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The metabolic fate of abalone: transport and recovery of *Haliotis iris* gills as a case study

Leonie Venter [a], Andrea C. Alfaro [a], Jeremie Zander Lindeque [b] and Peet J. Jansen van Rensburg [b]

[a]Aquaculture Biotechnology Research Group, School of Science, Auckland University of Technology, Auckland, New Zealand; [b]Human Metabolomics, North-West University, Potchefstroom, South Africa

ABSTRACT

Abalone is a gourmet seafood with a high commercial value, particularly when obtained as a live product. During live transportation, abalone encounter stressors causing biochemical modifications to tolerate the changes. Using semi-targeted metabolomics, this study characterised the left and right gill metabolite profiles of Blackfoot abalone, *Haliotis iris*, following transportation (48 h) and recovery (48 h). This study reports the association between left and right gill metabolites, to enhance our physiological understanding of the interplay between gills. The left gill metabolites are mainly active following transportation, while both gills partake in the metabolite response following recovery. Transportation necessitated increased metabolites linked to the glycolysis pathway, the Krebs cycle, amino acid, and nucleotide metabolism, for energy production, achieved via aerobic and anaerobic pathways. The recovery phase supported the replenishing of glycogen, triglycerides, and protein stores, albeit metabolic homeostasis was not achieved following two-days of water immersion recovery. This study showcases the well-adapted metabolic mechanisms implemented by *H. iris* in response to transportation stress and show that metabolites are in the process of returning to the same concentrations as measured pre-transport stress. The findings herein can be applied to improve animal health during transport and subsequent survival, which in-effect supports profitability.

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Introduction

Abalone (*Haliotis* spp.) are one of the most valuable fishery products in tropical and temperate waters around the world (Gao et al. 2023). Typically, these marine molluscs are sold as frozen, canned, dried, cooked or fresh (live) products (Bagarinao et al. 2020). Live markets tend to receive the largest animals at a premium price (Suleria et al. 2017; Maulidya et al. 2021), necessitating the best quality product. The price of live

CONTACT Andrea C. Alfaro andrea.alfaro@aut.ac.nz
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abalone of various species in the international market can range from US$60-80 per kilogram (personal communication). Various methods to minimise stress during abalone transport have been investigated, such as the supplementation of oxygen and temperature management (Bubner et al. 2009), supplemental humidity (Sawangwong et al. 2019), the application of anaesthetics (Yasa et al. 2021) and pre-transport cooling treatments (Kurnaningtyas et al. 2022). Generally, during live transport, abalone are placed in insulated humified containers which are often supplemented with oxygen (Brown et al. 2008). As a result of transport stress there is a reduction in the quality of the live product [i.e. change in colour, water content, texture, taste and smell (Morash and Alter 2016)], subsequently reducing the market value (Wingerter et al. 2013). Mortalities encountered along the transport chain also affect the profitability (Kurnaningtyas et al. 2022). A series of physiological responses, such as changes within respiration, metabolism and reproduction occur due to transport stress, which in effect make abalone more vulnerable to disease or morality (Moltschaniwskyj et al. 2014). Abalone have physiological functions to tolerate periods of hypoxia in water and air (Morash and Alter 2016), without harmful effects, yet they have minimal resistance to air exposure (Song et al. 2007).

Metabolomics is a research tool, which can be applied to identify the metabolic pathways underlying abalone responses in the face of environmental stressors, such as oxygen poor conditions (i.e. transport or hypoxia) (Nguyen et al. 2022b). For example, metabolites from muscle and haemolymph metabolomic analyses revealed osmoregulation and oxidative stress functions in *H. iris* post-transport (Alfaro et al. 2021). Then again, metabolites from gill tissues from *H. fulgens* (Tripp-Valdez et al. 2017), and *H. midae* (Venter et al. 2018a) showed a higher energy demand following environmental hypoxia. Transport of *H. lavigata* resulted in significant increases of lactate (Kurnaningtyas et al. 2022), supporting previous findings made in *H. midae* where both lactate and tauropine were considered to be indicators of abalone condition during transport (O'omolo et al. 2003).

Despite reasonable advances made within the field of metabolomics and the application to abalone research, we have a limited understanding of the metabolic flexibility of abalone gills under transport stress. Abalone contain a pair of bipectinate gills with elaborate cartilaginous support (Ragg and Watts 2015), which are in direct contact with seawater, making them a target tissue for adhesion of bacteria (Pichon et al. 2013), but also a source of organic compounds to the host (Mizutani et al. 2020). Abalone gills play a crucial role when oxygen levels vary greatly, for example under unstressed conditions the right gill is generally perfused, while the left gill is chronically under-perfused. In scenarios of increased oxygen demand (stressed conditions), perfusion rates in the left gill increase to effectively support the metabolic scope of abalone (Ragg and Taylor 2006). Arguably the gills are a valuable tissue to infer physiological responses and advance our understanding of the interplay between the gills under normal, stressed and recovery conditions. Additionally, research focusing on whole animal tissue investigations should take note of the different forms and functions of abalone gills and aim to clearly report which gill is under investigation.

