Maximising value by reducing stress-related mortality in wild harvested black-lip abalone (*Haliotis rubra*)

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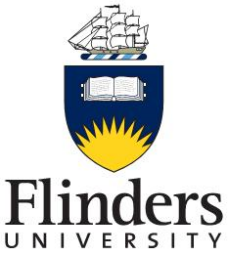

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1. Non-Technical Summary

2010/704 - Maximising value by reducing stress-related mortality in wild harvested abalone

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PROJECT OBJECTIVES:

1 Quantify changes in stress levels in abalone from the time that they are removed from the reef to the point of export from the processors

2 Quantify how the magnitude of stress and the capacity to recover from stress is affected by time spent on the deck prior to packing in bins post-harvest, the extent and use of seawater immersion on the boat, and the timing and frequency of water changes during transport.

OUTCOMES ACHIEVED

- Informed the Code of Practice to increase the value of product by reducing the post-harvest mortality of animals prior to live shipping.
- Identified cost-effective strategies to limit stress of abalone during the boat transit phase of the harvest supply chain.

LIST OF OUTPUTS PRODUCED

- Contributed to and informed the Abalone Council QA Code of Practise for the abalone industry in Australia.
- A decision tree that identifies the appropriate boat transit methods

Post-harvest mortality in wild caught abalone, particularly in the processor tanks affects the amount of product available for the valuable live export market. While abalone that die post-harvest can be canned, the value of this product is less than if exported live. Mortality of blacklip abalone, *Haliotis rubra,* in the processor tanks (typically <48h of harvesting) has been attributed to stress facilitated by factors such as harvest practices, practices during transport from the reef to the processor, the length of the road transport phase, and the time of the year. Minimising stress during the harvest supply chain (reef to processor) has the capacity to reduce post-harvest mortality and increase the value of the harvested abalone. This project aimed to quantify changes in stress levels in abalone from the time that they are removed from the reef to the point of export from the processors, and to quantify how the magnitude of stress and the capacity to recover from stress is affected by time spent on the deck prior to packing in bins post-harvest, the extent and use of seawater immersion on the boat, and the timing and frequency of water changes during transport.

Three manipulative experiments were carried out examining practices of managing blacklip abalone, *Haliotis rubra*, during the boat phase of the harvest supply chain; Tasmania summer, Tasmania winter and NSW summer. Recovery from the boat transit phase in the processor tanks was explored in three experiments; two in Tasmania and one in NSW. To complement manipulative experiments a diver survey using a questionnaire was used to determine the range and prevalence of handling

practices on the boat by the divers including; use of dousing, stacking practices, and time of the boat transit phase. Stress in abalone was assessed using haemolymph parameters of haemocyte phagocytosis, haemocyte density, glucose and lactate concentration, and haemolymph pH. Baseline measure of these parameters were obtained from abalone

Across all the experiments and the manipulations no single practise during boat transit phase was optimum for reducing the stress in abalone during the harvest supply chain. The temperature at which the abalone are transported, either in or out of water, appeared to be a major contributor to amount of stress that *H. rubra* experience. Abalone that experienced less stress during the harvest supply chain recovered faster in the processor tanks than those animals experiencing warmer temperatures and greater handling. Maintaining *H. rubra* within its thermal optima throughout the harvest supply chain will be essential for successful preparation of abalone for live transport internationally. Currently thermal optima for *H. rubra* are derived from experiments seeking to identify optimal temperatures for growth and survival for cultured populations held on Tasmania's east coast. However, no information is available about the spatial differences in thermal optima for minimising stress during transport of adult *H. rubra* out of water, which limits the scope of recommendations for harvest industries over the spatial distribution of *H. rubra*.

ACKNOWLEDGEMENTS:
Field and laboratory technical support was provided by Kylie Cahill and Michael Porteus; support by abalone fishers and processors in Tasmania and NSW; and many volunteers who assisted with laboratory work and fieldwork throughout the project. Thanks to Kirsten Benkendorff for providing advice about the phagocytosis assay.

2. Introduction and Background

In this project we addressed the problem of post-harvest mortality in wild caught abalone, a problem which has been attributed to stress facilitated by a number of factors including: harvest practices, practices during transport from the reef to the processor, the length of the road transport phase, and the time of the year (influence of temperature and reproductive condition of the abalone). Stress in animals manifests as a series of physiological responses affecting biological systems, including respiration, metabolic rates, and reproductive systems, all of which influence vulnerability to disease and increase rates of mortality (Malham *et al.* 2003, Bubner *et al.* 2009). The practice of harvesting, transporting, and holding live abalone exposes abalone to stressors beyond that which they are adapted to endure, such as extended periods of emersion and short term increases in temperature. The cost of failing to manage the extended periods of stress, which affect the health and survival of individuals, is a decrease in the quality and number of harvested animals available for the high value live product export market. By reducing the period during which abalone experience excessive stress through better handling, transport, and holding techniques it should be possible to reduce post-harvest mortality and increase the value of harvested abalone. Ultimately, maximizing the value of the wild harvest abalone through consistent delivery of high quality live abalone to the consumer requires understanding, managing and reducing stressors that compromise condition and survival of animals during the supply chain.

Abalone removed from the reef by divers are carried in a coarse net bag which holds 20-50 abalone, depending on the size of abalone. Divers clip net bags containing abalone to a weighted dropline which is hauled to the surface by the deckhand. Empty net bags are usually delivered to the divers via the same dropline. Retrieval/replacement of net bags occurs at regular time intervals (~ 10-15 mins), depending on sea conditions, catch rate, and personal preference. Deckhands empty net bags of abalone hauled to the surface onto the floor of the vessel and stack abalone on their sides (respiratory holes facing upwards) into plastic bins (Figure 2.1). Each bin may contain 1-2 layers of abalone (Tasmania) or one vertical layer and one horizontal layer of abalone (NSW). Bins are then covered with damp hessian sacking and may be stacked 3-6 bins high depending on conditions and size of bins (Figure 2.1). Several NSW fishers will interleave a thin plastic sheet between each row of abalone, to provide a smooth surface on to which the abalone can adhere. The types of bins used and the stipulated stacking densities vary across processors and states.

There are three broad types of vessels used in abalone fisheries; small runabouts (~6m in length), mid-size single or multi-hull vessels (6.5m – 8m), and mother boats (~>12m). Harvesting only occurs from small runabouts and mid-size vessels, whereas mother boats are used to access fishing grounds in remote areas of the state such as the west coast or northern offshore islands, and transport abalone live in wet well holding tanks back to port. No mother boats are in use in New South Wales. During single day trips are typically undertaken by small and mid-sized vessels, with abalone transported back to port on the same day. During multi-day trips on mother vessels, catch is transferred from the small runabout to the mother-boat one or more times each day. A small number of fishers have fitted wet well holding tanks in their multi-hull midsized vessels, although these tanks must be drained during transit between fishing grounds or during transit from the fishing grounds to the boat ramp.

Figure 2.1.1. Deckhand checking abalone for legal size and stacking in processor supplied bins. The abalone are stacked on their sides with the respiratory pores upwards and the abalone are being allowed to attach to the shell of the abalone against which they are stacked. On the left side of the photo bins that contain stacked abalone are stacked on top of one another and each bin is covered with damp hessian sacking.

Thus, the harvest supply chain for abalone involves the following sequence of events; chipping abalone from reef ➔ holding animals in mesh bags ➔ landing animals on the deck of the boat ➔ stacking the abalone and holding them in bins ➔ transfer of bins with abalone to processor transport vehicle ➔ transfer from transport vehicle to processor holding tanks. Where mother boats are in use, there is the additional transfer from the catcher vessel to the mother boat live holding tanks. There are conflicting views on the optimum methods of holding and transporting abalone, and for example whether for animals that are held out of water if the bins of animals should be doused with a bucket of saltwater regular or flooding of draining bins using a deck hose assists with minimising stress and maximising the health of animals arriving the processors.

Several factors both pre- and post-harvest potentially contribute to and influence the capacity of the animals to tolerate harvest and live shipment to international destinations. Pre-harvest factors that affect the stress levels and condition of the abalone, e.g. water temperature, nutritional history, parasite load, age, weather, reproduction. Post-harvest management factors include the type, nature and extent of handling both in the water and on the deck of the boat; the temperature animals experience during boat transit phase; time between harvest and arriving at the processors, and the nature and extent of using water to cool and hydrate the animals. Stress may also be an issue in live transport from processor to the market, but it is the opinion of the processors that if the stress levels in live animals can be minimised prior to arriving at the processors that this will increase successful delivery of live animals to the consumer.

Tasmania (representing approximately 57% of Australia' production) requires landing all abalone live, irrespective of the capacity of animals to survive conditions experienced during transport from the reef to the processor. Further, Tasmania exports approximately 70% of harvested abalone live to Asia, requiring a significantly greater quality of product than was expected in previous decades. Given the range in operation types; multiday trips using large mother boats to single day trips using small fast boats, there is a diversity of handling and transport practices, some of which are determined by the logistic and costs of managing live animals at sea, a sense of what is good for the animals, and biosecurity regulations regarding movement of water around the state. However, there is a distinct knowledge gap about the relationship between handling and transport practices and resulting stress in abalone. We need to quantify the relationship between stress levels and the reproductive status of animals, the way that animals are handled during the harvest supply chain; from the reef to the processor, and the holding conditions at the processor, with a view to minimising the magnitude and length of time of exposure to stressors. Recognising which handling protocols minimise stress levels allows delivery of live abalone to processors that will survive to the market place. This will ensure that the value of harvest resources is maximised by managing the condition and resilience of animals during transport from the reef to the processor.

Stress is physiological response to the conditions being experienced by the organism. For the wild abalone harvest industry the sequence of events during the harvest supply chain described above each step changes the conditions experienced by the animal and will elicit a physiological response. The fishers have greatest control over the time taken to stack abalone in bins and the conditions in which abalone are held during while they are on board the boat. Factors that contribute to and influence the capacity of the animals to tolerate the process of collection are: handling on the boat, transport to the processor including management of stock post-harvest, the biological condition of animals, and stress levels of abalone prior to harvest (due to the environment e.g. temperature). Post-harvest management factors include: the type, nature and extent of handling both in the water and the boat; the temperature animals are exposed to during transportation; time between harvest and the processor; and the nature and extent of using water to cool and hydrate the animals.

There is definitive evidence from abalone processor records that mortality events occurring within 24 hours of the animals arriving at the processor are more frequent over recent years. These mortality events are highly correlated with warmer than average water temperatures during summer and are concurrent with spawning in the processor tanks. However, we do not know if harvested animals are in an advanced stage of maturity and spawning induces death, or if stress responses accelerate final maturation of gametes and animals release gametes prior to dying in a last ditch attempt to reproduce before death. Either way there has been little success by processors with advanced and sophisticated re-circulating seawater facilities to modify conditions to prevent spawning of stressed animals. Biological condition of the animals, in particular the reproductive condition and its relationship to pre-harvest water temperature are a function of when animals are harvested (i.e. winter vs summer). Stress may also be an issue in live transport to the market but it is the opinion of the processors that if the stress levels in live animals can be minimised prior to live shipping that this will increase successful delivery of live animals to the consumer. Therefore, the focus of this project was identify ways to reduce stress in live animals early in the harvest supply chain process, rather than explore the development of shipping protocols of severely compromised animals, especially since the air freight regulations limit the options to change shipping conditions.

Abalone display a number of physiological responses to a stressor; reduced immune response and changes in the haemolymph (blood) chemistry, including pH, lactate and glucose concentrations, and osmolality (Hooper *et al.* 2007, Day *et al.* 2010). Abalone haemolymph pH becomes more acidic when animals are exercised (Baldwin *et al.* 1992), held out of water (Baldwin *et al.* 2007a, Bubner *et al.* 2009), or exposed to different concentrations of dissolved oxygen (Cheng *et al.* 2004). Acidosis, the decrease in haemolymph pH occurs when there is a switch to anaerobic respiration, and as the haemolymph become more acidic the affinity of the haemocyanin for oxygen increases, effectively reserving oxygen for organs requiring oxygen (Wells *et al.* 1998b). Lactate is a by-product of anaerobic metabolism, and build-up occurs when abalone are removed from water, thus the concentration of lactate can be used as an indicator of physiological costs of being out of water (Baldwin *et al.* 1992, Wells *et al.* 1998a). Given that abalone are primarily transported on the fishing boats out of water, it was expected that lactate concentrations and haemolymph pH would respond, but it was not clear the shape of the response curve with time out of water and if the changes in these parameters would differ seasonally and affect recovery in the processor tanks. Elevated glucose in the haemolymph is another stress response (Carefoot 1994, Cheng *et al.* 2004), while this is not a commonly used stress measure, mobilisation of glycogen as an energy substrate may be used under periods of anaerobic metabolism. If glycogen mobilisation is important to support anaerobic metabolism then the stores of glycogen which differ seasonally will affect the stress response. Suppression of the immune activity in response to stressors by abalone has been well documented and was a logical metric in this study given the mortality of animals in the processor tanks and assessment of immune response will provide an indicator of animal health (review by Hooper *et al.* 2007). Immune response is assessed by quantifying the activity of the haemocytes in fresh haemolymph that are introduced to inert particles. The protocols for this method have typically been developed for laboratory experiments close to laboratories. The challenge in this project was to use a protocol that could be used on a boat with limited facilities, as a result, two approaches to estimating immune response were used and trialled both in the laboratory and on the boat.

The aims of this project were to identify which current holding and handling practices increase stress levels in live abalone and when stress reaches levels from which animals cannot recover. Furthermore, we needed to place existing harvest and transport practices in the context of naturally occurring events and processes e.g. increased water temperature. We sought to reduce mortality by developing handling and transport protocols that reduce stress levels in abalone prior to arrival at processors.

2.1. **Need**

The Abalone Council of Australia (ACA) has clearly identified in their Strategic Plan (2007-2017) goals to have an Australian national wild abalone brand driven by a national Quality Assurance and Product Integrity Program, and to increase the industry GVP (in real terms) by 25% to $268 million in 2012 and by 50% to $321 million by 2017.

These goals are supported by this project, particularly through a desire to increase the value of the abalone, by ensuring that animals in the best possible physiological condition are provided to the processor for live export. This project also encompasses the development of practices that ensure marine environmental sustainability, because animals which are not suitable or which cannot be transported to maintain optimal physiological status will not be harvested. The development of handling and transport protocols delivers into the desire for the industry to develop uniform Codes

of Practice and product standards. Ultimately this will be about effectively managing harvest and the integrity of product going to market. This project will support ACA's vision to work with fishers, processors and value adders to establish a whole-of-chain approach to creating premium Australian products and servicing consumers.

This project aligns with the Objective 3 of the Strategic Plan: Develop techniques to increase marketable yield per fish under the Harvest Optimisation Platform of the ACA Strategic Plan 2007-2017.

2.2. Objectives

1 Quantify changes in stress levels in abalone from the time that they are removed from the reef to the point of export from the processors

2 Quantify how the magnitude of stress and the capacity to recover from stress is affected by time spent on the deck prior to packing in bins post-harvest, the extent and use of seawater immersion on the boat, and the timing and frequency of water changes during transport.

3. Methods

3.1. Tasmania Abalone Diver Survey

More than 100 Tasmanian abalone divers were invited by letter and phone to participate in a survey that sought to generate an industry wide picture of the practices used in the industry when handling wild harvest abalone from the time they arrive on the deck of the vessel until they are picked up by the processors at the jetty or boat ramp. The survey sought to obtain industry-wide information on practices of handling and stacking wild harvested abalone in the catcher vessel.

The questionnaire (Appendix 1) asked fishers to answer a maximum of 21 questions (depending if they operated a mother boat or a day boat) about how abalone are managed on board their boat, in particular how the animals are stacked in the bins and how animals are kept cool and wet from the time they are landed on the boat to when the processor picks them up. The data was analysed using χ^2 frequency analyses.

This survey was approved by the University of Tasmania Human Ethics Committee; Ethics Ref No: H0012820, Project title: Maximising value by reducing stress-related mortality in wild harvested abalone

3.2. Haemolymph Indicators of Stress & Physiological Status

Haemolymph Sampling

Haemolymph (approximately 2 ml) was drawn from the cephalic arterial sinus on the dorsal side of foot of *Haliotis rubra* using a syringe (Hooper *et al.* 2007, Hooper 2013). For each abalone, if a haemolymph sample could not be obtained within 5 min of the first attempt then that individual was rejected. Each abalone in the experiments had haemolymph taken only once and marked with waterproof crayon to indicate that they had been handled for drawing of haemolymph. Haemolymph was either stored on ice until back in the laboratory or processed immediately. Once in the laboratory, haemolymph pH was recorded and haemolymph centrifuged for 2 mins before supernatant was transferred to sterile Eppendorf tubes and frozen at -20°C for determination of concentrations of glucose and D-lactate. All frozen samples were analysed within 12 months of sampling.

To assess changes in haemolymph parameters post-harvest it was necessary to establish baseline values of abalone using pre-harvest values. Haemolymph parameters were likely to change rapidly once the abalone are removed from the reef. Blood samples from fish have been successfully obtained within minutes of capture by carrying out the procedure underwater (Pankhurst 1990). Therefore, using the same approach haemolymph was extracted from abalone within 2 mins of the animal being removed from the rock.

D-lactate & Glucose Concentration

Both D-lactate and glucose concentration in the haemolymph was estimated using colorimetric assay kits (Biovision). In the case of D-lactate, the D-lactate is oxidized by D-lactate dehydrogenase and the optical density of the resulting colour is read at 450 nm. Glucose was oxidized to generate a product which reacts with a dye to generate colour, whose intensity is proportional to glucose concentration and is also read at 450 nm.

Percentage Phagocytosis

Percent of haemocytes undertaking phagocytosis was estimated by incubating a sample of haemolymph with inactive Congo red stained yeast cells. The proportion of haemocytes undertaking phagocytosis of yeast cells was then scored under a high powered microscope (x400). Two slightly different assays were used throughout the project – tube incubation and slide incubation. The tube incubation method (modified from Li *et al.* 2007) was used in the Tasmania Summer, Tasmania Winter, and Tasmania Processor Recovery experiments. However, substantial variability among slides and animals was deemed unacceptable, and for the Motherboat Recovery and NSW Summer 2014 Experiment the slide incubation method was used (Hooper *et al.* 2014).

Tube incubation method – within 30-60 mins of haemolymph extraction, 600 µl of haemolymph was pipetted in to an Eppendorf tube and 40 µl of a Congo red stained yeast suspension (1.0 x 10^8 cells/ml) was added, vortexed, and incubated for 15 mins in the dark at room temperature. After the first incubation the tube was gently vortexed for 5 s before ~60 µl of the solution was pipetted onto a glass slide (this was done in triplicate). The slides with the haemocyte-yeast solution were incubated in the dark at room temperature for 15min, after which time the samples were mounted with coverslips and each slide read. Phagocytosis activity was determined by scoring the first 30 haemocytes encountered for the presence or absence of yeast cells.

This is first time that haemocyte phagocytosis has been assessed in wild abalone or used in a field experimental setting and there were delays of up to 60 mins in getting haemolymph to the laboratory for processing. Given that when the tube incubation method has been used in the past there has been no delay in the incubation of haemolymph with the yeast cells (Stone *et al.* 2013) we conducted a validation experiment to assess how rates of phagocytosis were affected by the delay in incubation. Six large adult *Haliotis rubra* collected from the wild were held in seawater when being transported from the field to laboratory and then held in flow-through seawater system for 24h at ambient temperatures (Hobart June 11 & 12 2013). It was assumed that these animals were minimally stressed. Haemolymph was drawn from each abalone at 1100 on June 12 and placed on ice, and at 10, 30, and 70 mins post-sampling an aliquot of haemolymph from each of the six animals was transferred to eppendorf tubes for incubation with yeast cells and processed as described above.

Slide incubation method– within 1-2 mins of haemolymph extraction ~60 µl of haemolymph was spread onto a glass slide and incubated for 10 mins in the dark at room temperature (done in duplicate). After which time 50 µl of a Congo red stained yeast suspension (1.0 x 10^8 cells/ml) was added to each slide and allowed to incubate for a further 30 mins in the dark at room temperature. Slides were then washed with filtered seawater and fixed in 10% formaldehyde in filtered seawater for 20 min to fix slides, before coverslips were placed on the slide. From each slide the first 50 haemocytes encountered were scored for the presence or absence of yeast cells.

Two comparisons were undertaken to assess the nature of the difference between the two assays using wild caught abalone caught in NE Tasmania, transported to seawater facilities at Institute of Marine and Antarctic Studies, University of Tasmania, and held for 24 h in a flow through seawater system before a haemolymph sample was drawn. In the first comparison between the two methods, haemolymph was drawn from 10 abalone within minutes of them being removed from the seawater tank. In the second comparison, three abalone were exposed to 60 mins aerial exposure stress before haemolymph was extracted, while over the same time three animals remained in seawater before haemolymph was drawn. All haemolymph was assayed using the two methods; for the slide incubation method there was no delay in the processing of

the haemocyte, while for the tube incubation method there was a 15-30 min delay in the start of the incubation assay as per the delay that occurs when using the method in the field.

Haemocyte Density

Haemocyte density was estimated from formalin fixed sampled (200 µl 6% formalin solution added to 400 µl haemolymph) using a haemocytometer. When there were delays of more than a few minutes in fixing the haemolymph there was substantial clumping of the haemocytes which prevented densities from being estimated. Haemocyte density data was only available for the Motherboat Recovery and NSW Summer 2014 Experiments when haemolymph was fixed within 5 min of the haemolymph being drawn.

Haemolymph Osmolality

Haemolymph osmolality was obtained using a Vapro© Model 5520 vapour pressure osmometer (Wescor Inc., Logan, Utah, USA) for abalone in the Tasmania Summer 2012 Experiment to examine the effects of time of deck before stacking.

3.3. Environmental Parameters

In all experiments non-submersible temperature/humidity loggers (Hobo U23 Pro V2) were placed in bins stacked with *Haliotis rubra* held in drained bins and covered with a damp hessian sack and doused with a bucket of seawater. Submersible temperature loggers (Hobo U22-001 Water Temp Pro) were placed in bins in which the stacked abalone were continuously submerged in seawater with flow-through circulation.

Maximum air temperature data for that region were obtained from the nearest Bureau of Meteorology station to the study site, which was Dover for Tasmanian experiments and Green Cape for New South Wales Experiments (http://www.bom.gov.au/climate/data/). Seasonal sea surface temperatures (SST) were obtained from http://www.seatemperature.org/australia-pacific/australia/, which provides a web-interface for daily SST observations provided by NOAA.

3.4. Experiments

A series of five experiments were carried out in summer in Tasmania (2012) and NSW (2014), and winter in Tasmania (2013). A specific processor based recovery trial was carried out in Tasmania during summer 2013, while a processor recovery period was included during the NSW summer experiment in 2014. Details for each experiment are provided below.

The treatments in each experiment differed slightly as new knowledge was accrued and we focussed on those handling and transport practices that have the logistic and policy capacity to be modified and altered based on the information derived during the research, e.g. practices that are in place to deal with biosecurity are not open for change and were not considered.

Tasmania Summer 2012 Experiment

This experiment sought to examine the effects of “time on deck” prior to stacking, use of dousing in drained bins, and the time that the abalone of held drained at the end of the boat transit phase while waiting for the processor truck on the stress levels in black lip abalone. This experiment was carried out over three consecutive days in February 2012 (14th, 15th and 16th) using *Haliotis rubra* collected from the George III Rock

research reserve (43°32'19.02"S, 146°58'17.44"E), southern Tasmania. On each day haemolymph was drawn from 10 replicate abalone underwater within 2-3 mins of animals being removed from rock to obtain baseline measures of all haemolymph parameters.

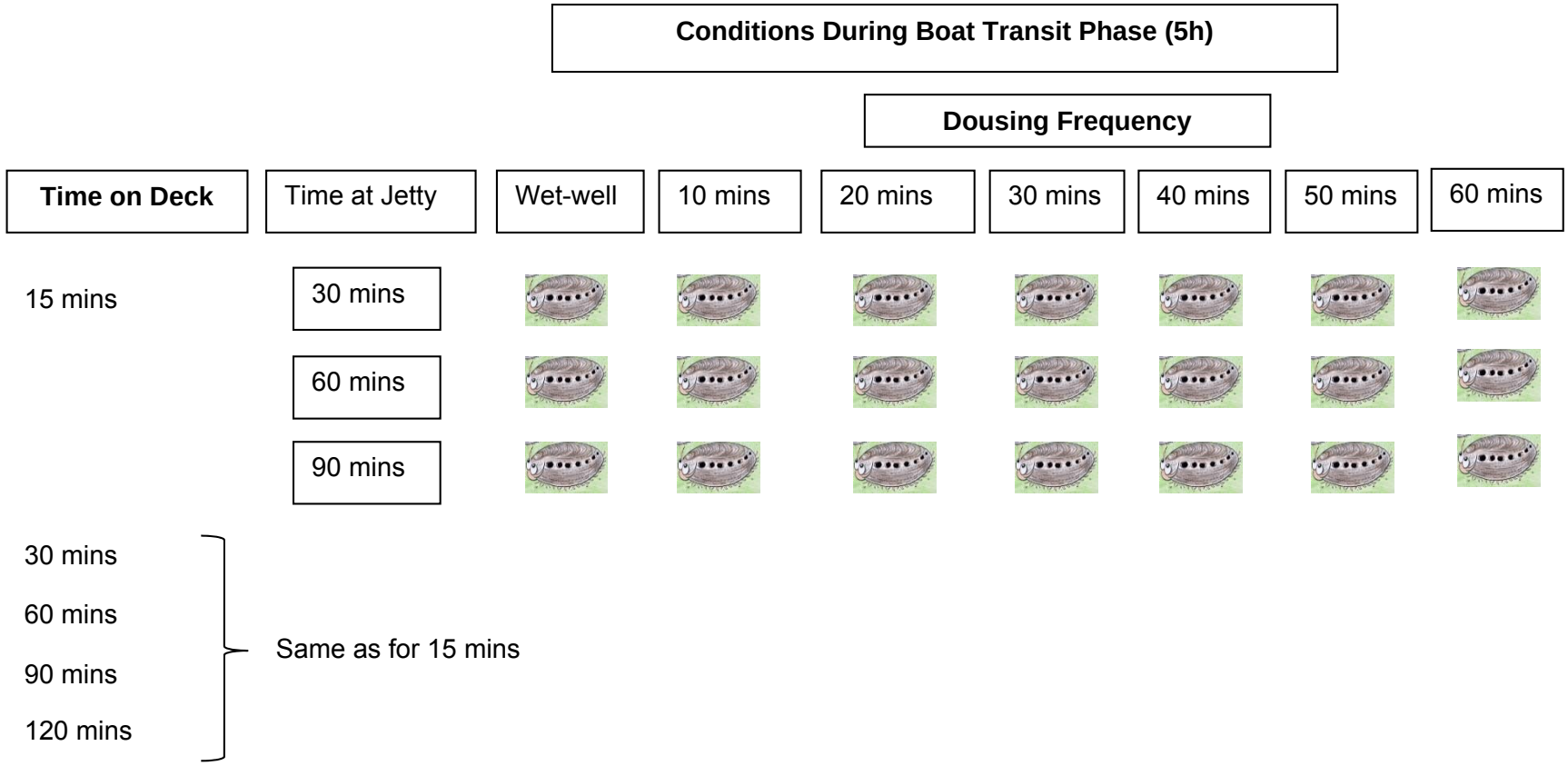
Abalone (20-30) were stacked vertically (holes up) into a fish bin (502 x 338 x 300mm) and allocated to one of three treatments during the 5 h boat transit phase and the 1.5 h post boat transit phase. The factors that could potentially affect stress levels in abalone were:

a) Time on deck. To assess the relationship between the time that animals spent on deck before being stacked in bins and stress responses, haemolymph was drawn from abalone that spent 0, 15, 30, 60, 90 or 120 mins on deck (**Error! Reference source not found.**).
b) Douse frequency. During a 5 h boat transit phase the effect of the frequency of dousing the abalone stacked in drained bins and covered with wet hessian sacks was examined. The following seven treatment levels were used: doused with a bucket of fresh seawater every 10, 20, 30, 40, 50, or 60 mins, plus wet-well bins with abalone continuously submerged in circulating seawater (**Error! Reference source not found.**).
c) Time at jetty. To assess the stress levels of abalone waiting for the processor truck we drew haemolymph from abalone immediately at end of boat transit, then at 30, 60, and 90 min post-completion of the 5 h boat transit phase (**Error! Reference source not found.**).

