the tubule.

	A few of the tubules were easily distinguishable between each other, however majority were difficult to distinguish. the distance of the lumen within the tubules increased, as well as the detachment of the tubule from the base. Deterioration of the epithelial cells increased significantly and majority of the epithelial cells have been deteriorated and lost from each tubule.

4.3.7.2 Digestive gland grading score

There was no significant difference of the digestive gland damage grading score between the different treatments, post the 42-h transport simulation. Animal digestive gland damage score ranged between 1.38 to 1.58 between the 16 different treatments (Table 4.7).

Table 4.7: Digestive gland damage grading score histopathology of animals, post the 42-h transport simulation (Multifactorial ANOVA, at p<0.05).

Factor 1 – Temperature	Chilled	Ambient	F statistics	P value
	1.54 ± 0.09	1.42 ± 0.09	$F_{(1, 16)} = 0.77$	p = 0.39
Factor 2 – Purge days	2 Day Purge	3 Day Purge	F statistics	P value
	1.53 ± 0.09	1.43 ± 0.09	$F_{(1, 16)} = 0.50$	p = 0.49
Factor 3 – Ice volume	1 ice sheet	2 ice sheets	F statistics	P value
	1.49 ± 0.09	1.47 ± 0.09	$F_{(1, 16)} = 0.02$	p = 0.90

Factor 4 – Size class	50 – 60g	90 – 110g	F statistics	P value
	1.38 ± 0.09	1.58 ± 0.09	$F_{(1, 16)} = 2.02$	p = 0.17

4.3.7.3 Digestive tubule epithelial cell degradation

There was no significant difference of the degradation of the epithelial cells of the digestive gland tubule, post the 42-h transport simulation. Degradation of the epithelial cells ranged from 7.79 % to 10.50 % between the 16 different treatments (Table 4.8).

Table 4.8: Percentage of the degradation of the epithelial cells within the digestive gland of abalone, post the 42-h transport simulation (Multifactorial ANOVA, at p<0.05).

Factor 1 – Temperature	Chilled	Ambient	F statistics	P value
	10.04 ± 0.02	8.25 ± 0.02	$F_{(1, 16)} = 0.53$	p = 0.48
Factor 2 – Purge days	2-day purge	3-day purge	F statistics	P value
	10.50 ± 0.02	7.79 ± 0.02	$F_{(1, 16)} = 1.20$	p = 0.29
Factor 3 – Ice volume	1 ice sheet	2 ice sheets	F statistics	P value
	8.47 ± 0.02	9.82 ± 0.02	$F_{(1, 16)} = 0.30$	p = 0.59
Factor 4 – Size class	50 – 60g	90 – 110g	F statistics	P value
	8.35 ± 0.02	9.93 ± 0.02	$F_{(1, 16)} = 0.41$	p = 0.53

4.3.8 Right kidney

Within the right kidney tubules, a total of three changes to the right kidney was observed, which were classified as stress indicators, including the observation of enlarged tubules (Figure 4.34), increased distance or dilation of the lumen (Figure 4.34), and degraded epithelial cells of the tubules (Figure 4.35).

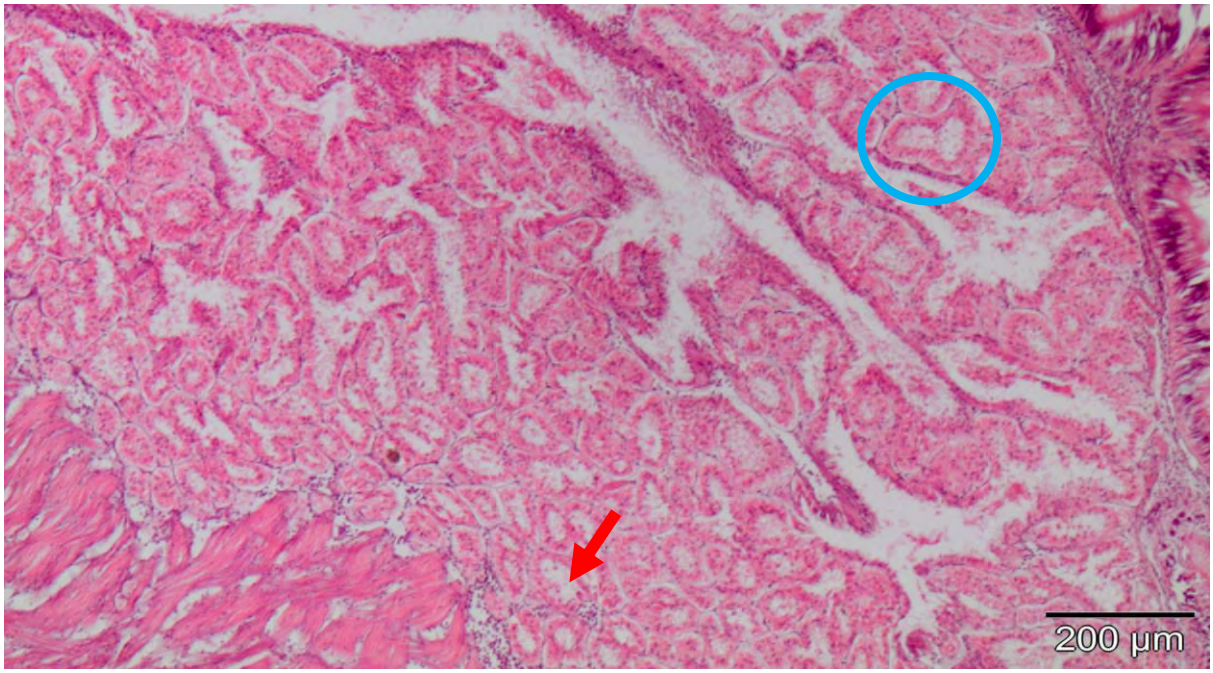


Figure 4.34: Abalone right kidney histology, post a 42-h transport simulation, under x400 magnification. Red arrow indicates the increased dilation of the lumen from different tubules, and blue circle indicates the enlarged tubule.

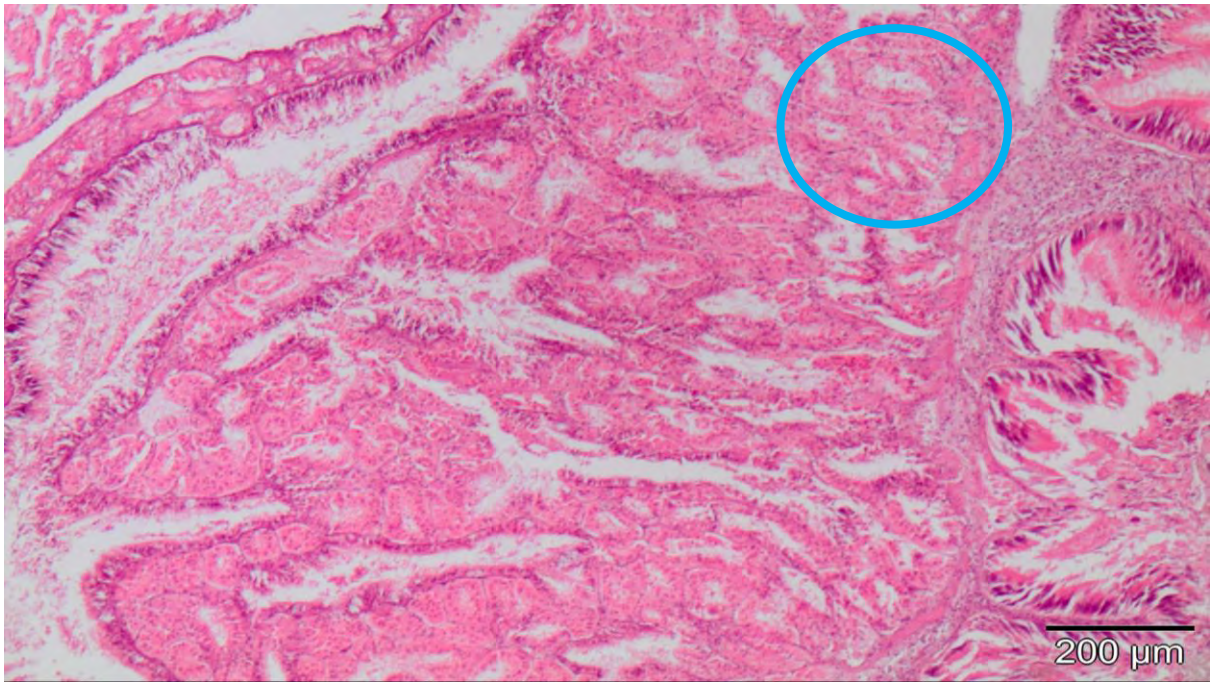
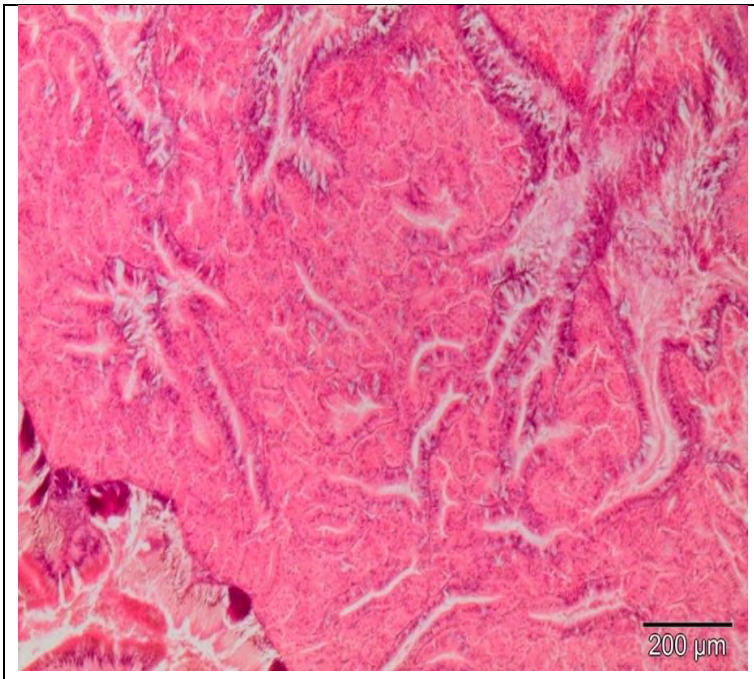
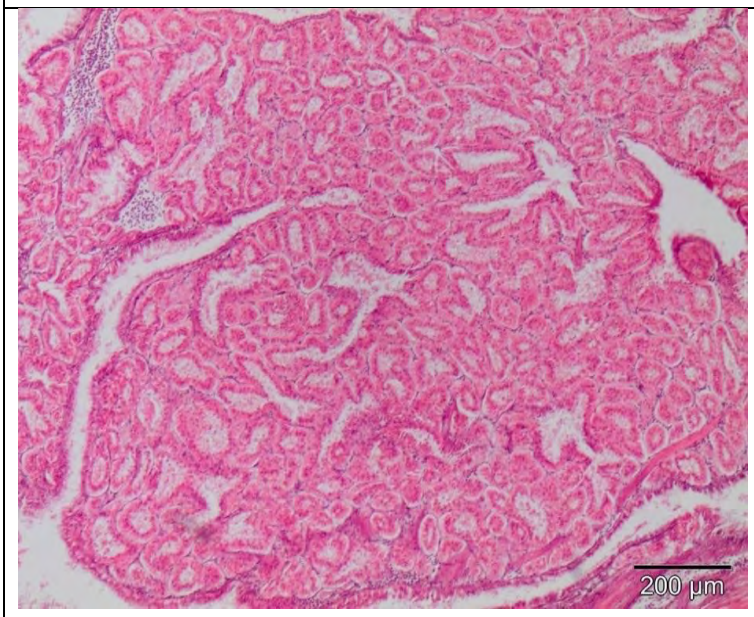