Ultimately, abalone that are less stressed during transport, recover faster post-transport and easily move to the next step of the market chain (Moltschaniwskyj et al. 2014). If we know how abalone respond during transport, and what the recovery phase looks like, we can intervene, by administering feed or enhancing the water

quality to support optimal organismal functioning. This study was carried out to measure the metabolite response of *H. iris* left and right gills following transport and recovery. This research has application to the abalone aquaculture industry, as it has the potential to improve animal health during transport and subsequent survival, which in-effect supports profitability.

Methods

Field sampling

Wild adult abalone of similar size were collected from Ascots Beach, Chatham Islands, New Zealand (44°00'59.0"S 176°23'11.7"W) by commercial divers under special permit (720, client number 9791209) issued by Fisheries New Zealand. On site, 10 abalone (control group) were weighed and measured, shucked and both left and right gill tissues were collected by making an incision in the mantle on the left side, under the shell pores. The left and right gills were individually removed with forceps from the left and right wall of the mantle cavity, respectively. All gill tissues were placed in individual microcentrifuge tubes and snap frozen using liquid nitrogen. All samples were shipped to the laboratory on dry ice and stored at −80°C until metabolomics analyses. A group of 30 abalone were transported from the collection site to a holding facility (1 h travel time) and placed in an on-site flow-through system for interim housing (48 h), whereafter the animals were packed in 4 polystyrene containers (10 animals per 50 L container; humid air 6°C) and airfreighted to Auckland. Following 48 h of transport, a subgroup of 10 abalone (transport group) were sampled upon arrival at the laboratory, as described above. The remaining animals were placed into a recirculating seawater system at Auckland University of Technology for 48 h. After two days of recovery (without being fed), 10 abalone (recovery group) were removed from the system and sampled as described above. No mortalities or unusual abalone behaviour were observed during both the transport and recovery phases of this study.

Metabolomics analyses

Frozen gill tissues were freeze-dried overnight and ground using a mortar and pestle. Approximately 10 mg of gill tissue were co-extracted with an internal standard (2-acetomidophenol, final concentration of 20 µg/mL) using a two-step methanol:water pre-blend extraction solvent mixture, resulting in extracts for liquid chromatography tandem mass spectrometry (LC-MS/MS) analyses. Quality control (QC) samples were prepared by pooling a mixture of gill tissue and analysed amongst the biological samples to measure repeatability and to identify any potential batch effects in the data. Metabolomics analyses were performed on an Agilent 1260 LC coupled to an Agilent 6470 triple quadrupole (QQQ) mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Agilent MassHunter Workstation Data Acquisition (V 10.0) was used for compound calibration and data acquisition with reference to the Agilent's G6412AA Metabolomics dynamic multiple reaction monitoring (MRM) database and method. The LC-MS/MS data were pre-processed with Agilent MassHunter Workstation QQQ Quantitative Analysis Software (V 10.0) (Azizan et al. 2023). Two unique transitions

were monitored per individual metabolite to provide spectral matching in addition to retention time, resulting in metabolite identities with the highest level of confidence (Sumner et al. 2007; Schymanski et al. 2014). Data were normalised using the internal standard and generalised log transformed (Venter et al. 2022).

Statistical analyses

The online webserver MetaboAnalyst (https://www.metaboanalyst.ca) was used to detect metabolic differences between the experimental groups per tissue (Chong et al. 2018). QC samples were assessed in terms of coefficient of variance percentages and data were evaluated for within batch effects. Focusing on a single tissue at a time, the control group was compared to the transport group, followed by comparisons of the control group to the recovery group. Univariate analyses (one-way ANOVA) were used to detect metabolites with statistical significance (Ellis and Steyn 2003) [p-value <0.05 (false discovery rate ≤ 0.1)], while the effect size was calculated to ensure practical significance [d-value >0.8, calculated by determining the absolute difference between the means of the two groups divided by the maximum standard deviation of the two groups (Venter et al. 2018b)]. Metabolites of importance are listed in the supplementary data. Multivariate analyses were utilised to provide an overview of the metabolic changes and covariance via principle component analyses (PCA) (Venter et al. 2021). An UpSet plot was generated using the Intervene online tool (https://asntech.shinyapps.io/intervene/) (Khan and Mathelier 2017). Schematic representations of the metabolite response in both the left and right gills post-transport and post-recovery were manually generated using the significantly detected metabolites.

