Aquaculture 547 (2022) 737438

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Review

Omics research on abalone (*Haliotis* spp.): Current state and perspectives

Thao V. Nguyen [a,b], Andrea C. Alfaro [a,*], Craig Mundy [c], Jillian Petersen [d], Norman L.C. Ragg [e]

[a] Aquaculture Biotechnology Research Group, School of Science, Auckland University of Technology, New Zealand
[b] NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Viet Nam
[c] IMAS Fisheries and Aquaculture Centre, College of Science and Engineering, University of Tasmania, Australia
[d] Centre for Microbiology and Environmental Systems Science, University of Vienna, Austria
[e] Cawthron Institute, Nelson, New Zealand

ARTICLE INFO

Keywords:
Omics
Abalone
Genomics
Transcriptomics
Proteomics
Metabolomics

ABSTRACT

The steady increase in abalone aquaculture production throughout the world has attracted growing interest in the application of new technologies, such as omics approaches for abalone research. Many omics techniques, such as genomics, transcriptomics, proteomics, and metabolomics are becoming established in abalone research and are beginning to reveal key molecules and pathways underlying many biological processes, and to identify associated candidate biomarkers of biological or environmental processes. In this contribution, we synthesize the published omics studies on abalone to highlight the current state of knowledge, open questions, and future directions. In addition, we outline the challenges and limitations of each omics field, some of which could be overcome by integrating multiple omics approaches – a future strategy with great potential for contributing to improve abalone production.

1. Introduction

The Family Haliotidae contains only one genus (*Haliotis*) which includes more than 70 abalone species (Geiger, 2000). Approximately 14 of these are economically important for fishery and/or aquaculture (Estes et al., 2005; Roodt-Wilding, 2007). Production of farmed abalone has grown from negligible amounts in the 1970s to 203,374 mt in 2019 (FAO, 2021) (Fig. 1). China is currently the world's largest abalone producer, with over 180,267 mt in 2019 (mainly *H. discus hannai* and *H. diversicolor*) (FAO, 2021). With 18,436 mt of *H. discus hannai* in 2019, the Republic of Korea is the second largest abalone producer. It is followed by South Africa with 1675 mt (*H. midae*) and Australia with 424 mt (*H. rubra*, *H. laevigata* and a hybrid of these two species). Other countries where abalone are farmed include Taiwan (*H. diversicolor supertexta* and *H. discus hannai*), Thailand (*H. diversicolor*), Philippines (*H. asinina*), USA (*H. rufescens* and *H. fulgens*), Chile (*H. rufescens*), Mexico (*H. fulgens*) and New Zealand (*H. iris*). In addition, there are also smaller commercial abalone aquaculture operations in Europe, Iceland, Canada, Oman and some Pacific Rim countries (FAO, 2017; Ponder et al., 2019). Despite the fast growth of abalone farming in many countries and high price for this seafood products worldwide, farmed abalone account for a relatively small percentage of global seafood production. In general, the abalone aquaculture production faces several challenges, such as the slow growth rate, diseases (e.g., abalone viral ganglioneuritis), lack of reasonably priced juveniles, high cost of production and infrastructure, technical barriers in hatchery and grow-out systems (Hannon et al., 2013; Wu and Zhang, 2016; Corbeil, 2020). Hence, there has been an increase in abalone research in the last few decades with a significant emphasis on optimizing production in farming systems (Wu and Zhang, 2016; Grandiosa et al., 2018; Grandiosa et al., 2020; Masoomi Dezfooli et al., 2021). Many researchers have also employed modern omics technologies to characterize different aspects of abalone biology and study the responses of abalone to external stress (Fig. 2).

Omics is the general term used for biological disciplines including a range of specialized fields, such as genomics, transcriptomics, proteomics and metabolomics (Vailati-Riboni et al., 2017). Omics fields aim to identify, characterize and quantify all biological molecules (e.g., genes, proteins, metabolites) to understand their structure, function and dynamics in a biological system (cell, tissue, organ, biological fluid or organism) (Vailati-Riboni et al., 2017). Hence, omics approaches are extremely powerful for biomarker discovery and understanding the complex interactions between genotypes and phenotypes, and between the host, associated microorganisms and the surrounding environment.