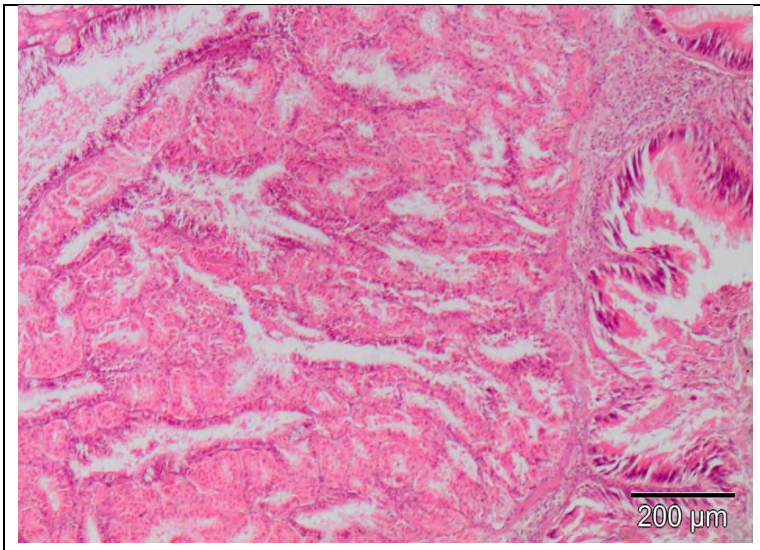
Figure 4.35: Abalone right kidney histology, post a 42-h transport simulation, under x400 magnification. Blue circle indicates a cluster of deteriorated or degraded tubules and epithelial cells.

4.3.8.1 Qualitative histological results of the right kidney

The deterioration of the epithelial cells was classified in the same manner as the digestive gland tubules, where each image was classified into three different criteria, based on the visibility of each tubule (Table 4.9).

*Table 4.9: Epithelial cell degradation levels observed of the right kidney tubules of South African abalone (*H. midae*) after subjected to 42-h transport simulation.*

	Majority of all tubules were easily distinguished between each other. Tubules were closely compacted together and no lumen were visible. Only ducts were present and visible No distance of the tubule lumen was observed or if observed, distance was minimal.
	Majority of tubules were also easily detectable and distinguishable between each other. Tubules were also closely compacted together. majority of the tubule lumen had an increased distance compared to criteria 1.

	Majority of the tubules were not easily detectable from each other and distinguishing between the different tubules was more challenging. Distance of the lumen within each tubule ranged, however an increased distance is observed compared to criteria 2.

4.3.8.2 Right kidney grading score

There was a significant inverse interaction of the right kidney damage grading score with both the different purge days (Factor 2) and the different acclimating temperatures (Factor 1) post the 42-h transport simulation (Multifactorial ANOVA, $F_{(1, 16)} = 8.55$, $p = 0.010$, Figure 4.36). All animals from all different treatment reached a damage grading score of one, which included low variations of enlarged tubules and increased distance of the lumen from different tubules. The right kidney grading score was influenced differently depending on the purge day and the temperature at which abalone were held ahead of transport. When animals were chilled, the right kidney score increased with an increase in the number of purge days, whereas when animals were kept at an ambient temperature ahead of transport, the right kidney score decreased with an increase in the number of purge days (Figure 4.36).

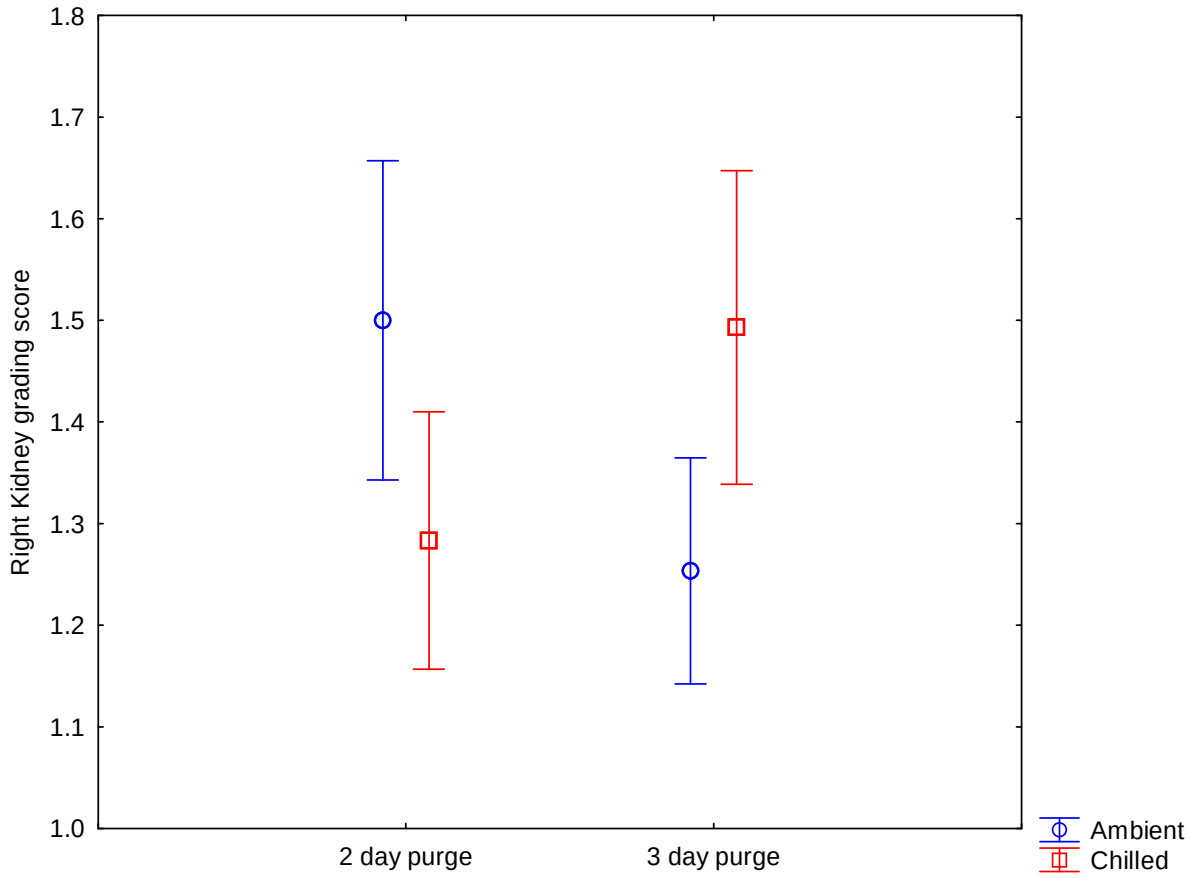


Figure 4.36: Mean (± 95 confidence intervals) right kidney grading score of animals subjected to both a chilled purging temperature (n = 131) and ambient purging temperature (n = 137), with both a shorter purge (two days, n = 126) and longer purge (three days, n = 142) (Factor 2), post the 42-h transport simulation. (Multifactorial ANOVA, $F_{(1, 16)} = 8.55$, $p = 0.009$).

4.3.8.3 Right kidney tubule size

Although there was no significant interaction, there was however a significant difference of the size of the right kidney tubule with both size classes (Factor 4) and different acclimation temperatures (Factor 1) (Factorial ANOVA, at $p<0.05$, Figure 4.37 and Figure 4.38). Larger animals (90 – 110 g) had a significantly bigger right kidney tubule width of 74.15 ± 2.00 µm compared to smaller animals (50 – 60 g) with a width of 66.42 ± 2.00 µm, after the 42-h transport simulation. This was similar with animals subjected to a chilled purging environment, which had a bigger tubule width compared to smaller animals.