Results

The abalone utilised for metabolomics analyses (n = 30) had an average shell length of 129 ± 5 mm (mean ± SD) and wet weight of 437 ± 45 g (mean ± SD). In total, 10 males and 20 females were identified based on gonad colour. Sex and size were investigated as confounding factors, but the metabolic response showed no differences between male and female abalone, or between weight and length measures (results not shown). From the LC-MS/MS analyses, a total of 205 metabolites were detected (Supplementary file 1). A total of 52 metabolites were significantly affected in the left gill and two metabolites in the right gill tissues, collected from the transported abalone group. Within the recovery group, 19 metabolites were detected as significant in the left gill tissue and 18 metabolites in the right gill tissue (Table S1). The total number of significantly affected metabolites across treatments are illustrated by the Upset plot in Figure 1D. The upset plot displays the metabolites uniquely detected in a specific group. For example, a total of 40 metabolites were detected only in the left gill of the transport group. Within the recovery groups, the right gill showed nine metabolites and the left gill eight metabolites only detected in that group. Also, metabolites detected in multiple groups are linked together. An overview of the significantly different metabolites (rows) is depicted by the 2D colour heatmap, grouping the average metabolite abundance per treatment (columns). Colouring varies from high (red) to low (green) metabolite responses using an online numeric scale from 2 to −2 (Figure 1A). The PCA score

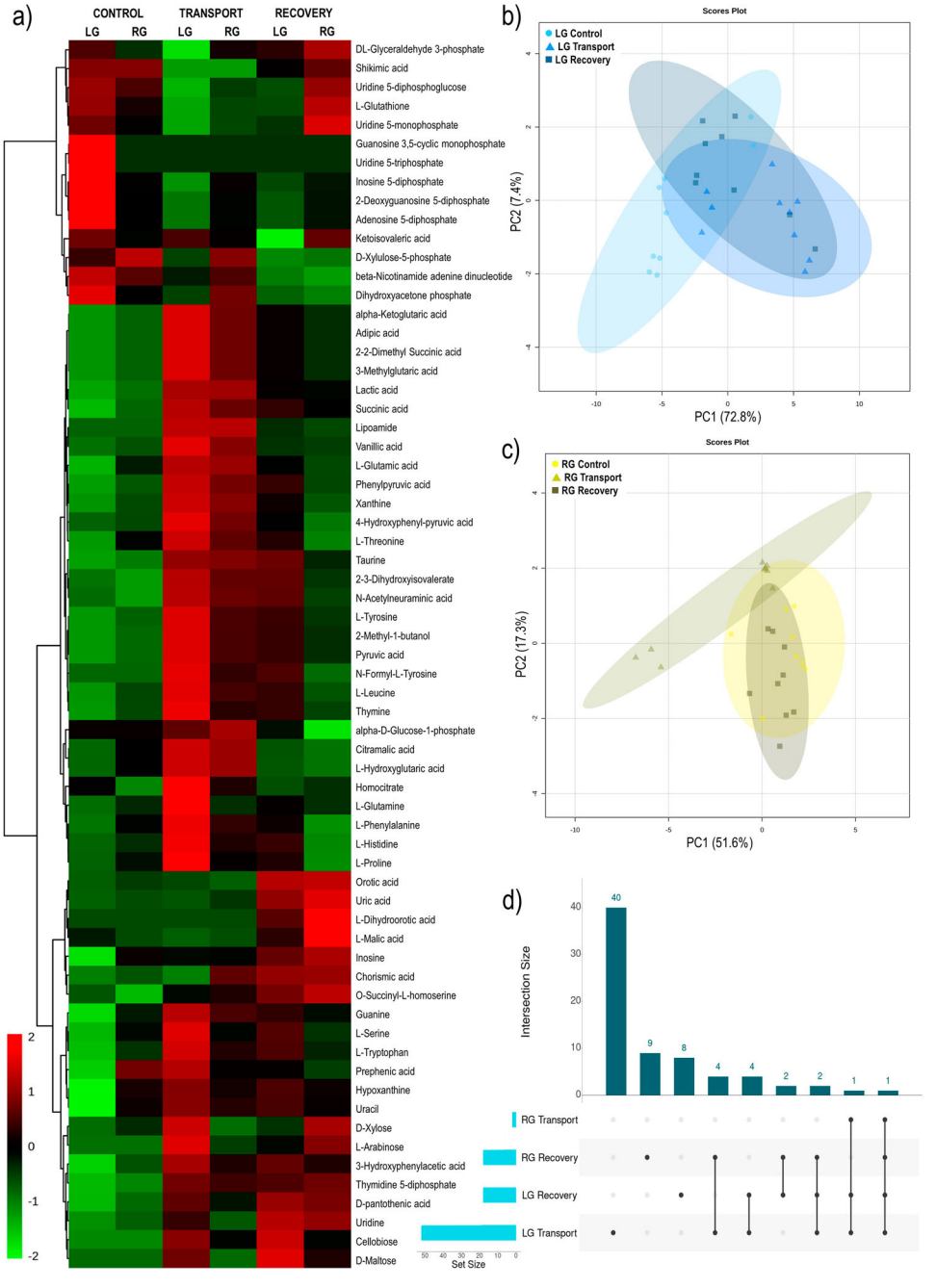

Figure 1. The metabolic response of left, and right gills of abalone subjected to transport and subsequent recovery. **A**, Heatmap visualisation of significant metabolites per treatment (red – high, green – low metabolite concentrations), **B**, PCA score plots of the metabolite groupings from the control treatment (●), transport treatment (▲) and recovery treatment (■), in the left gill, **C**, PCA score plots of metabolite groupings in the right gill, and **D**, Upset plot presenting the overall number of metabolites detected (intersection size) between the different treatments, along with the number of metabolites within a specific group (set size).