Haemolymph was drawn from 1-2 randomly selected abalone prior to stacking at the end of “time on deck”, at the end of the 5 h boat transit, and at the end of “time at jetty” (**Error! Reference source not found.**). Once a haemolymph sample was taken, abalone were marked with waterproof chalk to ensure that they were not repeatedly sampled, and the animal was returned to the treatment bin to maintain the density of animals in the experimental bins. The experiment was run three times over three days and days blocked to provide replicate animals in each treatment. All haemolymph was placed on ice for 30-60 min before being processed for haemocyte phagocytosis (tube incubation method).

To identify the most important factors contributing to the stress of the abalone, post-harvest stress parameters measured in the haemolymph were analysed using multiple regression, as all the explanatory variables are continuous, i.e. time or frequency.

Figure 3.4.1. Schematic of the sampling design used to examine the stress response of abalone due to the time the abalone are left on the deck of the boat prior to stacking in bins (time on deck), the frequency with which the abalone stacked in the bins are doused with clean seawater during the 5h boat transit phase (dousing frequency) and time the abalone in drained bins are waiting at the jetty prior to pick up by the processors truck (time at jetty). One abalone was sampled from each treatment with time on deck.



Tasmania Winter 2013 Experiment

This experiment sought to examine the effects of "time on deck" prior to stacking, and the use of continuous immersion, periodic immersion and dousing of abalone during the boat transit phase on the stress levels in black lip abalone. This experiment was carried out over three consecutive days in August 2013 (13th, 14th and 15th) using *Haliotis rubra* collected from the George III Rock research reserve (43°32'19.02"S, 146°58'17.44"E), southern Tasmania. On each day haemolymph was drawn from 10 replicate abalone underwater within 2-3 min of animals being removed from rock to obtain baseline measures of all haemolymph parameters.

The levels in each of the two factors:

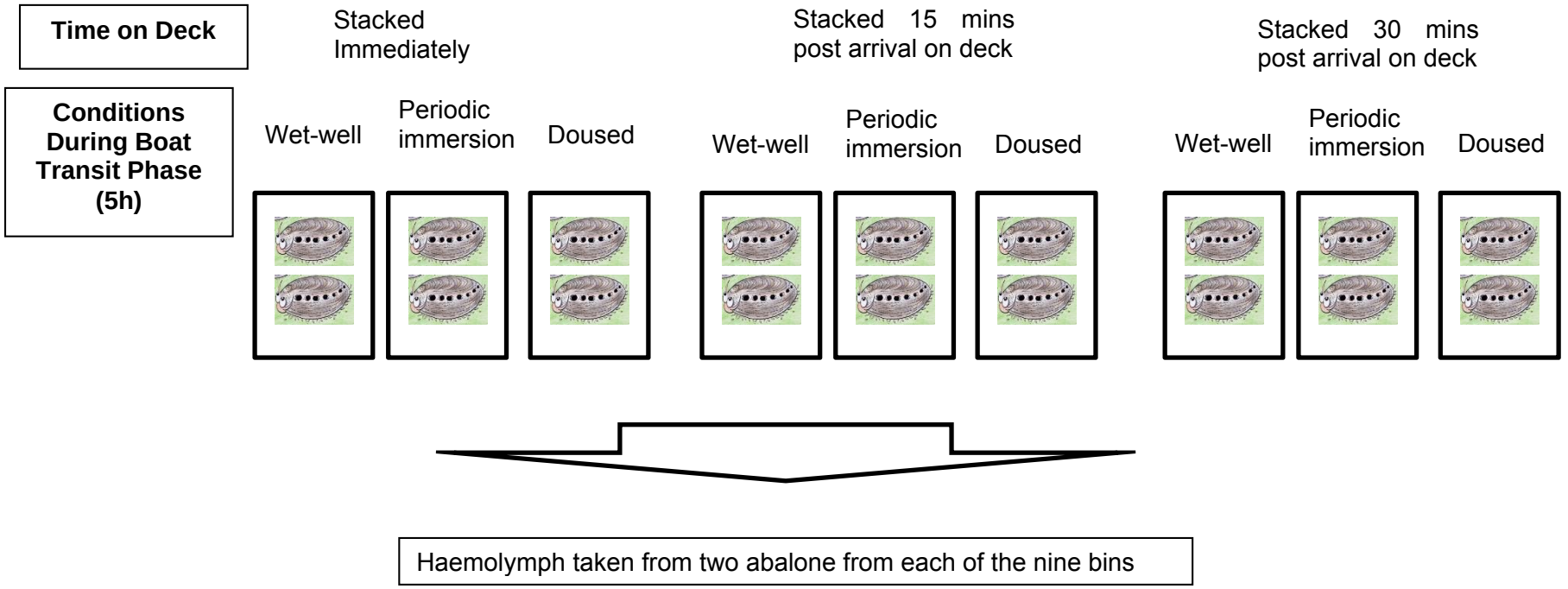
a) Time on Deck. Three times were used; abalone were stacked either immediately (0 mins), at 15 mins, or 30 mins after the bags arrived on the deck of the boat (Figure 3.4.2).
b) Conditions During Boat Transit Phase. Three methods were explored in this experiment (Figure 3.4.2):
    i. Wet-well - abalone were stacked vertically with holes up and each layer of abalone allowed to attach to the surface of another abalone; and the abalone were continuously submerged in flowing seawater
    ii. Periodic Immersion – abalone were stacked vertically with holes up and each layer of abalone allowed to attach to the surface of another abalone; these bins were drained and covered in damp hessian sack, every 60 mins they were filled with fresh seawater and left for 15 mins before draining.
    iii. Doused – abalone were stacked vertically with holes up and each layer of abalone allowed to attach to the surface of another abalone; these bins were drained and covered in damp hessian sack, and doused with a bucket of fresh seawater every 60 min.

On each day a single bin (180mm wide x 180mm high x 350mm long) containing 6-9 abalone was used for each of the nine combinations of the two factors ("time of deck" and "boat transit method"). The experiment was run three times; once each day, with day blocked to provide replicate animals in each treatment (Figure 3.4.2).

Abalone were exposed to boat transit conditions for 5 h before the experiment was terminated and haemolymph samples taken from two individuals from each of the nine bins. Once a haemolymph sample was taken, abalone were marked with waterproof chalk to ensure that they were not repeatedly sampled, and the animal was returned to the treatment bin to maintain the density of animals in the experimental bins. Haemolymph was placed on ice for 30-60 minutes before being processed for haemocyte phagocytosis using the tube incubation method.

Stress parameters measured in the haemolymph were analysed using a two-way ANOVA. Terms in the model that explained significant amounts of variation in each parameter were explored using either planned contrasts or Tukey's HSD post-hoc test.

Figure 3.4.2. Design of the experiment carried out in Tasmania Winter Experiment carried out in August 2013, which examined the stress response of abalone stacked at one of three times on deck and held in one of three boat transit methods. Two abalone were sampled from each of the nine bins (180mm wide x 180mm high x 350mm long) which held 6-9 abalone each. This experiment was done on three different days, to provide a total of six replicate abalone for each of the nine combinations of the two treatments; “time on deck” and “boat transit method”.



Tasmania Processor Recovery Experiment

This experiment was designed determine how quickly abalone recovered in the tanks of a commercial processor as a function different treatments during the boat transit phase. This experiment carried out early in the austral autumn, on March 19 2013, started pre-harvest, moved through the boat and transit phases post-harvest, and ended 61 h after transferring *Haliotis rubra* into the processor tanks. This experiment tracked the changes in haemolymph parameters through each phase and ultimately recovery of the abalone post-harvest. Animals were sourced from the George III Rock research reserve (43°32'19.02"S, 146°58'17.44"E), southern Tasmania. Haemolymph was drawn from 10 replicate abalone underwater within 2-3 mins of animals being removed from rock to obtain baseline measures of all haemolymph parameters.

Two treatments ("time on deck" and "boat transit method") were explored in this experiment (Figure 3.3):

a) Time on Deck. Animals were stacked either immediately upon landing (<15 mins) or after 30 mins (>30 mins) on landing in the boat (Figure 3.4.3).
b) Conditions During Boat Transit Phase. Abalone were all stacked vertically with holes up and allocated to one of four treatments (Figure 3.4.3);
    i. Wet-well bins – abalone in these bins were fully immersed in circulating seawater
    ii. Periodic immersion bins – abalone were held in drained bins, covered in damp hessian sack, that were filled with seawater and left for 15 mins before draining; this periodic immersion was repeated every 60 mins post draining.
    iii. Doused bins with abalone attached – abalone were held in drained bins and doused with a bucket of fresh seawater each hour, covered with hessian sack and abalone were allowed to attach to a flat plastic surface. This treatment was included as in NSW fishers vertically stacked abalone in a bin with a plastic separator.
    iv. Doused bins with abalone unattached – abalone were held in drained bins and doused with a bucket of fresh seawater each hour, covered with hessian sack and abalone were allowed to attach to the surface of another abalone.

Abalone were exposed to boat transit conditions for 5h after which time two randomly chosen abalone from each combination of the treatments (Figure 3.4.3). Once a haemolymph sample was taken, abalone were marked with waterproof chalk to ensure that they were not repeatedly sampled, and the animal was returned to the treatment bin to maintain the density of animals in the experimental bins.

After the boat transit abalone were taken by road, the "road transit phase", for 4h to a commercial live abalone processor. During the road transit phase each bin was drained of water, and covered with a wet hessian sack and canvas sheet before being loaded onto an unrefrigerated vehicle. On arrival at the processors haemolymph was drawn from two randomly chosen abalone in each of the treatment combinations.

Recovery in Live Processor Tanks. Once at the live abalone processors the abalone were transferred from the bins used for the boat and road transit into the aluminium mesh crates used at the processors, then placed in the processor tanks with circulating seawater at 12-13^{o}C. For each of the eight combinations of "time on deck" and "boat transit method" there was one mesh crate of 40 abalone (Figure 3.4.4). Crates were removed from the water to allow a sample of haemolymph to be drawn from two randomly selected individuals from each crate at 4, 15, 25, 39, 47, and 61 h post-

transfer to processor tanks (Figure 3.4.4). Abalone from whom a haemolymph sample was taken were removed from the experiment to ensure no repeat sampling of the same individual

All haemolymph was placed on ice for 30-60 minutes before being processed for haemocyte phagocytosis (tube incubation method). Stress parameters measured in the haemolymph were analysed using a three-way ANOVA. Terms in the model that explained significant amounts of variation in each parameter were explored using either planned contrasts or Tukey's HSD post-hoc test.

Figure 3.4.3. Design of the experiment carried out in Tasmania Processor Experiment carried in March 2013 which examined the stress response and recovery in the live abalone processor of abalone stacked at one of two times on deck and held in one of four boat transit methods. Two abalone were sampled from each 40L bin, each of which held 50 abalone. At the end of the 5h boat transit phase, all abalone were transferred to the live abalone processor facility by road.

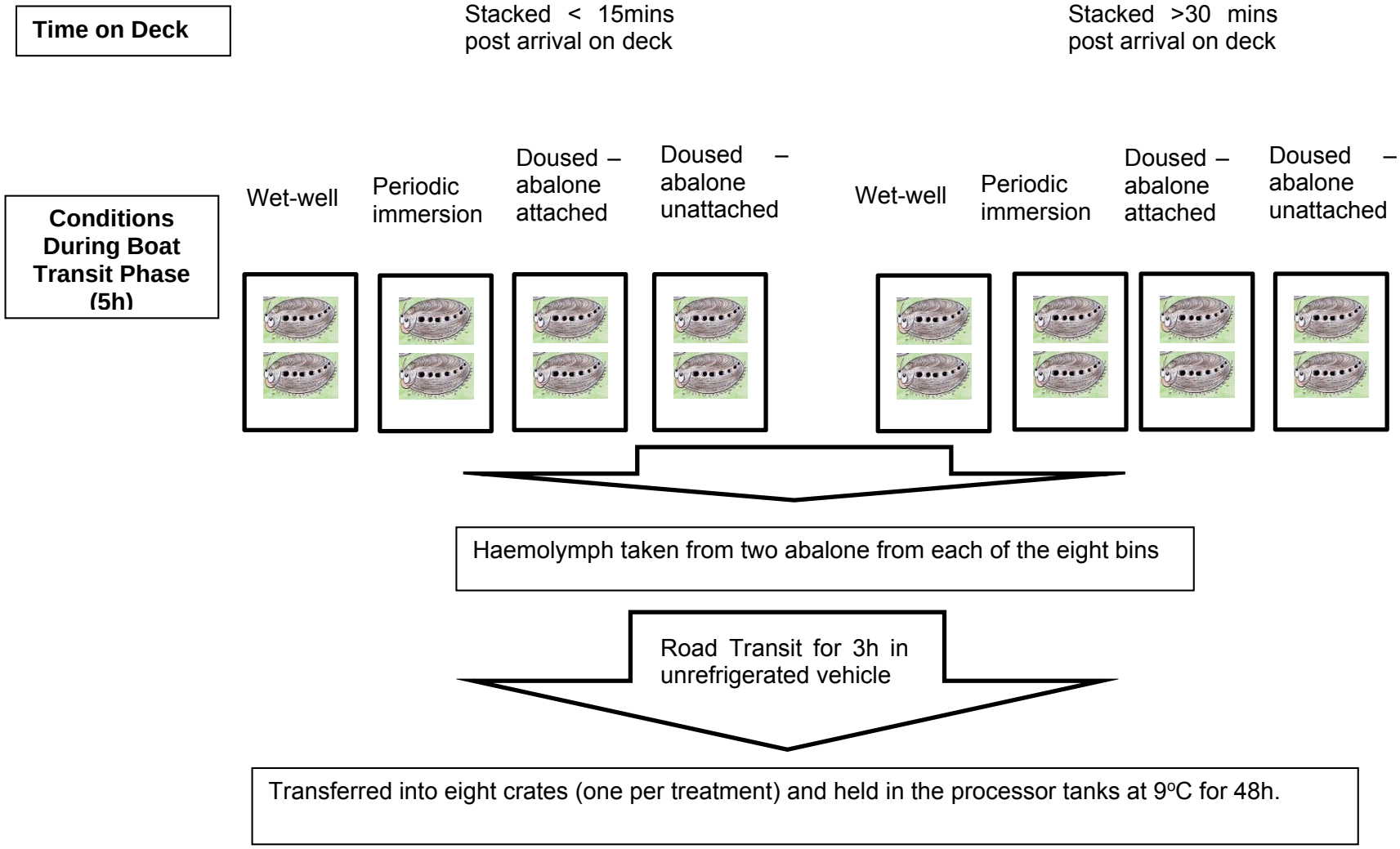
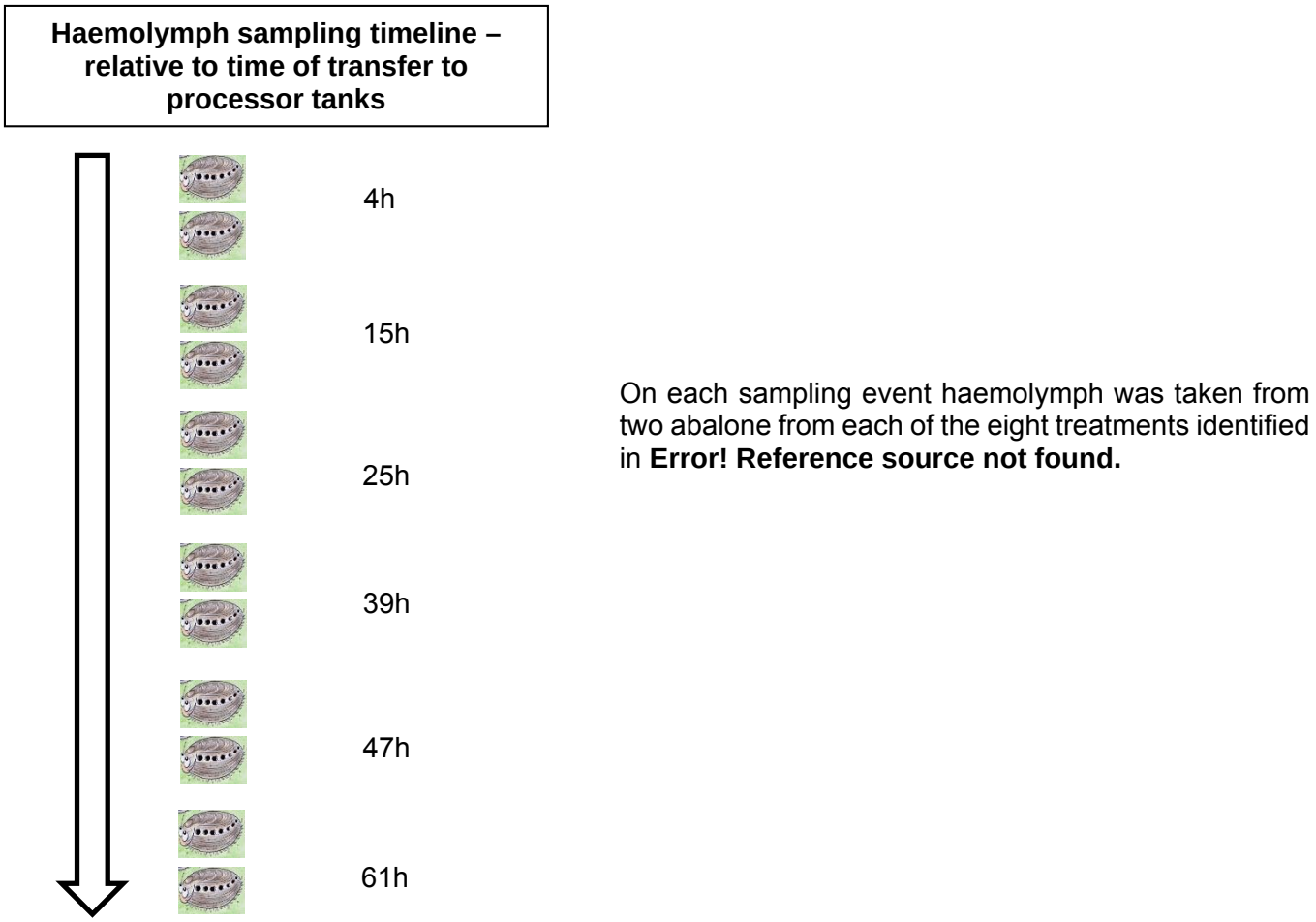


Figure 3.4.4. Sampling timeline to assess the recovery of abalone in the Tasmanian live abalone processor facility.



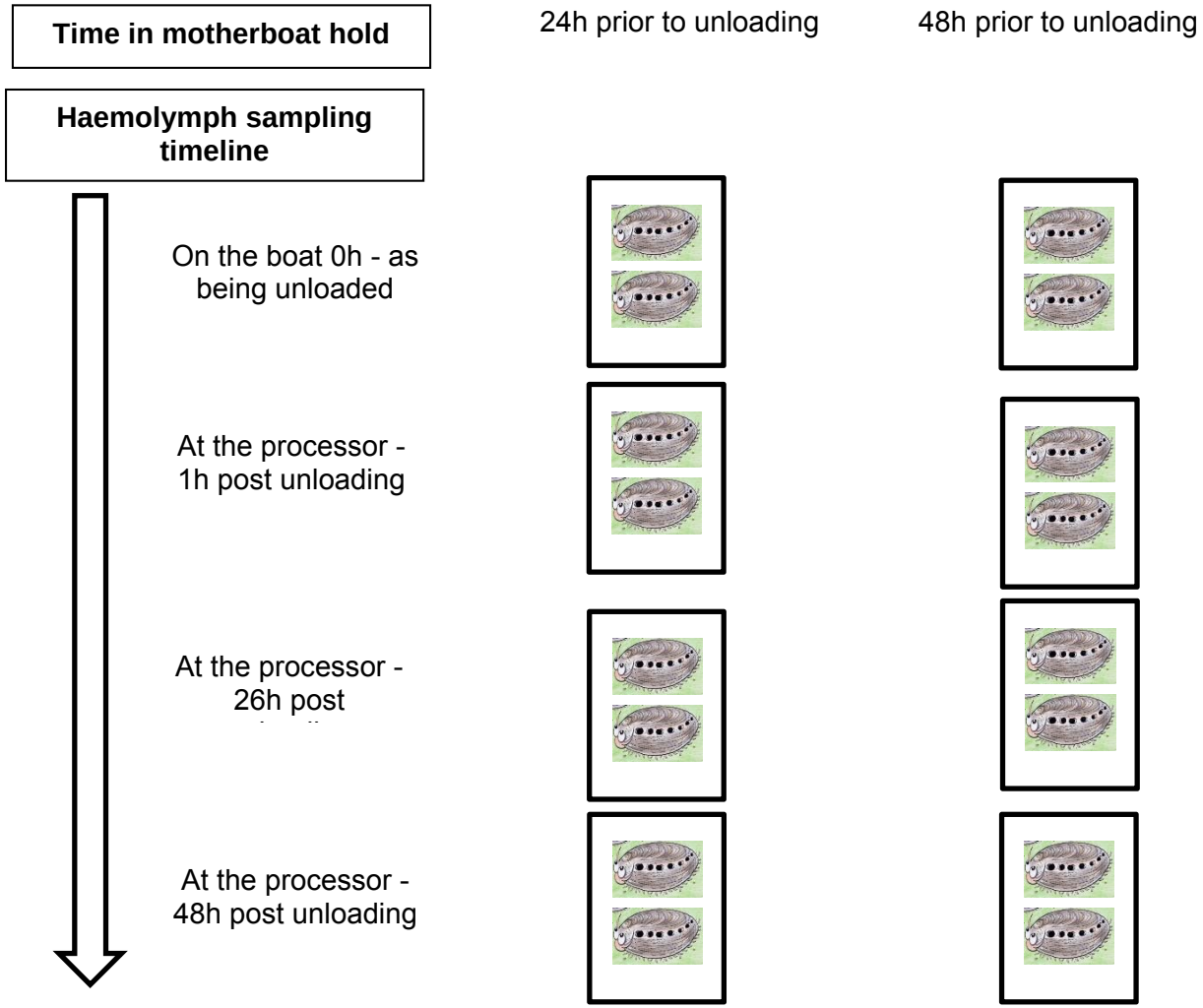
Tasmania Motherboat & Processor Recovery Experiment

Sampling of harvested abalone was undertaken to determine the stress levels of abalone that had been transported on a motherboat. On January 16th, 2014 a motherboat with abalone harvested from the west coast of Tasmania landed *Haliotis rubra* at the jetty in Margate, Hobart at approximately 0900h. Abalone on the motherboat were identified as having been caught on either January 14 (48 h prior to landing at jetty) or January 15 (24 h prior to landing at jetty). From the motherboat, abalone were transferred from the sea-going bins to smaller plastic bins for transport via truck to the processors. Once at the processor the abalone were transferred by hand from the small plastic bins to the large cages used to hold abalone in the processor tanks.

To assess the condition of the abalone being landed at the jetty and their recovery in the processor tanks, haemolymph was drawn from five animals from each collection day as they left the boat to be loaded into the truck (0 h), at the processor shed before transfer to tanks (1 h), then 26 and 48 h after transfer to the tanks at the processors (Figure 3.4.5). No haemolymph was taken from abalone on the reef as this was part of a commercial, not a research, operation. All haemolymph was processed for haemocyte phagocytosis immediately upon being drawn using the slide incubation method.

Stress parameters measured in the haemolymph were analysed using two-way ANOVA. Terms in the model that explained significant amounts of variation in each parameter were explored using either planned contrasts or Tukey's HSD post-hoc test.

Figure 3.4.5. Design of the sampling carried out in January 2014 of abalone landed on a commercial abalone motherboat to examine the stress response and recovery in the live abalone processor of abalone collected on two different days. Two abalone were sampled at each sampling time point relative to the time that the abalone were unloaded from the motherboat.



NSW Spring 2014 and Processor Recovery Experiment

This experiment examined the effect of three different boat transit methods on the recovery of abalone in the processors in New South Wales. This experiment was carried out over three consecutive days in March 2014 (18th, 19th and 20th) using *Haliotis rubra* collected from the coast within 5 km of Eden, NSW (37° 4'15.60"S, 149°54'32.06"E). On each day haemolymph was drawn from five abalone underwater within 2-3 mins of animals being removed from rock to obtain baseline measures of all haemolymph parameters.

To assess the relationship between the time that animals spent on deck before being stacked in bins and stress responses, haemolymph was drawn from 24 abalone that spent between 0 and 30 mins on deck (**Error! Reference source not found.**).

Only boat transit treatments were explored:

Conditions During Boat Transit Phase. Abalone were stacked within 15 mins of landing into one of three transport treatments (**Error! Reference source not found.**);

i. Wet-well - vertically stacked with holes up and continuously immersed in water
ii. Doused vertical – abalone were vertically stacked in the bins, covered with a hessian sack, and doused with a bucket of fresh seawater every 60 min
iii. Doused horizontal – abalone were horizontally stacked, covered with a hessian sack, and doused with a bucket of fresh seawater every 60 min. The use of horizontal stacking in the NSW experiment was included because some fishers place a single layer of abalone horizontally over the vertically stacked abalone in a bin with a plastic separator.

For each of the three treatments there were two bins (180mm wide x 180mm high x 350mm long) with 6-9 abalone per bin. Abalone were exposed to boat transit conditions for 5 h and haemolymph samples taken from two abalones in each bin at the end of the boat transit phase (Figure 3.4.7). This experimental design was repeated on each of the three days. Once a haemolymph sample was taken, abalone were marked with waterproof chalk to ensure that they were not repeatedly sampled, and the animal was returned to the treatment bin to maintain the density of animals in the experimental bins. All haemolymph was processed for haemocyte phagocytosis immediately upon being drawn using the slide incubation method.