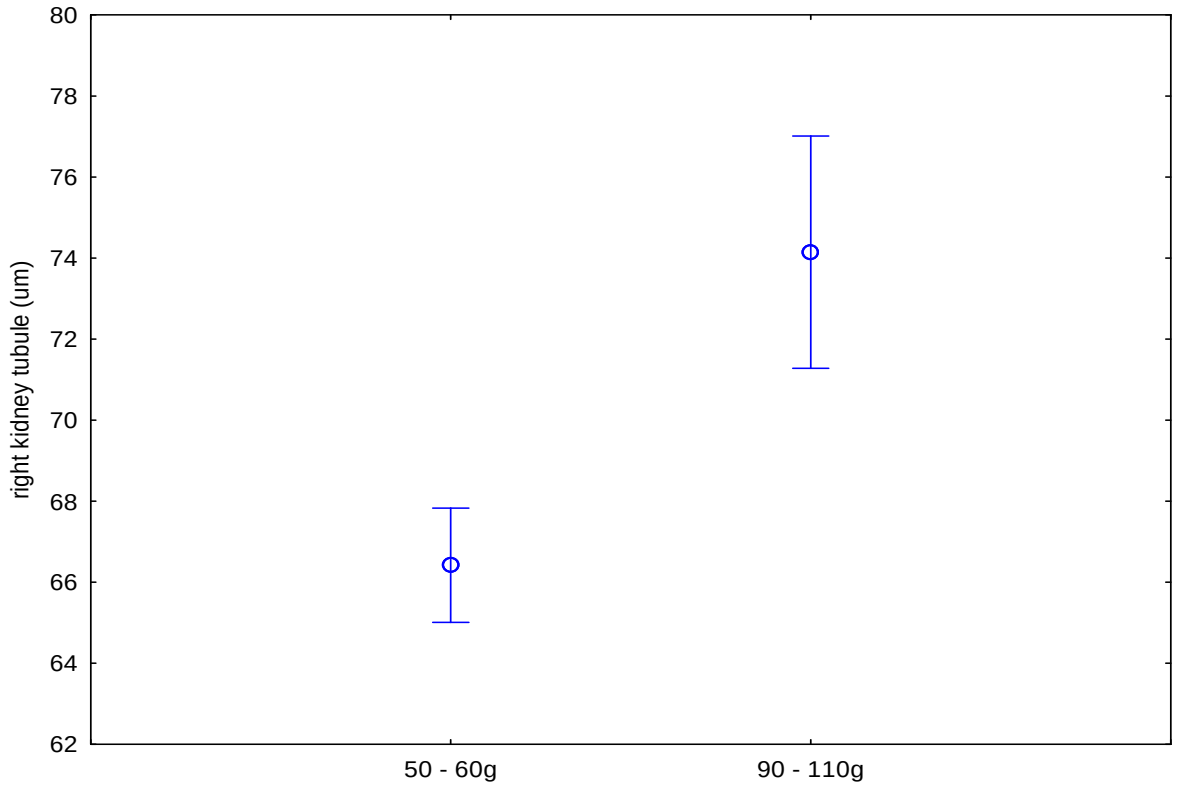


Figure 4.37: Mean (± 95 confidence intervals) of the right kidney tubule width (µm) of 50 – 60 g (n = 16) and 90 – 110 g (n = 16) size classes, post the 42-hour transport simulation. (Factorial ANOVA, $F_{(1, 16)} = 7.44$, $p = 0.015$).

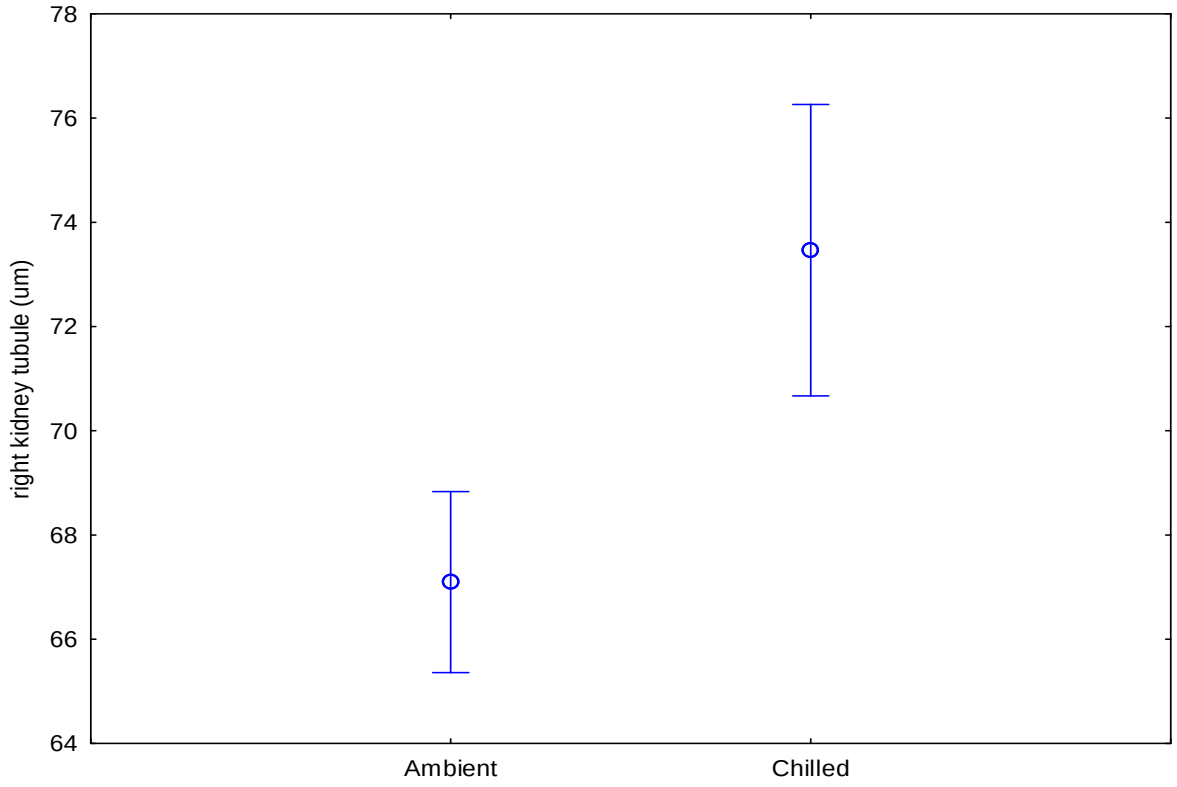


Figure 4.38: Mean (± 95 confidence intervals) right kidney tubule width (µm) of animals subjected to a chilled purging temperature (n = 16) and ambient puring temperature (n = 16), post the 42-h transport simulation. (Factorial ANOVA, $F_{(1, 16)} = 5.06$, $p = 0.04$).

4.4 Discussion

4.4.1 Water quality

During the summer months, when increased sea temperatures prevail, dissolved oxygen depletion (deoxygenation) and acidification was commonly observed as temperature is correlated with the diffusion ability of both dissolved oxygen and pH (Song *et al*., 2020; Kale, 2016; Pintilie *et al*., 2014). This was observed within this trial, where dissolved oxygen, within the purging tanks, in ambient sea temperature, during the summer months, had a higher O_2 depletion by 0.59 mg/L compared to DO levels during the winter months, where average concentrations reach 7.58 mg/L, 94 % for DO, and 7.86 for pH. This was similar to results from Yang *et al*., (2021) that found a 0.31 – 0.55 and 0.96 – 2.10 μmol d^{-1} decrease rate of dissolved oxygen within the summer months of bay scallops. In previous studies, it was shown that DO concentrations, within seawater, that were lower than 94.0 and 62.5 μmol 1^{-1} resulted in variation of fish and different benthic organism survival. It was then deduced that these concentration levels were the threshold of low oxygen and hypoxia conditions (Yang *et al*., 2021). This was also observed within this trial, where a mortality was observed in the high ambient purging condition, which might have been due to the lower oxygen supply within the tanks.

The pH concentration levels (H^+) distributed within this trial for ambient tanks were higher compared to pre-cooled tanks. Under normal circumstances, pH levels decrease with an increase in temperature (Yartsev, 2015), where for this trial, a higher pH within the ambient tanks was observed. This could be due to both the biological and physiochemical process during purging. At increased temperatures, metabolic activity from abalone, increases and in turn produce a higher volume of carbon dioxide, which also changes the pH of the water column (Hu, *et al.,* 2021). As abalone produce carbon dioxide, it forms carbonic acid (H_2CO_3), which dissociates in water to release hydrogen ions (H^+), which subsequently contribute to lowering the pH of the tanks (Woyke, *et al.,* 2022).

Export stress, which include periods of handling and purging, affects the metabolism and in turn their mortality rates (Alfaro *et al*., 2021). This effects the CO_2 build up during transport, where Novak *et al*. (2024) found that a prolonged exposure of a low pH, of around 6.8 – 6.4, resulted in sublethal levels for abalone. The prolonged exposure to CO_2, combined with low oxygen levels suppresses respiration, which could induce CO_2 hypoxia during transport

(Steckbauer *et al*., 2015). Hypercapnia has been shown to have severe negative effects on the physiology of marine animals, where different calcification species, including molluscs are more sensitive to acidification due to the added energetic cost of calcification (Novak *et al*., 2024).

4.4.2 Post transport-simulation

The best outcome in terms of abalone performance, by maintaining their weight during transport, improved survival upon arrival and during recovery, was influenced mostly by purge days, prior to transport. Animals, which are fed an artificial feed (Abfeed®, Marifeed Pty Ltd, South Africa), with a longer purge resulted in a better performance, specifically in the maintenance of their net weight, their weight loss, survival upon arrival and during recovery. Results from this trial further enhance and elaborate on the insights of the current proposed suggestions and recommendations, of a 2-3 day purging period, by Bubner *et al*. (2009) and Sales (2001).

Abalone performance was also mostly influenced by the animal's internal and external temperature, either through the amount of ice packed or through the process of pre-cooling. Animals that had more ice, during transport, had a better performance on the maintenance of their individual weight, the amount of excess biomass retained from their individual weight, and finally their survival during recovery. Pre-cooling, on the other hand, influenced mainly survival during recovery, behaviour, and majority of the right kidney histology, including the damage grading score and the size of tubules, post the transport simulation.

These more optimal and combination of different pre-transport conditions, including more ice in the boxes, pre-cooled animals, and a longer purge prior to transport, also demonstrate a more optimal temperature regulation method of the boxes during transport. This was seen in this trial during the transport simulation, where boxes exposed to these different conditions had a better drop in internal temperature at the initial start of transport and maintaining the temperature regulation up until the end of aerial transit. However, the rate of temperature increase, post aerial transit, was more determined by the amount of ice within the boxes compared to the other transport conditions. These results expand over a range and long duration of ambient air temperatures, which is important as temperatures within different international markets exceed 30 °C (Bubner *et al*., 2009). However, ambient air temperatures for this trial ranged from 13.94

to 21.76 °C throughout the simulation, which is lower compared to actual export temperatures within different international markets. The process of chilling animals during transport is a highly effective method of preserving and maintaining high quality products and reducing spoilage (Ninan, 2017). This method can be achieved either through cold air storage, chilled seawater or freshwater storage, or inclusion of ice during packing, where the method of ice storage is the most widely employed. It was found by Ninan (2017) that the use of ice for thermal regulation of fish was more advantageous as ice has a high latent heat of fusion and is capable of removing large amounts of heat. A supply of one kilogram of ice can absorb 80 k cal of heat, which is sufficient enough to cool around three kilograms of fish. This was observed in this trial, where the temperature of the boxes had a higher success of thermal regulation throughout transport with the use of ice compared to pre-cooling animals prior to transport.