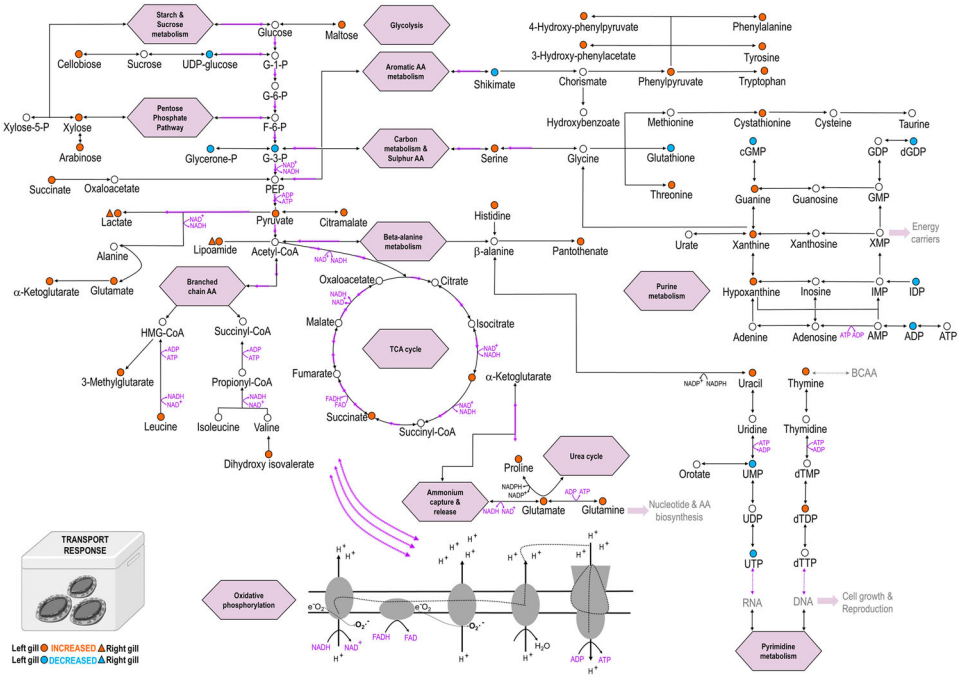

Figure 2. Metabolic overview of the effect of transport on the metabolism of *H. iris*, as detected in both the left (●) and right (▲)gills as an increase (blue) or decrease (orange) in metabolite response. Other metabolites (with white symbols) serve as general links within the metabolic overview and were not of significance for this study.

plots of left gill (Figure 1B) treatment groups show a larger overlap between the transport and recovery treatments, while the score plots from the right gill (Figure 1C) shows larger grouping of the control and recovery group. In both instances PC1 accounts for the largest variation in the data, namely 72.8% within the left gills and 51.6% in the right gills. The metabolite response implemented by abalone following transport (Figure 2) and a recovery period (Figure 3) involves a range of metabolic pathways which include starch and sucrose metabolism, the glycolysis pathway, the pentose phosphate pathway, aromatic amino acid metabolism, the central carbon metabolism, sulphur containing amino acids, branched chain amino acids (BCAA), lysine metabolism, ß-alanine metabolism, purine metabolism, pyrimidine metabolism, the tricarboxylic acid cycle (TCA), the urea cycle and oxidative phosphorylation.

Discussion

When confronted with oxygen poor conditions, abalone implement physiological modifications, such as an increase of oxygen uptake or an increase of oxygen transport to tissues as survival mechanisms (Shen et al. 2021). The metabolic modifications implemented by *H. iris* following an oxygen deficit transport scenario with subsequent recovery is herein reported, focusing on how the left and right gills support metabolic outputs.