Processor Recovery Phase

On the first two days of the experiment at the end of the boat transit phase the remaining abalone in the experiment were transferred to the tanks of a local abalone processor (< 2 min drive from jetty) for a 24-48 h recovery phase. In the processor tanks (11-12^{o}C), abalones were placed in plastic mesh crates with the boat transit treatment bins pooled i.e. one processor crate per boat transit treatment. Two abalone from each transport treatment collected on the 18th March had haemolymph drawn 24 and 48 h after transfer to the processors, while two abalone from each boat transit treatment collected on the 19th March had haemolymph drawn 24 h after transfer (Figure 3.7).

Stress parameters measured in the haemolymph were analysed using a two-way ANOVA. Terms in the model that explained significant amounts of variation in each parameter were explored using either planned contrasts or Tukey's HSD post-hoc test

Figure 3.4.6. Design of the experiment carried out in NSW in March 2014 examined the stress response and recovery in the live abalone processor of abalone stacked within 15 minutes of landing and held in one of three boat transit methods. Two abalone were sampled from each 5L bin, which held 6-9 abalone each. At the end of the 5h boat transit phase, all abalone were immediately transferred to the live abalone processor facility.

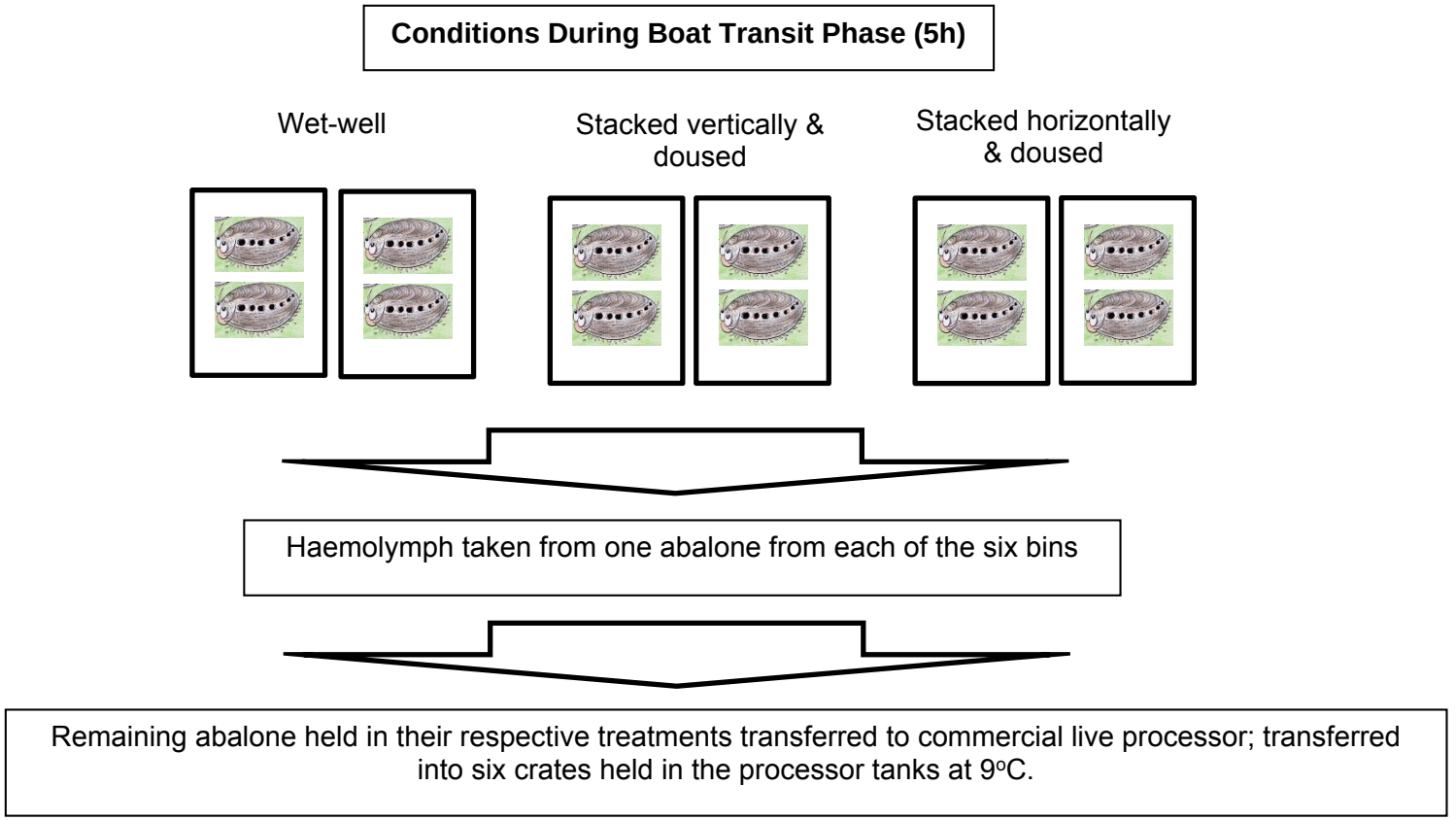
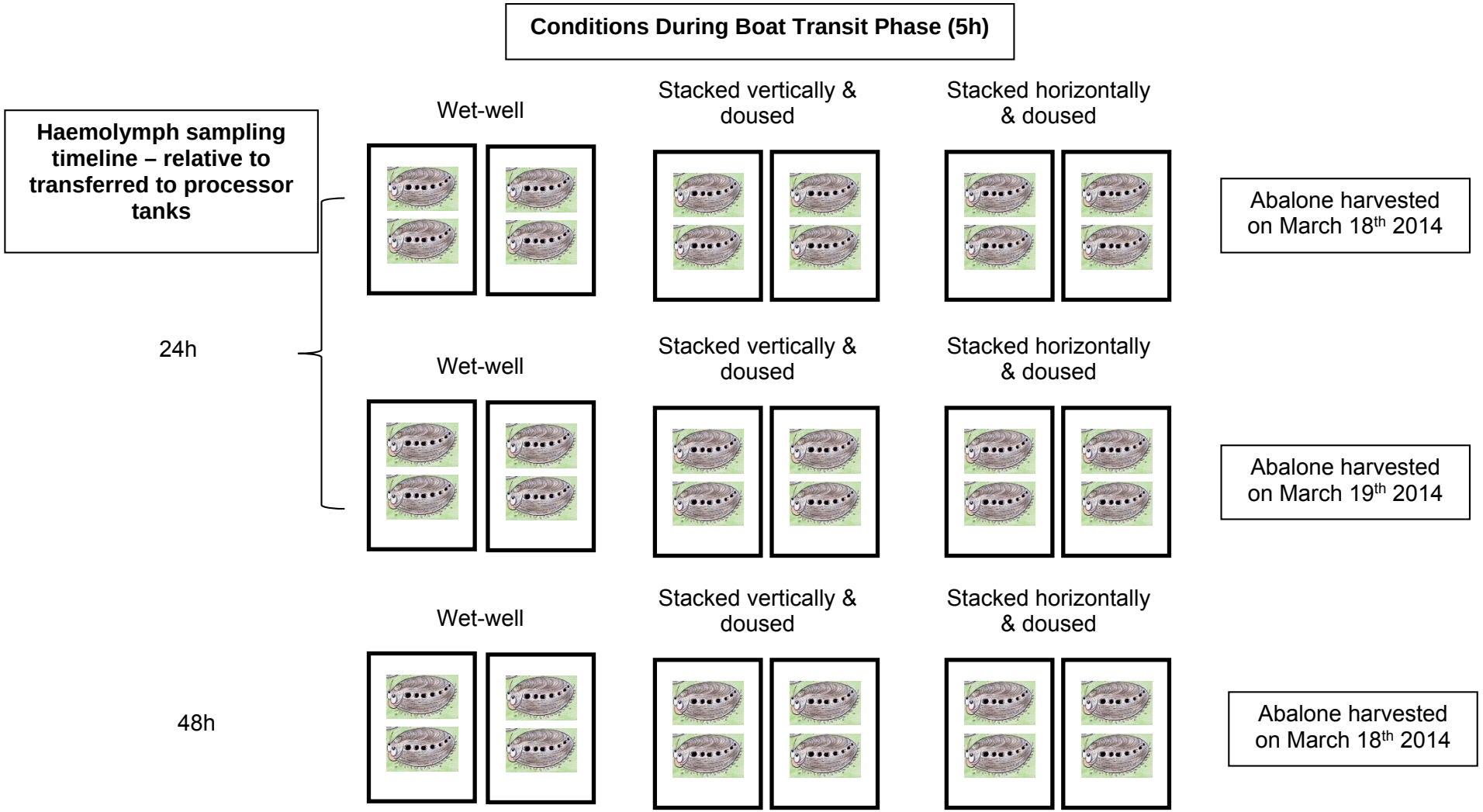


Figure 3.4.7. Sampling timeline to assess the recovery of abalone in the NSW live abalone processor tanks. Abalone were transferred immediately upon completion of the boat transit phase (5h). Haemolymph was removed from two abalone from each treatment bin at each time point post-transfer to the processor tanks



4. Results

4.1. Abalone Diver Survey

A total of 37 Tasmanian abalone fishers returned questionnaires from the approximately 100 distributed by the Tasmanian Abalone Council and University of Tasmania. All fishers preferred to complete the questionnaire themselves rather than complete the questionnaire over the phone.

Day boat trips were between 4-10 hours long, with most fishers estimating that trips are typically 6-7 hours (Figure 4.1.1). There was no evidence that the length of day trips differed among the regions (χ^2= 3.43 df 12, p=0.992) or between seasons (χ^2= 12.28, df 18, p=0.832).

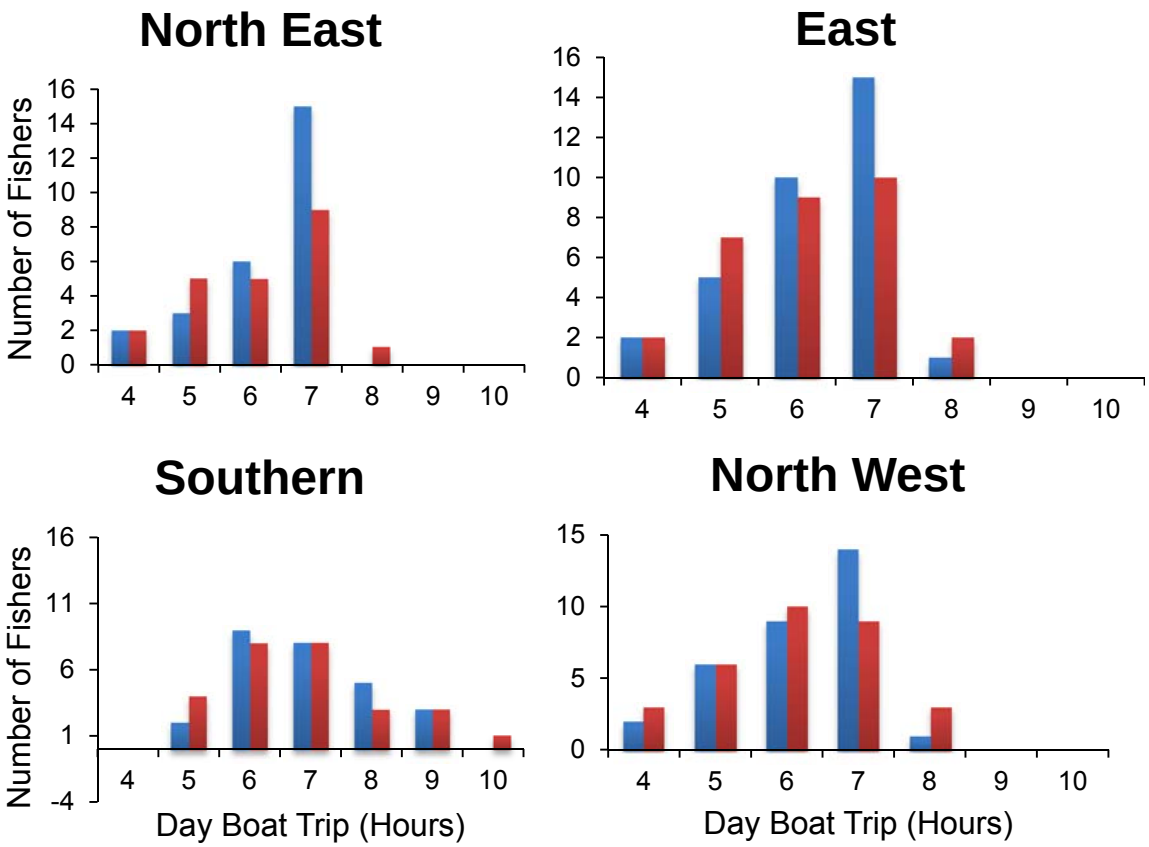


Figure 4.1.1. Frequency distribution of the length of day trips (hours) undertaken by abalone fishers in the four fishing regions of Tasmania in summer (red) and winter (blue).

Bags of abalone can be left in the water for up to 30 mins before they are retrieved and landed on the deck, but for most fishing operations (74%) the deckhands retrieve the bags within 15 mins (Figure 4.1.2). The time within which abalone are stacked into bins after landing is <40 mins, but most fishers will stack abalone within 10 mins of them being landed on the deck of the boat in both summer (64%) and winter (62%) months (Figure 4.1.2).

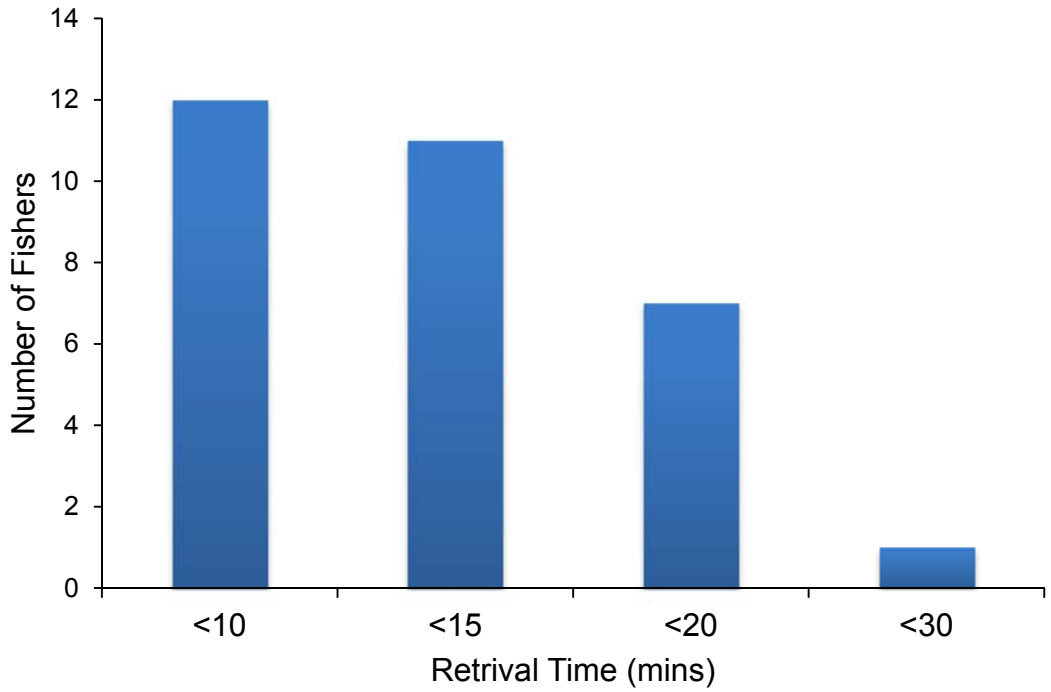


Figure 4.1.2. The frequency distribution of the time that bags of harvested abalone are left in the water before being landed on the deck of the boat.

Abalone are stacked into bins after landing on the deck of the boat within 10 mins, and rarely extending out to 40 mins (Figure 4.1.3). When there is a single diver in the water, the abalone are stacked within 5 mins of landing on the deck, and within 10 mins when there are two divers in the water (Figure 4.1.3). The frequency distributions are very similar for winter and summer (Figure 4.1.3).

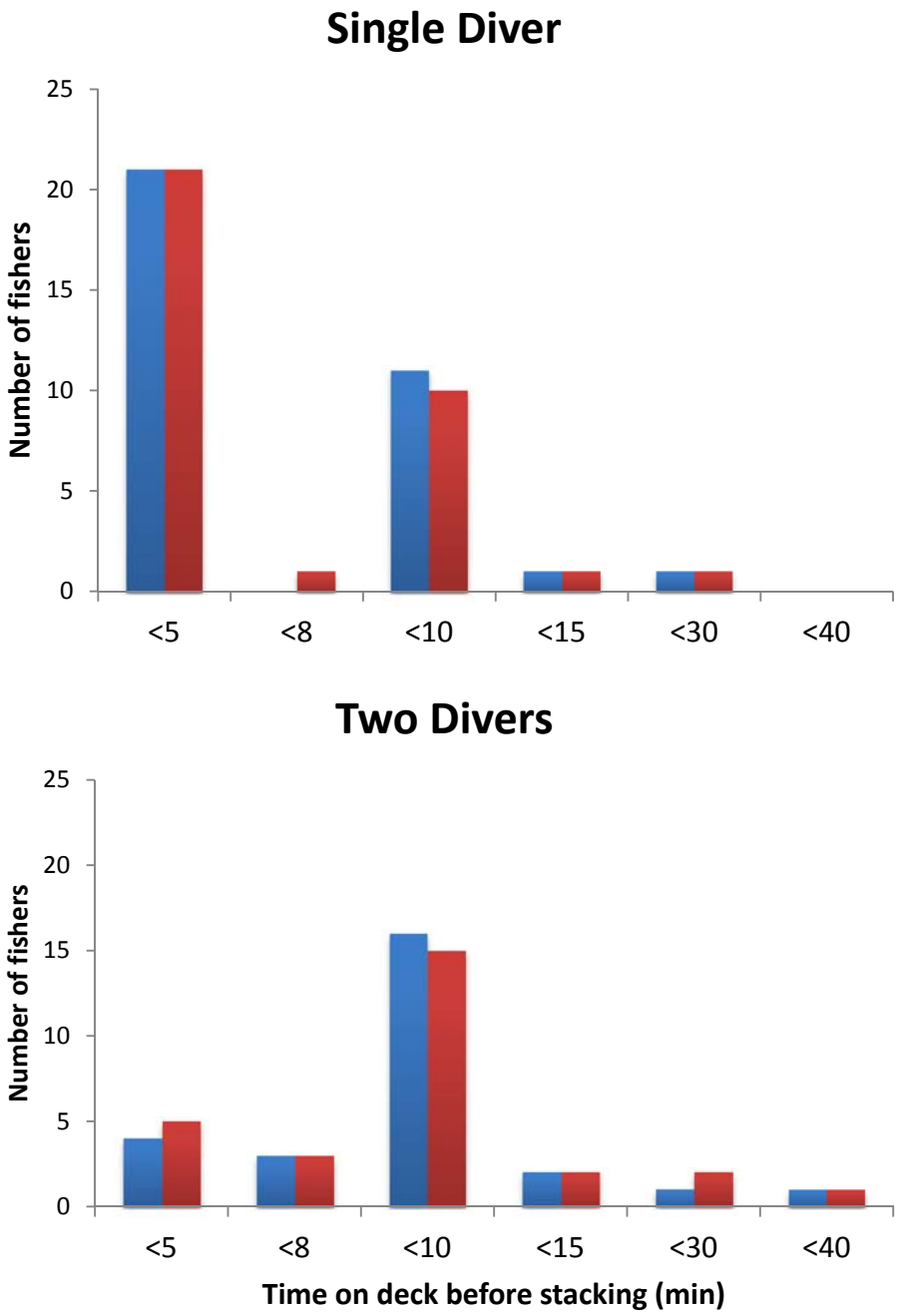


Figure 4.1.3. Seasonal differences in the frequency distribution of time that abalone are left on deck before being stacked into bins for dive operations involving one or two divers in the water at a time in winter (blue) and summer (red).

Cleaning of the shell by manually scraping the outside of each shell with a knife is very common practice throughout the industry with >90% of fishers cleaning the abalone prior to stacking. Once the abalone shell has been cleaned all the day-boat fishers stack the abalone on their side in the bins and >90% of fishers stack the animals with the holes up (Figure 4.1.4).

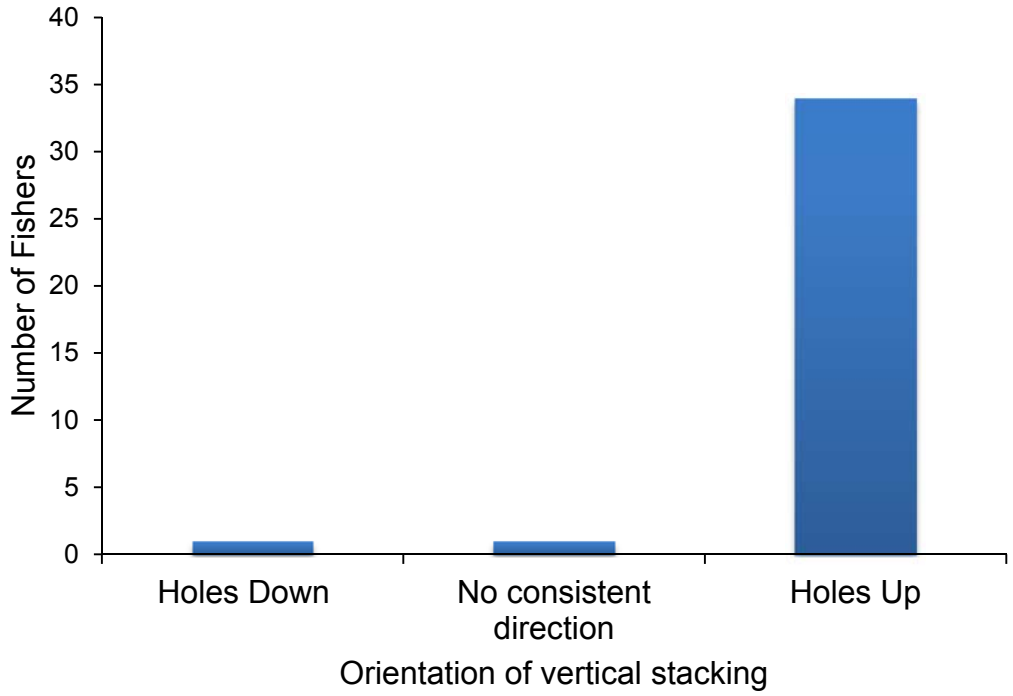


Figure 4.1.4. The frequency of times that harvested abalone are stacked in bins with the hole up or the hole down during boat transit.

Most fishers stack approximately 20 kg or less of abalone in a bin (Figure 4.1.5) and 67% of fishers stack the abalone across two layers. Six fishers indicated that processors prescribe the number of stacking layers in the bin. There was a significant difference between the seasons in the densities that abalone were stacked into the bins, (χ^2=14.097, df 7, p=0.049); in the summer more fishers stacked bins with 20-24kg of abalone than in the winter months (Figure 4.1.5).

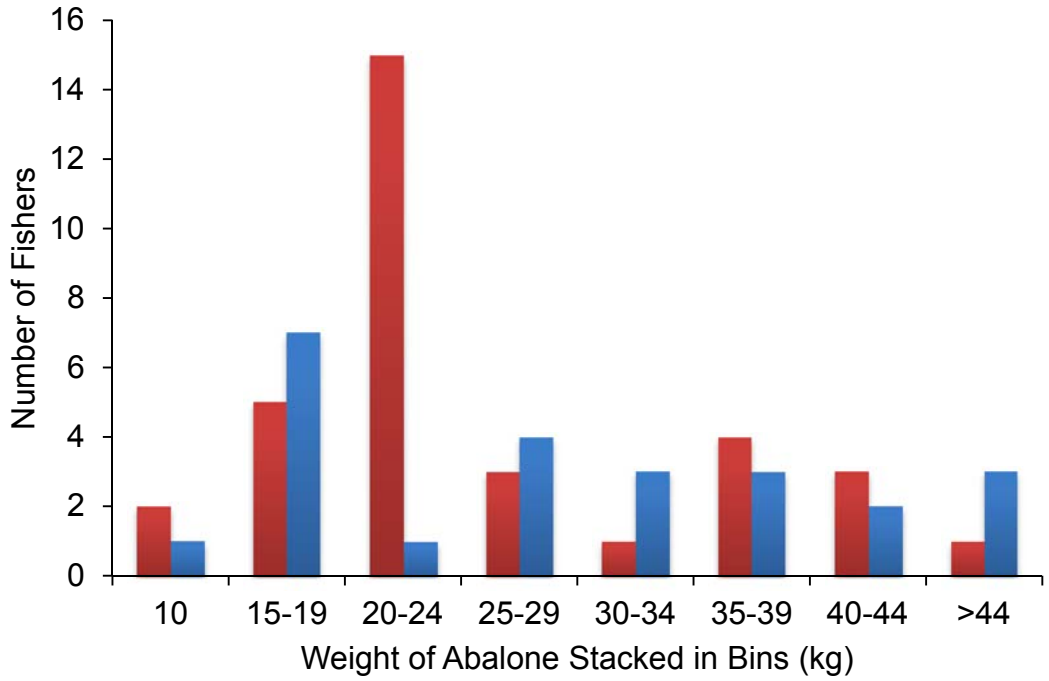


Figure 4.1.5. Frequency distribution of abalone weight stacked in each bin layer in summer (red) and winter (blue).

More than 90% of day boat divers hold the abalone in drained bins, with limited evidence that the animals are flushed for any period of time during the transit period, covered with a hessian sack, or doused with seawater when the animals are in transit. Approximately 50% of fishers provide some shade for the bins of animals during transit.

Once at the boat ramp, the processor trucks are there within 30 mins of arrival, and while often there is no wait, one diver had to wait up to 3 h (Figure 3.1.6). During this time fishers cover the animals with a hessian sack and douse the animals.

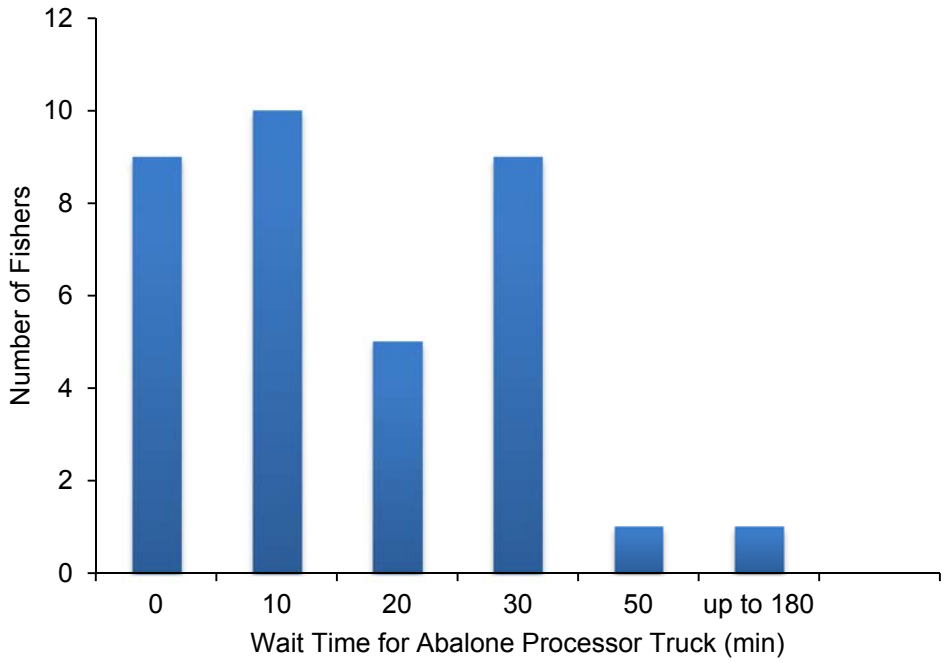


Figure 4.1.6. Frequency distribution of the time that fishers wait at the jetty for the processor's truck.