Thermal regulation was also influenced by purge days in this trial, as animals which were subjected to a longer purge resulted in a lower temperature drop at the initial start of transport and also throughout the different phases of transport, with the exception of thermal regulation upon arrival. Mørkøre *et al*., (2008) found that a longer starvation period of five weeks, of Atlantic salmon, prior to harvest, resulted in improvement of acute stress due to reduced spending of stored energy from the intestinal and muscular lipid stores. This could indicate the ability of the abalone to efficiently use their energy reserves, during transport, with a longer purge. This is due to abalone's digestive process, including duration and enzymatic activity, like other "stomach-foot" gastropods, which are affected by different surrounding environmental factors, including temperature, water quality, and stress (Rogers-Bennet, *et al*., 2010; Morash & Alter, 2015; Currie, *et al*., 2015; Frederick, *et al*., 2022). The abalone's gastrointestinal evacuation time (GIE), or the rate at which food passes, and nutrients are absorbed (Currie *et al*., 2015), through the intestinal tract has been tested for *H. midae* and used in order to determine optimal purging durations for export (Currie *et al*., 2015). A period between 12 to 60 h post feeding, during purging, results in majority of faeces being voided from the animal, which suggest that a longer purge of at least 72-h is required for a full or most optimal expel of the gut content during purging. This could be due to less digestible algae species remaining in the stomach for more than 48 hours, compared to desirable algae species, which normally digested within 24-h (Day and Cook, 1995). Thermal regulation with a longer purge was in turn more beneficial as it has been shown by Waagbø *et al*., (2017) to reduce

metabolic rate, physical activity, and stress during transportation, which was also observed within this trial.

Since South African abalone (*Haliotis midae*) are temperate species that are mainly farmed across the west and south-west coast, average temperatures range between 13.5 °C until 16 °C (Vosloo *et al*. 2013), however during summer months temperatures may peak at 22 °C for extended weeks. This in turn can have negative physiological effects due to a prolonged exposure to temperatures higher than 20 °C, such as increased mortality (Vosloo *et al*., 2013), and decreased behavioural response (Madeira *et al*., 2016). This was observed within this trial post purging, where animals subjected to ambient acclimation conditions, at temperatures higher than 21 °C for the entire purging process, were less active during each simulation event, where mortality was also observed during the packing process of the last simulation event. However, from when animals were packed in their export boxes and then post the 42-h transport simulation, only a longer purging period resulted in both a better maintenance of net weight (g) during transport, a higher net weight upon arrival (lower weight loss %) and higher initial survival rate (%) upon arrival.

During the peak summer months, when increased warm waters prevail, due to prevailing westerly winds, and extend for a period of time across the coast (Vosloo and Vosloo, 2010; Vosloo *et al*., 2013), added weight (g) of animals, during harvest, is adjusted and calculated for (Shaw *et al*., 2023) to mitigate for the extra weight loss (%) animals experience during purging within the warmer summer months. Minimum excess weight (g) for smaller animals (50 – 60 g) was 9 grams and 20 grams for bigger animals (90 – 110 g) during grading. After purging and the transport simulation, during the summer months, both small and large animals lost an individual weight of 8.24 g and 9.51 g, respectively. This weight change, was dependent on the amount of ice provided in the boxes during packing. Larger animals packed with more ice, resulted in a lower change in individual weight (g) by 4.71 g, post the transport simulation, and smaller animals with less ice had a smaller change in individual weight by 3.13 g. Chilling animals, through the provision of ice has been to shown to reduce enzyme activity and also in turn reduce bacterial activity (Ninan, 2017). Reduction in the enzymatic activity, which is due to a reduced metabolic rate through the provision of ice, allows for a reduced cellular energy demand and in turn energy reserves are preserved (Marshall and McQuiad, 2020).

Abalone, like other gastropod molluscs, are capable of using metabolic rate depression (hypometabolism), where animals reduce their cellular energy demand, through reducing rates of membrane ion pumping and macromolecule synthesis, which reduces energy demand (Marshall and McQuiad, 2020). This process, which is coupled with behavioural isolation, is generally activated during episodes of prolonged reduced oxygen uptake during hypoxic events that facilitates estivation during prolonged air exposure (Marshall and McQuiad, 2020). Animals adjust their physiological, morphological, behaviour, and enzyme activity in order to cope with rapid changes in ionic, water, and thermal balances (Chukwuka and Ejere, 2019), which was seen within this trial as animals subjected to a longer purge were able to preserve and retain their net weight during transport, which resulted in a higher net weight upon arrival. Chukwuka and Ejere (2019) found that during episodes of estivation, enzyme activity was significantly higher in order to regulate biochemical fuel reserves efficiently, which could explain the higher net weight retention with a longer purge. Animals which had a longer purge were more able to efficiently adjust their enzyme activity in order to regulate their energy reserves. Animals which had a shorter purge were not capable of expelling their gut content, which in turn could have led to complications of digestion during hypoxic stressful events, which can increase oxygen demand and initiate changes in the enzymatic activity, including amylase activity, β-glucosidase activity, maltase activity, and alanine-aminopeptidase activity (Chabot *et al*., 2015; Frederick *et al*, 2022). A longer purge may have also resulted in a reduced bacterial load during transport within this trial, as Martinez *et al*., (2009) found that port periods of depuration, high loads of gut bacteria, including vibrio, salmonella, and Aeromonas, were greatly reduced.

More ice, during transport, also resulted in a higher percent of excess biomass of animal's body weight, after the export process, for older animals. Since the ability of abalone to retain water is based on their shell aperture (Gorfine, 2001), which is also influenced by animal age, bigger and older animals were able to retain more water of 80 g compared to small juvenile animals during this experimental trial. Pan *et al*., (2018) also found that reduced UV combined with low temperatures conditions significantly reduced the bacterial count in molluscs, as animals upon arrival had a low accumulation of waste and bacterial loading (Kijewska, *et al*., 2023).

Animal age can also affect water retention, where different temperatures can also influence the GIE rate differently, for different abalone species (Currie *et al*., 2015; Frederick *et al*., 2022).

This was observed in this trial where bigger and older animals were more affected in terms of their individual weight upon arrival, 42-h post the transport simulation. Bigger animals, which had a shorter purging period, and less ice during transport, resulted in the lowest individual weight upon arrival.

Under rapid environmental change, including prolonged functional hypoxia and climate change, understanding the mechanisms that aquatic organisms use to adapt to hypoxic stress, is of importance in order to optimally conserve and utilize the animals' biological resources and ensure survival (Dai *et al*., 2024). Previous studies have shown that hypoxia affects vital "functional traits, including respiration, excretion, behaviour and immune systems" (Gurr *et al*., 2018; Shen *et al*., 2020; Hou *et al*., 2023). In South African abalone (*H. midae*) functional hypoxia is a stress condition that is produced from increased muscle activity, which is also aggravated with increased temperatures, which also initiates the start of anaerobic metabolism, where abalone are heavily reliant of this process during oxygen deprived episodes (Venter *et al.*, 2018). During prolonged air exposure, during transport, animals also experience desiccation (Alfaro *et al*., 2021), which further decreases the ability of the gut of the abalone to optimally conduct their enzymatic activity (Frederick *et al*, 2022). Animals that are able to more optimally expel the content of their guts, prior to functional hypoxia, by using a longer purging period, within this trial, were able to retain the absorbed feed in the body for longer compared to animals which were exposed to hypoxia conditions during digestion. This was seen for all weight parameters, with the exception of the change in individual weight during transport. This in turn lead to further complications as digestion results in increased oxygen demand (Chabot *et al*., 2015), and animals under heat stress have been shown to have elevated metabolic rates and changes to the digestive enzyme activities (Frederick *et al*, 2022). During elevated temperatures, or heat stress, the digestion, specifically the digestive enzyme activities on the gut, could be reallocated to other resources, including reproduction.

Organisms that are also capable of using alternative metabolic pathways, (including aerobic and anaerobic respiration), specifically during prolonged hypoxic episodes, ensure a higher chance of survival until recovery is possible after the hypoxic episode (Venter *et al*., 2018). Bursts of muscle activity, during prolonged hypoxic episodes, deplete cellular energetic reserves resulting in an increase in oxygen demand, which also limits adenosine triphosphate (ATP) production (Venter *et al*., 2018). This was seen in Kurnaningtyas *et al*. (2022) study, which conducted a transport simulation black lip abalone (*H. rubra*) and found that the

provision of ice increased the survival rate and animals lost less weight (g) upon arrival by 10 % compared to the 13% when ice was not present, after a 40-h transport simulation. This was also seen within this trial where survival rate was influenced by both the amount of ice packed and the purge days animals were exposed to prior to transport. Animals which had more ice packed had a higher survival rate during recovery as animals which had a reduced metabolic rate were able to use their energy reserves more efficiently for thermal regulation (Marshall and McQuiad, 2020).

Survival rate from Kurnaningtyas *et al*. (2022) study showed that animals which were conditioned to pre-cooling prior to being subjected to a transport simulation, had no mortalities upon arrival and animals subjected to an ambient pre-acclimation condition reached a 7 % mortality rate upon arrival. This was also seen within the recovery duration of this trial, where coupled with abalone mortalities being delayed, which can last a couple of weeks (Ragg and Watts, 2015), survival differed throughout each of the different recover days. Animals subjected to a chilled purging environment had a 14 % lost compared to ambient animals which had a 22% lost. This was also seen within Lorennzo *et al,* (2020) study that found a higher survival of crabs during recovery when animals were subjected to a pre-cooled condition prior to transport.

Similar results were observed for animals which had both a longer purging period and a higher volume of ice during transport, which had a lower 13% loss compared to animals which had shorter purging period and a lower volume of ice, which had a 23% loss of animals. An incomplete gastro evacuation process can result in a lower survival rate due to the amount of food available for energy turnover, and in turn impacting the metabolic processes and pathways, post the transport simulation (Anestis, *et al*., 2007). This is due to temperature influencing the health of aquatic animals, through decreasing their metabolic rate, which reduces oxygen demand and consumption (Shin and Yang, 2005; Lorenzo *et al., 2020*).

4.4.3 Behaviour

Overall behavioural response was negatively affected by the 42-h transport simulation within this trial, which was similar to results from Chapter 2, where overall abalone behavioural response, was negatively affected. Abalone righting time (coupled with the side-to-side movement), adhesive force, and speed, having the most prominent undesirable behaviour.

As righting behaviour is a good indication of their energy metabolism (Baldwin *et al*., 2007), specifically their energy resilience, animals subjected to this entire trial lost a significantly high amount of energy, due to different prolonged stress conditions, which resulted in all animals not being able to right themselves. However, animals which were subjected to a chilled purging environment prior to transportation resulted in stronger response during their righting behaviour by the animal's side to side movement, which is an indication of a better energy reserve through a reduced metabolic rate, and in turn a better stress resilience and a higher chance of survival during recovery (Baldwin, *et al*., 2007; Lachambre *et al*., 2017).

