



Survival and Physiological Responses of Greenlip Abalone (*Haliotis laevis*) under Simulated Live Transport

Frederika Niken Restu Kurnaningtyas^{1,2}, Ishaq Saputra^{1,2*} and Eko Hendri Gunawan¹

¹Fish Quarantine and Inspection Agency, Gedung Mina Bahari 2, 6th Floor, Jl. Medan Merdeka Timur 16, Jakarta 10110, Indonesia

²School of Molecular and Life Sciences, Curtin University, Kent Street, Bentley, Western Australia 6102

*Correspondence :
isaputra.6m2@gmail.com

Received : 2021-03-01

Accepted : 2021-09-28

Keywords :

Abalone, Live transport, Physiology,
Haliotis laevis

Abstract

Greenlip abalone (*Haliotis laevis*) are high-value marine bivalves. To preserve the quality, most edible aquatic animals were transported in live conditions. In addition, keeping the condition at a low temperature is considered the best way to reduce the stress level of the animal. However, this method is still poorly understood in greenlip abalone. The present study examines the effects of pre-cooled (± 14 °C) and non-precooled (± 21 °C) temperature treatments on survival and physiological responses such as total haemocyte count, phagocytic assay, lysosomal assay, and lactate of live abalone during transport simulation at time intervals of 0, 12, 24, 36 and 48 hours. Results indicated that pre-cooling treatment before transport increased the survival rate and minimized weight loss. The lactate levels were significantly higher in the abalones that were not cooled before transport. Both animals that were pre-cooled and those that were not cooled before aerial transport simulation showed increases in total haemocyte count and a decrease in phagocytic activity, which indicates that live transport has an impact on their immunity. The present study indicated that the importance of pre-cooling treatment before transportation to preserve the condition of live abalone.

INTRODUCTION

The growing market demand for greenlip abalone (*Haliotis laevis*) has led to the establishment of a land-based greenlip abalone hatchery and grow-out farm near Bremer Bay, Western Australia. To improve profitability in the export market for abalone, it is crucial to minimize the mortality of the product during shipment. In general, aquatic animal live transport involves maintaining a low temperature during transport. Although it has been practiced in some species, the research of pre-cooling

treatment in *H. laevis* remains unclear. For blacklip abalone (*H. rubra*), chilling to approximately 5- 10 °C is recommended to improve survival rate (ACA, 2013). In fact, chilling immersion before transport is currently being used by the Ocean grown abalone industry to reduce mortality during transport.

During live transportation, animals typically experience typical stressors such as air exposure, salinity shock, physical injury, heat changes, and ammonia poisoning (Wingerter *et al.*, 2013). During

aerial exposure, abalone respiration can be jeopardized because of gill impairment which impedes the exchange of oxygen to the blood, and if prolonged aerial exposure occurs, this can result in permanent tissue damage (O'Omolo *et al.*, 2003; Ragg and Watts, 2015). During aerial exposure, lactate can accumulate in the haemolymph of abalone (Baldwin *et al.*, 2007). Other parameters implicated in the stress responses during transport simulation involving abalone are subsequent haemocyte density and phagocytosis after harvesting from the wild to the processing facility. The study of physiological stress from harvest to the processor found that phagocytosis, haemolymph lactate, and pH declined following aerial exposure and temperature shocks (Bubner *et al.*, 2009).

Abalone has physiological functions to tolerate hypoxia conditions without any deleterious effects. However, abalone has minimal resistance to air exposure (Song *et al.*, 2007). These findings clearly show that exposing abalone in different temperatures followed by air exposure may affect the physiological condition, which in turn is likely to affect immunity, which can lead to mortality (Malham *et al.*, 2003). Although the immunological responses of wild and ranched *H. laevigata* has been evaluated (Setyaningsih *et al.*, 2020), no studies have been reported which investigate the physiological response of *H. Laevigata* specifically focusing on temperature treatment prior to transport, following aerial exposure. The study of the impact of temperature treatments prior to aerial exposure on the physiological response of abalone remains to be determined. This topic has high relevance to the abalone culture industry, as it has the potential to boost the survival of abalone during transport, and hence, improve profitability.