4.2. Haemocyte Phagocytosis – Assessment of Incubation Methods

Investigation of the tube incubation method for estimating haemocyte phagocytosis activity revealed time delays in commencement of the analytical process could influence the outcome of experiments. Changes in haemocyte phagocytosis declined with delays in starting the tube incubation assay. At the first time point, i.e. 10 mins past sampling, the percent phagocytosis was 11-59%; when the animal with the smallest phagocytosis value was dropped the next smallest value was 30%. This amount of phagocytosis was very similar to values we obtained for the reef abalone in the Tasmania Summer Experiment (31-52%), but less than the reef abalone in the Tasmania Processor Recovery Experiment (51-71%). At 30 mins post-sampling the average change in phagocytosis (increase in mean of 5% standard error 6%) was not significant (t=-0.93, df 5, P=0.197). The percentage of haemocytes undertaking phagocytosis activity was 34-76%; with two abalone displaying a decrease in phagocytosis activity. However, by 70 minutes post collection of haemolymph phagocytosis activity was 13-60% across the six animals; activity in four individuals declined from the 10 min value by 9-33%, on average this was a decline of 13% (standard error 6%) which was significant (t=2.04, df 5, P=0.048). This suggests that a delay of up to 30 mins delay in starting the tube incubation is unlikely to result in a significant effect on the estimate of percentage of phagocytosis, but after 60 mins the tube incubation method significantly underestimated of the percentage of haemocytes undertaking phagocytosis.

The comparison of the two incubation methods using 10 minimally stressed abalone found the average difference between the percent phagocytosis was 12% (range of differences 10%-41%), and there was no evidence of a significantly consistent difference between the two methods (paired sample t-test, t=-1.82, df 9, p=0.051). However, the variability among animals was five times less for the slide incubation (s^2=93.38) than for the tube incubation (s^2=554.89).

There were differences between the two methods when abalone were more stressed (paired sample t-test t=-17.01, df 2, p=0.002). The average difference between the two methods for stressed animals was 45%, with the tube incubation method estimating phagocytosis to be five times greater than the slide method. While estimates of phagocytosis between stressed and unstressed using the tube incubation method were no different (t=0.57, df 3, p=0.305), phagocytosis in stressed animals using the slide incubation method were six time less than in unstressed animals (t=-10.37, df 4, p=0.002).

This suggests that the tube method may have been over-estimating the rates of haemocyte phagocytosis of the stressed animals, but not of the unstressed animals. Given this outcome and that the incubation method was not consistent among experiments there was limited capacity to make direct comparisons of percent phagocytosis among experiments.

4.3. Tasmania Summer 2012 Experiment

Environmental Parameters

Day time air temperatures over the three days were variable, but typical for the south-east Tasmania region in February. Records at the closest Bureau of Meteorology weather station (Dover), showed maximum temperature was coolest on the first day (14th Feb) at 21.6°C, increasing to 29.4°C on the third day (16th Feb), and is reflected in the pattern of temperature increase recorded in the drained bins over the duration of the experiment (Figure 4.3.1, Figure 4.3.2). Temperature change was gradual, with the exception of periodic spikes associated with the interval of dousing (Figure 4.3.1, Figure 4.3.2). Humidity quickly reached ~ 90% in the drained bins covered with hessian sacks and was stable over the course of each day, and did not appear to be affected by the periodic dousing. The average SST during this experiment was 16.6°C, which was slightly warmer than the average for south-east Tasmania in February (16.5°C).

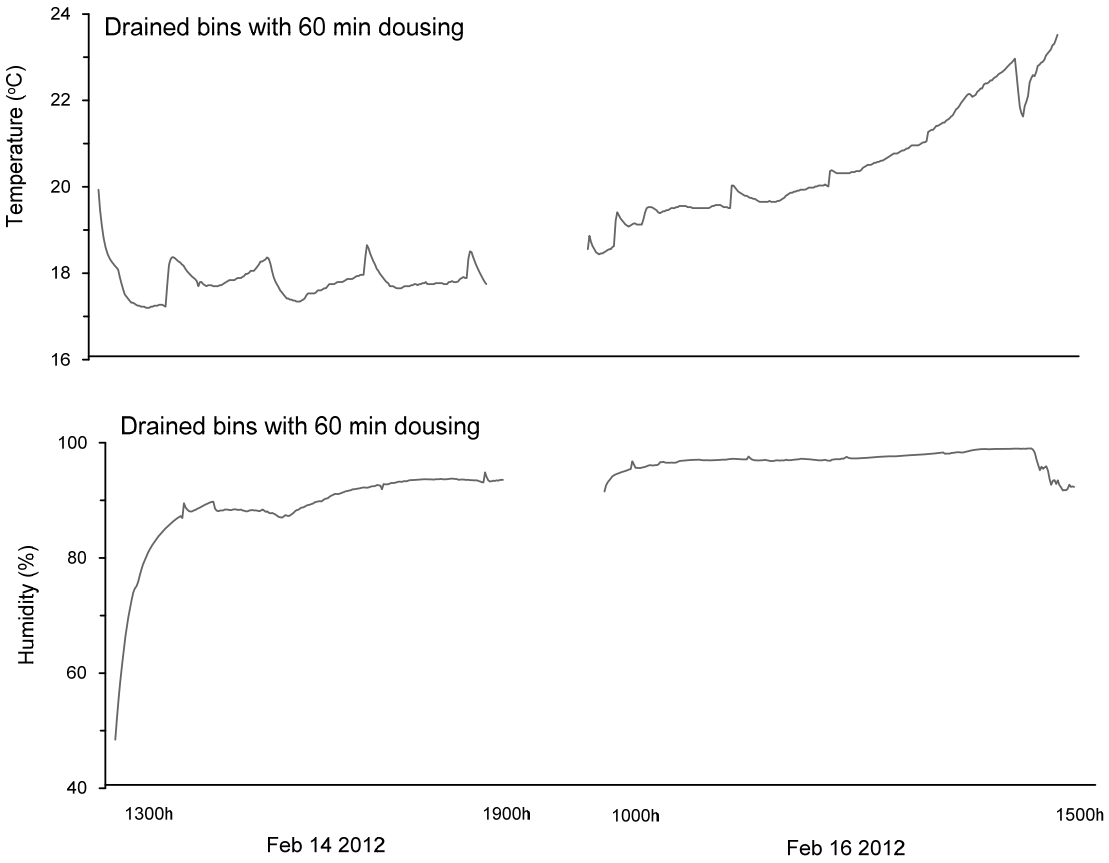


Figure 4.3.1. Temperature and relative humidity in drained bins doused with fresh seawater every 60 min over the 5 h boat transit phase (14th Feb – 16th of Feb). Sampling frequency for both temperature and humidity was once per minute.

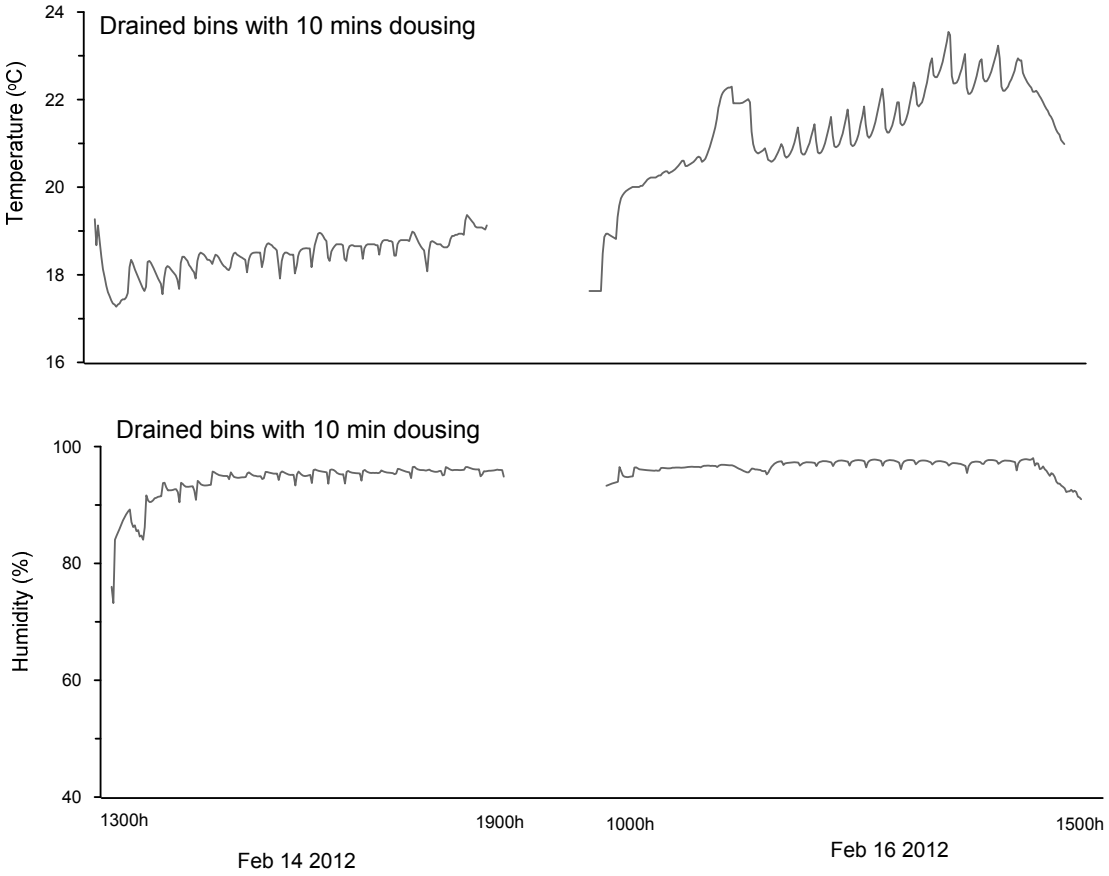


Figure 4.3.2. Temperature and relative humidity in drained bins doused every 10 min over the 5 h boat transit phase (14th Feb – 16th of Feb). Sampling frequency for both temperature and humidity was once per minute.

Haemolymph pH

Haemolymph pH was highly variable (mean = 7.05; s^2 = 0.099, n=328) and 30% of this variability could be explained as a function of frequency of dousing, length of time the abalone are on deck before stacking, and time at the jetty ($F=42.056$, df 3, 282, $P<0.001$). Haemolymph pH dropped quickly after the abalone were landed on deck and showed substantial variability across sampling intervals (Figure 4.3.3).

Haemolymph pH was significantly lower (more acidic) in abalone held in drained bins that were doused periodically with fresh seawater than the abalone held in wet-well bins ($F=55.41$, df 6, 271, $p<0.001$), however there was no evidence that the frequency that abalone were doused with fresh seawater affected haemolymph pH (Figure 4.3.4).

Haemolymph pH also decreased as a function of increasing time that the abalone spent at the jetty (i.e. waiting for processor truck) ($b=-0.001$, SE 0.001, $p<0.001$). Importantly, the rate haemolymph pH changed was similar for both abalone held in wet-well conditions during the boat transit then drained at the jetty and those abalone doused during boat transit (Figure 4.3.5). The relationship between haemolymph pH and time on the deck of the boat prior to stacking in bins was significant, although weak ($t=3.993$, df 282, $p<0.001$).

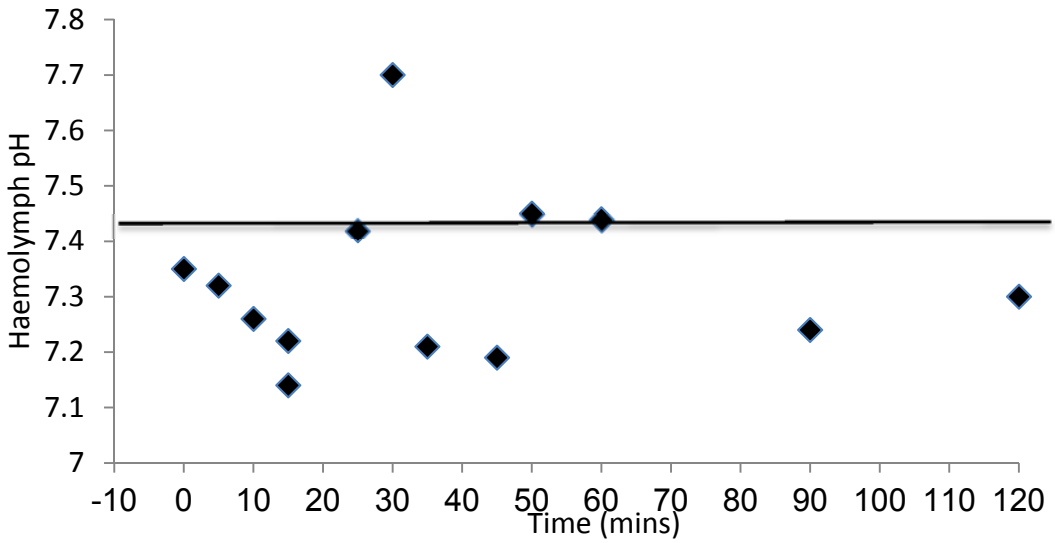


Figure 4.3.3. Abalone haemolymph pH of individuals held for 0 -120 mins on the deck of the boat prior to being stacked in bins ready for boat transit. The solid horizontal line is the mean haemolymph pH of abalone sampled on the reef.

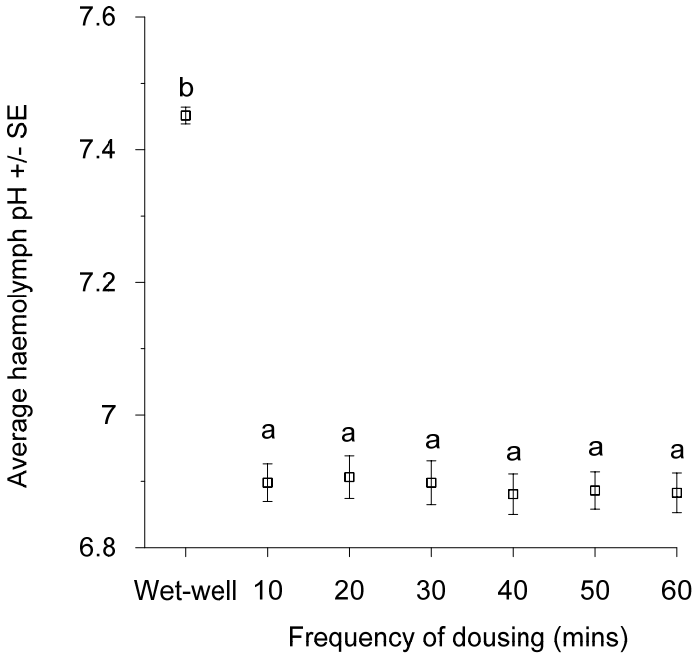


Figure 4.3.4. Mean haemolymph pH of abalone at the end of a simulated 5 h boat transit phase. Abalone in the douse treatments were stacked in free draining bins and doused at different frequencies during the boat transit phase. Abalone in the wet-well were continuously immersed in seawater. Means with different letters are significantly different from one another. n=40 for each mean, abalone were pooled across "time on deck" and "time at jetty".

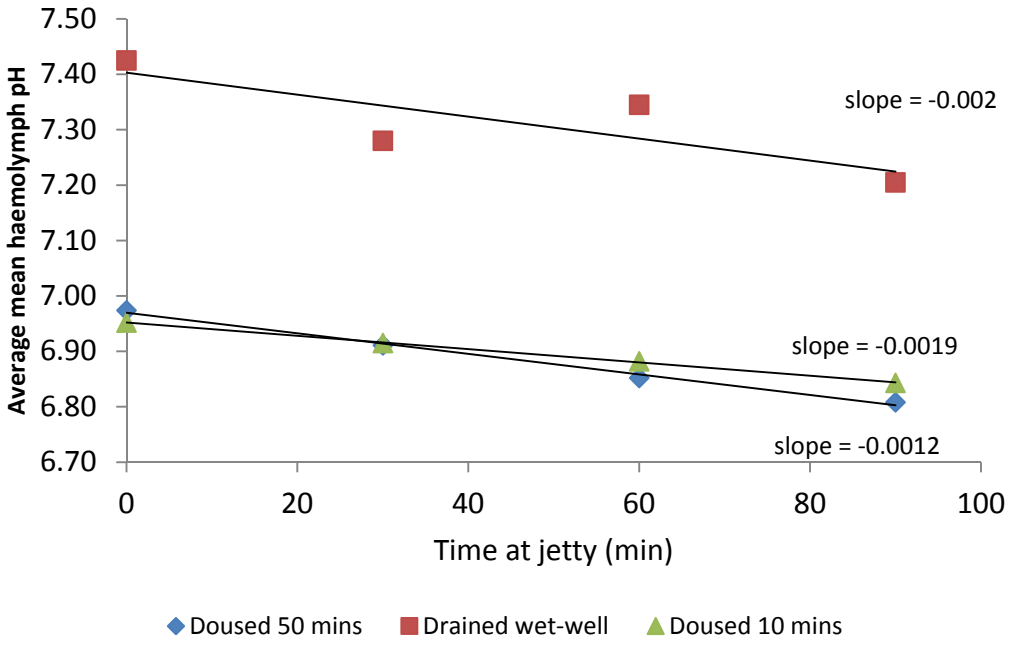


Figure 4.3.5. Change in haemolymph pH in abalone with time at the jetty for the animals held in wet-well conditions and drained (drained wet-well), the animals held in drained bins and doused either every 10mins or 50 mins during the boat transit phase.

Haemocyte Phagocytosis

Haemocyte phagocytosis (tube incubation) ranged from 11-76% across all abalone sampled (n=42). The average haemocyte phagocytosis across all abalone sampled was 31.18% (s^2 = 234.51), with none of the variation explained by the treatments, including time at the jetty. Haemocyte phagocytosis differed between the abalone held in wet-well and drained bins at the end of the 5 h boat transit phase (F=8.10, df 2, 39, p=0.001). Abalone held in drained bins with dousing had levels of haemocyte phagocytosis approximately 1.5 times less that of the reef abalone (Figure 4.3.6), while abalone in the wet-well treatment had levels of haemocyte phagocytosis intermediate, but no different, to both abalone held in drained bins or from the reef (Figure 4.3.6).

Time on deck had a dramatic effect on haemocyte phagocytosis which dropped linearly after 20 mins (F=14.997, df 1,6, p=0.008) at a rate of 0.3% phagocytosis for each minute on the deck of the boat (Figure 4.3.7).

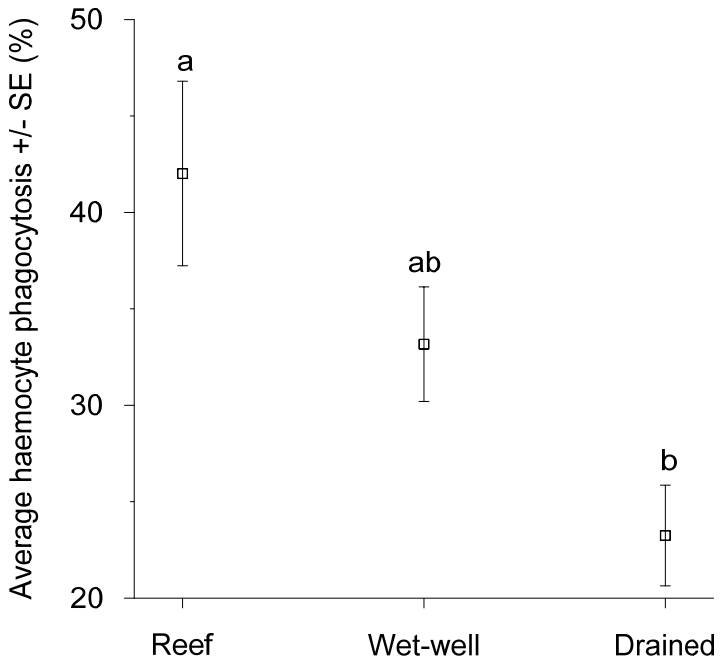


Figure 4.3.6. Mean haemocyte phagocytosis of abalone sampled in the reef and those sampled at the end of a 5 h boat transit phase where abalone were stacked and either held in wet-well bins or drained bins with dousing. Means with different letters are significantly different from one another and n=9-20

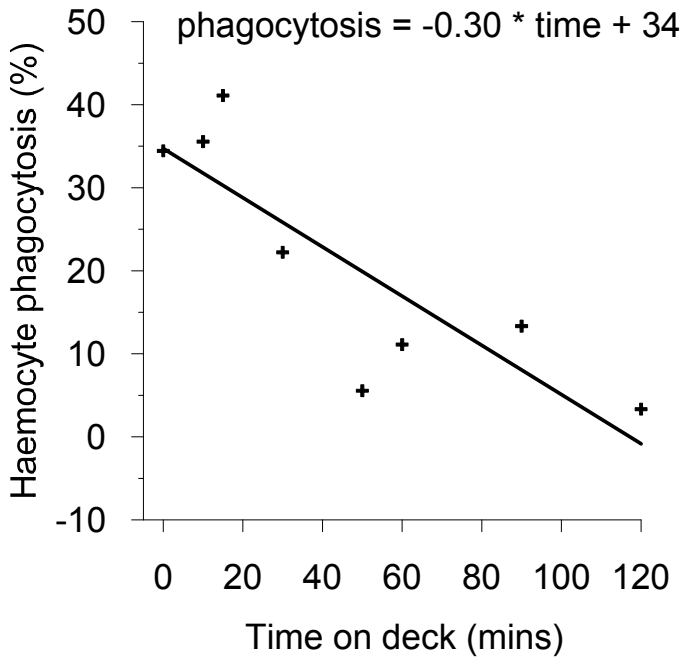


Figure 4.3.7. The rate of decline in percent phagocytosis with time on the deck of the boat before being stacked into bins for the boat transit phase.

Haemolymph Lactate

Baseline haemolymph D-lactate concentration was undetectable for all unstressed abalone on the reef, and close to undetectable for animals held in wet-well like conditions. In contrast, animals held in drained bins had haemolymph D-lactate concentrations of approximately 120 μg/ml, and as high as 515.9 μg/ml (Figure 4.3.8). Thirty-eight percent of the variability in haemolymph D-lactate concentration was explained by the dousing frequency ($F=63.33$, df 1, 100, $P<0.001$). Haemolymph D-Lactate concentration continued to increase during the “time at the jetty” for abalone in the douse bins, but abalone in wet-well bins and drained at the start of the “time at jetty” experienced a very small increase in haemolymph D-lactate (Figure 4.3.9).

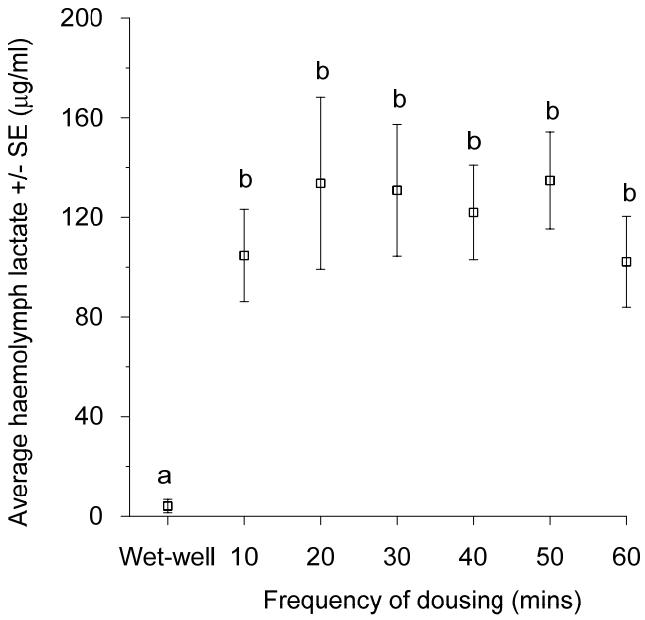


Figure 4.3.8. Mean haemolymph D-lactate concentration of abalone at the end of a 5 h boat transit phase. Abalone in the douse treatments were stacked in drained bins and doused with a bucket of fresh seawater at different frequencies during the boat transit phase. Abalone in wet-well bins were continuously immersed in seawater. Means with different letters are significantly different from one another. All the abalone sampled on the reef had no detectable haemolymph D-lactate. n=40 for each mean, abalone were pooled across “time on deck” and “time at jetty”.

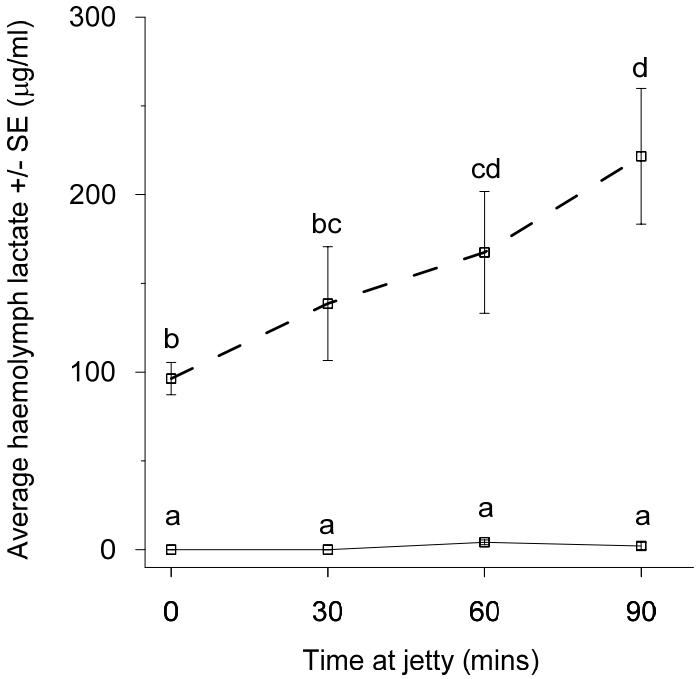


Figure 4.3.9. Changes in haemolymph D-lactate concentration of abalone held in douse bins during the boat transit phase (dashed line) or in wet-well conditions (solid line) and drained at the start of the “time at jetty” phase

Haemolymph Glucose

Haemolymph glucose concentration ranged from 0.12-957.57 μg/ml across all the abalone sampled (n=84). The average haemolymph glucose concentration differed significantly between the abalone held in wet-well and drained bins at the end of the 5 h boat transit phase ($F=9.98$, df 2, 69, $p<0.001$). While abalone held in bins with dousing had haemolymph glucose concentrations approximately 4-5 times greater than the reef abalone, abalone in the wet-well treatment had very similar glucose concentration to the reef abalone (Figure 4.3.10).