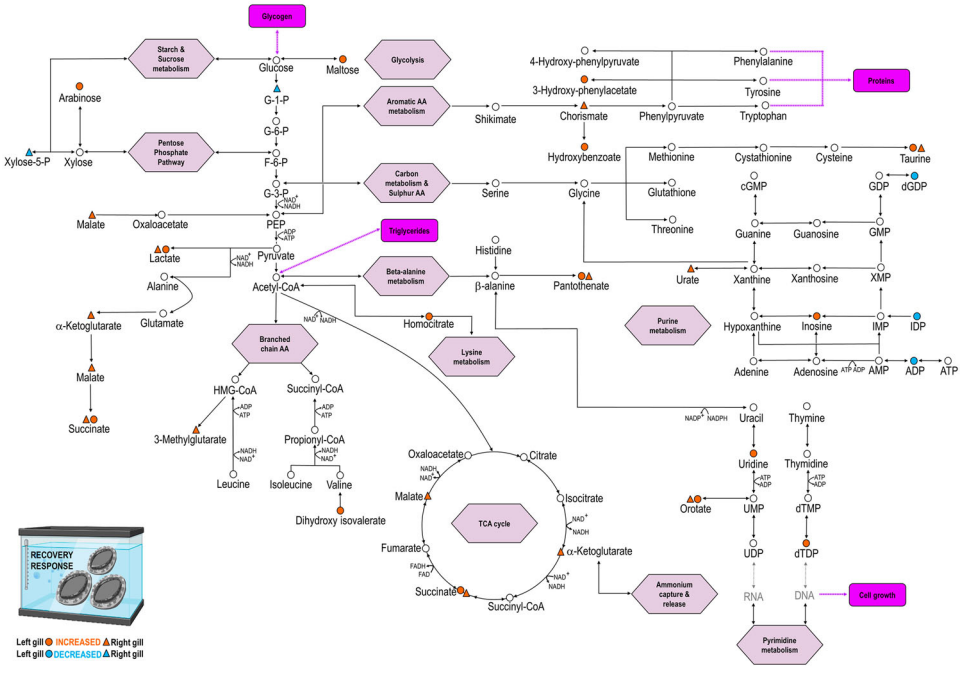

Figure 3. Metabolic overview of the metabolism of *H. iris* following a recovery period, as detected in both the left (●) and right (▲) gills as an increase (blue) or decrease (orange) in metabolite response. Other metabolites (with white symbols) serve as general links within the metabolic overview and were not of significance for this study.

It is common for abalone physiological studies to report the response of abalone gills as a whole (as opposed to left and right gill separately), as seen in *H. diversicolor* exposed to thermal and hypoxic stresses (Lu et al. 2016), *H. fulgens* exposed to hypoxia and hypercapnia (Tripp-Valdez et al. 2017) and *H. discus hannai* X *H. fulgens* exposed to thermal stress (Zhang et al. 2022). Yet, from a physiological viewpoint, abalone gills show diverse responses (Ragg and Taylor 2006). Under standard conditions, abalone utilise primarily the right gill for oxygen uptake, with the left gill only utilised during periods of hypoxia (oxygen need) to enhance the surface area and successive oxygen uptake (Morash and Alter 2016). The implementation of the left gill under anaerobic conditions is again confirmed in the current study when evaluating the metabolomics data. Firstly, the number of metabolites significantly affected was the highest in the left gill of abalone subjected to transport (Figure 1D). A similar response has been previously described in *H. midae* where hypoxia resulted in more metabolic changes within the left gill than in the right gill tissue (Venter et al. 2018c). Secondly, most of the metabolic changes in the left gill of the abalone within the transport group resulted in increased metabolite levels (Figure 1A), supporting either a buildup of metabolic intermediates or increased influx (breakdown) of metabolites to support energy homeostasis. Likewise, both haemolymph and muscle samples of *H. iris* showed increased metabolite concentrations in a group of abalone subjected to transport stress (Alfaro et al. 2021). Next, the metabolites of significance detected in the left gill showed a larger overlap in the PCA score plots between the transport and recovery groups, than between the transport and control

groups (Figure 1B). Indeed, this can indicate that the metabolic changes implemented by the left gill during transport has not returned fully to pre-transport concentrations after recovery have been allowed. In contrast, when considering the response of the right gill tissue, the recovery and control groups are grouped together hinting at recovery to control concentrations (Figure 1C). Arguably, the metabolites within the left gill were more affected by transport (hypoxia) than those of the right gill. Lastly, the changes within specific sections of metabolism are largely ascribed to significant changes within the left gill during transport (Figure 2), while both the left and right gill attributed to the response recorded during recovery (Figure 3).

The metabolic response of H. iris *gills due to transport stress*

The process of transportation leaves abalone with limited available oxygen, which subsequently affects energy metabolism, as previously reported in *H. iris* (Alfaro et al. 2021). In the current study, abalone utilised various metabolic pathways to ensure energy supply as a vital mechanism to survive the period of transport stress (oxygen need) (Venter et al. 2018d). An increase in metabolites (mainly sugars – maltose, cellobiose, xylose, and amino acids – serine, threonine, phenylalanine, tyrosine, tryptophan) feeding into the glycolysis pathway enabled the regeneration of cytoplasmic nicotinamide adenine dinucleotide (NAD^+) for the synthesis of adenosine triphosphate (ATP) directly from the phosphorylation of adenosine diphosphate (ADP) (Venter et al. 2018a). Also, the accumulation of TCA cycle intermediates (α-ketoglutarate and succinate) suggest an influx of metabolites to assist with the generation of NADH and flavin adenine dinucleotide ($FADH_2$), which transfer electrons to the electron transport chain with ATP generation as an outcome. The increase of TCA cycle intermediates can also be linked to a disruption in aerobic metabolism as previously reported in heat-stressed *H. iris* (Nguyen et al. 2022a). Hence, a shift between aerobic and anaerobic metabolism comes into play to avoid a metabolic shutdown and ensures ATP production. Typically, during periods of oxygen need, energy levels are somewhat sustained by reducing energy consuming reactions and also by activating oxygen independent metabolic pathways, such as phosphagen breakdown and anaerobic glycolysis (Venter et al. 2018a). Results from the current study do not show the breakdown of phosphagens as a means to fulfil energy constraints, but the implementation of anaerobic metabolic pathways to maintain cellular homeostasis are supported within *H. iris* following transportation.