Therefore, this research was carried out to investigate the effects of temperature treatment prior to simulated transport and the effects of time duration on the physiological status of abalone. The survival rate, mass loss, lactic acid, total

haemocyte count, and phagocytic assay were the parameters of interest. This study aimed to optimize transport time of abalone by using different seawater temperature treatments prior to transport and examining subsequent recovery at different temperatures in holding tanks. The parameters mentioned above will be measured and compared to observe their sensitivity to temperature and transport.

METHODOLOGY

Place and Time

The simulation transport was performed in November 2015, took place at Curtin Aquatic Research Laboratory, Western Australia. The live abalone was collected from the wild water of Flinders Bay, Augusta, Western Australia.

Research Materials

In the present study, several equipment and materials were used, including 50 L polystyrene fish tanks, medium-sized net scope, 20 L aquaria, chiller (Hailea, Guandong, China), water quality equipment (Handy Polaris 2, OxyGuard Germany), nitrite test kit (API, USA), polystyrene boxes (40 L), absorbent pads, polyethylene plastic bags (50 L) and ice packs (500 g). Also a temperature data logger (Onset HOBO Pendant Temperature Data Logger, USA), syringes (25G, Terumo, Philippines), haemocytometer (Neubauer, Germany), pH meter (Waterproof EC-PCS Testr20, Oakton, USA), micro-tube 1.5 mL, light microscope (Motic, China), lactate reagent set (Pointe Scientific L7596), centrifuge (5804R, Eppendorf, Hamburg, Germany), UV-VIS Spectrophotometer (Shimadzu, Japan), zymosan (Sigma#Z4250), May Grunwald Giemsa stain solution, and microplate reader (BioTek Synergy HT Multimode, New Jersey, USA).

Research Design

Cultured greenlip abalone (n=480, whole wet weight 89 ± 6.38 g) was collected by Ocean Grown Abalone divers from the lease site in Flinders Bay,

Augusta, Western Australia. The abalone was held in eight aquaria at the Curtin Aquaculture Research Laboratory at Technology Park, Bentley, WA. Approximately 45-60 abalone were transferred to 50 L tanks, each with aerated circulating seawater at $21 \pm 1.5^\circ\text{C}$, for ten days. During this acclimation period, the abalone was purged. At the end of the acclimation period, the abalone was randomly sampled from the eight tanks and physiological parameters were analyzed.

The effect of two different chilling treatments on the abalone prior to packing was investigated over 48 hours of simulated live transport. After first acclimating the experimental abalone, four aquaria were exposed to a pre-cooling treatment of 14°C for two days and the other four aquaria were exposed to a non-precooling treatment of 21°C . The temperature in the pre-cooled tanks was reduced to 14°C . The temperature was maintained at 14°C using a chiller (Hailea, Guandong, China) and the abalone was labeled the “pre-cooled” group. Over this same period, abalone in four other aquaria was held at a non-precooled temperature close to ambient seawater temperature ($21 \pm 1.5^\circ\text{C}$). The water temperature in this group was maintained at 21°C using a chiller, with this abalone group labeled “non-precooled” group. During the

experiment, the water quality was measured twice daily. Dissolved oxygen and temperature were recorded using a Handy Polaris 2 (OxyGuard, Germany). Ammonia was measured by using an ammonia test kit and Nitrite was measured using a nitrite test kit (API, USA).

Work Procedures

Live Transport Simulation

The live transport simulation in this study was based on current industry practice which uses polystyrene boxes (40 L), absorbent pads, polyethylene plastic bags (50 L), and ice packs (500 g). The complete process of this simulation is presented in Figure 1. All the abalone in the three pre-cooled treatment aquaria and three non-precooled treatment aquaria were carefully detached from the tank walls using a metal spatula and placed into trays. Each tray was weighed to determine weight loss before and after the 48 h period of simulated transport. To do this, the abalone was arranged in the trays in rows, one on top of another, with the radula region of the shell facing upwards. The time taken to arrange the abalone in this order was less than two minutes. Another tank of abalone in the pre-cooled and non-precooled group was used as a control.