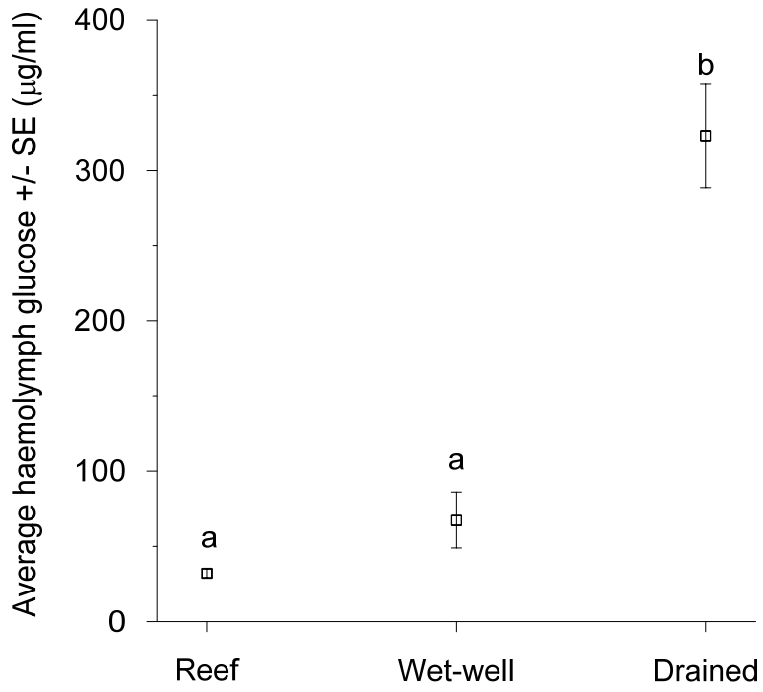


Figure 4.3.10. Average haemolymph glucose concentration of abalone sampled in the reef and those sampled at the end of a 5 h boat transit phase where abalone were stacked and either held in wet-well bins or drained bins with dousing. Means with different letters are significantly different from one another and n=9-48.

Haemolymph Osmolality

Haemolymph osmolality ranged from 992-1081 mmol/kg, with no evidence that this range of values was a function of the time that abalone spent on the deck of the boat prior to stacking (Figure 4.3.11).

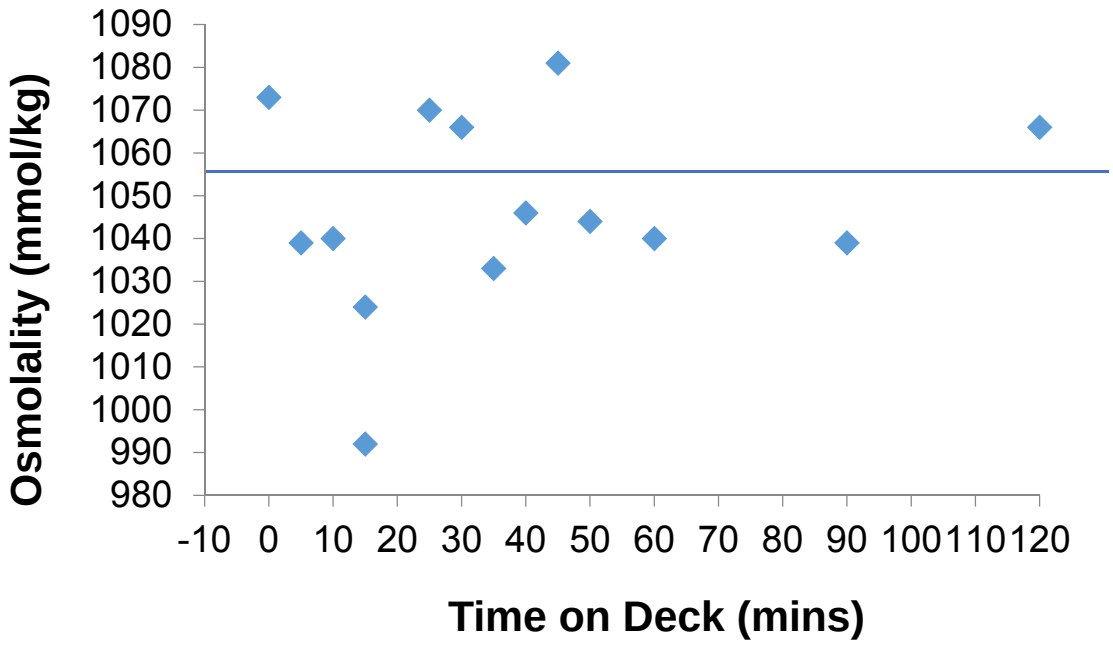


Figure 4.3.11. Abalone haemolymph osmolality of individuals held for 0-120 mins on the deck of the boat prior to being stacked in bins ready for boat transit. The solid horizontal line is the mean haemolymph osmolality of abalone sampled on the reef.

4.4. Tasmania Winter 2013 Experiment

Environmental Parameters

The temperatures in the wet-well bins over the three days ranged from approximately 11 to 12.4^{o}C. Replicate wet-well bins showed very similar patterns in temperature during each of the three days, although one of the wet-well bins on Aug 13 appeared to cool late in the day, dropping a degree over a 60 min period (Figure 4.4.1). The average SST for south-East Tasmanian in August is 12.6^{o}C, and temperatures were close to average during the experiment. The SSTs were consistent with an upward spike in water temperature in the experimental bins associated with periodic water exchange, as temperatures in the bins were cooler than ambient SST.

The abalone in the drained bins that were periodically doused with fresh seawater experienced cooler and more variable temperatures over the three days compared with the wet well bins. Maximum air temperatures at the closest Bureau of Meteorology weather station (Dover) for these three days were very similar (13, 13.2, 13.8^{o}C for Aug 13, 14, & 15th respectively). There was also evidence of more than one degree difference between the replicate bins, which is most probably associated with the position of the bin on the deck of the boat (Figure 4.4.1).

In this experiment, the temperature changes associated with the dousing treatment (once an hour) are clearly visible with temperatures in the bins increasing by 1-2^{o}C (Figure 4.4.1). In addition to a substantial rise in temperature, the dousing action exposed the abalone to substantial temperature shift within a short time frame (Figure 4.4.2). Within a few minutes after the dousing, temperatures returned to ambient temperature, but at a comparatively slower rate, and 30 minutes after dousing, temperature change was negligible (Figure 4.4.2). Temperature also changed in the wet well treatments when water exchange occurred, but at a slower rate and smaller magnitude of change.

Humidity inside the drained bins that were periodically doused with fresh seawater bins averaged about 95% over the three days and while there were small increases and concomitant drops associated with the dousing these changes were relatively small (Figure 4.4.3). There was an increase in humidity seen over the first 60-90 minutes of the experiment indicating that there was a delay in generating a moist environment in the bin (Figure 4.4.3). This is consistent with the pattern observed in the summer experiment.

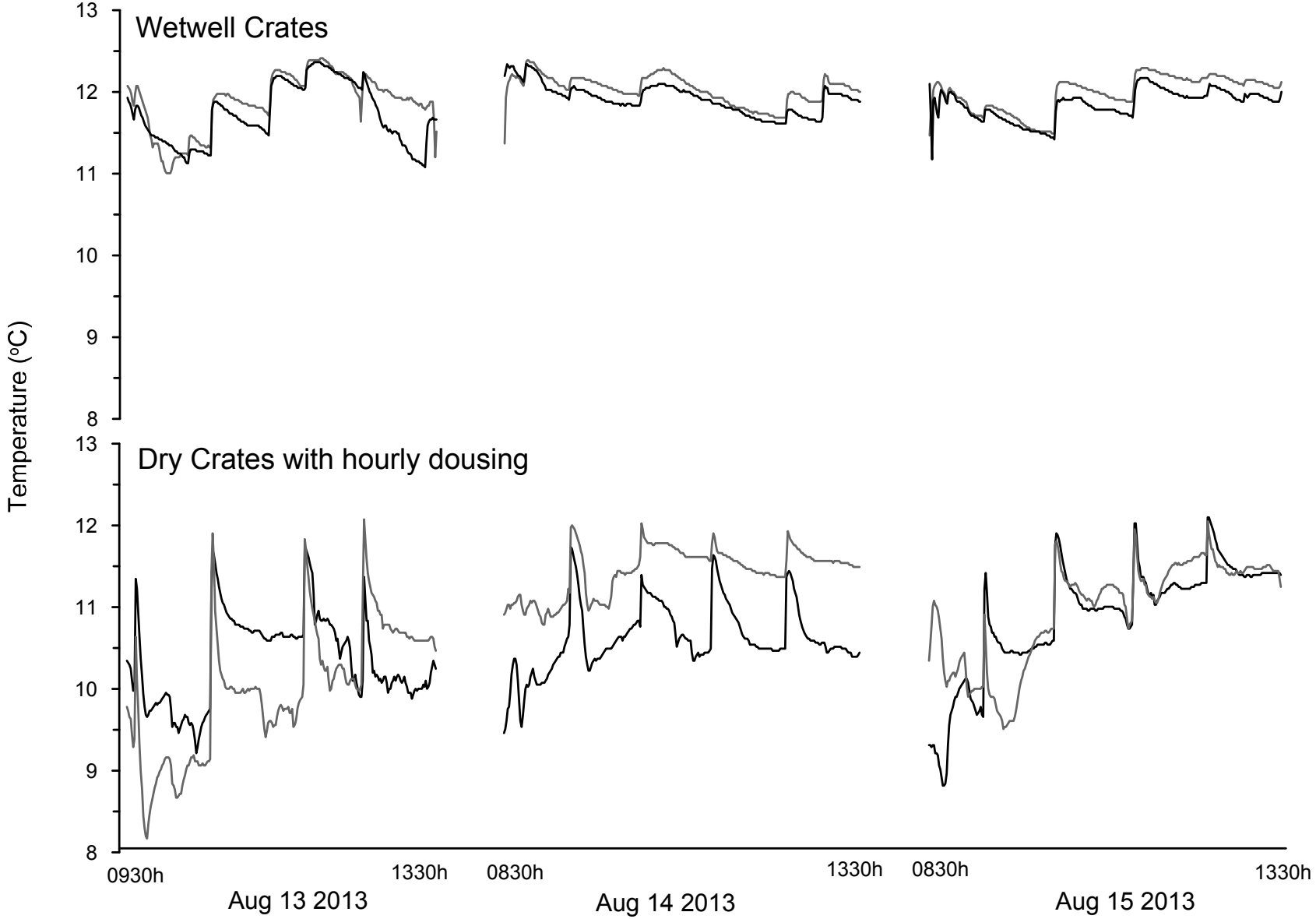


Figure 4.4.1. Temperature experienced by abalone in the wet-well bins and drained bins periodically doused with fresh seawater on each of three days the experiment was done. The black and grey lines refer to the two replicate bins in the experiment.

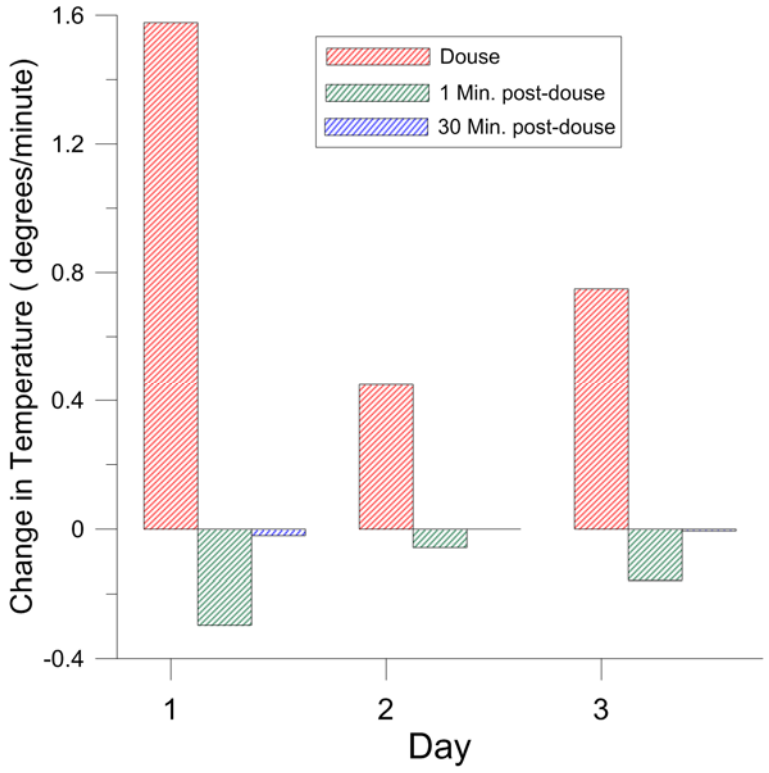


Figure 4.4.2. Rate of temperature change (°C/Minute) associated with dousing of drained bins. Red bars indicate rate of change at dousing, green bars indicate mean change one minute after dousing, and blue bars indicate mean change 30 minutes after dousing.

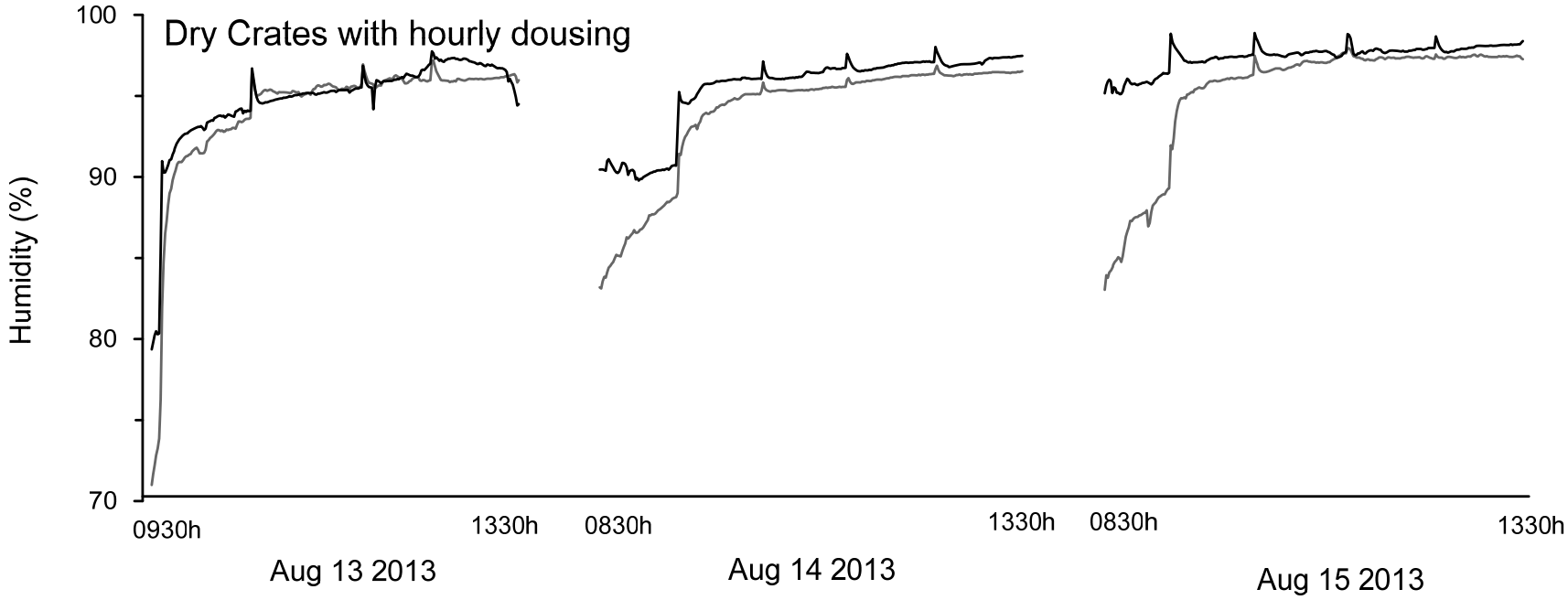


Figure 4.4.3. Humidity experienced by abalone in the drained bins with dousing on each of three days the experiment was done. The black and grey lines refer to the two replicate bins in the experiment

Haemolymph pH

Abalone haemolymph pH levels at the end of the boat transit phase were affected by the amount of time they were left on the deck prior to stacking, but this difference depended upon the transit methods (F = 2.91, df 9, 49, p = 0.008). By the end of the boat transit phase abalone in the wet-well and douse transit methods had a lower haemolymph pH than reef abalone regardless of how long they spent on deck before stacking (Figure 4.4.4). This response of lowered haemolymph pH was also seen for abalone who experienced periodic immersion and spent 0 or 15 mins on deck before stacking, but abalone left on deck for 30 mins prior to stacking had similar pH values as the reef abalone (Figure 4.4.4).

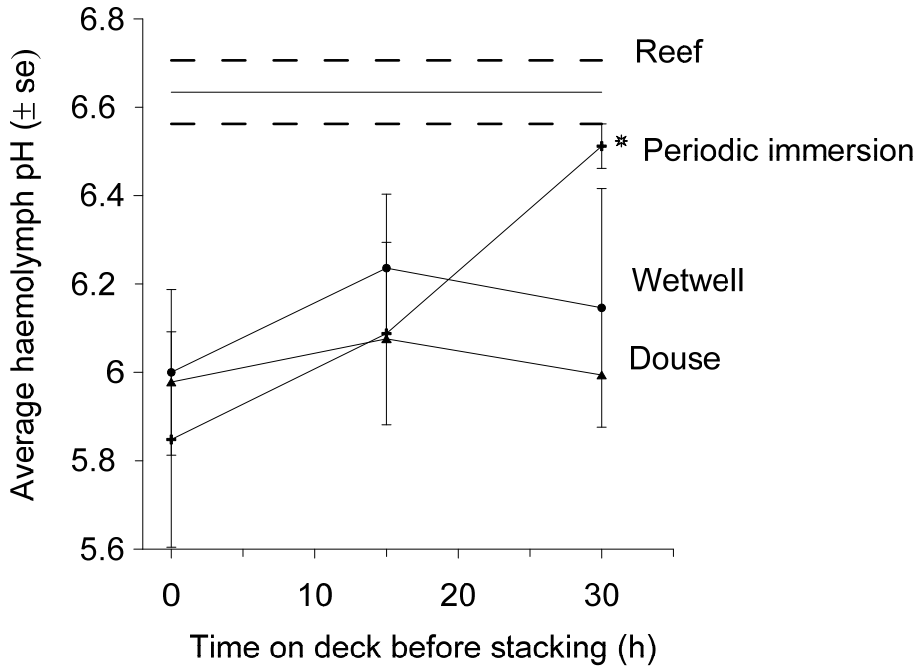


Figure 4.4.4. Mean haemolymph pH sampled from wild caught abalone sampled immediately after removal from the rock (reef) and at the end of a 5 h boat transit in each of the three boat transit treatments; periodic immersion bins were immersed in clean seawater for 15mins each hour, wet-well bins had continuous flowthrough of clean seawater, and douse bins were drained and a bucket of clean seawater poured through every 60 mins. . Starred means are not-significantly different from the mean for the reef abalone, n = 5-15.

Haemocyte Phagocytosis

Mean percent haemocyte phagocytosis (tube incubation) differed based on the time abalone spent on deck of the boat before being stacked into bins (F = 5.89, df 2,36, p = 0.006). While there was evidence that animals left on deck for 15 mins before stacking had the least haemocyte phagocyte activity while the animals left on deck for 0 and 30 mins had the greatest haemocyte phagocytosis activity; which was similar to the abalone on the reef (Figure 4.4.5).

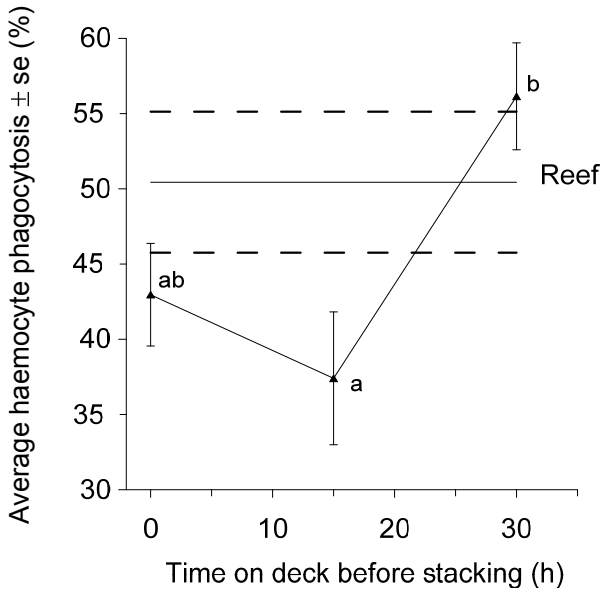


Figure 4.4.5. Average percent haemocyte phagocytosis for abalone that spent different times on deck before being stacked into bins. The reef animal average (solid horizontal line) and SE (dashed horizontal lines) are shown. Means with different letters are significantly different from one another. n=3-15.

Abalone in different boat transit treatments did not differ in their mean percent haemocyte phagocytosis ($F = 0.072$, df 2,36, $p = 0.930$). Abalone sampled on the reef had an average of haemocyte phagocytosis rate of 50.4% (sd 17.01), and all the treatments had haemocyte phagocytosis rates of a similar magnitude. However, there was substantial variability in percent haemocyte phagocytosis across all treatments, with a range of 15-69% and an average of 44.38% (sd 17.08).

Haemolymph Lactate

Haemolymph D-lactate concentrations were very small for all abalone in this experiment, with no evidence of significantly elevated haemolymph D-lactate concentrations as a function of any of the treatments (all $p \geq 0.05$). Abalone on the reef had no detectable haemolymph D-lactate and on average abalone across all treatments post-harvest had an average haemolymph D-lactate concentration of 1.46 μg/μL (standard error 2.58).

4.5. Tasmania Processor Recovery Experiment - Spring 2013

Environmental Parameters

The abalone in replicate wet well bins experienced very stable temperatures during the boat transit phase, averaging approximately 16.5^{o}C (Figure 4.5.1). However, during the road transit there were dramatic differences in the temperatures in the two bins (which were now drained). One bin of abalone reaching a peak of 28^{o}C shortly before the abalone were transferred to the processor tanks, while the second bin barely attained 17^{o}C (Figure 4.5.1).

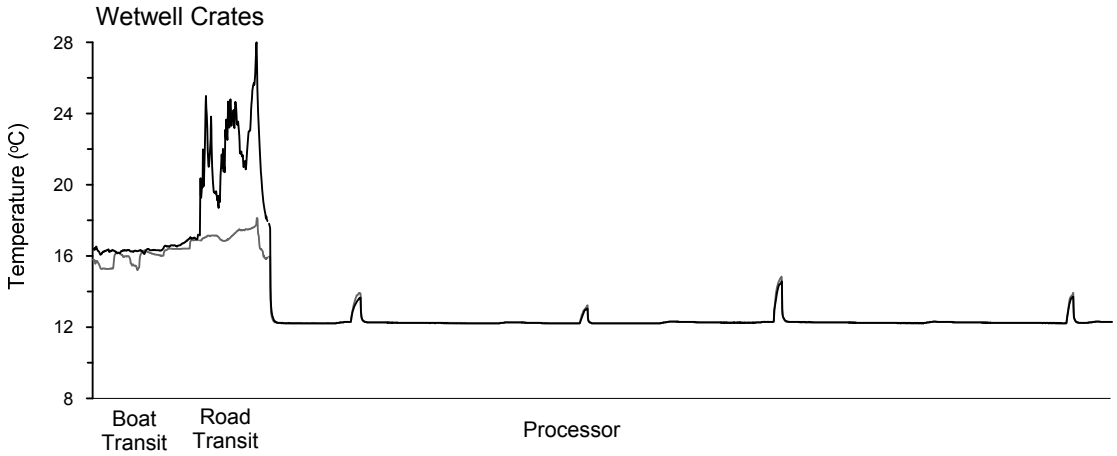


Figure 4.5.1. Temperature in the wet well bins during the boat transit when animals were fully submerged in seawater; followed by a road transit phase where abalone were drained and covered with wet hessian sacks. Following immersion of abalone in crates at the processors, crates were periodically removed from the water to allow sampling. The black and grey lines refer to the two replicate bins in the experiment.

Abalone in bins periodically doused with seawater experienced a gradual increase in ambient air temperature from approximately 14.5-16^{o}C during the 5 h boat transit phase (Figure 4.5.2). During road transit the ambient air temperature also increased, reaching almost 18^{o}C in one bin, but barely 16^{o}C in the other (Figure 4.5.2). The humidity in the bins took approximately 40 mins to reach over 90% during the boat transit phase, but this humidity was maintained and increased slightly during both the boat and road transit phases (Figure 4.5.2).

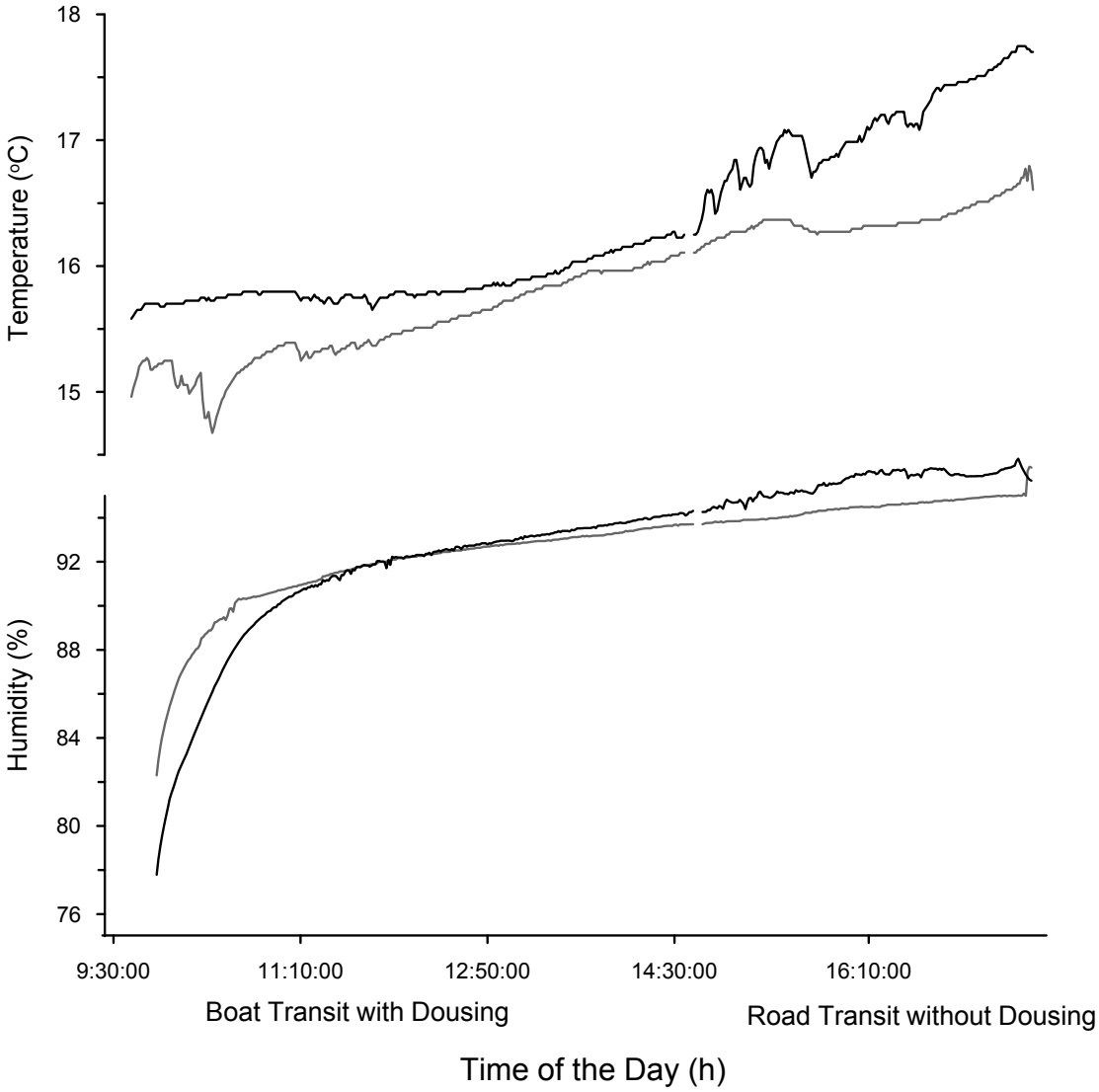


Figure 4.5.2. Temperature and humidity in drained bins with animals stacked, covered with wet hessian sack, and doused with seawater every 60 mins during the boat transit and not doused during the road transit phase. The black and grey lines refer to the two replicate bins in the experiment.