* Corresponding author at: Aquaculture Biotechnology Research Group, School of Science, Faculty of Health and Environmental Sciences, Auckland University of Technology, Private Bag 92006, Auckland 1142, New Zealand.
E-mail address: andrea.alfaro@aut.ac.nz (A.C. Alfaro).

https://doi.org/10.1016/j.aquaculture.2021.737438
Received 23 April 2021; Received in revised form 31 August 2021; Accepted 2 September 2021
Available online 6 September 2021

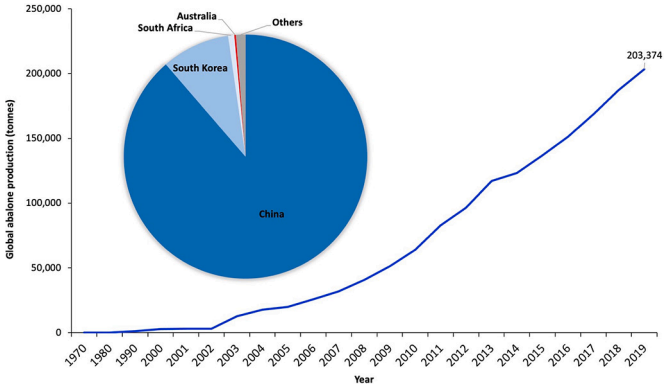

Fig. 1. Global abalone aquaculture production and major abalone producers.

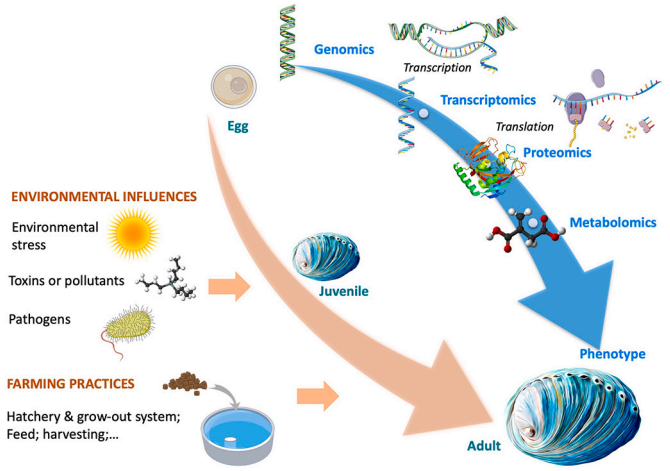

Fig. 2. Omics applications for abalone research. The omics techniques are shown on the blue arrow, while the orange arrow demonstrates the abalone development which influenced by internal (e.g., life stages, species) and external factors (environmental stressors and farming practices). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Recent advances in high-throughput analysis of biological molecules and bioinformatics for data interpretation have led to the widespread application of omics techniques across life sciences (Vailati-Riboni et al., 2017; Abid et al., 2018; Aizat et al., 2018; Long et al., 2019). Omics applications in abalone production began in the mid-2000s and have increased in prevalence in recent years (Tables 1, 2, 3, 4). The use of high-throughput omics technologies has significantly enhanced our knowledge of abalone biology and physiology, and the molecular processes underlying the interactions between abalone and environment stressors or pathogens. Such critical information would be important for current and future investigations in hatchery methods, grow-out technologies, disease control, fishery management and wild population conservation. This contribution aims to provide a systematic and comprehensive review of the present state of omics research and impacts on abalone production. This, in turn, will provide perspectives for future applications of omics in abalone research. The paper focuses on four main omics approaches currently used in abalone research - genomics, transcriptomics, proteomics and metabolomics. For each omics field, we provide an overview of the technique, and review its advantages and applications for abalone research. In addition, we discuss the challenges as well as future perspectives of omics applications in abalone research in both aquaculture in fisheries.