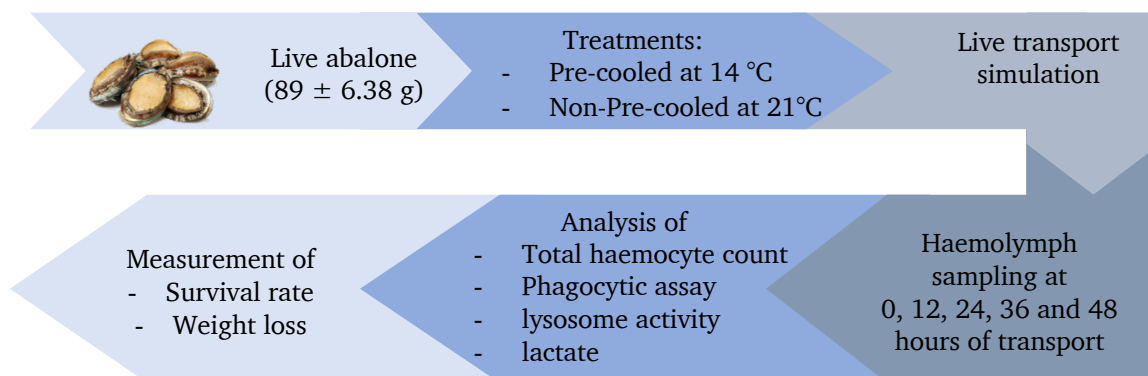


Figure 1. Live transport simulation process diagram.

The animals were then placed into a plastic bag on top of an absorbent pad. Each row of abalone was separated with cardboard wrapped in plastic. A damp soft

absorbent pad that had previously been soaked in 14°C seawater was used to cover the abalone inside the plastic bag. Pure oxygen was pumped into the plastic

bag until inflated. The bag was then sealed with an elastrator, to ensure that there would be no leakage of oxygen. Two ice packs were placed in between the plastic bags to chill the abalone during simulated transport. The polystyrene box lids were sealed with packaging tape and were stored at room temperature for 48 hours. Every 12 hours, the sealed boxes were opened to check for mortalities and to take blood samples from 1 abalone. After each sampling episode, the plastic bag was replenished with pure oxygen and resealed. Abalone that had been sampled for their haemolymph was not returned to the polystyrene transport boxes. Temperature data loggers were attached inside the polystyrene boxes during simulated transport. The temperature inside the boxes was recorded every 60 minutes using a temperature data logger (Onset HOBO Pendant Temperature Data Logger, USA).

Survival Rate and Weight Loss

The survival rate of abalone was measured according to Setyaningsih *et al.* (2020) while weight loss was measured according to Bubner *et al.* (2009) as follows:

$$SR = \frac{N1}{N0} \times 100\%$$

Where:

SR = survival rate (%)

N1 = final number of survived abalone

N0 = initial number of abalone

$$WL = \frac{W0 - W1}{W0} \times 100\%$$

Where:

WL = weight loss (%)

W1 = final wet weight (g)

W0 = initial wet weight (g)

Physiological Responses

Haliotis laevigata haemolymph collected from the beginning of the experiment, during transport time was assessed for total haemocyte count (THC), phagocytic assay (PA), lysosome activity, and lactate. Approximately 0.2 mL of fresh haemolymph was drawn, using a non-destructive technique, from the cephalic

arterial sinus (Travers *et al.*, 2008) which is located at the anterior of the animal between the foot and mantle. This procedure was performed using a pre-cooled sterile syringe and a 25G needle kept on ice. The haemolymph samples were discharged from the syringe to pre-cooled sterile microtubes (Eppendorf, Germany) 0.2 mL. Haemolymph pH, muscle pH, and muscle temperatures were measured using a pH meter (Waterproof EC-PCS Testr20, Oakton, USA). The probe was calibrated at the start of each measurement session. The probe was inserted into a micro-tube (1.5 mL) containing haemolymph. Muscle pH and the temperature were recorded by inserting the probe into the muscle. The reading was recorded until the value was stable.