Haemolymph pH

Mean haemolymph pH changed through time, with the pattern of change determined by the time spent on the deck of the boat and was consistent for all boat transit treatments ($F = 3.79$, df 7, 64, $p = 0.002$). There was little evidence that haemolymph pH was affected by the boat transit conditions with pH not significantly different to the pH of reef abalone (Figure 4.5.3). By the end of the road transit phase, haemolymph pH was less than observed for the reef abalone for all abalone (Figure 4.5.3). However, abalone that spent < 15 min on deck before stacking had haemolymph pH levels similar to reef abalone within 4 h of transfer to the processor tanks, while the abalone who spent longer on deck took longer than 4h in the processor tanks for their pH levels to return to those seen for the reef abalone (Figure 4.5.3).

Abalone in the different boat transit treatments experienced different patterns of change in haemolymph pH during the boat and road transit and recovery in the processor tanks ($F=2.40$, df 21,73, $p=0.003$). The abalone in wet-well boat transit conditions did not differ in their haemolymph pH throughout the experiment (Figure 4.5.4). In contrast, abalone in the other boat transit treatments did see a change through time; while at the end of the boat transit phase haemolymph pH did not appear to decrease, there was a significant decline in haemolymph pH by the end of the road transit phase (Figure 4.5.4). Once abalone were transferred to the processor tanks, there was evidence of haemolymph pH increasing to become similar to the haemolymph pH levels seen in the reef animals, but there were differences in the rates of increase. The abalone stacked in bins where they were attached to a smooth surface and doused with fresh seawater very 60 mins increased their haemolymph pH within 4 h of transfer to the processor tanks (Figure 4.5.4). Abalone in both the periodic immersion and doused/unattached treatment also improved their haemolymph pH within 4 h of transfer to the processor tanks, although this was achieved more slowly (Figure 4.5.4).

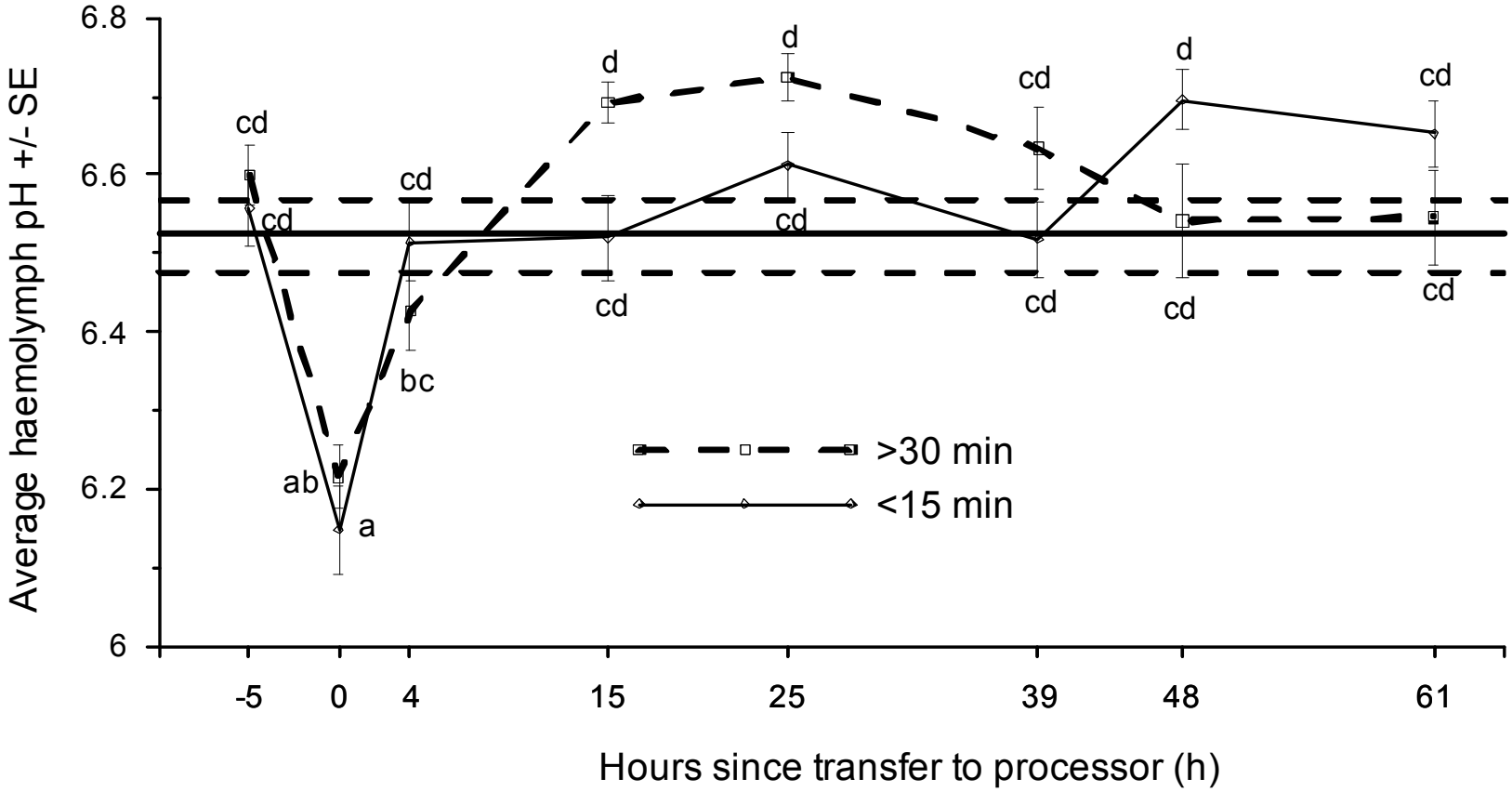


Figure 4.5.3. Mean haemolymph pH (solid horizontal line) ± standard error (dashed horizontal line) for abalone on the reef and at the end of the boat (-5 h) and road transit (0h) periods and 4, 15, 25, 39, 48, and 61 h post transfer to the processor tanks for individuals that spent <15 mins or >30 mins on the deck of the boat before being stacked in bins. Means are pooled for boat transit treatments and means with different letters are significantly different from one another.

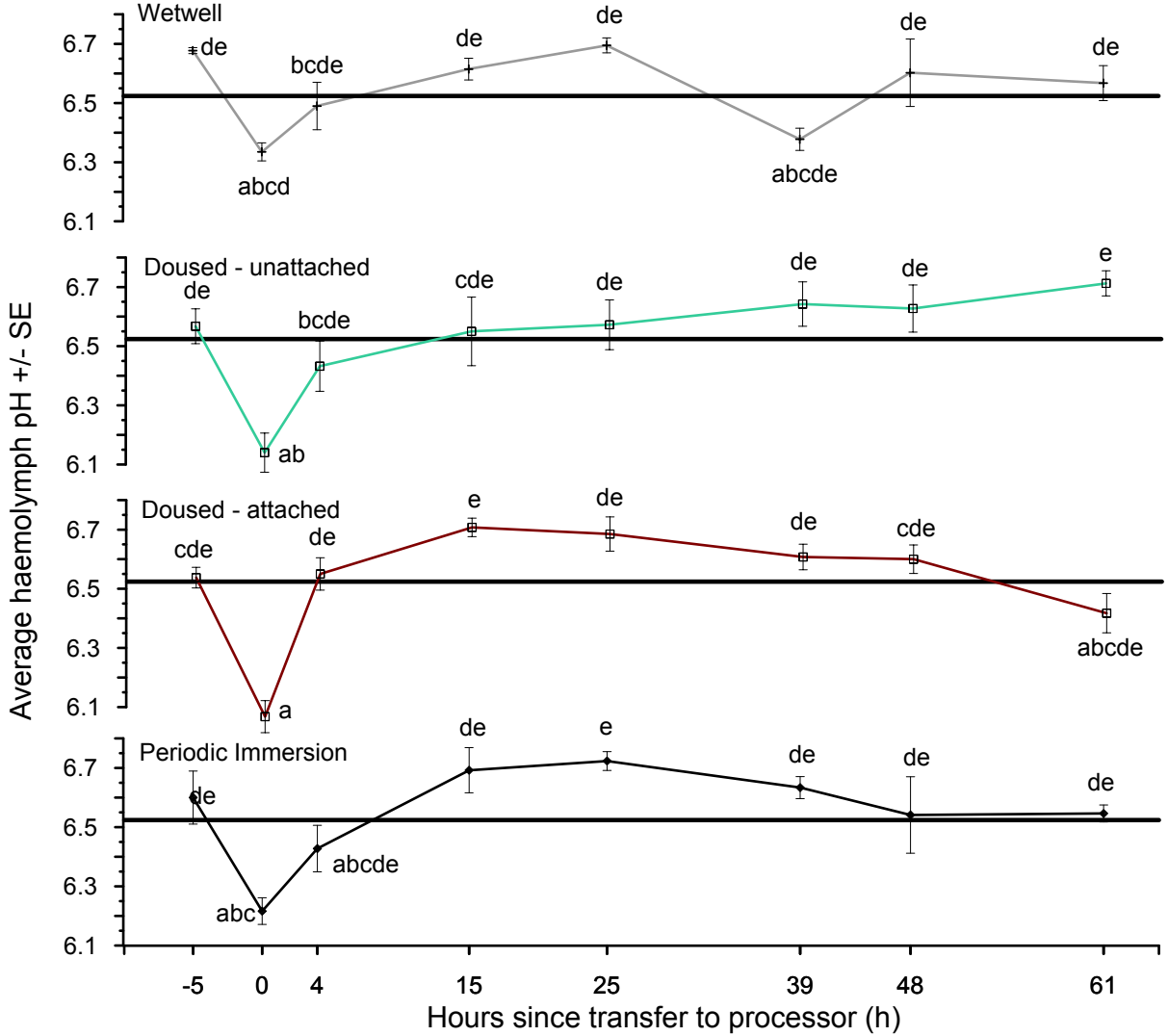


Figure 4.5.4. Mean haemolymph pH for abalone on the reef (solid horizontal line) and at the end of boat (-5 h) and road transit (0 h) periods and time post-transfer to processor tanks for different boat transit conditions. Means with different letters are significantly different from one another.

Haemocyte Phagocytosis

Mean percent haemocyte phagocytosis differed through time, and the pattern of change through time was consistent for all boat transit treatments and time on deck treatments ($F = 2.42$, df 8, 129, $p = 0.02$). At the end of the 5 h boat transit phase, there was no evidence that the levels of haemocyte phagocytosis had changed compared with the reef abalone (Figure 4.5.5). However, mean percent haemocyte phagocytosis showed an average decrease of 20% at the end of the road transit phase (Figure 4.5.5). Subsequently, mean percent haemocyte phagocytosis increased within 4h of the abalone being transferred to the processor tanks, and while not returning to base line levels, the level of haemocyte phagocytosis observed in the tank recovery phase were not significantly different from abalone sampled on the reef (Figure 4.5.5).

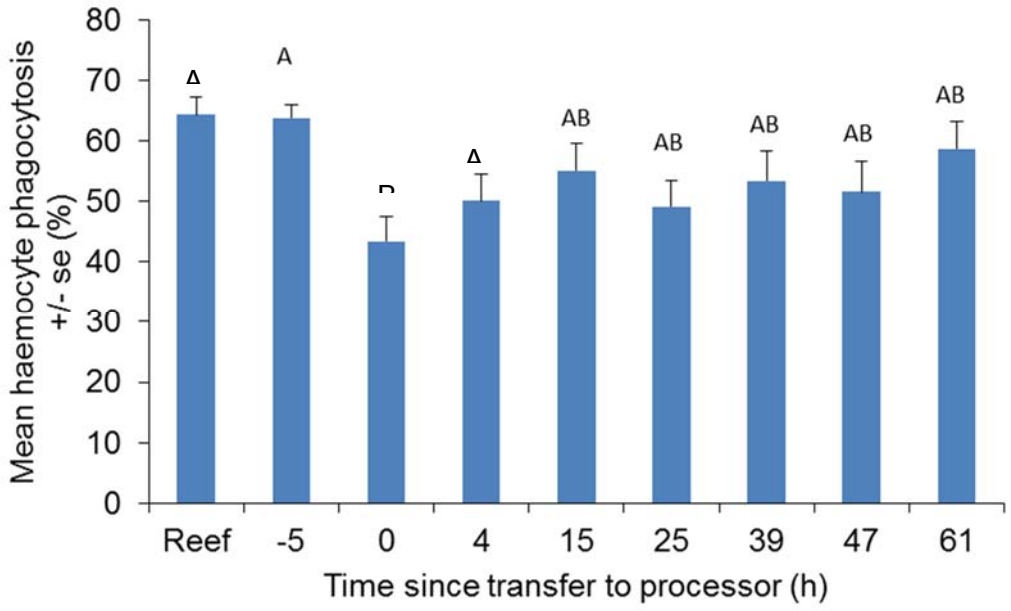


Figure 4.5.5. Mean percent haemocyte phagocytosis for abalone on the reef and at the end of the boat (-5 h) and road transit (0 h) periods and 4, 15, 25, 39, 48, and 61 h post transfer to the processor tanks. Means are pooled across the boat transit treatments and means with different letters are significantly different from one another.

Haemolymph D-Lactate

Mean haemolymph D-lactate concentration changed through time, but the nature of the change depended upon the boat transit treatment ($F=1.908$, df 19, 53, $p=0.034$). Abalone in wet-well boat transit conditions did not differ in their haemolymph D-lactate concentrations throughout the experiment and were no different from the reef abalone haemolymph D-lactate concentrations (Figure 4.5.6). In contrast, abalone in the other boat transit treatments showed significant changes in D-lactate through time. At the end of the 5 h boat transit phase there was no increase in haemolymph D-lactate concentrations and they were not significantly different from haemolymph lactate concentrations in abalone on the reef. However, by the end of the road transit phase abalone in treatments other than wet-well had elevated haemolymph D-lactate concentrations (Figure 4.5.6).

Once abalone were transferred to the processor tanks, there was evidence of haemolymph D-lactate concentrations decreasing to concentrations measured in the reef animals, but there were differences in the rates at which the concentration of D-lactate in the haemolymph decreased. Abalone in the doused/attached bin and periodic immersion bin decreased their haemolymph D-lactate concentrations within 4h of transfer to the processor tanks (Figure 4.5.6), while haemolymph D-lactate concentrations of abalone from the doused/unattached boat transit treatments decreased more slowly, within 15h of being transferred into the processor tanks (Figure 4.5.6).

Haemolymph Glucose

Haemolymph glucose concentration across all boat transit treatments changed during the fisher supply chain, including the wet-well abalone ($F=14.41$, df 3,33, $p<0.001$). By the end of the 5 h boat transit phase the haemolymph glucose concentration had increased to levels four times greater than measured in the reef abalone (Figure 4.5.7). During the 3h road transit phase, haemolymph glucose concentration of the abalone increased by a further 15 μg/ml (Figure 4.5.7). However, once transferred to the processor tanks, haemolymph glucose concentration decreased and within 39 h of transfer was comparable to the reef abalone (Figure 4.5.7).

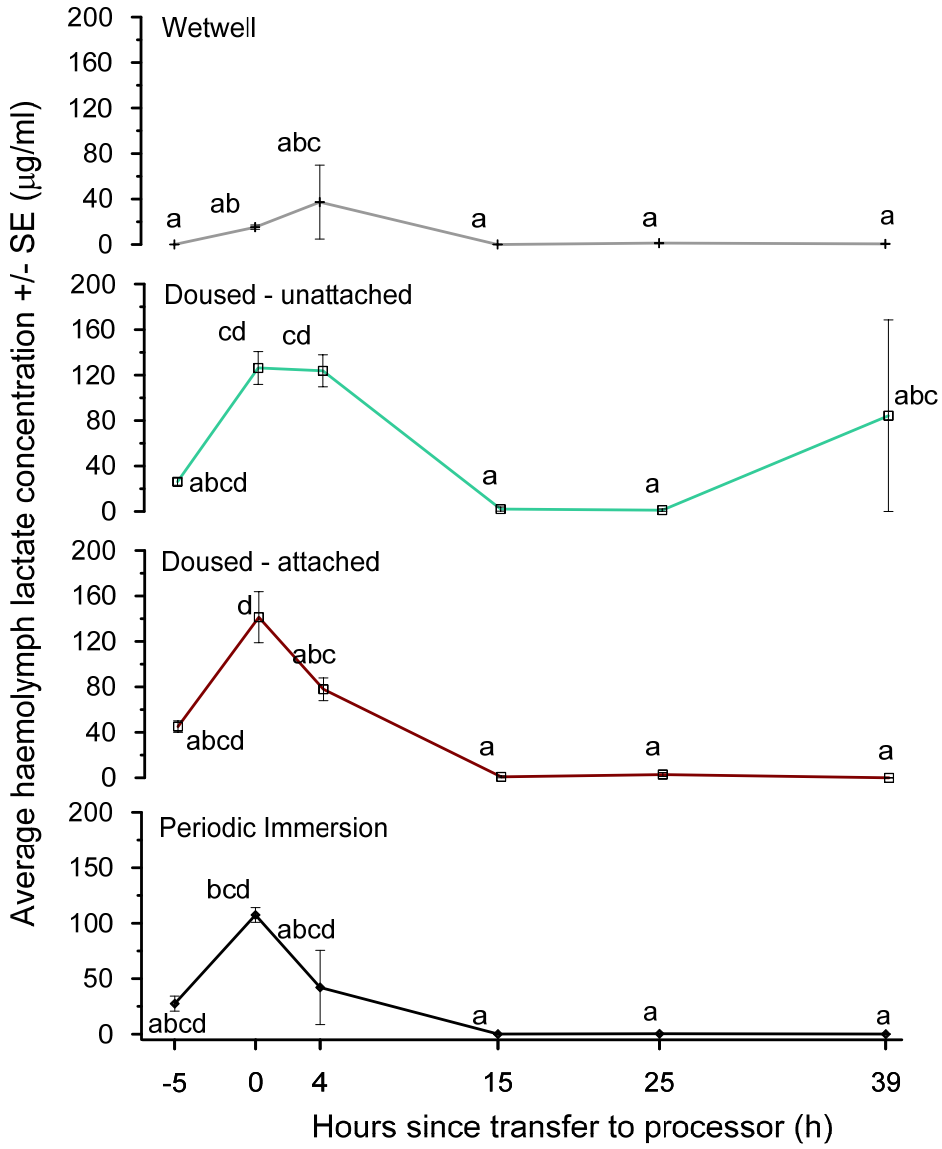


Figure 4.5.6. Mean haemolymph D-lactate for abalone on the reef and at the end of boat (-5 h) and road transit (0 h) periods and number of hours post transfer to processor tanks for different boat transit conditions. The D-lactate concentration for all reef abalone was undetectable. Means with different letters are significantly different from one another.

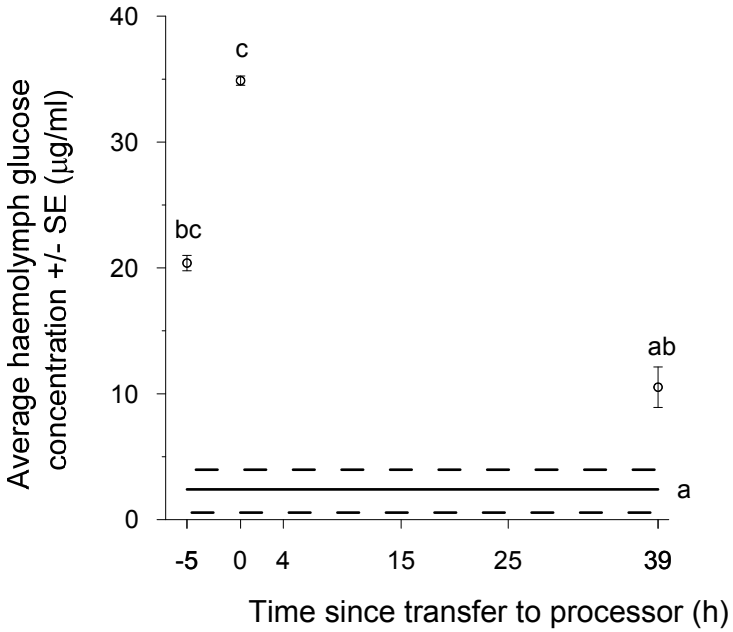


Figure 4.5.7. Mean haemolymph glucose for abalone on the reef (solid line mean ± SE) and at the end of boat (-5 h) and road transit (0 h) periods and 39 h post transfer to processor tanks, with boat transit treatments pooled. Means with different letters are significantly different from one another; n=10-16.

4.6. Tasmania Motherboat & Processor Recovery Experiment

Haemolymph pH

Mean haemolymph pH declined after the abalone were transferred to the processor tanks ($F = 27.95$, df 2, 24, $p<0.0001$). The decline occurred within 24 h of transfer and even after 21h transfer there was no evidence of change in haemolymph pH (Figure 4.6.1). This pattern of change post transfer to the tanks was consistent for abalone harvested either 24 or 48 h prior to landing at the jetty ($F=1.512$, df 2,24, $p=0.241$), and abalone collected 24 h or 48 h before landing did not differ in their haemolymph pH ($F=1.120$, df 1, 24, $p=0.300$).

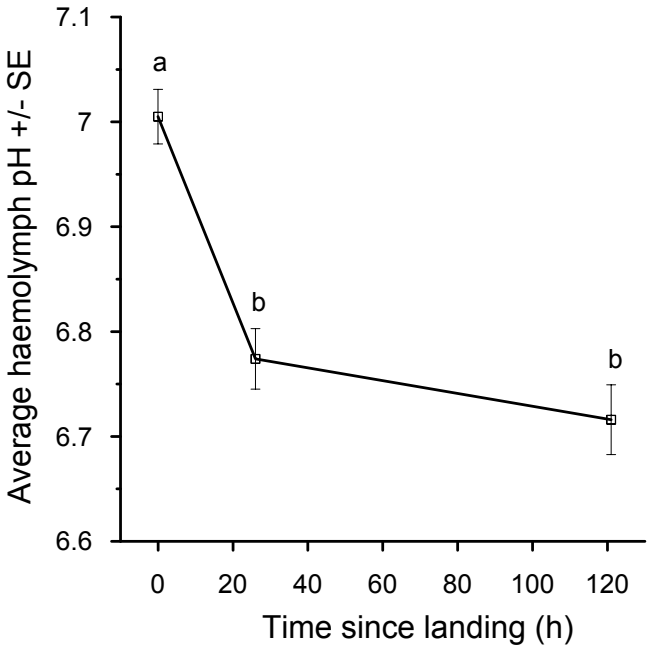


Figure 4.6.1. Changes in mean haemolymph pH of abalone after landing at the jetty. Abalones were transferred to the processor via a truck and from the truck into the tanks within 1-2 h of being landed at the jetty (0 h). Means with different letters are significantly different from one another. Means for the two groups of abalone harvested on two different days are pooled.

Haemocyte Phagocytosis

Mean haemocyte phagocytosis differed among post-arrival times at the dock ($F = 88.63$, df 2, 23, $p<0.0001$). This pattern of change was consistent for abalone harvested 24 h or 48 h before landing ($F=0.697$, df 2,23, $p=0.508$), and abalone harvested 24 h or 48 h before arrival at jetty did not differ in their haemocyte phagocytosis ($F=2.167$, df 1, 23, $p=0.155$). Haemocyte phagocytosis of abalone from both the 24 and 48 h harvest times on transfer from mother-boat to the processor truck (0 hours) were the lowest, with increases in the percentage of haemocyte phagocytosis occurring over the 121 h in the processor tanks (Figure 4.6.2).

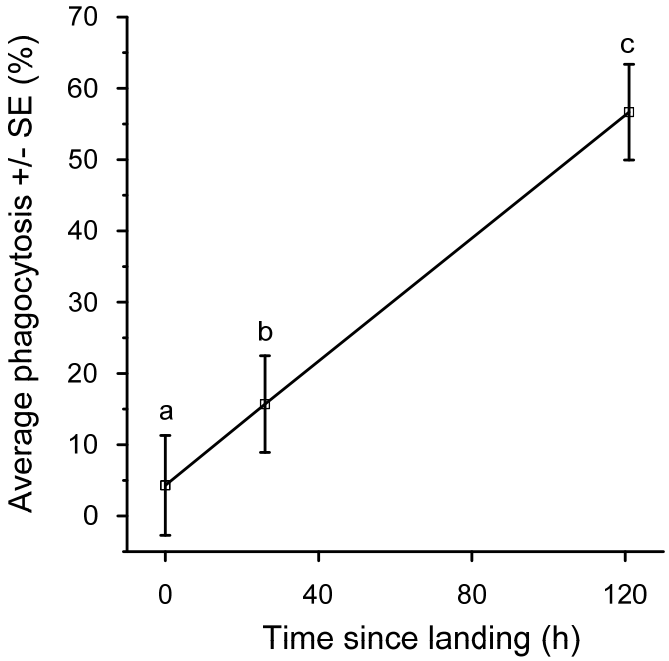


Figure 4.6.2. Changes in percent mean haemocyte phagocytosis of abalone after landing at the jetty. Abalone were transferred to the processor via a truck and from the truck into the tanks within 1-2 h of being landed at the jetty (0 h). Means with different letters are significantly different from one another. Means for the two groups of abalone harvest on two different days are pooled.