2. Abalone genomes

Genomics is the earliest form of omics, which aims to study the genome - the complete set of genetic material in an organism. The first draft genome in the Family Haliotidae was sequenced in *H. discus hannai* using multiple sequencing platforms (Nam et al., 2017). The final genome assembly consists of 1.86 Gb with 35,450 scaffolds (>2 kb) and contained 29,449 genes (Table 1). This first genome also represents the longest abalone genome to date. The proportion of identified total repeat elements in *H. discus hannai* is 30.76%, which is almost six times larger than that of *Lottia gigantea* of the same genome size. Subsequently, draft genomes of *H. rufescens* (Masonbrink et al., 2019), *H. laevigata* (Botwright et al., 2019) and *H. rubra* (Gan et al., 2019) were sequenced (Table 1). So far, the genome of *H. rufescens* (1.498 Gb) is the most complete genome of abalone with a scaffold N50 of 1.9 Mb (Masonbrink et al., 2019). The annotation of the *H. rufescens* genome resulted in 57,785 gene models, which is almost twice as in *H. discus hannai* (29,449 gene models) (Nam et al., 2017). The draft genome of *H. laevigata* is 1.76 Gb genome (expected 1.54 Gb) in length (Botwright et al., 2019), which was comparable to that of *H. discus hannai* (Nam et al., 2017). A total of 55,164 genes were putatively identified in the *H. laevigata* genome, which belongs to the top five biological processes, including cellular, metabolic, single-organism, localization and biological regulation. A total of 22,180 orthologous groups of which 6652 were core ortholog clusters in the 1.38 Gb genome of *H. rubra* were identified as those in three marine gastropod genomes (*L. gigantea*, *H. rufescens*, *H. discus hannai*), and 3689 were exclusively shared by three abalone genomes (*Haliotis rubra*, *H. rufescens*, *H. discus hannai*) (Gan et al., 2019). These haliotid genomes not only advance our understanding of abalone genetics, but also provide valuable references for genomics and other omics applications in abalone. These approaches would contribute to understanding the relationships between physiology, function and development and the underlying genotype for key research fields, such as broodstock enhancement techniques, larval quality and overall aquaculture production.

Additionally, several complete mitochondrial genomes have been reported for *H. rubra* (Maynard et al., 2005), *H. diversicolor* (Xin et al., 2011), *H. discus hannai* (Yang et al., 2015; Guo et al., 2019) and *H. laevigata* (Robinson et al., 2016) (Table 1). The mitochondrial genomes of abalone species are quite similar which are approximately 16,000 bp length, 40% GC, 13 protein-coding genes (Table 1). The available mitochondrial genomes are useful to assess the phylogenetic relationship of abalone, determine the maternal contribution to hybrid populations, investigate population structure and stock-enhancement effectiveness (Robinson et al., 2016; Guo et al., 2019). For example, the mitochondrial genome of *H. laevigata* shares 92% nucleotide sequence identity with that of *H. rubra* (Maynard et al., 2005; Robinson et al., 2016), while the sequences of *H. discus hannai* in China shared 98.54% identities with that of Korean population (Yang et al., 2015; Guo et al., 2019).

Overall, several *Haliotis* draft genomes and mitogenomes have been reported recently, but the current genome assemblies are still incomplete. There is demand for full genome sequencing of other commercially important species (e.g., *H. iris*). The decrease in costs for full genome sequencing will likely lead to more genome and transcriptome studies for abalone and other aquaculture species. Abalone genomics could take advantage from the current cutting-edge genomics techniques such as single-cell RNA sequencing technologies (ten Hacken et al., 2020) and CRISPR-Cas9 gene editing (ten Hacken et al., 2020). The more complete genomics resource available for abalone will hopefully lead to successful and effective implementation of genomic selection in abalone aquaculture through the development of new markers-based models for genetic evaluation and novel breeding programs.

Table 1
Publicly-available abalone genomes.