Total haemocyte count was conducted as following established protocol (Setyaningsih *et al.*, 2020). A 50 μ L sample of fresh haemolymph was mixed with anticoagulant (1% glutaraldehyde in 0.2 mol L⁻¹) in pre-cooled Eppendorf tubes and kept on ice to prevent the haemocytes from clumping. The haemolymph samples were vortexed before being estimated in duplicate with a haemocytometer (Neubauer, Germany) under a microscope (Motic, China) at 100-fold magnification. THC is calculated as cell x 10⁴ per ml.

Lactate was determined using lactate (liquid) reagent set (Pointe Scientific L7596) with temperature modification. The supernatant of fresh haemolymph was collected by centrifugation at 5000g for 10 min at 5 °C using a centrifuge (5804R, Eppendorf, Hamburg, Germany). Briefly, a 12 μ L sample of fresh haemolymph was mixed with 600 μ L reagent 1 and incubated at 20 °C for 30 seconds. The haemolymph solution then was added with 400 μ L reagent 2 and incubated at 20 °C for 5 minutes before measuring the absorbance at 546 nm using UV-VIS Spectrophotometer (Shimadzu, Japan). Results were presented as mg/dL lactate.

The phagocytic assay was measured as the procedure for abalone (Day *et al.*, 2010). A 40 μL sample of fresh haemolymph was placed onto a poly-L-Lysine glass. The slides were incubated for 10 minutes at room temperature. After incubation, the excess haemolymph was poured off and 40 μL of zymosan (Sigma#Z4250) was added to the haemolymph. The smears were incubated for 30 minutes then placed into a 10% neutrally buffered formalin in seawater solution for 15 minutes. The smears were then stained with May Grunwald Giemsa stain and the cover slipped. Every 100 haemocytes in each smear were counted and the number of haemocytes that phagocyte zymosan was recorded. Duplicate slides were created for each abalone sampled. The phagocytosis was expressed as (phagocytic haemocytes/total adhered haemocytes) $\times 100\%$.

Lysozyme activity was measured using a 96-well microplate reader. A 50 μL sample of fresh haemolymph in duplicate was pipetted into a microplate. The sample was incubated for 15 minutes at room temperature. 50 μL of 0.02% *Micrococcus lysodeikticus* bacterial suspension (Sigma M3770) in 0.1M KH_2PO_4 (pH 6.2) was added to each well. The decrease in absorbance was measured at 2-minute intervals for 20 minutes at 450 nm using a microplate reader (BioTek Synergy HT Multimode, New Jersey, USA). Negative and positive controls, without haemolymph, containing 0.02% *Micrococcus lysodeikticus* bacterial suspension and 0.1M KH_2PO_4 buffer were run in parallel. Results were expressed as Enzyme Unit (EU) per mL (EU. mL^{-1}).

Data Analysis

All statistical analyses were performed using SPSS 20. An independent t-test was performed to determine the mean difference of temperature profile inside the boxes between the two treatment groups (pre-cooled and non-precooled). The assumption of variance of homogeneity was investigated using Lavene's equal variance test. All data

regarding the effects of pre-cooling treatments and period on haemolymph lactate, phagocytic rate, lysosomal activity was performed using one-way analysis of variance (One way ANOVA) to determine the significant difference between five transport times. The value of the statistical tests among time durations and during recovery time of less than 0.05 was considered as significant.

RESULTS AND DISCUSSION

In this study, results demonstrated that both temperature treatment prior to transport as well as transport duration affects the physiological status and stress condition of *H. laevigata*. Pre-cooling treatment prior to transport had a beneficial effect on all parameters that were considered. Pre-cooling treatment increases the survival rate during transportation. Abalone treated in pre-cooled water prior to transport did not result in any mortalities after transport from 0 to 48 h. However, after 36 h of transportation, a mortality rate of 7% was observed in abalone treated within the non-precooled seawater. Control specimens immersed in aerated seawater over the same period had no mortalities. This suggests that the latter group experienced less stress during transportation as a result of their conditioning prior to being packed.