Haemolymph Lactate

The nature of the changes in mean haemolymph D-lactate concentration through time after arrival at the jetty depended on the day that the abalone were harvested ($F = 4.301$, df 2, 27, $p=0.024$). Animals who had been harvested 24 h before arrival at the jetty did not have elevated haemolymph D-lactate concentrations compared with animals who had been harvested 48 h before landing at the jetty (Figure 4.6.3). However, the 24 h abalone experienced an increase in haemolymph D-lactate concentration within 0.5 h of arrival at the dock, resulting in means comparable to the 48 h abalone (Figure 4.6.3). While haemolymph D-lactate concentration of the 48 h abalone decline to undetectable levels within 26 h of being transferred to the processor tanks, haemolymph D-lactate in the 24 h abalone took 26-121h before their concentrations reached undetectable levels (Figure 4.6.3).

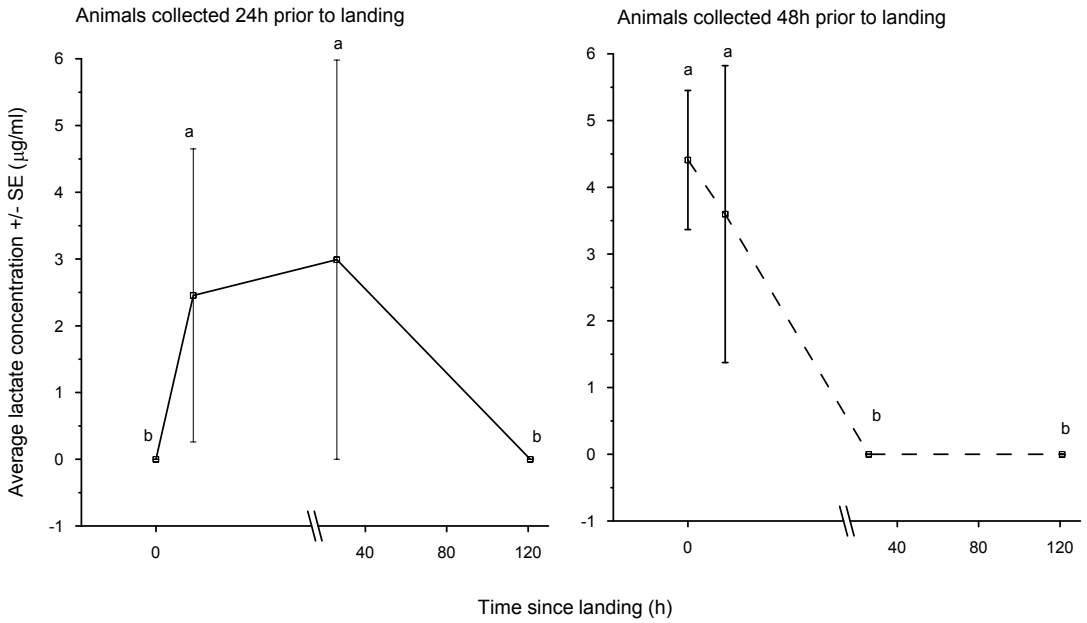


Figure 4.6.3. Changes in mean haemolymph D-lactate concentration in abalone post-arrival landing at the dock for abalone harvested 24 and 48 h prior to landing. Note, abalone were transferred to processor tanks within 1 h of landing at the dock (0 h). Means with different letters are significantly different from one another.

Haemocyte Density

Mean haemocyte density in all the abalone sampled in this experiment was 1.261×10^6 cells/ml (sd = 0.800×10^6). There was no evidence that the variation in mean haemocyte density among abalone was a function of either time since harvesting ($F=0.152$, df 1, 32, $p=0.699$) or time post-arrival at the jetty ($F = 0.364$, df 3, 32, $p = 0.78$).

4.7. NSW Spring 2014 Experiment

Environmental Parameters

Day time maximum air temperatures varied little over the duration of the experiment (Bureau of Metrology weather station Green Cape), and were not noticeably warmer (24.2 - 25^{o}C) than the Tasmanian Summer experiment. The mean SST at Eden in March is 21.1^{o}C (http://www.seatemperature.org/australia-pacific/australia/eden-march.htm), and was slightly warmer during the experiment.

The temperatures in the bins gradually increased during the day; the drained bins that were periodically doused with a bucket of freshwater every 60 mins experienced some cooling with each dousing (Figure 4.7.1). This pattern is expected given the experimental bins were warmer than the ambient SST (~21^{o}C). A rapid decrease in bin temperature occurred with each dousing, followed by a period of cooling for several minutes before the temperature returned to a pattern of gradually increasing (Figure 4.7.2). Humidity quickly reached ~ 90% in the drained bins and was remained at this level over the course of each day and was not affected by the periodic dousing.

Haemolymph pH

Average abalone haemolymph pH differed among the three experimental phases; animals on the reef, at the end of boat transit and during recovery in the processor tanks. However, the magnitude of the difference depended upon the boat transit treatment ($F=15.53$, df 9, 44, $p<0.0001$). Abalone stored in wet-wells during boat transit had haemolymph pH levels at either the end of the boat transit phase or during the processor holding phase that were no different from the abalone sampled on the reef (Figure 4.7.3). Both vertically and horizontally stacked abalone held in the drained bins periodically doused with fresh seawater showed a similar decline in haemolymph pH at the end of the boat transit (Figure 4.7.3), but abalone in both treatments increased their haemolymph pH to levels measured in reef abalone within 24 h of transfer to the processor tanks (Figure 4.7.3).

Haemocyte Phagocytosis

By the end of the boat transit phase abalone in all of the boat transit holding treatments had haemocyte phagocytosis percentages that were 30% less than for the abalone sampled on the reef (Figure 4.7.4).

Abalone held in the wet-wells had increased levels of haemocyte phagocytosis to a level similar to the reef abalone by 24 h in the processor tanks (Figure 4.7.4). The abalone stacked vertically in drained bins periodically doused with fresh seawater also had an increased percentage of haemocyte phagocytosis after 24 h in the processor tanks, but this declined again after 48 h in the processor tanks (Figure 4.7.4). In contrast, abalone stacked horizontally in drained bins periodically doused with fresh seawater did not show an increase in percent haemocyte phagocytosis until 48 h in the processor tanks (Figure 4.7.4).

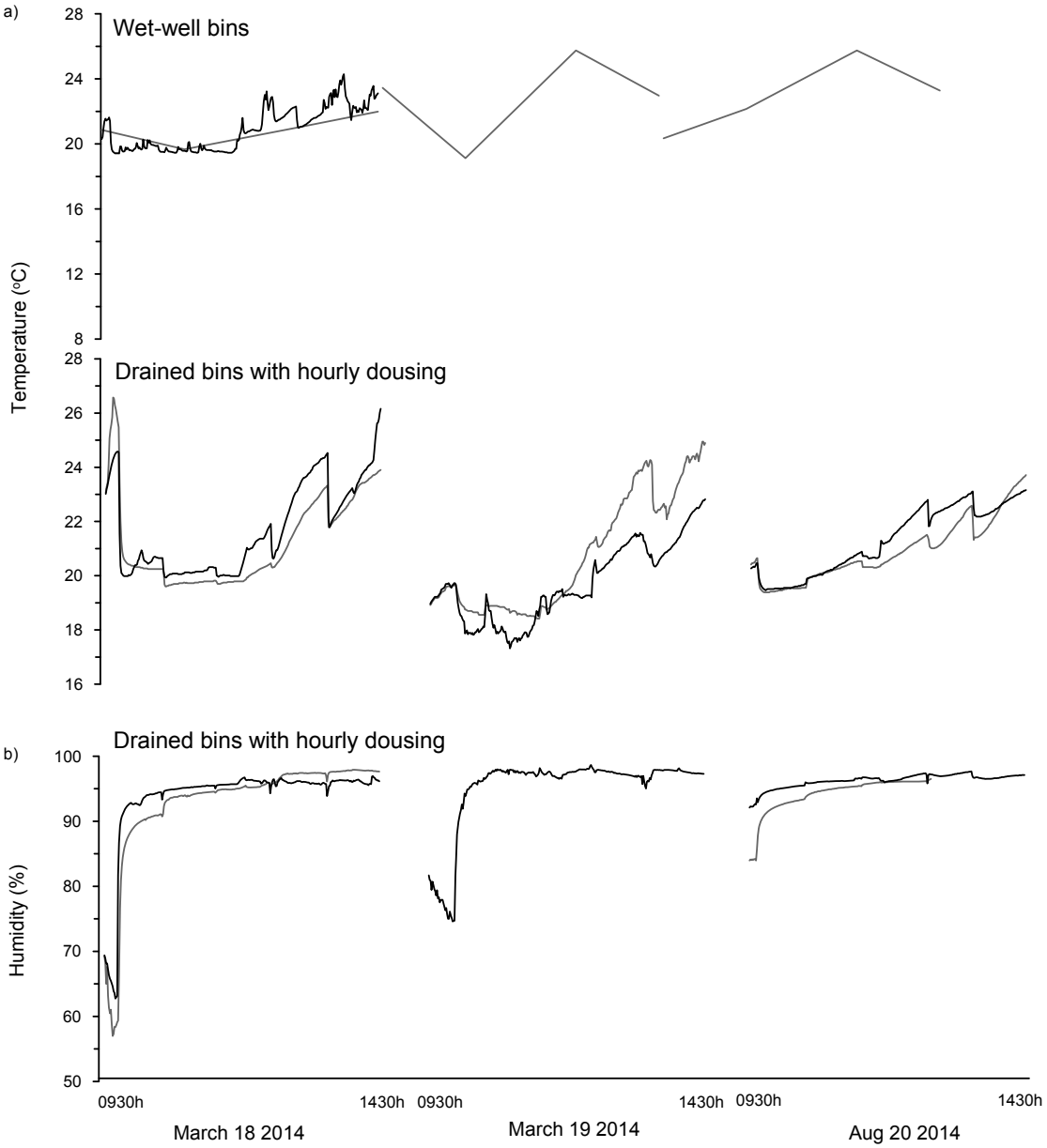


Figure 4.7.1. Patterns of a) temperature in the wet-well and the drained bins periodically doused with fresh seawater in each of the three days and b) humidity in the drained bins periodically doused with fresh seawater in each of the three days. The black and grey lines are the different bins in each treatment. Note that one of the wet-well temperature loggers was logging every 2 h compared with 1 min for the other three loggers. One of the wet well loggers was placed in the processor tank at the end of the 1st day, so data are only available for one logger in the wet-well bins over the 2nd and 3rd day of the experiment.

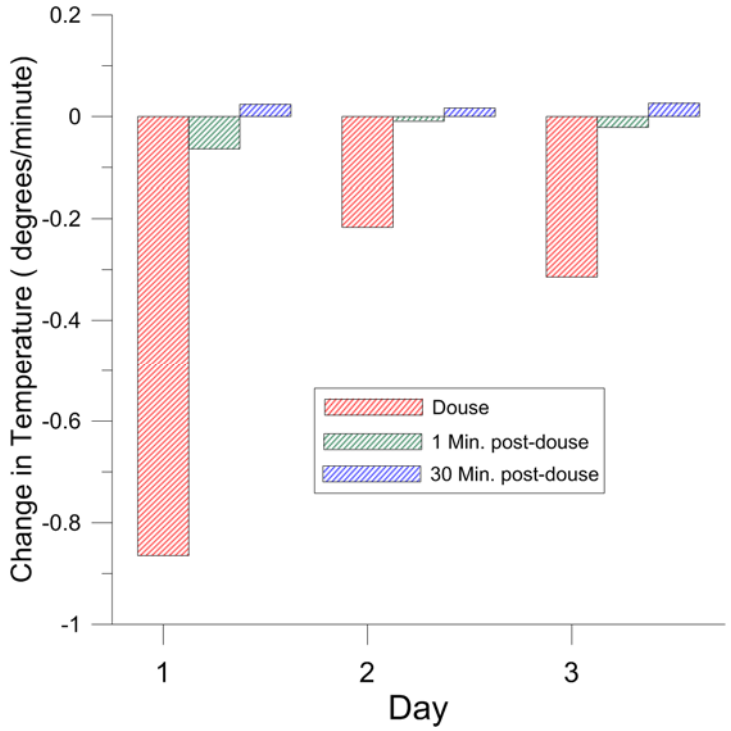


Figure 4.7.2. Rate of temperature change (°C/Minute) associated with dousing of drained bins. Red bars indicate rate of change at dousing, green bars indicate mean change one minute after dousing, and blue bars indicate mean change 30 minutes after dousing.

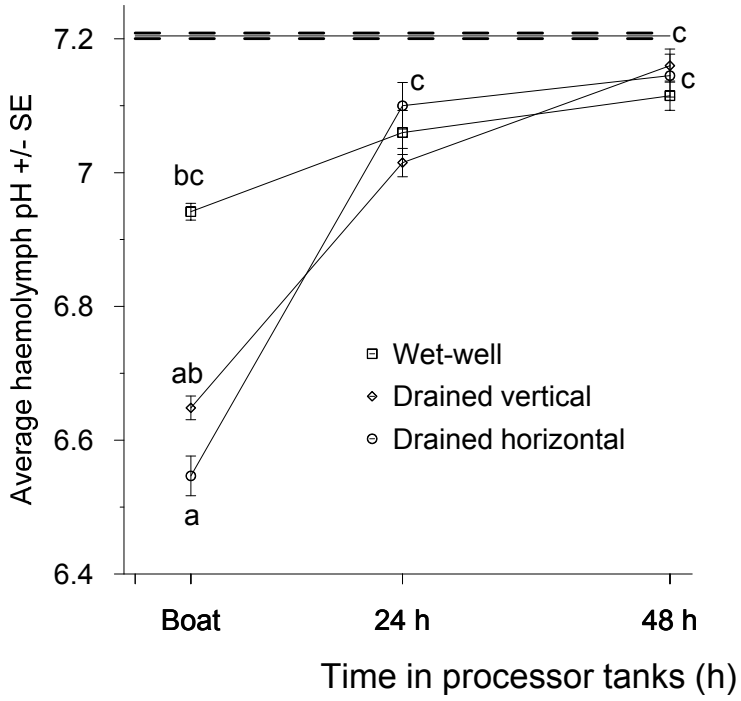


Figure 4.7.3. Mean haemolymph pH of abalone sampled on the reef (solid horizontal line with dashed lines showing SE), at the end of the 5 h boat transit phase (Boat), and time after transfer to processor tanks. n = 2-18. Means (or groups of means) with different letters are significantly different from one another; at 24 and 48 h in the processor tanks all means were the same.

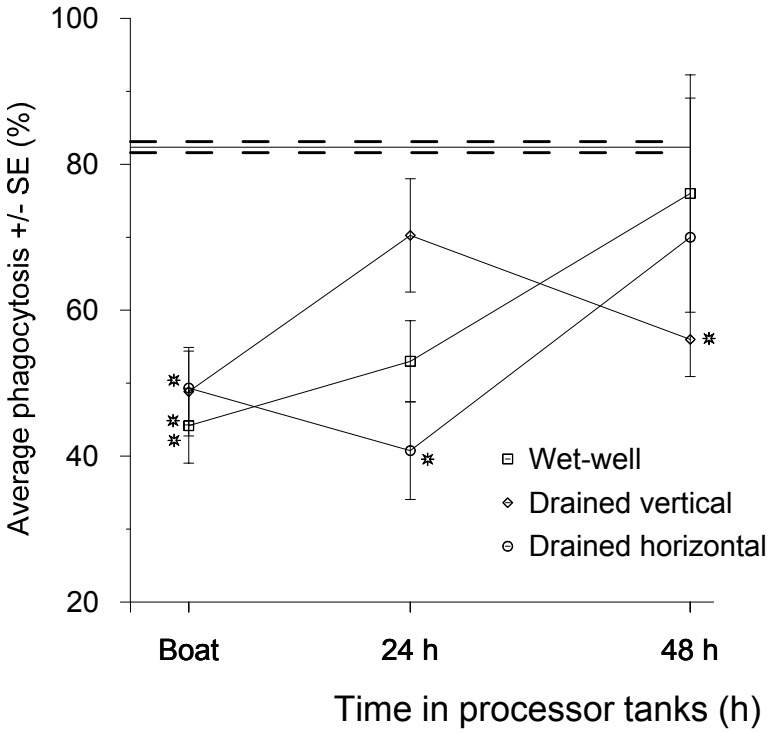


Figure 4.7.4. Average percent haemocyte phagocytosis for each of the boat transit conditions at the end of the boat transit and at two time points in the processor tanks. The reef animal average and SE are shown as the flat line at the top. Treatment means with stars are significantly different from the average of the reef abalone. n=2-6.

Haemolymph Lactate

Average abalone haemolymph D-lactate concentration differed among the abalone on the reef, end of boat transit and during recovery in the processor tanks and the pattern of difference depended upon the transit treatment ($F = 16.22$, df 9, 44, $p<0.0001$). At the end of the boat transit phase, abalone D-lactate concentrations increased significantly in all abalone regardless of the transit method (Figure 4.7.5). However, the increase in the wet-well abalone was almost an order of magnitude less than for abalone held in drained bins. D-lactate concentration for abalone held in drained bins periodically doused with fresh seawater was similar for both vertically and horizontally stacked abalone (Figure 4.7.5). Within 24h of being placed in the processor tanks abalone held in the wet-well and the doused/horizontal had no detectable D-lactate present, while the doused/vertical abalone had no detectable D-lactate within 48h (Figure 4.7.5).

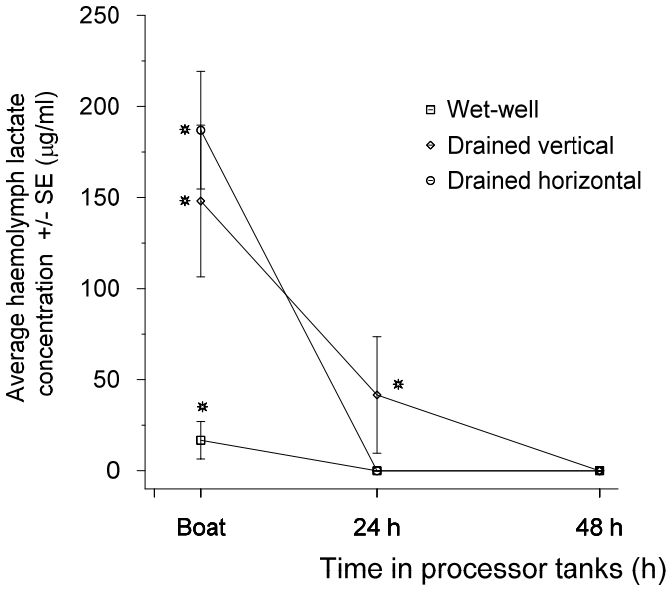


Figure 4.7.5. Mean haemolymph D-lactate concentration at the end of boat transit (Boat), and time after transfer to processor tanks in each of the three boat transit treatments. n = 2-18. The D-lactate concentration for all reef abalone was below detectable levels. Starred means are significantly different from the reef abalone.

Haemocyte Density

Average haemocyte density (slide incubation) differed among abalone on the reef, at the end of the boat transit, and during recovery in the processor tanks (F = 2.37, df 9, 44, p = 0.03). This difference was consistent for abalone across the three boat transit treatments. Abalone on the reef had consistently smaller haemocyte density than abalone in the three boat transit treatments at the end of boat transit phase and during recovery in the processors (Figure 4.7.6).

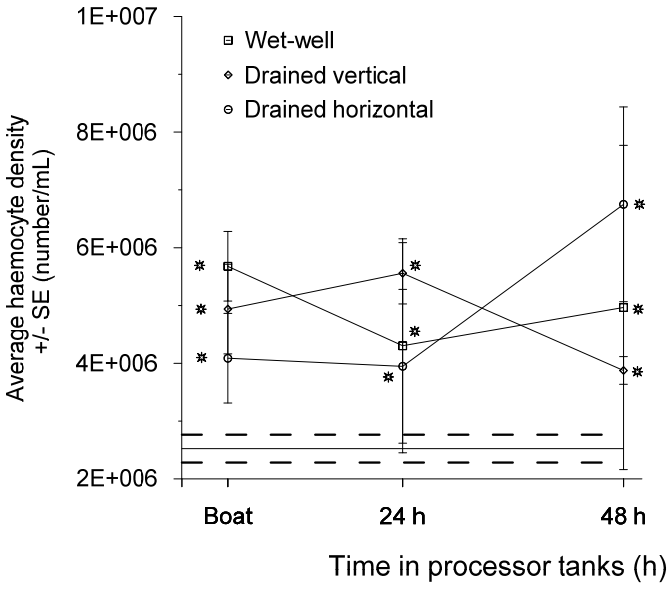


Figure 4.7.6. Mean haemocyte cell density in abalone in each of the three boat transit treatments at the end of the 5 h boat transit phase, and 24 and 48 h post-transfer to the processor tanks. The mean (solid horizontal line) and SE (dashed horizontal lines) for the abalone sample on the reef are shown. Starred means are not significantly different from the reef abalone mean. n = 2-18 abalone.

5. Discussion

Across five field-based experiments we effectively explored the role and contribution of aerial exposure on the magnitude of changes in physiological parameters used to assess stress in wild harvested abalone during the harvest supply chain, from reef to processor. The diver survey highlighted areas where practice was uniform across the respondents, but also identified areas where there was a divergence of approaches. While the process of removing *Haliotis rubra,* a sub-tidal haliotid, and transporting it out of water will necessarily result in a stress response, this is further compounded by transport conditions, transport period, and daily environmental conditions. We have integrated information obtained across the five experiments to identify new knowledge that will inform the nature and type of handling that might minimise the stress experienced by abalone and maximise survival post-harvest. While air temperature during the boat transit phase appeared to be a major factor explaining the differences in the response of *H. rubra* to boat transit conditions (Table 5.1.1), dehydration may contribute to the stress response. There was evidence that baseline condition of *H. rubra* on the reef also differed in the parameters used to asses stress. While, it was outside the scope of the project to explore the potential causes of these differences the implications for the stress response by abalone during the transit phase and recovery are substantial and warrant exploration.

5.1. Conditions During Boat Transit

Time before Stacking of Abalone in Bins

While most divers surveyed indicated that they stacked abalone within 10 mins of their arrival on the deck of the boat, the majority indicated that the shell was cleaned prior to stacking. Depending on the number of abalone in the bag and catch rate, the process of cleaning and stacking may mean that not all animals are stacked within 10 mins.

There was an ambiguous and inconsistent response by abalone to the effects of being on deck before stacking, due to a difference between two sampling times (January and August). In the summer experiment there was a very obvious effect within 15 mins of the abalone on deck in all parameters, while in the winter even 30 mins appeared to have little effect during the boat transit phase. However, it was evident recovery of abalone in the processor tanks reflects an accumulation of multiple stressor events, as was evident in haemolymph pH recovery took longer for abalone which spent longer on deck before stacking (Table 5.1.1). It is clear that as soon as the abalone are aerially exposed and forced to undertake anaerobic respiration that this stress parameter became worse. However, it appears that temperature is a driving factor in the sensitivity of this species to handling, and any time that the animals are likely to be exposed to warmer than optimal temperatures (Table 5.1.1).

Recommendation 1. ***Include in the industry COP that particularly in warmer months, where practicable, abalone should be stacked in bins within 15 mins of being landed on the deck of the boat.***

Some of the variability in response to “time on deck” in the parameters we measured could also be a function of what the abalone do when either in the bag on the deck or once emptied on the deck. For example, we would expect a slower response to aerial exposure from animals that were able to attach their foot to a surface vs abalone that were lying upside and unattached.

Table 5.1.1. Temperature of the water, air, and in drained bins in each of the four manipulative experiments and the mean and range of percent phagocytosis, haemolymph pH, and haemolymph D-lactate and glucose concentrations for wild *Haliotis rubra* sampled on the reef, and for wild-harvested animals held in wet-well bins (immersed in flow-through seawater) and drained bins (periodically doused with a bucket of fresh seawater) for a 5 h (simulating boat transit). * percent haemocyte phagocytosis assessed using slide incubation method and not tube incubation method.

	Treatments	Tasmania January 2012	Tasmania August 2013	Tasmania March 2013	NSW March 2014
Reef water temperature (°C)		16.6	12.6	16.3	21.1
Maximum air temperature (°C)		22-29 (range over 3 days)	13-14 (range over 3 days)	18 (single day)	24-25 (range over 3 days)
Average temperature in wet-well bins (°C)		No data	11.7-12.0 (range over 3 days)	16.0-16.4 (single day)	20.9-22.9 (range over 3 days)
Average temperature in drained bins (°C)		17.8 - 21.3 (range over 3 days)	11.0-12.0 (range over 3 days)	15.7-16.07 (single day)	19.7-21.7 (range over 3 days)
Percent haemocyte phagocytosis (mean and range)	Reef	37.6; 26.7-52.2	50.44; 0-67.8	64.33; 50-73.33	*80; 21-100
	Wet-well bins	31.5; 16.7-63.3	46.9; 11.1-66.67	62.5; 53.33-70	*44.27; 5-79
	Drained bins	23.1; 8.8-64.4	44.51;20-76.67	61.94; 46.67-78.89	*49.08; 4-90
Haemolymph pH (mean and range)	Reef	7.5; 7.42-7.8	6.63; 6.02-7.0	6.53; 6.3-6.72	7.20; 6.93-7.35
	Wet-well bins	7.46; 7.28-7.62	6.12; 5.27-6.65	6.68; 6.65-6.7	6.94; 6.57-7.1
	Drained bins	6.89; 6.28-7.59	6.02; 5.34-6.48	6.55; 6.47-6.72	6.60; 6.28-6.87
Haemolymph D-lactate concentration µg/mL (mean and range)	Reef	0.22; 0-1.54	Undetectable	Undetectable	Undetectable
	Wet-well bins	4.82; 0-50.73	0.77; undetectable-4.80	Undetectable	16.75; undetectable-58.70
	Drained bins	108.13; 12.28-274.08	2.10; undetectable-8.95	35.65; 18.98-54.97	167.51; 97.76-315.82
Haemolymph glucose concentration µg/mL (mean and range)	Reef	31.88; 0-119.55	10.99; 2.47-25.67	2.40; 0-5.57	28.80; 0-73.83
	Wet-well bins	210.20; 6.72-957.57	-	-	-
	Drained bins	11.23; 0-24.77	-	-	-

Dousing vs Wet-well

A consistent conclusion of this series of experiments is that aerial exposure has capacity to affect the haemolymph parameters measured, although there was not always a significant difference for all parameters in all experiments. At the end of the boat transit phase of the harvesting operation abalone in wet-well bins had haemolymph metrics that were generally more similar to abalone sampled on the reef than those abalone stacked in drained bins periodically doused.