Species	Common name	Sequencing Platforms	Genome representation/ Assembly level	Total length	Genome size	Scaffold N50 (Mb)	GC%	Genes	Protein-coding genes	Reference
Haliotis rubra	Blacklip abalone	N/A	Mitochondrial genome	16,907 bp	N/A	N/A	40	N/A	13	Maynard et al. (2005)
		Oxford Nanopore MiniION, Illumina NovaSeq 6000	Draft/Scaffold	1.38 Gb	1.24–1.31 Gb	1.23	40.52	47,928	44,137	Gan et al. (2019)
H. diversicolor	Multi-coloured abalone	N/A	Mitochondrial genome	16,186–16,266 bp	N/A	N/A	40.9	37	13	Xin et al. (2011)
H. discus hannai	Pacific abalone	Illumina HiSeq 2000	Mitochondrial genome	16,886 bp	N/A	N/A	39.6	37	13	Yang et al. (2015)
		Illumina Hiseq2000, Nextseq500 and Pacbio RS II	Draft	N/A	1.86 Gb	0.21	40.51	29,449	N/A	Nam et al. (2017)
		N/A	Mitochondrial genome	16,716 bp	N/A	N/A	39.6	22	13	Guo et al. (2019)
H. laevigata	Greenlip abalone	Illumina MiSeq and HiSeq	Mitochondrial genome	16,545 bp	N/A	N/A	N/A	N/A	N/A	Robinson et al. (2016)
		Illumina HiSeq 2500	Draft/ Scaffold	1.71 Gb	1.54 Gb	0.86	40	55,164	N/A	Botwright et al. (2019)
H. rufescens	Red abalone	Illumina HiSeq 3000 and Pacbio RSII	Draft/ Scaffold	1.498 Gb	N/A	1.90	38.9	57,785	N/A	Masonbrink et al. (2019)

3. Abalone transcriptomics

Transcriptomics is the study of transcriptomes and their functions. The transcriptome is the sum of all coding and non-coding RNA transcripts expressed from the genome of an organism or biological specimen under specific conditions such as developmental stage, physiological condition and physical and chemical conditions in the external environment (Nguyen and Alfaro, 2020). The introduction and rapid adoption of RNA sequencing (RNA-Seq) techniques in marine science has led to an abundance of transcriptomics studies for abalone (Table 2). These studies have successfully characterized transcriptomes of many abalone species and identified the differentially expressed genes (DEGs) involved in abalone responses to environmental stress and seawater contaminants, immunology, developmental processes and growth. Transcriptomics is often combined with other molecular approaches (e.g., qRT-PCR) to validate expression patterns of specific genes, such as growth-related, immune-related, sex-specific and stress-regulated genes. A particularly exciting development is direct RNA sequencing on nanopore arrays, which allows reverse transcription- and amplification-free transcriptome sequencing that is strand-specific and has the potential to identify RNA modifications (Garalde et al., 2018). These latest techniques have not yet been applied to abalone.

In the wild, abalone are exposed to a wide range of abiotic and biotic stressors, such as changes in water salinity, elevated temperature (thermal stress), toxins/pollutants, pathogens, lack of food and changes in water flow. Physiological responses to stresses are manifested by the changes in gene expression, which could be captured by transcriptome analyses. To this end, transcriptomics approaches, mostly RNA-Seq techniques, have been employed to compare abalone transcriptomes before and after stress exposure to identify and characterize DEGs and specific pathways involved in the stress responses (Table 2). Among abiotic stress factors, heat stress is the most well studied factor in these transcriptomic studies (Shiel, 2017; Shiel et al., 2017; Chen et al., 2018; Yao et al., 2019). As an example, a study in the abalone *H. discus hannai* found 8532 DEGs in the heat-stressed group compared to the control group (Yao et al., 2019). Enrichment analysis identified that temperature stress caused several disturbances, such as an increase in the synthesis of fatty acids and amino acids, misfolded proteins, mitochondrial dysfunction, DNA damage and apoptosis (Yao et al., 2019). Chen et al. (2018) compared the transcriptomes of heat-tolerant and the heat-sensitive abalone exposed to different temperatures and observed 3370 and 1351 DEGs between the control and the heat-stress temperature in heat-sensitive abalone and heat-sensitive abalone, respectively. The results suggest that the heat-tolerant abalone employed a more effective strategy to cope with heat stress than the heat-sensitive abalone, and its cardiac performance was less disrupted by high water temperature stress when compared to heat-sensitive abalone (Chen et al., 2018). Similarly, 487 DEGs were observed between the heat-resilient and heat-susceptible *H. laevigata* abalone (Shiel et al., 2020). Among these, DEGs associated with metabolism (e.g. Mitofusin 1) and immune process (e.g. Multiple epidermal growth factor-like domain 10, Lysozyme) may enable resilient individuals to endure heat stress or heat wave events. Overall, the findings from transcriptomics studies in abalone provide a better understanding of heat stress signatures and mechanisms in abalone. This fundamental information would be critical for selective breeding of better heat-tolerant lines.