The mass loss experienced by the abalone during simulated transportation in this study is similar to what has been recorded in a previous study on *H. laevigata* (Bubner *et al.*, 2009). The percentage of weight loss experienced by abalone over the 48 hours of simulated transport revealed that the weight loss was greater when abalone was held in the non-precooled seawater temperature prior to transportation (10.25%) compared to those subjected to the pre-cooled treatment (8.63%). In that study, the weight loss of abalone was greater after transporting the abalone for 35h in 60% of oxygen without ice conditioning compared to 60 % of oxygen with ice conditioning. In that study, adult abalone

(less than 90 mm) experienced a weight loss of 7 to 13% of body weight after 35 h of aerial transportation. The same study showed that the omission of ice during 35

h of transportation resulted in a greater mass loss (~13%) than when ice was used (~10%).

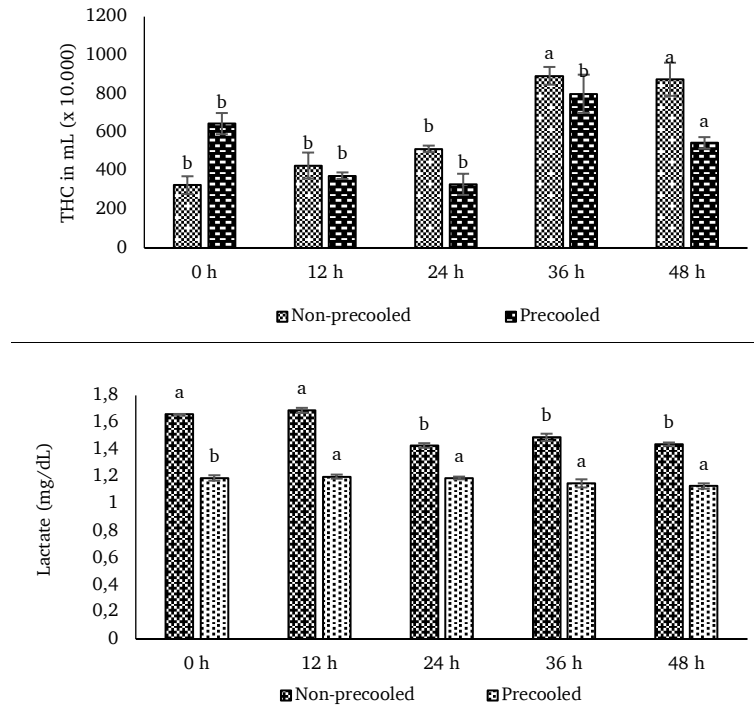


Figure 2. Total haemocyte count (10^4) and lactate level ($\text{mg}\cdot\text{dL}^{-1}$) of abalone under simulated live transport. Bars with different superscript (^{a,b}) indicate significant different ($P < 0.05$).

Air exposure is known to impact the immune response of *H. Laevigata* (Cardinaud *et al.*, 2013). In this current study, haemocyte counts in the non-precooled group increased faster over time than in the pre-cooled group. A sharp increase of haemocyte count in the abalone from the pre-cooled group was only observed after 48 h of transportation, whereas for the non-precooled group, this increase occurred sooner, after 36h. The mean THC for pre-cooled and non-precooled groups increased gradually as the transport period lengthened (Figure 1). A Scheffe test ($\alpha = 0.05$) indicated that there was no significant difference in THC of the pre-cooled group after 0 to 36 h, but there was a significant increase after 48 h. THC levels of the non-precooled group after being subjected to 24 h of simulated transport were not significantly different but were significantly lower than those

measured after simulated transport of 36 and 48 h. The increase of haemocyte cells is suggested to be due to the stimulation of haemocyte relocation from the tissues (Cajaraville *et al.*, 1996), and not by cellular propagation, which results in a different morphological type of haemocyte cell (Cardinaud *et al.*, 2013; Perez and Fontanetti, 2011). No differential haemocyte count data were available for this study, but the difference in haemocyte counts throughout simulated transport indicates that both treatment groups were experiencing high levels of stress.