The abalone has a strong adhesive force, where a single animal, at around 15 cm, is capable of generating an adhesive capacity of around 200 kg, which indicates a good survival skill when under stress, such as wave action, which in turn can produce survival activities, including crawling, predation, reproduction (Xi, *et al*., 2023). An abalone's adhesive force is also a good indication of their energy reserve and their hypoxia tolerance, as it is energetically costly (Li *et al*., 2018; Dai *et al*., 2024). An animal's adhesive force is used in order to strongly adhere to slippery rocks within their natural environment (Li *et al*, 2018) and also to successfully complete the righting behaviour performance (Zhang *et al*., 2020). Within this trial, animals lost a significant amount of energy, where within every treatment, the righting behaviour was unsuccessful. As the success of performing the righting behaviour is also based on the amount of force abalone are able to produce (Zhang *et al*., 2020), animals were not successful in performing the righting behaviour, in this trial, as all animals were not able to produce a minimum of 3.29 N force, post the 42-h transport.

Temperature has the ability to negatively influence the amount of strength an adhesive or bonding agent can have, where Banea *et al*., (2012) found that under increased temperatures, the strength of an adhesive reduces. This was observed within this trial, as both small/ young animals and bigger/ older animals, which had more ice during transport had the highest adhesive force of 0.08 N and 2.0 N compared to all the other treatments, which indicates a higher energy reserve (Baldwin *et al*., 2007). The animals body size and age has also been shown to influence and vary in energy allocation (Ganmanee *et al*., 2010), which was also observed within this trial as the highest adhesive force produced by the younger animals, of 0.75 N, was significantly lower than older animals, which had a higher adhesive force of 2.17 N.

Although temperature has been shown, in previous studies and within this trial, to influence adhesive strength, the number of days animals were purged for also influenced animal strength during transport. Within this trial, for small animals, a high adhesive strength was only observed when animals were subjected to a shorter purging period, and a higher adhesive strength for larger animals when subjected to a longer purging period. This coincides with results of the individual weight (g) upon arrival, as older animals, with a shorter purge resulted in a lower individual weight upon arrival. This could indicate that animals that were not able to fully evacuate their stomach content, prior to packing and transport, were under the most stress and energy reserves were at the lowest. This was also coupled with a higher external temperature, as animals, within this trial, which had less ice also resulted in a lower adhesive force upon arrival, which could indicate that a higher metabolic rate, through increased temperature, also reduces the adhesive strength of abalone, which also indicates a lower energy reserve (Baldwin *et al*., 2007; Banea *et al*., 2012).

Measuring an abalone's speed is a non-invasive method in determining their stress levels (Robinson *et al*., 2013). Abalone are capable of travelling at faster speeds compared to other crawling gastropods (Donovan and Carefoot, 1997). Abalone generate rhythmic waves on the ventral surface of their broad pedal muscle in order to locomote (Donovan and Carefoot, 1997). Within this trial, animals which had a longer purge, prior to the 42-h transport simulation, used a faster speed of 1.6 mm s^{-1} to move across half of the top plate, two hours after the 42-h transport simulation. Due to the both prolonged stressful conditions, from the transport simulation and animals exposed to sunlight, abalone which had a shorter purge and all other treatments used a significantly reduced speed to move across the rest of the top plate, where animals were all unsuccessful in escaping the top plate. This indicates that animals which had a longer purge were able to have a higher energy reserve, post the transport simulation. However, due to them being exposed to extreme stress for a prolonged period of time, all animals had significantly reduced energy reserves and compromised mobility, which under extreme stress can be seen in other abalone species (Claredon-Gurrola *et al*., 2023). This in turn reduces the animal's ability to escape from predators and other stressors, and lowering their likelihood of survival (Donovan and Carefoot, 1997; Calderon-Gurrola *et al*., 2023).

4.4.4 Histology

Since abalone are highly reliant on anaerobic metabolism during functional hypoxia, increased levels of different anaerobic end products have been observed, including lactate, alanopine,

tauropine, succinate, and alanine (Venter *et al*., 2018). Different abalone tissue, including the gills (both left and right), digestive gland, and kidneys, are pathways to different metabolic responses to functional hypoxia (Day *et al*., 2010; Bullon *et al*., 2023). Excessive changes in these different tissues indicates the ability of *H. midae* to adjust their energy requirements when utilizing anaerobic respiration during functional hypoxia (Venter *et al*., 2018).

The level of damage abalone gills experience during prolonged stress, can be indicated by changes in the multiple gill lamella, of each leaflet, including changes observed in the gill lamellae and as well as the epithelium layer of the lamellae, and changes observed in the goblet cells (Copedo *et al*., 2024; Tung, *et al*., 2011). During hypoxic conditions, gill lamellae have been shown to produce lesions, including hyperplasia within the epithelial layer (Mitchell *et al.*, 2012). The gill leaflets also have been observed to produce eosinophilia within the sinus fluid of different leaflets and goblet cells, which indicates a reflection of the protein level or haemolymph pooling due to the burst of the tissue cells of the haemolymph vessel (Hooper *et al*., 2014). This burst in tissue cells could be from damaged epithelial cells (Hooper *et al*., 2014), which is an indication of inflammation (Hooper *et al*., 2008). The animals overall damage grading score for the gills, within this trial was more affected solely on the age of the animal, where smaller animals had more mild changes in the gill structure compared to bigger animals. Larger animals reached a slight change in their gill structure; however, results did not differ between all 16 treatments (Holland *et al*., 2023). This is due to juvenile animals being smaller in size and in turn, internal organs and tissues also being smaller in size, which limits different physiological process, including the rate of oxygen consumption, excretion, and regulation capabilities (Barkai and Griffiths, 1988; Yearsly 2007).

The infiltration of haemolymph (blood) into the gill lamellae (Figure 4.40) has been observed in other studies, including Muznebin *et al*. (2021) and Hooper *et al*. (2008), where coupled with haemocytosis, disrupted and enlarged or swollen gills was also accompanied, which was also seen within this trial. For both large and small animals, haemolymph infiltration occurrence was higher with a longer purge, post transport, however results were not significantly different from each other. The pooling of haemolymph, which is commonly observed within the left gills of abalone, due to stress, may be caused by a compromised circulation which has also been described with severe bacterial infection (Venter *et al*., 2025). The infiltration of haemolymph is common due to the alterations of the gill epithelium as cells become damaged through accumulation of bicarbonate in the mucus layer (Burke *et al*., 2001).

This could indicate that during periods of air exposure, animals may experience compromised respiration due to damaged gills, which in turn results in gills collapsing (Song *et al*., 2007).

The size of the gill lamellae, when exposed to stress, can be altered and changed, which was seen within this trial. The gill lamellae of larger abalone that were packed with more ice were 4 % smaller compared to those that were packed with less ice. Small animals on the other hand, had the opposite effect and animals which had more ice, during transport, reached a bigger size in their gill lamellae by 8 % compared to animals which had less ice. This was similar to results from Zhang *et al.* (2022), that found a difference in the thickness of the gill lamellae, of *Naquetia cumingii*, at different temperatures. At 16 °C, gill lamellae were present on both sides of the gill filaments to allow for gas exchange, however at extremely low temperatures, between 0 to 8 °C, and at extremely high temperatures, between 24 to 28 °C, gill lamellae were negatively affected where lamellae were severely deformed (Zhang *et al.* 2022). Similar results were also seen in Ding *et al*. (2015) study, that found a higher accumulation of the total haemocyte count (THC) of juvenile *H. discus hanni*, when subjected to a lowered 8 °C environment compared to animals held at higher temperatures of 14 °C. Heat stress has been shown to result in the impact of tissues and damage to gill epithelium when abalone are exposed to air for 30 – 42-h during transport (Mouton, 2003). This was seen within this trial, where larger animals with less ice during transport, and in turn animals with a higher ambient external temperature reached a larger gill epithelium thickness post the 42-h transport simulation. This indicates that gill lamellae thickness was influenced by a reduced metabolic rate and a reduction in the severity of a stress indicator (increased thickness of gill lamellae) can be observed with a lowered temperature, specifically for larger animals.

Due to the difference in age of the two size classes, gill lamellae thickness was different between the two abalone sizes and older animals had a larger average gill lamellae thickness size of 38 µm compared to small animals, which had an average size of 31 µm (Gaty and Wilson, 1986; Muir, 2011). This is due to juvenile animals being smaller in size and in turn more sensitive to temperature, as internal organs and tissues are also smaller in size, which limits different physiological process, including the rate of oxygen consumption, excretion, and regulation capabilities (Barkai and Griffiths, 1988; Yearsly, 2007). This could indicate why smaller animals had a lower thickness with less ice packed as smaller animals are more

sensitive to thermal changes and physiological processes in order to adjust to stress (Pedler *et al*., 2019).

All animals during live transport are exposed to main major induced stress procedures, including purging and air exposure (functional hypoxia), which also greatly influences the change in the digestive gland histology. Different changes within the digestive gland tubules have been observed in many other studies, which has also been observed and identified within this trial. Different changes within the digestive gland tubules include an observation of enlarged tubules (Taylor *et al.*, 1998), increased dilation variations of the lumen of each tubule (Handlinger *et al.,* 2006), detachment of the tubule from the cytoplasm base (Hooper *et al*., 2014), accumulation of inorganic matter (brown pigmentation) within the tubules (Martinez *et al*., 2015), and the degradation and deterioration of the epithelial cells of different tubules from either the basal lamina or the lumen of different tubules (Johnston *et al*., 2005; Forsyth *et al*., 2006). All of these identified stress indicators made up the damage grading scoring system for the digestive gland. Within this trial, the higher observation of multiple stress indicators, with a higher frequency of occurrence was not influenced by any factors and damage score was influenced on the transport procedures. The low grading score of all treatments could be due to the low spread of the identified stress indicators across majority of the tubules within each treatment and as well as the low count of different observed stress indicators within each tubule. Due to animals still undergoing digestion by the time they were exposed to a prolonged hypoxic condition, animals change their digestive tissue morphology, which also modifies the metabolic processes and in turn affecting their overall health (Bullon *et al*., 2023). This could indicate that the process of transport was not drastically damaging to the abalone digestive gland. This could indicate that digestive physiology was not influenced by environmental conditions, such as temperature, starvation, or abalone size but rather stress.