The accumulation of lactate and succinate, herein reported, support the onset of anaerobic metabolism as described from metabolomics studies performed on *H. diversicolor* (Lu et al. 2016), *H. fulgens* (Tripp-Valdez et al. 2017), *H. midae* (Venter et al. 2018b), *H. iris* (Alfaro et al. 2021) and *H. laevigate* (Kurnaningtyas et al. 2022). In this context, accumulated pyruvate is converted to lactate (increased in both the left and right gills) producing NAD^+ to support anaerobic glycolysis (Venter et al. 2018d). An elevated NADH:NAD ratio is expected in hypoxic conditions due to the slowed electron transport chain activity. Then again, the elevated NADH levels and succinyl-CoA (detected only in hydrolysed form) inhibit citrate synthase (and 2-ketoglutarate dehydrogenase), leading to elevated pyruvate levels. Increased pyruvate can also couple to amino acids to produce imino acid derivatives (opines), resulting in the regeneration of cytoplasmic NAD^+ to support anaerobic glycolysis (Müller et al. 2012), albeit no opine

mass spectral transitions were monitored in the dynamic MRM method and is thus not included in the current study. Furthermore, the glucose-succinate pathway, using the reversal of the second half of the TCA also supports the synthesis of NAD^+ in marine invertebrates (Harcet et al. 2013), like abalone, and is highlighted as another mechanism utilised by *H. iris* to sustain energy production under stress. Resultantly, the metabolites detected in the left gill of *H. iris* under investigation are utilised to produce energy to support organismal survival during transport.

Apart from the energy producing pathways emphasised by the left gill metabolites, changes within amino acid metabolism are also showcased in the current investigation. Abalone cells are rich in amino acids, as seen in *H. discus hanna*i (Shen et al. 2021), and *H. iris* (Alfaro et al. 2021), where amino acids were regarded as metabolic fuels and are important for intracellular osmotic regulation. Free amino acids, such as glutamine and glutamate (Hatae et al. 1995), detected as increased in the current study, can fulfil osmoregulation roles, but can also serve as an energy source during periods of stress (Rosenblum et al. 2006), such as hypoxia induced by transportation. Glutamate ensures various metabolic processes, such as protein and cofactor synthesis, nitrogen assimilation and the production of nucleosides and amino acids (Walker and Van Der Donk 2016). Glutamate, along with other metabolites of the glutamate family (glutamine, proline, histidine, α-ketoglutarate) were all increased in the current study in the left gill of *H. iris* following transportation. The formation of glutamate results in an α-amino group which supports alanine production as a vital metabolite end-product during hypoxia (Fujimori and Abe 2002). Glutamate in turn yields glutamine, which is a precursor for the synthesis purine and pyrimidine bases (Ding et al. 2011). Proline can also be synthesised from glutamate and supports cell structure, anti-oxidative reactions along with energy metabolism (Venter et al. 2019). The upregulation of this part of the metabolism in *H. iris* likely provides carbon and nitrogen towards the biosynthesis of diverse precursors (Zhang et al. 2017), which also contributes to ATP production during transport stress.

Another metabolite related to osmolarity is succinate (increased in the left gills under investigation) as also seen in *H. diversicolor supertexta*. Interestingly, increased succinate reported in *H. diversicolor supertexta* is said to reflect an increase of reactive oxygen species (ROS) production (Zhou et al. 2015). The notion of ROS production in the current study is further supported when considering changes within the glutathione metabolism (glutathione, glutamate, cystathionine). Generally, glutathione can act as an antioxidant molecule, which scavenges excessive ROS production (Herath et al. 2017). Considering the decrease in glutathione, it can reflect the depletion (degradation or oxidation) thereof in the gills of *H. iris* subjected to transportation. Opposingly, an increase in glutathione was detected in the muscle of *H. iris* subjected to transport (Alfaro et al. 2021), demonstrating the varying functionality and metabolism of different tissues, as previously reported in *H. midae* (Venter et al. 2018c). Additionally, tyrosine is also seen as an antioxidant, where elevated tyrosine in this study may support *H. iris* to avoid cell damage in response to transportation (Li et al. 2019). The accumulation of amino acids detected in this study might also be associated with the storage of precursors for protein synthesis to prepare for rapid recovery following transportation.