Lactate concentration was significantly affected ($P < 0.05$) by temperature treatment prior to and during transportation. Lactate can accumulate in the haemolymph during emersion (Lorenzon *et al.*, 2008). (Wells and Baldwin, 1995) found that *H. iris* and *H. australis* demonstrated the significant

elevation of tauropine and D-lactate in the foot and adductor muscles after 24 h of air exposure. When oxygen is limited some animals can partially fulfill energy constraints by using anaerobic metabolism to maintain homeostasis (Urbina *et al.*, 2013). During aerial exposure, the oxygen is impaired, causing abalone to rely on anaerobic metabolism (Malham *et al.*, 2003). This allows energy production (ATP), but at the same time leads to metabolic acidosis (Urbina *et al.*, 2013) and generates by-products of anaerobic metabolisms, such as lactate. The present study clearly shows that lactate production in abalone was affected by temperature treatment prior to transport and transport duration. Abalone from the pre-cooled group exhibited lactate accumulation in the first 12 h. These

results were the same as previous work by (Barrento *et al.*, 2012; Wells and Baldwin, 1995), where elevated lactate levels were detected after air exposure. This indicates that transport simulation affects the metabolic process of abalone. This is likely to have been caused by the abalone being stimulated to commence anaerobic reaction from aerial exposure marked by lactate elevation in the first 12 h and possibly the production of other anaerobic end-products such as tauropine (O'Omolo *et al.*, 2003). The difference in results may be because the different species of abalone in that study were being acclimated to a higher temperature, whereas in this study the focus was on physiological responses during seawater immersion in a different temperature.

Table 1. The phagocytic and lysosomal activity of abalone (mean \pm SE) subjected to non-precooled and pre-cooled treatment under live simulated transport.

Parameters	Periods	Non-precooled	Precooled
Phagocytic assay (%)	0 h	55.21 \pm 0.57 ^a	71.66 \pm 0.88 ^a
	12 h	50.66 \pm 0.88 ^{ab}	57.66 \pm 1.45 ^b
	24 h	49.33 \pm 0.58 ^b	50.17 \pm 1.16 ^{cd}
	36 h	46.33 \pm 1.2 ^{bc}	53.66 \pm 0.88 ^{bc}
	48 h	42.33 \pm 1.2 ^c	46.67 \pm 1.67 ^d
Lysosomal activity (EU.mL ⁻¹)	0 h	33.03 \pm 9.30 ^b	27.79 \pm 3.30 ^c
	12 h	62.25 \pm 5.38 ^a	84.06 \pm 5.58 ^a
	24 h	24.69 \pm 5.98 ^b	52.67 \pm 2.77 ^b
	36 h	12.86 \pm 1.11 ^b	49.68 \pm 1.73 ^b
	48 h	10.42 \pm 0.84 ^b	42.14 \pm 1.23 ^{bc}

Data in a similar column with different superscripts (^{a,b,c}) indicates a significant difference (P<0.05).

Phagocytic activity declined following transport simulation. As transport duration lengthened, phagocytic activity decreased. Although the phagocytic activity for both groups of water treatment before transport decreased significantly compared to the control, the phagocytic activity of the pre-cooled treatment was significantly higher (p<0.05) than those of the non-precooled seawater treatment (Table 1). This result was similar to a study by (Cheng *et al.*, 2004) where air exposure decreased phagocytic activity in mussels. In a study on *H. tuberculata* (Cardinaud *et al.*, 2013), total haemocyte count and the phagocytic rate decreased after 15 minutes of

mechanical stress. The reduction in the phagocytic index following transport simulation may be explained as a decrease in the ability of haemocytes to phagocyte the dead cells. Such an explanation has previously been hypothesized by Cardinaud *et al.* (2013) for European abalone (*H. tuberculata*) during aerial exposure and heat shock. There was a dependent relationship between the aerial transport period and phagocytic activity for both groups. This collaborates with the findings of Pipe and Coles (1995) for mussels (*Mytilus edulis*), namely that phagocytosis can be a sensitive immune marker to aerial exposure.