All animals subjected to the transport simulation, experienced different changes (stress indicators) within the right kidney renal tubules. These different stress indicators, which include the observation of enlarged renal tubules, variation of the dilation of the lumen of different renal tubules, and the degradation of the epithelial cells of the renal tubules or collecting ducts (Taylor *et al*., 1998; Handlinger *et al*., 2006; Forsyth *et al*., 2006; Zhang *et al*. 2022). All these observed stress indicators contributed to the overall damage grading score of the right kidney tubule, where for animals within this trial, which were subjected to both different acclimation temperatures and different purge days had an inverse relationship

between each other. Animals which were subjected to a chilled purging environment, reached a lower damage score of 1.2 if they were also subjected to a shorter purge, where animals subjected to longer purge reached a higher score of 1.5. On the other hand, animals which were subjected to an ambient purging environment, and were subjected to a longer purge reached a lower damage grading score of 1.2 and animals with a shorter purging period reached a higher grading score of 1.5.

This was in contrast to a study by Zhang *et al*. (2022) who found no significant differences of the change in renal tubules, when subjected to different acclimation temperatures, however they did find a difference in the size of the columnar cells at a specific temperature of 16°C. This was seen within this trial for the size of the renal tubules, where animals subjected to chilled purging environment reached a larger tubule size compared to animals subjected to ambient purging environment. Ambient acclimated animals reached a higher damage score with a shorter purging period due to the shifted metabolic processes from an incomplete purging process. Due to the shift in the metabolic pathways and processes from the hypoxic episode, which caused a disruption or compromised circulation within the gills, but was also seen within the right kidney renal tubules. Within the renal circulation of the right kidney, the presence of a large vessel blockage is known to affect the blood flow and glomerular filtration in the post stenotic kidney (Gomez *et al*., 2009). The kidneys of aquatic animals are vital excretory organs that function by removing metabolic wastes from the body and to regulate the osmotic pressure (Miller, 1987). This might explain results from this trial, where animals which were subjected to a chilled purging environment reached a significantly larger size of the renal tubule, which could be due to thermal regulation (Holland *et al.,* 2023), as it was found within Marshall and Grosell (2006) study that rainbow trout with a lowered temperature, reduces the metabolic rate and in turn alters their renal function.

Other stressors, including a compromised water quality, specifically a lowered pH, has been observed to cause the size of the kidney tubule and lumen to become enlarged (Yearsly, 2007). It might also be due to the reduced water quality, during purging, which is expected, as it was also shown within Souza *et al.,* (2020) study that fish histology of the right kidney was affected by sludge accumulation. However, for the animals that were chilled during acclimation, a higher concentration of suspended solids was found within each replicate event, which is due to the semi-recirculating system.

Similar to results from the size of the gill lamellae, the size of the right kidney tubule can also be attributed to the age range of each selected size class range. Older animals have a larger tubule size, which aids in maintaining internal metabolic processes for a longer duration compared to juveniles or young animals.

The accumulation of different end products, which include lactate, octopine (main metabolites produced), strombine, alanopine, and tauropine, forms when condensation reacts between pyruvate and the amino acids arginine, glycine, alanine, and tauropine, respectively (O'omolo *et al*., 2003). Therefore, the accumulation of both the D-lactate and tauropine in the muscles of abalone can provide a useful index of the level of metabolic stress, which can influence the quality and in turn effect the food value for export (Baldwin et al., 1992).Although this was not done within this research, future work could be done on this with abalone transportation.

4.5 Conclusion

Sea temperatures increase to above 21 ºC in the summer months, and prevailed for extended weeks, which can reduce dissolved oxygen levels and increase pH. This further decreases the efficiency of the purging process prior to transport. Pre-cooling tanks allowed for tanks to increase dissolved oxygen levels to winter conditions, where export processes were more successful. Optimal export water quality dissolved oxygen ranges were between 7.53 mg/L to 8.2 mg/L and 95 % to 99 %. Similar results for pH values were also found, with pre-cooling tanks, with the most ideal export ranges between 7.2 and 8.1, however, a higher accumulation of sludge at the bottom of the tanks was also found, which might influence ammonia concentration in the tanks.

Animals were most affected by the purge days and the amount of ice packed within boxes during transport, as a longer purge and higher volume of ice resulted in a better performance of abalone during transport and a better outcome upon arrival, where a minimum purge of 3-days is recommended for a routine export. A longer purge resulted in a better and more optimal outcome of animal's internal temperature during transport and the animal's ability to maintain their weight during transport. A longer purge also resulted in a lower weight loss and a higher survival upon arrival and during recovery after the transport simulation. The animals' behavioural parameters, including their speed and adhesive force ability, was also more positively impacted with a longer purge, where animals had a higher adhesive strength upon

arrival, which increased their likelihood of survival, and also used a faster speed in order to escape off the top plate. However, animals were not successful in completely escaping the top plate, which could indicate exhaustion after transport. Animals' right kidney parameters, including the damage grading score and the width of the renal tubules, were also influenced with different purge days. Both a longer purge and shorter purge had a positive and negative influence on the damage score of the right kidney, which was dependent on the acclimation temperature animals were subjected to. Chilled animals which had a shorter purge had a more positive outcome in terms of their damage score, where ambient animals with a longer purge resulted in a more optimal outcome of their right kidney damage score.

The provision of ice also greatly affected the animal's performance during transport and as well as post the transport simulation. Animals' internal temperature within boxes during transport, was affected positively with more ice packed, where a minimum provision of two ice sheets was required for an optimal export procedure. Animals had a higher drop in temperature with more ice, where internal temperatures were also maintained more optimally during transport, with more ice. The animal's ability to maintain their individual body weight, during transport, was also influenced with different volumes of ice packed within boxes. Boxes which had more ice packed resulted in a higher maintenance of their individual body weight, for older animals. This was also confirmed with the amount of excess biomass animals produced after the transport simulation. Older animals, which had more ice within the boxes, also reached a higher excess biomass after the 42-h transport simulation. Survival during recovery was also influenced positively with more ice. Animals, which had more ice within the boxes, were able to have a higher survival rate throughout the twelve days during recovery. Animals' behaviour, upon arrival, which consisted of their adhesive force, was more positively influenced by animals which had more ice during transport. Animals with more ice were able to adhere to a substrate with more force, however, results were also based on abalone size, where older animals had the highest adhesive strength with more ice. Smaller animals also had a higher adhesive strength with more ice within the boxes, however, this was only seen if animals were subjected to a shorter purge. Animal gill lamellae thickness was the last abalone parameter which was affected by the provision of ice during transport. Animal gill lamellae width between abalone sizes differed, where larger animals which had more ice within the boxes resulted in a smaller width of the gill lamellae upon arrival. Smaller animals on the other hand, had a less desirable outcome and animals which had more ice resulted in a larger width of the gill lamellae upon arrival.

The pre-cooling process of animals, prior to transport, also resulted in improved survival during recovery, where animals which were pre-cooled prior to transport, resulted in a higher survival throughout recovery. Pre-cooling also resulted in animals being more active on arrival, which was confirmed with their side-to-side movements. Alterations observed within the right kidney was affected negatively by pre-cooling, as the right kidney renal tubules were larger in size upon arrival if animals were subjected to a pre-cooled environment, prior to transport. This was also confirmed with the right kidney damage score as animals which were pre-cooled, prior to transport, resulted in a higher damage score. However, these results were also based on the days animals were purged for, and animals which had a longer purge resulted in the higher damage grading score, where the optimal pre-cooling temperatures were between 15 to 17 °C within the summer months.

As detailed water quality parameters of purged tanks were not determined, alterations of the right kidney were not determined if it was caused by pre-cooling alone, or due to changes in the water quality prior to transport due to the semi-recirculating system.

Chapter 5: Concluding discussion

All three objectives within this thesis were fulfilled by (1) determining the level of stress South African abalone (*H. midae*) experience during a transport simulation, (2) developing a method in order to pre-cool abalone prior to transport during elevated sea temperatures in the summer, and (3) assessing the most optimal conditions to transport abalone during peak summer months when sea temperatures rise. This chapter brings the findings of each objective together to fulfil the overall aim and points towards recommendations for abalone production and avenues for future research.

5.1 Purging water quality

The water quality parameters, including dissolved oxygen (mg/L and %) and pH, were all within the optimal range for purging, according to farm practices. Under standard farm practices, dissolved oxygen concentrations and saturation reach 7.58 mg/L and 94 % (Experiment 1), which is similar to concentration and saturation levels observed within pre-cooled conditions (Experiment 3) under increased sea temperatures in summer months. Dissolved oxygen reached 7.57 mg/L and 96 %. Tanks that were cool in temperature, either through ambient conditions during the winter months or through pre-cooling, gave the most optimal oxygen conditions. With a low temperature, oxygen diffusion into the water was higher, where a lower oxygen demand was also found to contribute to the high oxygen affinity during low temperatures (Vosloo *et al*., 2013). This was confirmed as the most optimal oxygen concentration levels from Experiment 1: *Farm condition transport simulation* was collected within the winter months when sea temperatures remained low between 11 – 16 °C, and oxygen levels collected from Experiment 3: *Summer live export transport simulation*, were all from the pre-cooled treatment.

The pH levels during standard farm practices reach 7.86 in concentration which is similar to levels found in the ambient purging conditions in Experiment 2*: Development of cooling acclimation methods prior to summer live transport* and Experiment 3, with levels reaching 7.86 and 7.80. High temperatures can decrease the pH of the water through molecular vibrations, which promotes the formation of H^+ ions. This can also be exacerbated through animals increased metabolic rate, which also promotes the increased formation of H^+ ions (Alberti and Cuthbert, 1982). Cooled tanks were able to significantly reduce the formation of

H^+ ions (Bandura and Lvov, 2006) within the tanks, where it was concluded that the most optimal pH concentration levels were from a decreased acclimation temperature, from pre-cooling, or from ambient conditions during the winter month, as concentration levels for both conditions were similar. These two environmental conditions reached and produced a lower pH (H^+) concentration during purging, in Experiment 1 and Experiment 3, when animals were subjected to a simulated transport under farm conditions and under different pre-transport conditions.

Both oxygen and pH levels within the pre-cooled conditions from Experiment 2 were relatively higher in concentration levels of 8.2 mg/L, 95 %, and 8.43 of pH, respectively, compared to both other experiments due to the trial being conducted during winter months. During this seasonal period, sea temperatures were low, and in turn attained more oxygen within the tanks due to the higher oxygen affinity with a lowered temperature (Kepenyes and Varadi, 2021). Higher pH within the tanks could be due to the semi-recirculating system, which had a higher accumulation of sludge, which could affect ammonia levels within the tanks (Beem *et al.*, 2017).