Air temperature appeared to be a major factor explaining the differences in the response of *H. rubra* to different transit conditions among the experiments (Table 1). In summer all stress measures indicated that abalone became stressed during the boat transit phase, particularly abalone stacked in drained bins (Table 1). However, there was no evidence of a similar impact of aerial exposure on abalone during winter (Table 1). It is possible that the cooler air temperatures in August compared with January (13.0-13.8^{o}C vs 21.6-29.4^{o}C) may have slowed the metabolic rates of abalone (Bubner *et al.* 2009) and reduced the impact of aerial exposure during the boat transit phase. This is supported to some degree in the Tasmania March 2013 experiment when compared to the abalone on the reef no additional stress was expressed during the boat transit phase, on a day that was slightly cooler than during the Tasmania January 2012 experiment (19^{0}C vs 21.6-29.4^{o}C). What this does highlight is that there is a threshold in temperature tolerance, beyond which adverse effects become pronounced. Wild *H. rubra* show seasonal differences in their antiviral and antibacterial activity that are strongly correlated to water temperatures, with antibacterial activity reduced when water temperatures were warmest (Dang *et al.* 2012). Mortality of wild abalone increases with temperature changes of only 1˚C when animals are exposed to temperatures near their upper limit (Travers *et al.* 2009). *Haliotis rubra* growth is maximal at 8-17^{o}C, and their critical thermal maximum is 26.9^{o}C (Gilroy and Edwards 1998). So small changes in temperature for abalone near the upper end of the thermal tolerance may explain why abalone in the Eden March 2014 experiment, held in both wet-well and drained bins showed similar levels of stress, particularly displaying reduced immune activity (Table 5.1.1). What appears to be the pattern here is that in times of elevated temperatures there is an immediate and short term impacts on disease resistance due to post-harvest handling. Care in warmer summer months needs be taken to reduce the period between harvest and arrival at processor tanks, and minimise harvest activities when air temperatures exceed 21^{o}C.

Like the fishers, we made no attempt to control the temperature of the bins being used to transport the abalone during the boat phase, and this would be logistically impossible for the small boat operators. While we did use wet hessian sacks to cover the drained bins, unlike many fishers we did not use any additional shelter over the bins of abalone, so some of the experimental bins will have experienced different degrees of shading and sun exposure during the 5 h boat transit phase. Shading of bins during boat transit and use of refrigerated trucks by processors would assist in reducing the temperature in the bins. Cheap disposable temperature loggers attached to bins holding abalone during the harvest supply chain may also inform processors of mortality or disease risks associated with temperatures during the harvest supply chain.

There was strong evidence for an increase in D-lactate concentration with aerial exposure for all experiments, except for Tasmanian Winter Experiment, when very little lactate production occurred in the abalone aerially exposed. Increased lactate during aerial exposure is indicative of a build-up of metabolic by-products due to anaerobic metabolism and glycolysis (Baldwin *et al.* 1992) that can occur either during aerial exposure (Wells and Baldwin 1995), sustained exercise (O'Omolo *et al.* 2003), or when immersed with no oxygen (Cheng *et al.* 2004). The results of this study are more likely

attributed to slower metabolic rates in cooler temperatures, although cooler temperatures can increase oxygen uptake in abalone out of water (Bubner *et al.* 2009).

Increased haemolymph glucose concentration during aerial exposure suggests that abalone mobilise stored energy reserves (glycogen) as part of their anaerobic physiology. This is a similar response of glycogenolysis to that expressed by vertebrate animals when stress is experienced, where catecholamines stimulate vertebrates' responses. Use of stored energy reserves during successive periods of aerial exposure or when stored energy reserves are limited, e.g. mature females (Webber 1970) may jeopardise the capacity of abalone to tolerate extended period of aerial exposure. Therefore, at times of the year when glycogen reserves may be low, e.g. periods leading up to, during and immediately after spawning, then periods of aerial exposure should be as short as possible. Increased haemolymph glucose concentration may also arise as a direct result of elevated metabolism during warmer temperatures, but since these two effects occur in the same season, then this will be a result of the two compounding effects. However, recommendations about harvesting strategies around accumulation and use of glycogen stores cannot be provided without further knowledge of glycogen reserves, and the pattern and rate of release of glycogen in response to energy demands.

One parameter that did provide clear results was pH haemolymph. This parameter declines in response to aerial exposure (Bubner *et al.* 2009) and reduced oxygen availability (Cheng *et al.* 2004), as a result of both aerobic and anaerobic respiration, and will increase the haemocyanin-oxygen binding affinity as it become reduced (Wells *et al.* 1998a). In the Tasmania and NSW Summer experiments abalone held in wet-well conditions had haemolymph pH levels no different from reef abalone, while the doused bin abalone experienced decreasing pH levels. It is highly likely that this decline in pH is reflective of faster metabolic rates in warmer temperatures. This effect is further compounded by the added restriction on respiratory gas diffusion imposed by emersion severely limiting their ability to void excess products of respiration, such as CO_2 from aerobic respiration, and H^+ from anaerobic respiration. However, abalone doused during boat transit in the Tasmania Winter and Tasmania Processor experiments did not experience such a decline in haemolymph pH during the boat transit phase, although they did during the road transit phase. Haemolymph pH for *Haliotis* spp. is approximately 7.24-7.61 in control animals used in manipulative experiments (Baldwin *et al.* 1992, Cheng *et al.* 2004, Baldwin *et al.* 2007b, Bubner *et al.* 2009). An examination of the haemolymph pH of the abalone sampled directly after removal from the rock shows a difference among the sampling times and locations (Table 5.1.1). There is some evidence in *Haliotis iris* that animals experiencing greater wave exposure experience energetic differences from animals in sheltered water (Wells *et al.* 1998b). It is possible that abalone in our experiments during the winter experiments had experienced greater wave, currents, and turbulence due to the prevailing environmental conditions, making them expend greater amounts of energy to maintain attachment and thereby resulting in decreased haemolymph pH levels.

Haemocyte phagocytosis, a measure of immune capacity, provided more difficult results to interpret the influence of aerial exposure on abalone during the harvest supply chain. Abalone in the Tasmania Summer Experiment had depressed phagocytosis rates when doused with seawater, and declining phagocytosis rates with time on deck. Crate temperatures were high for this component (17-23˚C). Abalone from the Tasmanian Winter experiment did not show comparable declines in phagocytosis rates with time on deck, when crate temperatures were low (11-12˚C). In the Tasmanian Processor Experiment, boat transit did not affect phagocytosis, but road transit did (up to 28˚C for one bin), although this effect was gone within 4 h. The poor immune status of abalone landed in the motherboat was unexpected as these animals are stored below decks in wet-well conditions. No temperature data was available, but conditions in the drained tanks indicates the abalone were experiencing high levels of stress. Phagocytosis rates at the end of the boat transit period were very

low, presumably due to the time frames involved, and regained normal levels within 120 h, although temperature data is not available for this phase of the transport process. In the NSW Summer Experiment, at the end of the boat transit period the abalone showed reduced phagocytic activity, which returned to normal levels within 48 h. Temperatures were high in this part (18-26˚C), although this stock would have adaptation to higher temperatures than the Tasmanian stock.

Abalone in drained bins were doused with seawater during the boat transit phase of the harvest supply chain. While dousing is not extensively used by fishers, it is used to cool animals as suggested by the Abalone Council of Australia Code of Practice. Changes in the temperatures associated with the dousing bins were obvious, although the direction and magnitude of the temperature change was dependent on the experiment. In the Tasmanian experiments, dousing with surface seawater resulted in a warming of the temperature in the bin, while in the NSW experiment, dousing cooled the internal environment of the bin. Such a difference is most probably due to the differences in evaporative cooling between the warmer NSW and the cooler Tasmanian air temperatures. As the water doused over the abalone is not retained in the bin but drains away it is unlikely that intermittent dousing results in a whole body temperature change. However, abalone gills are external and only protected from the environment by the shell, therefore it is likely that the gills were directly in contact with the seawater doused over the animals. This raises the potential for respiratory stress to occur with each dousing of seawater in the doused bins.

Humidity inside the doused bins covered with wet hessian sacks took some time (at least 2-3 h) to reach 95%, and it was not clear if this was attributed to the wet hessian sack and/or the dousing during the day. While dousing early in the transit phase after stacking may have assisted in generating a moist environment, there was no evidence that repeated dousing later in the transit period helped, and the delay in increasing the humidity may contribute to the stress of aerially exposed animals.

Recommendation 2. ***Maintain temperatures in bins during boat and road transit phases to <20^{o}C using shading during boat transit phase and refrigerated trucks for road transit phase. If this is not possible then Harvesting should be avoided on days with air temperatures predicted to reach >20^{o}C; or identify that these animals may be unsuitable for live transport.***

Recommendation 3. ***Where practicable, abalone should be held in wet-well conditions during the boat transit phase of the harvest supply chain until transferred to the processor, unless SST are >20^{o}C, at which point abalone bins should be drained and bins kept at <20^{o}C. There may be a need to work with resource managers, marine police, and biologists to identify a solution to the issue of obtaining accurate weight of wet or recently drained animals***

Recommendation 4. ***Over summer months attach small disposable temperature loggers and display on bins, and crates in wet wells to provide immediate read-out to the fishers and provide information to the processors about the conditions experienced by the abalone during.***

Recommendation 5. ***Immediately after stacking abalone to be held in drained bins during transit, the bin is filled with water for 10-15 mins, then drained and covered with wet hessian sack to maximise and maintain humidity in bins during boat transit; hessian be kept wet.***

Recommendation 6. ***Reconsider the risk rating of "low" for the quality-related activity of draining tanks prior to landing as this is dependent upon the bins being kept cool.***

Recommendation 7. ***Given that the capacity to tolerate aerial exposure requires the use of stored glycogen (sugar), harvest strategies need to consider temporal and spatial patterns of somatic and reproductive condition. As little is known about the patterns of glycogen accumulation and use as a function of reproduction and environmental factors by Haliotis rubra, further research is required.***

Orientation & Attachment

Stacking procedures, including the weight of abalone stacked in a bin stacking orientation, are often prescribed by the processor for whom the abalone are being harvested. Stacking also differs among states, e.g. NSW has a top layer of abalone stacked horizontally on a vertically stacked abalone, with a stiff plastic sheet between the layers. In these experiments the stacking density of the abalone was not controlled, but abalone were stacked in such a way as to prevent animals moving around and so they would remain vertically stacked. In all the experiments, vertically stacked abalone were orientated with holes up as per the Code of Practice for the industry. Once stacked, the abalone held in drained bins with dousing did not move much, which contrasts with the wet-well animals that were inclined to move about inside the bin.

There were no differences among treatment in the stress parameters measured during the boat transit phase of the NSW experiment, although the horizontally stacked abalone recovered more quickly in the processor tanks. Horizontally-stacked abalone had a more rapid reduction of lactate in the haemolymph than vertically stacked abalone, but the orientation the abalone were stacked in bins did not contribute to recovery of haemolymph pH.

The vertical stacking procedure, with the respiratory holes upwards, is believed to offer some physiological advantage to the abalone. Their gills, in the absence of support from water, will not be squashed by their body mass if stacked with the respiratory holes down. Holding abalone in a high humidity environment offers some potential respiratory gas exchange, at least in terms of oxygen, with atmospheric gases. Carbon dioxide's high level of solubility in water would mean that it will still be accumulated even when diffusion of oxygen occurs (Bubner *et al.* 2009).

Recommendation 8. ***There is no reason that practice of stacking of the top layer of abalone horizontally in bins in NSW should be stopped. However, in times when temperatures are elevated, this procedure may need to be modified.***

5.2. Recovery in Processor Tanks

There was some difference in recovery rates among the three experiments that explored this; the recovery of abalone transported from the Tasmania west coast via motherboat and abalone in the NSW experiment took >24 h to show recovery, while abalone in the Tasmanian researcher simulated harvest experiment recovered more quickly (4-15 h). The three experiments were not identical in the handling of abalone during the boat phase, however it was expected that the wet-well animals in the NSW and motherboat would have recovered faster than 24 h. It is likely that again temperature of the abalone in the bins, or in the drained tanks could have played an important role in the recovery. While some of the abalone in the Tasmania Processor

experiment did experience temperatures >20^{o}C during the road transit phase, this does not appear to have compromised recovery rate.

While in some experiments the stress parameters measured did not immediately indicate that wet-well transit resulted in less stress (see Tasmania Processor Recovery Experiment), the advantages of using wet-wells during the boat phase were evident during the road transit phase (Tasmania Processor Recovery Experiment), when a stress response in the abalone held in wet-wells was either non-existent or was followed by very rapid recovery in the processor tanks (Tasmania Processor Recovery Experiment).

Measures of stress in the haemolymph did not increase once the abalone were transferred to the processor tanks, and in most cases full recovery or progress to full recovery occurred within 24 h of transfer. Therefore, it appears that like *Haliotis lamellosa* (Gäde 1988), *Haliotis rubra* is able to return to aerobic metabolism relatively quickly once returned to water. However, depending how stressed the abalone became during the boat and road transit phases will determine how quickly abalone will return to the state they were on the reef. Return of most of the haemolymph parameters to values similar to those of abalone sampled on the reef was faster for the abalone which were in wet-well bins during the boat transit phase, with only haemocyte phagocytosis and haemolymph glucose during the Tasmanian Recovery Experiment showing no difference in recovery between the wet-well and drained bins periodically doused, indicating that handing alone is enough to affect these parameters (Hooper *et al.* 2011).

5.3. Other Practices Contributing to Stress during the Supply Chain

Removal of Growth Prior to Stacking

The majority of fishers remove growth from the abalone shell after harvesting and this practice as an additional source of stress has been raised by fishers. As this practice appears to be necessary and there is little option to modify the practice, we suggest that assessing the contribution of this practice to the stress of abalone is not required currently. However, we did not remove growth from abalone in our experiments and as a result the measures of the haemolymph parameters that we used as indicator of stress may have underestimated the impacts of typical post-harvest processes.

Stacking Densities and Layers in Bins

Stacking densities were highly variable among fishers, and although in the summer fishers were more likely to stack 20-24 kg per bin, there was no consistent stacking density in the winter. In our experiments we stacked the maximum number of animals in a single layer vertically, while the actual weight stacked in an experimental bin was not controlled and depended on the size of the abalone. Many fishers stack the abalone in two layers in the bin. We did not explore this aspect of stacking density.

6. Benefits and Adoption

The wild abalone harvest sector (both fishers and processors) are the direct beneficiaries of this research, with the investment sector secondary beneficiaries. Adoption of key recommendations from this study could be achieved with minimal infrastructure change or capital investment. Investment in new engineering solutions requiring substantial capital is unlikely to be adopted in the short to medium term, and adoption will occur primarily through changes in harvest practice, and low-cost solutions to maintaining cool temperature and high humidity.

Inclusion of revised handling protocols in the abalone QA-COC to minimise dehydration, and minimise the transport time in drained bins will make immediate improvements to the quality and condition of abalone on arrival at abalone processing facilities. Increasing the proportion of abalone harvested in association with motherboats and transported in wet wells will also lead to a reduction in the risk of landing abalone in poor condition during the warmer months. This latter option however could have economic consequences, as costs associated with motherboat based harvesting are greater than shore based operations in day boats. At the time of writing, two abalone fishers had installed large wet wells in the pontoons of their large catamarans (> 7.5m). However, few fishers are likely to pursue this option, either because of the cost of installation, or their boat size is insufficient to accommodate live wells of sufficient size to be effective. In jurisdictions where motherboats are not active such changes will require significant capital investment and may inhibit adoption in those jurisdictions.

The beneficiaries, benefits and recommendations provided here are directly in line with those proposed in the application. The magnitude of financial benefit is difficult to determine and must be considered in light of the cost of revised protocols. A complicating factor is that the benefits are largely restricted to the processing and investment sectors, the costs, although minimal will largely be borne by the harvest sector. Nevertheless, a reduction in the proportion of abalone harvested and sold to processors for the live market, but diverted to the canned market due to quality and condition factors, from 10% (current levels) to approximately 3% (levels previously experienced) will result in an annual increase in value of $7million.

Currently the Australian Wild abalone harvest is approximately 5% of world abalone production. At that level, there is scope for a boutique premium product and maintaining a supply of robust, high quality abalone into the market place will greatly assist achieving a higher premium for the live wild product.

7. Further Development

Several interacting factors appear to influence the degree of stress response observed in abalone transported in drained bins after harvest. Seasonal differences in air and SST temperatures, energy reserves, metabolic rate, and desiccation are more than likely prime determinants of the extent of stress and rate of recovery post transport. Seasonal factors cannot be changed, but practices could be altered according to the season of harvest if quantitative background data were available to map risk potential across seasons.

Dehydration in drained bins could be better managed by maximising humidity and preventing exposure of external organs to desiccation. Simple engineering solutions could be explored to rapidly achieve and maintain high humidity on small catcher vessels.

Spatio-temporal patterns in energy reserves and reproductive activity are poorly understood, but are important for a range of issues in sustainable management of abalone. The relative frequency of post-harvest spawning across sex during the

troublesome months of December to March is unknown, but spawning of males in processor tanks is more likely to be problematic than spawning of females. These males may be individuals that have not participated in natural spawning events and are responding to stress cues associated with transport in drained bins. Given the density and number of animals held in processor tanks, if a fraction as little as 1% of animals spawned, negative effects would be observed. A better understanding of seasonality in reproductive activity across different regions around Tasmania would assist with scheduling of harvest to minimise interaction with periods of high spawning activity.

This project has focused on practices during harvest supply chain up to and prior to arrival at the processor tanks that are likely to result in a reduction in condition and quality of abalone. While recommendations largely apply to the harvest phase, many of the findings also relate to the road transport phase. Modification of transport fleets to reduce handling, provide cooler air temperatures, and maximise humidity could slow the rate at which abalone become stressed and increase rates of recovery in the processor tanks.

Principal outcomes of this research were presented at the 6th National Abalone Convention in Queenstown, NZ, Aug 6-8 2014.

8. Planned Outcomes

A key proposed outcome was to inform the abalone wild harvest industry about potential sources of stress that are compromising the vulnerability of post-harvested animals to disease and death. This knowledge can then be used to reduce the percentage of animals purchased for the live market being diverted to the canned market due to quality and condition factors associated with transport practices. The nature of the live market is that it is principally a fishing-to-order harvest strategy, with high demand during the months when warmer conditions are most likely to compromise the stress and survival of abalone transported in drained bins (e.g. Chinese New Year).

The outcomes of this research enable the construction of a decision tree, as to preferred harvest practices for a given season and region (Figure 5.3.1). Physiological indicators of stress informed us that abalone harvested on cooler days tolerated the boat transit phase, in wet-well or drained bins, better than during warm days. Winter fishing can continue to supply abalone in good condition to processors, with few modifications to current practice. Greater physiological stress occurred in the experiments in both NSW and Tasmania when water temperatures and/or air temperatures were >18^{o}C. Under these warmer temperatures the stress response of the black-lip abalone can be minimised by adopting of one or more strategies, depending on the size of the vessel and proximity of the fishing grounds to the processing facility (Figure 5.3.1). As practices and infrastructure vary across processors, it will be up to each processor to incorporate the recommendations of this project into their operational plan.

An unexpected outcome of this research was to provide a scientific basis for the rationale held by some fishers that dousing of abalone in drained bins has negative rather than positive consequences. Several experienced fishers indicated it was a long held assumption that dousing was not a good idea, but would not have known the reason why.

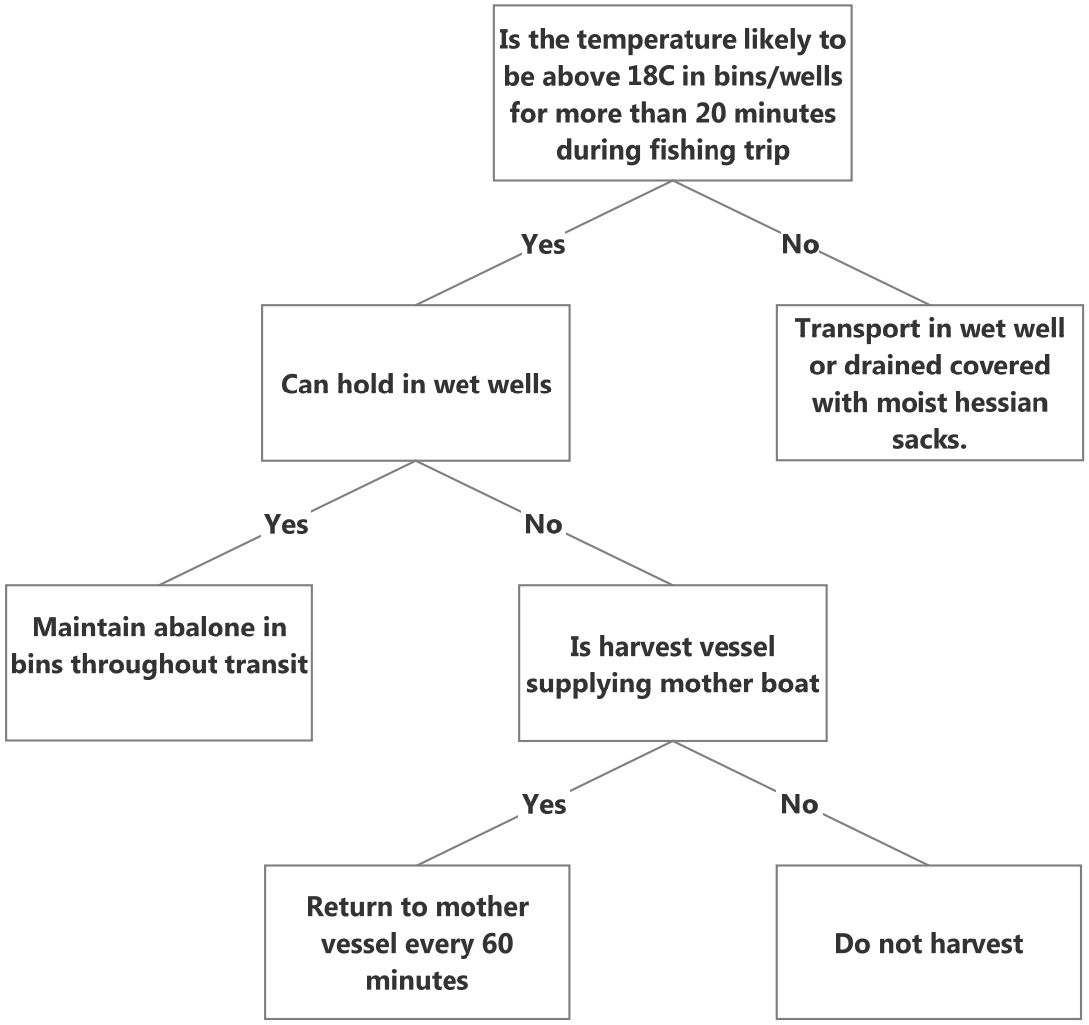


Figure 5.3.1. A decision tree identifying whether harvest should go ahead and under what harvest-chain conditions for black-lip abalone harvested in New South Wales and Tasmania. Temperature was identified as a critical factor around reducing stress of abalone during the harvest supply chain and maximising survival of abalone in the processor prior to live transport.

9. Conclusion

In conclusion this project identified that the temperature at which the abalone are transported, either in or out of water, is most probably the strongest predictor of the amount of stress that *H. rubra* will experience. Abalone that experienced less stress during the harvest supply chain recovered faster than those animals experiencing warmer temperatures and greater handling. Maintaining *H. rubra* within its thermal optima throughout the harvest supply chain will be essential for successful preparation of abalone for live transport internationally. Currently thermal optima for *H. rubra* are derived from experiments seeking to identify optimal temperatures for growth and survival for cultured populations held on Tasmania's east coast. However, no information is available about the spatial differences in thermal optima for minimising stress during transport of adult *H. rubra* out of water, which limits the scope of recommendations for harvest industries over the spatial distribution of *H. rubra*.

For animals that will be transported out of water at any time during the harvest supply chain, the reliance on glycogen (sugar) energy reserves is critical to supporting anaerobic metabolism. While maintaining animals in cooler conditions will slow metabolic rates and minimise the use of glycogen during the harvest supply chain, the

quantity of glycogen reserves will determine the capacity of the abalone to survive multiple or extended periods of aerial exposure. When glycogen stores are depleted, such as following reproduction, during anaerobic metabolism, extended periods of exercise such as during storms, or reduced feeding periods, success of transporting *H. rubra* out of water will be reduced. The timing of harvesting and transporting *H. rubra* needs to be better informed about the spatial and seasonally patterns of glycogen stores and to what degree these are affected by biological and environmental factors.

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11. Appendices

Appendix 1. Tasmanian Abalone Diver Survey

Internal Use Only Questionnaire Number:

Abalone Fishing – Day trips

Years of experience diver has had in industry:

Do you operate a day boat?
Yes – go to Section 1
No – go to Section 2

Section 1 – Day Boat Operation

Question 1. What is the approximate length (in hours) of a day trip ie from start of fishing to return to the boat ramp for:

(record a whole number or NA if not applicable)

Location	Summer (ie warmer months)	Winter (ie cooler months)
North east		
North west		
South		
East		

Question 2. What is the average time interval between retrieval of each net-bag (provide range in minutes)?

Question 3. What is the approximate time in minutes (provide range) from landing abalones on the boat to stacking in bins in:

	One diver	Double up
summer		
winter		

Question 4. Do you ever clean the growth off the abalone shell? Yes/No

Question 5. How are abalone stacked in the bins?

i. "Foot down" OR "Foot to the side" OR "Changes with Trip"

ii. If "Foot to the side" are abalones stacked:

i. HOLES DOWN
ii. HOLES UP
iii. No consistent direction

Question 6. When stacking the abalone in a bins how many are stacked in each layer (approx):

iii. in summer? KG:

iv. in winter? KG:

Question 7. How many layers of abalone are stacked in a bin:

v. in summer?

vi. in winter?

vii. Depends on processor

Question 8. Do you hold the abalone in wet wells ie bins of abalone are fully submerged in seawater with fresh seawater provided?

Yes – go to Question 9
No – go to Question 12

Question 9. Before travelling are the wet wells drained of water?

No (go to Question 11)

Yes (answer the next 4 questions)

a. Do you stop to flush fresh seawater through the bins?

b. How many hours between flushing stops?

c. For approximately how many minutes do you flush seawater through the bins of animal?

d. How many minutes before arrival at the boat ramp is the wet well drained?

Question 10. If abalone are not submerged in seawater once stacked in bins (ie no wet well), do you place hessian sacking over:

Each layer OR Top layer only

Question 11. If abalone are not submerged in seawater once stacked in bins (ie no wet well), do you have a deck pump flushing the bins continuously?

Yes – go to Question 14
No

Question 12. If abalone are not placed in a wet well, or have continuous flushing water, approximately how frequently is water poured over the bins in:

i. Winter?
ii. Summer

Question 13. Do you have a shade structure on your day boat to keep the sun off bins? Yes/No

Question 14. Once you arrive at the landing point ie jetty or boat ramp what is the average time that you wait for the processors truck to pick up the abalone?

Question 15. How are abalone kept cool and damp while waiting for the processors truck?

a. Just damp hessian sacks?
b. Hessian sacks and dousing with buckets of water?
c. Wet wells with water flushing through the bins?

Question 16. Do the processors provide specific instructions about how you are to look after abalone?

Section 2 – Mother Boats

Question 17. For how many hours do the smaller boats fish before returning to Mother vessel:

Summer:
Winter:

Question 18. How long before landing abalone at port are the wet wells drained?

Question 19. Do you know the flushing rate of the live well tanks (e.g. how frequently do the pumps replace the water in each live well)?

Appendix 2. Staff

Staff engaged on the project:

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Co-investigators
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