In the present study, lysosomal assays ranged from 10.42 to 84.06 EU.mL⁻¹. A Kruskal-Wallis test indicated that the median lysosomal assay of non-precooled and pre-cooled treatment groups was significantly different (Asymp.sig=0.012 and 0.011). The median lysosomal assay decreased as the simulated transport duration increased. Neutral red retention assays were performed mostly to measure the membrane function and lysosomal activity in cell cultures (Ivanova and Uhlig, 2008). Neutral red retention has been used more commonly for aerial stressors than lysosomal assay. However, this study was unable to collect the NRR data due to limits of time to observe the exact time when the membrane was failed to retain red dye. This may indicate that the lysosomal assay was not a sensitive parameter for aerial exposure investigation.

CONCLUSION

This study has shown that the pre-cooling live abalone at ± 14 °C before transport can prolong the survival of abalone during shipment. Overall, the stress parameters of the pre-cooled group were stable. Abalone that had not been pre-cooled prior to simulated transportation showed a more significant lactate accumulation than those that had been pre-cooled. Furthermore, a higher proportion in a moribund state during recovery of abalone that had not been pre-cooled prior to simulated transport compared to those that had been pre-cooled. This resulted in them having high mortality at the end of the trial. The present study also clearly shows that both temperature treatment prior to transport and transport duration affects physiological conditions during recovery, and pre-cooling treatment before transport has a beneficial effect on abalone during recovery.

ACKNOWLEDGMENT

Foremost, We would like thanks to Australia Award Scholarship program as

the sponsor of the first author. We also would like to express our gratitude to Rowan Kleindienst and all staff for technical assistance at Curtin Aquatic Research Laboratory.

REFERENCES

- ACA (Abalone Council Australia), 2013. *A Quality Assurance Code of Practice for the Australian Wild Abalone Fishery*. http://www.abalonecouncil.com.au/public/69/files/Australian-Wild-Abalone-Quality-Assurance-Code-of-Practice-Master-Manual_June-2013-compressed.pdf
- Baldwin, J., Elias, J.P., Wells, R.M.G. and Donovan, D., 2007. Energy metabolism in the tropical abalone, *Haliotis asinina* Linné. *Journal of Experimental Marine Biology and Ecology*, 342(2), pp.213-225. <https://doi.org/10.1016/j.jembe.2006.09.005>
- Barrento, S., Marques, A., Vaz-Pires, P. and Nunes, M.L., 2012. Physiological changes during simulated live transport of *Cancer pagurus* and recovery in holding tanks. *Aquaculture Research*, 43(10), pp.1415-1426. <https://doi.org/10.1111/j.1365-2109.2011.02943.x>
- Bubner, E.J., Harris, J.O. and Bolton, T.F., 2009. Supplementary oxygen and temperature management during live transportation of greenlip abalone, *Haliotis laevigata* (Donovan, 1808). *Aquaculture Research*, 40(7), pp.810-817. <http://doi.org/10.1111/j.1365-2109.2009.02167.x>
- Cardinaud, M., Offret, C., Huchette, S., Moraga, D. and Paillard, C., 2013. The impacts of handling and air exposure on immune parameters, gene expression, and susceptibility to vibriosis of European abalone *Haliotis tuberculata*. *Fish & Shellfish Immunology*, 36(1). <https://doi.org/10.1016/j.fsi.2013.09.034>
- Cheng, W., Hsiao, I.S., Hsu, C.H. and Chen, J.C., 2004. Change in water temperature on the immune