Changes within the left gill metabolites were additionally evident in the purine and pyrimidine metabolic pathways. Broadly, purines serve as metabolic signals to provide

energy, support cell growth, and contribute to coenzymes and phosphorylation reaction. Then again, pyrimidines partake in detoxification processes and the production of lipids and proteins (Fumagalli et al. 2017). Indeed, the synthesis of nucleotides require energy consumption (Zeng et al. 2022), a process which is already compromised in *H. iris* subjected to transportation. Resultantly, decreased equivalents of purine (cGMP, dGDP, IDP, ADP) and pyrimidine (UMP, UTP) metabolites were detected in the current study, suggesting an increased use of these metabolites to support cell maintenance and to tolerate transport stress. Then again, purine bases (guanine, xanthine, hypoxanthine) were increased in the transport group reflecting a possible decrease in purine salvage reactions (Zhu and Thompson 2019). Research on other marine invertebrates has shown that purines are important for the survival of hypoxic scenarios, allowing a pathway for ATP degeneration (Haider et al. 2020). Thus, the use of purines to support cell proliferation (likewise ATP production and survival) are herein reflected in *H. iris* following transportation induced hypoxia. Similarly, increased pyrimidine bases (uracil and thymine) were also detected in *H. iris* as in the case of purines, likely for use in nucleic acid biosynthesis or use in carbohydrate and lipid metabolism (Okesli et al. 2017). Yet, decreased pyrimidine nucleotides (UMP, UTP) hints at the usage of these for the formation of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) (Huang and Graves 2003), which ultimately supports cell growth and the transfer of genetic material to proteins.

The metabolic response of H. iris *gills following a recovery period*

The recovery period post-transport (hypoxia) is essential to ensure that energy reserves are restored and that accumulated metabolic end-products are removed from organisms (Maciel et al. 2008). Yet, the recovery phase may also pose a scenario of oxidative damage due to reoxygenation which might hinder the recovery proses (Welker et al. 2013). The results from this research indicate that the metabolism of *H. iris* did not fully recover after 48 h, with the concentration of many metabolites in both the left and right gill increased. These increased metabolites support mechanisms to recharge energy, protein, and triglyceride stores, while also promoting cell growth. The processes of anaerobic end-product removal are well documented within invertebrates (Ellington 1983). Yet, in the current investigation, both lactate and succinate end-products were increased in both the left and right gills following recovery. Apart from indicating that a level of anaerobiosis is still being experienced as these metabolites have not returned to pre-transport levels (Zammit et al. 1978), it should be highlighted that lactate and succinate can fulfil metabolic replenishing functions. For example, lactate can serve as a gluconeogenic metabolic precursor (Ellington 1983) to support gluconeogenesis in the hepatopancreas, as previously reported in *H. diversicolor supertexta* (Zhou et al. 2010). Increased succinate in the recovery group can be utilised to support oxaloacetate formation (using the upregulated malate), which in turn can be used as a precursor for the biosynthesis of amino acids, purines and pyrimidines (Fasulo et al. 2012). Other studies on the metabolic recovery of abalone also describe the restoration of energy sources and removal of end-products following exposure to stressors. For instance, following recovery from anaerobiosis, the adductor and foot muscle of *H. lamellose*, showed energy charge and phosphagen pool levels similar to an aerobic state (Gäde 1988). Furthermore, the haemolymph of *H. iris* following

a recovery period post transportation showed reduced lactate and succinate (Alfaro et al. 2021). Also, in the haemolymph of *H. discus hannai*, glucose levels decreased during the recovery phase following exposure to various environmental stressors (Lee et al. 2023).

Curiously, the abundance of increased amino acids seen during the transport phase was not present in the recovery group, indicating that amino acid concentrations have recovered during the recovery period. Yet, metabolites which serve as precursors of certain amino acids or support amino acid biosynthesis were detected as increased in this study. Aromatic amino acids, such as tyrosine, phenylalanine and tryptophan partake in protein synthesis (Parthasarathy et al. 2018). Considering that their precursors, 3-hydroxy-phenylacetate, chorismite and hydroxybenzoate were increased in the recovery group, it can be reasoned that amino acids are being utilised for protein synthesis in *H. iris* during recovery. A similar outcome is seen when accessing the metabolic changes within branched chain amino acids (BCAA). Here, 3-methylglutarate and dihydroxy isovalerate were increased in the recovery group of abalone, likely to assist with the oxidation of leucine and valine (Neinast et al. 2019). Additionally, BCAA are transaminated with α-ketoglutarate (also increased in the current study) to yield glutamate (Mann et al. 2021), which is a key component of nitrogen removal for all amino acids and support glutamine production, and in effect purine and pyrimidine synthesis (Wu 2009). As for amino acids themselves, taurine was an increased amino acid detected in both gills of the recovery group. Taurine has been described as the most abundant amino acid in abalone (Yun et al. 2021), and likely contributes to osmoregulatory functions as previously described in *H. rufescens* (Rosenblum et al. 2005). The possibility of taurine being released from the anaerobic end-product tauropine when metabolic homeostasis is achieved remains a possibility and an interesting question for future research.