Although pH levels were within farming ranges for an optimal purging environment, sediment and sludge build up was observed within both treatment and control tanks within every purging duration for Experiment 1 (Chapter 2), Experiment 2 (Chapter 3), and Experiment 3 (Chapter 4). However, pre-cooled tanks accumulated more sludge and sediment by the end of each purge duration, which was due to the dynamics of the recirculating system. Aquaculture waste consists of animal faeces, expelled and uneaten food, and bacterial growth, which can result in increased suspended solids. The use of a recirculating system produces concentrated waste sludge, which within a standard RAS system, different wastewater treatments are available for removal of organic matter, which was not available for this trial (Chen, 1997). Due to this, a larger presence of sludge was observed within the pre-cooled tanks for both Experiment 2, during the development of the pre-cooling methods and within Experiment 3, where animals were acclimated to different pre-transport conditions. Therefore, farms would benefit from further research with regard to whether there is a significant effect of more accumulated sludge on animal health in the case of implementation of a recirculating system.

5.2 Transport simulation

Abalone were exposed, through a routine export procedure, to five different phases that included truck transit to the airport, animals checked in and waiting to be loaded onto the plane, boxes loaded onto the plane, aerial transit, where duration is dependent on destination, and boxes upon arrival (Alfaro *et al*., 2021). The importance of a good thermal regulation within boxes is needed, as a more optimal thermal regulation would result in a higher survival (Bubner *et al*., 2009). Temperature loggers were placed within a number of boxes (1 – 4) per size class for Experiment 1, under farm condition export, and boxes within an actual live export scenario, and within all the boxes within Experiment 3, under different pre-transport conditions. Under standard exporting conditions, the drop in animal temperature across each export phase, for both size class ranges were affected differently. Within the routine export procedure, bigger animals had a higher temperature drop across the first four phases of export, during truck transit, check in, when animals were loaded onto the plane, and during aerial transit. This could be due to boxes being exposed to a low temperature-controlled room, when animals were within the truck, and during aerial transit, which could have reduced the speed of the temperature decrease within boxes. A high amount of ice packed within the boxes could have also decreased the internal temperature. This in turn could have assisted with thermal regulation during phases where boxes were exposed to higher ambient air temperature as animals adjust their body temperature to the surrounding environment (Gardner, *et al*., 2024). Temperature regulation upon arrival was significantly reduced, which was due to the high ambient temperature over an extended period of time (Figure 4.8). However, these results were based on the assumption that a higher provision of ice would result in a lower temperature drop, where there was a gap in information on the effect of different pre-transport conditions on the different export phases. Within Experiment 3, where animals were transported under different pre-transport conditions, three different conditions were found to affect the rate of the drop in temperature across the first four export phases. Although the pre-cooled conditions and a higher volume of ice affected the drop in temperature during truck transit and aerial transit, respectively, a longer purging duration affected all four export phases more significantly. A low internal animal temperature, either through pre-cooling or the provision of a higher volume of ice, affected the rate of temperature decrease. This occurred under conditions where there was a control in air temperature within a temperature-controlled room. The same was concluded for a longer purging duration within a temperature-controlled room. However, the

rate of the temperature decrease under ambient air temperature conditions were also affected positively with a longer purging duration during the summer months.

Animal temperature upon arrival within standard exporting scenarios during Experiment 3, where animals were transported under different pre-transport conditions, showed that temperatures increase and were similar to the surrounding air temperature. This was with the exception of animals which had a larger volume of ice, where temperature upon arrival was relatively lower. It was then concluded that the best temperature regulation lasted within the first 36 h of transport for all treatments, however the inclusion of more ice will give a lower temperature of animals upon arrival. It was also concluded from both Experiment 1, and 3, where animals were transported under farm conditions and under different pre-transport conditions, respectively, that transportation has a direct effect on the variation in both survival and weight retention. Animals within Experiment 3, under different transport conditions, had a higher variation in net weight and individual weight upon arrival due to the simulation. This was not the case within Experiment 1, under farm condition transport simulation, as animals were not subjected to a transport simulation and boxes were left untouched until the end of the simulation. During transport, animals experience a combination of different stressful conditions, including air exposure, temperature shifts and handling, and this may reduce the coping mechanisms of ectothermic animals (Morash and Alter, 2015). In turn, animals exposed to multiple stressors were shown to experience a decrease in overall coping range by Portner and Knust (2007). As a result, it is recommended that farms could benefit with a more accurate mock pack scenario if boxes were handled similarly to boxes during an actual export.

5.3 Post-transport simulation

Transport can lead to a decrease in abalone weight due to different physiological and environmental stressors (Alfaro *et al.*, 2021). During transport, different sizes of abalone experience a reduction in multiple characteristics, including their weight, survival, behaviour upon arrival, and reduced health. However, the different pre-transport conditions within this research have been shown to help mitigate these stressful conditions and increase the likelihood of a better outcome upon arrival.

Within every export simulation, the change in weight and the weight upon arrival varies, however from both Experiment 1 and Experiment 3, where animals were subjected to both a

farm condition and simulated transport scenario, animal size or age greatly affected the amount of weight lost. As the body size of the animal influences the amount of water that can be retained within the body during stress (Gorfine, 2001), all smaller animals within both experiments lost a greater amount of weight compared to the larger animals. This was seen within the net weight and water weight within Experiment 1, where animals were transported within farm conditions, and in Experiment 3, where animals were transported within different pre-transport conditions. This was due to smaller animals having an increased metabolic rate during stress, compared to larger animals, which in turn resulted in a higher production of mucus (Kuanpradit *et al*., 2012). This could have also been aggravated due to the higher surface area to volume ratio smaller animals have, which could cause faster evaporation during air exposure (Simmons, 2007).

Purging duration had the greatest effect on the initial survival upon arrival, both immediately following the simulation, and as well across the different days during recovery for Experiment 3, where animals were transported under different treatment conditions. Upon arrival, a longer purging period provided a higher chance of survival and also a lower variation, between boxes. This was also seen when animals were within recovery and a longer purging period resulted in a higher survival twelve days following the simulation.

Although a lowered body temperature, either through pre-cooling or the provision of more ice, did not have an effect on the ability of abalone to maintain their net weight during transport, it did have a positive influence on their recovery. Survival is a great indicator of the success of a trial (Minh *et al*., 2010) and Experiment 3 concluded that pre-cooling animals would result in a higher survival rate for a week, if animals were placed within optimal environmental conditions post-transport.

The provision of a higher volume of ice resulted in a higher survival, throughout the twelve days of recovery. The provision of ice showed to have a greater effect on the change in individual body weight of animals during transport. From both Chapter 2 and Chapter 4, animals were shown to lose additional body weight with their water weight, which was also mainly influenced by their size. Larger animals within Experiment 1, within farm transport conditions, lost more weight during transport compared to smaller animals. This was due to their larger body size, which was based on the animal's shell aperture, which was also determined by animal size/age (Gorfine, 2001). However, as shown in Experiment 3, where

animals were transported under different transport conditions, larger animals were also more sensitive to prolonged stress. The inclusion of more ice and a longer purging period, however, positively influenced a better individual weight during transport and upon arrival. In contrast, smaller animals were negatively influenced by a longer purge and more ice.

5.4 Behaviour

The ability of the abalone to right themselves is an indicator of their survival rate (Zhang *et al.,* 2020) and it can be concluded from Experiment 3, where animals transported under different pre-transport conditions, that the export procedure was metabolically straining and leads to exhaustion. However, from both Experiment 1 and 3, where animals were transported under farm conditions, and subjected to different pre-transport conditions, a reduced internal temperature of animals upon arrival can mitigate the physiological constraints due to extreme stress from transport (Venter *et al*., 2023). Animals that were pre-cooled had a greater ability of righting themselves, which was similar to animals that were successful in completing the righting behaviour within Experiment 1 (Chapter 2). This could be due to animals having a lower body temperature because trials were conducted during the winter months where average sea temperature ranged between 13 – 16 °C. This was similar to results from Young *et al.*, (2006), that found a more optimal righting behaviour of temperate isopods in low environmental temperatures. This could be due to these animals being temperate species, which limits behavioural response if temperatures exceed optimal ranges (Young *et al.*, 2006).

From both Experiments 1 and 3, larger animals had a higher metabolic capacity as they achieved the highest adhesive force both through un-transported or simulated transport scenarios. In agreement with Xi, *et al*., (2023), animals which were able to adhere more efficiently to a substrate had a higher chance of survival, even during periods of stress, and is an indicator of their survival skills, and in turn their energy reserves. Temperature has been shown to have an effect on the adhesive ability of abalone (Yu, *et al*., 2021) and during this trial a higher provision of ice resulted in a higher adhesive force for both larger and smaller animals. The duration of the purging process, however, would determine the most optimal condition of animals upon arrival. As animal adhesion duration can be based on temperature, animal tolerance to fluctuating temperatures can be determined by their adhesive duration (Yu, *et al*., 2021). Yu *et al.*'s (2021) study showed that a higher adhesive duration through higher temperatures indicates a higher plasticity and durability of abalone during fluctuating

temperatures. This could indicate why cooling animals during transport resulted in a higher adhesive force within this trial, as more optimal physiological responses are associated with a colder temperature for South African abalone (*H. midae*) due to them being found mainly within temperate conditions (Young *et al.*, 2006; Vosloo and Vosloo, 2010).

Both old and young animals presented an inverse relationship with the purging duration, where during a longer purging process larger animals would show a higher adhesive force and smaller animals, with a shorter purging process, would display a higher adhesive force post transport. Purging duration affects digestion and animals with a less efficient expel of the gut content prior to transport, resulted in a lower performance. This was due to enzymatic activity shifting during transport in order to regulate their energy reserves in conditions of stress from air exposure and digestion (Chabot *et al*., 2015; Frederick *et al*, 2022). This also resulted in animals not using their energy reserves efficiently (Currie *et al*., 2015; Chukwuka and Ejere, 2019). This could explain why larger animals with a shorter purge were noted to have a lowered adhesive strength upon arrival. The opposite effect was observed with smaller animals, where a shorter purge resulted in a higher adhesive strength upon arrival.

Abalone have the capability of increasing their speed when stressed in order to escape undesirable environmental conditions, but also demonstrate reduced locomotion abilities when under extreme stress (Donovan and Carefoot, 1997; Calderon-Gurrola *et al*., 2023). This was observed within Experiment 1 and 3, where it was deduced that the transport process was extremely stressful and would result in a significantly reduced locomotion mobility. However, although it can be mitigated slightly with a longer purging period which would result in a faster speed, animals would still be exhausted and their mobility abilities greatly hindered. This could potentially affect their feeding during the first day of recovery because feeding may be reduced due to exhaustion (Zhang *et al*., 2020).