- response of Taiwan abalone *Haliotis diversicolor* supertexta and its susceptibility to *Vibrio parahaemolyticus*. *Fish & Shellfish Immunology*, 17(3), pp.235-243. <https://doi.org/10.1016/j.fsi.2004.03.007>
- Day, R., Hooper, C., Berkendorff, K., Slocombe, R. and Handlinger, J., 2010. *Investigations on the immunology of stressed abalone* (FDRC Project No.2004/233). Retrieved from Melbourne.
- Ivanova, L. and Uhlig, S., 2008. A bioassay for the simultaneous measurement of metabolic activity, membrane integrity, and lysosomal activity in cell cultures. *Analytical Biochemistry*, 379(1), pp.16-19. <https://doi.org/10.1016/j.ab.2008.04.035>
- Lorenzon, S., Giulianini, P.G., Libralato, S., Martinis, M. and Ferrero, E.A., 2008. Stress effect of two different transport systems on the physiological profiles of the crab *Cancer pagurus*. *Aquaculture*, 278(1-4), pp.156-163. <https://doi.org/10.1016/j.aquaculture.2008.03.011>
- Malham, S.K., Lacoste, A., Gélébart, F., Cueff, A. and Poulet, S.A., 2003. Evidence for a direct link between stress and immunity in the mollusc *Haliotis tuberculata*. *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, 295A(2), pp.136-144. <https://doi.org/10.1002/jez.a.10222>
- O'Omolo, S., Gäde, G., Cook, P.A. and Brown, A.C., 2003. Can the end products of anaerobic metabolism, tauroxine and D-lactate, be used as metabolic stress indicators during transport of live south African Abalone *Haliotis midae*? *African Journal of Marine Science*, 25(1), pp.301-309. <https://doi.org/10.2989/18142320309504019>
- Perez, D.G. and Fontanetti, C.S., 2011. Hemocytical responses to environmental stress in invertebrates: a review. *Environmental Monitoring Assessment*, 177, pp.437-447. <https://doi.org/10.1007/s10661-010-1645-7>
- Pipe, R.K. and Coles, J.A., 1995. Environmental contaminants influencing immunefunction in marine bivalve molluscs. *Fish & Shellfish Immunology*, 5(8), pp.581-595. [https://doi.org/10.1016/S1050-4648\(95\)80043-3](https://doi.org/10.1016/S1050-4648(95)80043-3)
- Ragg, N.L.C. and Watts, E., 2015. Physiological indicators of stress and morbidity in commercially handled abalone *Haliotis iris*. *Journal of Shellfish Research*, 34(2), pp.455-467. <https://doi.org/10.2983/035.034.0229>
- Setyaningsih, S.R., Fotedar, R., Melville-Smith, R., Saputra, I., Fernandez, N. and Adhikari, S., 2020. The effects of transportation on immune modulation of wild and ocean-ranched greenlip abalone (*Haliotis laevigata*). *Indonesian Aquaculture Journal*, 15(1), pp.33-41. <http://dx.doi.org/10.15578/iaj.15.1.2020.33-41>
- Song, L., Li, X., Bott, K., Wang, T., Clarke, S. and Zhao, W., 2007. Effects of air exposure on the lysosomal membrane stability of haemocytes in blacklip abalone, *Haliotis rubra* (Leach). *Aquaculture Research*, 38(3), pp.239-245. <https://doi.org/10.1111/j.1365-2109.2007.01655.x>
- Urbina, M.A., Paschke, K., Gebauer, P., Cumillaf, J.P. and Rosas, C., 2013. Physiological responses of the southern king crab, *Lithodes santolla* (Decapoda: Lithodidae), to aerial exposure. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 166(4), pp.538-545. <https://doi.org/10.1016/j.cbpa.2013.08.006>
- Wells, R.M.G. and Baldwin, J., 1995. A comparison of metabolic stress during air exposure in two species of New Zealand abalone, *Haliotis iris* and *Haliotis australis*: implications for the handling and shipping of live

animals. *Aquaculture*, 134(3-4),
pp.361-370. [https://doi.org/10.1016/0044-8486\(95\)00027-Y](https://doi.org/10.1016/0044-8486(95)00027-Y)

Wingerter, K., Betty, M. and Miller-Morgan, T., 2013. *Development of live shellfish export capacity in Oregon*. Oregon State University.