The metabolites which were decreased from the recovery group are linked to glucose and purine metabolism. The reduced sugars (xylose-5-P, G-1-P) can indicate that glycogen stores have been depleted following transportation and that during recovery, these metabolites are still being utilised to fill the stores. Glycogen has been described as an energy store in *H. discus hannai* (Koyama et al. 2020) and *H. diversicolor* via glucogenic amino acids (Lu et al. 2016). Considering that ATP cannot easily be stored in cells, carbon sources for ATP production by means of glycogen or triglycerides are required for energy maintenance (Bonora et al. 2012). The increased maltose and arabinose detected in the current study feeds into the glycogen pathway and can be linked to a glycogen replenishing function. Branching to acetyl-CoA, pantothenate (vitamin B5) were detected as elevated in both the left and right gill of *H. iris*. The biological importance of pantothenate is linked to fatty acid oxidation, carbohydrate metabolism, pyruvate degradation, amino acid catabolism and more (Sampedro et al. 2015). Yet, from the current study, increased pantothenate likely supports fatty acid elongation, thereby ensuring energy storage as triglycerides, which have arguably been depleted following transportation.

The depletion of ATP sources, typically as seen in the current study following transportation can activate the purine metabolic pathway, with uric acid (increased) produced as an end product (Furuhashi 2020). Uric acid has been described as an antioxidant and radical scavenger in humans (Wright 1995), but the role hereof in abalone has not been clearly defined. Additionally, inosine was increased potentially as a product of purine degradation, indicating that a high rate of ATP turnover (Pechlivanis et al. 2010) is

still present after the recovery period. Additionally, decreased concentrations of dGDP, IDP and ADP were recorded in the left gill of *H. iris*, likely as these were utilised by *de novo* synthesis pathways that are regenerating following transportation stress (Hossain et al. 2020). When considering pyrimidine metabolism, the metabolites orotate, uridine and dTDP were increased in the recovery group of abalone. The functions of these metabolites can be linked to increased pyrimidine synthesis and assist with pyrimidine recycling (Löffler et al. 2015). Ultimately, purine and pyrimidine nucleotides are directly involved in nucleic acid synthesis, which in effect provides an energy source for the synthesis of carbohydrates, lipids, peptides and secondary metabolites (Das et al. 2017). From the current metabolomic results, it is evident that transportation affected *H. iris* DNA and RNA turnover and repair, which is still compromised after 48 h of recover.

Conclusions

In this study, we applied metabolomics as a research tool to examine the metabolic response of the left and right gills of *H. iris* following a period of transportation and subsequent recovery. The process of live transportation and corresponding biochemical adaptations are unavoidable when shipping live product to international markets. This study provides metabolic evidence that the left gill of *H. iris* is predominantly active during transportation stress, mainly to ensure the production of energy via aerobic and anaerobic metabolic pathways. Changes in amino acid metabolites possibly assisted with intracellular osmolality, served as precursors for ATP production, and supported antioxidant functions. Transport stress also affected purine and pyrimidine metabolites possibly to increase nucleotide synthesis. The two-day recovery period allowed in this study was not sufficient time to ensure metabolic homeostasis to pre-transport conditions, with both the left and right gill of *H. iris* increasing metabolite concentrations to refill glycogen, triglyceride, and protein stores. It is hypothesised that increased anaerobic end-products can support glycogen production, fatty acid oxidation, triglyceride storage and amino acids protein synthesis in *H. iris*. Additionally, changes within purine and pyrimidine metabolism reflect the replenishing of nucleic acids supporting DNA and RNA turnover and repair. This research also proves that the use of right and left gills of abalone should be considered as separate entities for physiological research, and reporting thereof should clearly indicate the usage of the left or right gill. In studies where a stressor is implemented the use of the left gill is advised to capture the signature of the stressor. While the use of the right gill is likely to be sufficient as tissue in studies concerning baseline profiling.

Ultimately, metabolism is governed by a homeostatic setpoint, determined by a specific organism in a particular environment and the ability to effortlessly transition between periods of stress and normality. The target for live export is to ensure that abalone in the best possible physiological condition are provided to the processer for live export. Studies profiling the metabolic state of abalone during various stages of the live export chain, (i.e. purification/purging methods, types of packaging, distribution practices, retail display environments, extent of consumer handling) are lacking creating an opportunity for researchers and industry to co-develop experiments with application outcomes. Once these parameters have been defined for the abalone species in question,

strategies can be put in place to monitor and manage the metabolic state of abalone to achieve the homeostatic setpoint as quickly as possible post-transport. Even though abalone have well adapted metabolic mechanisms enabling them to survive transport periods and to thrive following recovery periods, the aim should be to metabolically prepare abalone for oxygen-poor scenarios and enhance recovery by comprehending the mechanisms at play.

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Data availability statement

Raw metabolite data is presented as supplementary material with this manuscript.

ORCID

Leonie Venter http://orcid.org/0000-0003-0019-3722
Andrea C. Alfaro http://orcid.org/0000-0003-0543-7212
Jeremie Zander Lindeque http://orcid.org/0000-0001-8017-4278
Peet J. Jansen van Rensburg http://orcid.org/0000-0001-6911-1866

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