5.5 Histology

Heat-stressed animals may experience tissue damage, including within the gill lamellae of an abalone, which was seen within the results from Hooper *et al*., (2014) that showed that heat-stressed gill epithelium was too stressed and resulted in no recovery within the first seven days. During transport, within Experiment 3, where animals were transported under different transport conditions, various abalone tissues were affected differently and independently,

which was also influenced by the size and temperature of animals. Similar to results by Hooper *et al.*, (2014), this research showed that older animals were more negatively affected by an increased temperature, which resulted in a thicker size of the gill lamellae after the transport simulation. Smaller animals, on the other hand, were more negatively impacted overall by a lowered internal temperature induced through the provision of more ice. This could be due to smaller animals being more sensitive to changes in water temperature and limited oxygen supply compared to larger, adult abalone (Vosloo *et al*., 2009).

Alterations within the digestive gland were observed within all treatments following the transport simulation. However, abalone size, different acclimation temperatures, different purge days, and different ice volumes had no effect on the extent of damage to the digestive gland. Although abalone digestive gland enzymatic activity can be affected by starvation and temperature, digestibility due to these stressors cannot be significantly changed or altered (Frederick *et al*., 2022). This could indicate that, although animals were susceptible to alterations either due to the purging process or transport or a combination of both, digestibility might not be heavily impacted. This could be shown through animals displaying a low overall damage score across all treatments.

Within the right kidney, animals were majorly affected by pre-cooling prior to transport and cooling within the duration of purging. Animals that had a longer purge tended to display a worse condition upon arrival, where pre-cooling would aggravate this. Inversely, a shorter purge coupled with a pre-cooled condition would result in the most optimal condition upon arrival. This could be due to a combination of stressors, including temperature and ammonia concentrations during purging. As pre-cooled tanks had a higher accumulation of recirculated discharged water, ammonia concentration levels might have differed between tanks. This could have resulted in a worse outcome for the right kidney with a longer purge because accumulation of low levels of ammonia can induce inflammation of the right kidney of different fish species (Harris *et al*., 1998). This could be exacerbated by a low temperature as ammonia concentration within a low temperature environment can be difficult to remove (Lee *et al*., 1999).

It was then concluded that due to longer exposure to starvation, animals were more prone to experience different negative histological effects, however, due to data only being collected after the 42-h transport simulation, a comparison of the histological effects, between post purging and during recovery, was not applicable. A histological comparison between the

different exporting events would provide a more detailed depiction of the effects of starvation itself on the animal and as well as the combined effect of starvation and transportation on abalone health.

5.6 Recommendations for the abalone industry

The following three recommendations are suggested for implementation in abalone farms to increase production and, thereby, profit. First, in order to ensure higher survival rates and keep shipping costs relatively low, implementation of a pre-cooled system would ensure a constant supply of optimal water quality all year round, even during periods of temperature spikes. A constant supply of optimal water quality would include ideal temperature ranges between 12 to 16 °C, proper aeration and high dissolved oxygen concentration levels between 7.00 to 7.90 mg/L, and an ideal pH range between 7.00 to 8.10.

Second, as animals were more likely to display better performance with a longer purge, it would be recommended to purge animals for a minimum of 72 hours prior to transport. This would increase the likelihood of animals fully expelling their gut content and other waste prior to transport. This would, in turn, increase the potential of animals having a better overall outcome upon arrival and, thereby, increase revenue and meet satisfactory quality of meat upon arrival.

Third, the collection of histological data recorded during recovery would provide farms with a more detailed depiction of the pace of recovery after exposure to extreme stress. This could enable farms to improve management of feeding protocols during recovery.

5.7 Suggestions for future research

While this research project has important findings that will influence practices involved in the export of abalone, these findings were limited by the temporal scope of the study. Five limitations to the data were identified and will guide suggestions for future research. First, while the inclusion of ice and a longer purge resulted in a better method of transport for larger abalone, the understanding of the degree to which this can be applied is limited. Therefore, further research into the effect of higher volumes of ice and a longer purging period on larger animals could be beneficial. Further testing could reveal the optimal cooling and purging for weight retention during transport. This is important as “exporters are paid based on the landed weight” (Bubner *et al*., 2009) and increased weight retention could increase revenue.

Second, for smaller animals, farms may also benefit from more research on the detailed effects of starvation, lowered temperature, and transportation on animal health and weight. This is because younger, smaller animals were affected differently to larger, older animals. This may reveal a successful method of transporting smaller animals that would produce similar outcomes to those of larger animals.

Third, the implementation of a pre-cooling system was shown to improve purging methods during the summer months, however, pre-cooling was shown to have no significant effect on the ability of animals to maintain their weight during transport. This could be due to the pre-cooling system being limited in its ability to reach more than a three-degree difference from the incoming ambient temperature and this needs further investigation. It would be beneficial for farms to conduct additional research on the ability of the pre-cooling system to decrease the temperature of the tanks to lower temperatures than measured within this research. Future research could also investigate the combined effect of the pre-cooling system and other pre-transport conditions on both younger, smaller and older, larger animals as both were affected differently with respect to different parameters.

Fourth, because speed, righting behaviour and adhesive force data were only collected on the first day of recovery, the abalone's full behavioural recovery abilities were not documented. Future research should look into the recovery capabilities of abalone through behavioural observation and quantitative analysis. This would provide farms with a more detailed timeline of when animals start to recover. In addition, this would provide details on the optimal times to feed animals in order to eliminate unnecessary feeding or wasted feeding when animals are too stressed. This would save revenue from feed purchases and, thereby, increase profitability.

Fifth, abalone metabolites and meat composition data were not collected within this research. This means the research could not confirm the energy reserves abalone use during transport and the metabolic pathways animals use during episodes of hypoxia. Metabolite research would also provide insight on the detailed effect of the metabolic pathways of the gills, digestive gland, and kidney, during transport of South African abalone. In connection to this, research providing a biochemical understanding of the energy reserves used during transport with the extent of tissue damage, could provide a detailed understanding of the metabolic shift during transportation. In addition to metabolite study, research would also benefit from assessing the

microbial loading of abalone during transport. Other studies from Jager *et* al., (2024) have found that Ulva acts as a biofilter and reduces the bacterial load in abalone effluent, where more research would be beneficial to estimate if and how different transport conditions could potentially affect the amount of bacterial load of abalone during transport.

Lastly, further research would benefit from investigating the measurement of other water quality parameters, including ammonia and CO_2 accumulation in the packages containing abalone. These compounds have been found to accumulate and have been shown to be among the main drivers of mortality during transport (Steckbauer *et al.*, 2015; Novak *et al.*, 2024). These water quality parameters were not tested in this study and could form the basis for further research.

5.8 Conclusion

The findings showed that abalone were more prone to experience multiple and extreme stress during transport, under both favourable and unfavourable environmental temperature conditions. However, their performance was reduced under unfavourable environmental conditions within the summer months. Animals' performance during export was influenced positively and negatively by four interrelated factors. These factors include water quality during purging, purging duration, the internal temperature of animals during transport, and animal age and size.

First, water quality was shown to be more positively influenced by a lowered water temperature, as more dissolved oxygen would accumulate within the water and increases the chance of survival during purging and ultimately transport. This confirms the findings of Yang *et al*. (2021). In addition, pH was more positively influenced by a decreased temperature because higher temperatures can result in a higher accumulation of H^+ ions that can further reduce the performance of abalone during the purging process. During unfavourable and higher environmental temperature conditions within the summer months, oxygen depletion and increased pH was shown to aggravate negative effects on animal physiology during purging. This aligns with the findings of Barrento *et al.* (2013) and can also result in a further reduction of abalone performance during transport. The implementation of a pre-cooling method, with a semi-recirculating system, was shown to have a positive effect on the water quality and,

therefore, animals post-purging within the summer months. Pre-cooled tanks were able to reach similar oxygen levels and pH levels that were found within winter conditions.

Second, purging duration was shown to affect the performance of abalone. This is due to the fact that animals without a completely expelled gut content displayed the catalyzation of their enzymatic activity during hypoxia. This could lead to a lower net weight upon arrival and a lower survival rate upon arrival and during recovery. This weight loss and low survival rate is costly for exporters as profits of abalone are based on landed weight (Vosloo and Vosloo, 2006) and, therefore, necessitates modification of existing transport protocols.

Third, performance was shown to vary in terms of animal age and size when subjected to the different pre-transport conditions. Larger animals performed better due to their higher ability to mitigate stressful conditions for a prolonged period of time in comparison to smaller animals (Pedler *et al*., 2019). As more numerous studies on physiology adaptation during stress have been conducted on larger and older animals, less information is available on the impacts on prolonged stress and multiple stressors on small and juvenile abalone. Therefore, more research is needed to fully understand the effects different procedures during export have on juvenile and smaller animals in order to maximise their performance.

Fourth, this research has the potential to make a significant impact on the optimisation of abalone transport through pre-cooling methods and pre-transport conditions during the summer months. The implementation of a pre-cooled system has been shown to be beneficial in terms of survival, behaviour and histological characteristics of abalone during the summer months. However, these findings are limited and the optimal method of pre-cooling still needs further investigation to maximise overall performance on both larger and smaller animals. Different transport conditions within the summer months were tested and these were coupled with a pre-cooling method in order to assess the most optimal condition for transport. Results indicated that a combination of a higher inclusion of ice and a longer purge resulted in the best pre-transport method, which would increase the likelihood of a more optimal condition of abalone during transport and upon arrival. Pre-cooling was also beneficial to abalone during recovery and contributed to animals being more active upon arrival. The method of cooling selected by farms – either through the use of a pre-cooling method or the provision of more ice is dependent on costs and seasonality. During packing, the use of ice is common during transport to maintain temperature control, but greater amounts of ice also increase the costs of shipping (Ninan,

2017). The pre-cooling method prior to transport is especially beneficial during summer months as it could reduce the amount of ice used during transport and, therefore, the cost of shipping. Within the summer months, farms have been found to typically use greater amounts of ice during shipping mitigate higher temperatures. While the provision of more ice could be a more beneficial in terms of cooling animals with minimal cost to abalone physiology and health, the use of greater amounts of ice has the potential to increase shipping fees to around 10 to 40 US dollars, per shipment (Bowes, 2023).

The methods used for pre-cooling within this research, however, were based on a semi-recirculating system, which provides additional complications to abalone physiology because the system partially recirculates discharged water. This could lead to the accumulation of ammonia within the tanks and have a negative effect on abalone physiology. If a pre-cooled method were to be implemented, therefore, more studies would need to be conducted to determine the different methods of implementing a pre-cooled system in order to ensure minimal implications to abalone physiology and histology during purging.

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