Similar to heat stress, hypoxia caused several negative effects on the abalone physiological functions, such as disturbance of redox balance, induction of cell death/apoptosis and suppression of DNA metabolic activity (Shen et al., 2019). In addition, the effects of simultaneous exposure to multiple stressors such as thermal extremes under hypoxia and hypercapnia in gill and muscle of *H. fulgens* have been investigated (Tripp-Valdez et al., 2019). Differences between combined stress and individual drivers were observed in mitochondrial activity and mRNA levels in which a warming-induced strengthening of citrate synthase gene expression in both tissues only under combined hypoxia and hypercapnia (Tripp-Valdez et al., 2019). Thus, future studies will need to be carefully designed to account for the effect of combined multiple stress factors to reflect the realistic conditions that animals experience in their natural aquatic environment, where exposure to a single, isolated stress factor is likely to be rare. A few authors have investigated toxic effects of pollutants (e.g., tributyltin, copper) on abalone using transcriptomics approaches and identified DEGs between control and exposed animals (Jia et al., 2011; Silva-Aciares et al., 2011). Some of these DEGs were validated via PCR and could be good biomarkers for environmental monitoring of pollutants.

Infectious diseases caused by pathogens (e.g., bacteria, virus) are common concerns in aquaculture settings (Chang et al., 2005; Hooper et al., 2007; Sawabe et al., 2007). In order to understand the molecular responses of abalone to these diseases, RNA-Seq has been used to

Table 2
Transcriptomics investigations for abalone (*Haliotis* spp.)

Approaches/ Techniques/ Platforms	Species	Tissues	Study field (Stress factors/Comparison parameters)	Key Findings	Reference
ESTs	*H. discus discus*	Digestive gland (adult)	N/A	- A total of 841 clones (122 clusters and 510 singletons). 278 putative novel transcripts.	Munasinghe et al. (2006)
Roche 454 pyrosequencing and Illumina-SBS (sequencing-by-synthesis).	*H. midae*	Various tissues	N/A	- Identification of miRNA transcriptome.	Picone et al. (2017)
cDNA microarrays	*H. asinina*	Whole larvae	Preproduction and development	- 144 genes as candidates for a role in competence and/or metamorphosis.	Williams et al. (2009)
Pyrosequencing/454 sequencing	*H. diversicolor*	Whole larvae	Preproduction and development	- 35,415 unigenes of which 7566 were assigned GO terms - A batch of specific genes that are indispensable during embryonic development	Huang et al. (2012)
454 pyrosequencing	*H. rufescens*	Mature reproductive tissues	Preproduction and development (male vs female)	- 79,877 and 133,850 high-quality reads for females and males. - 2793 and 10,354 contigs, 8581 and 32,175 singletons for males and females, respectively. 20% of the DEGs involved in sex-specific patterns.	Valenzuela-Muñoz et al. (2014)
Iso-Seq protocol of the PacBio RSII platform	*H. discus hannai*	Pooled tissues: ganglia, gills, intestine, hepatopancreas, muscle and gonads	Preproduction and development (male vs female)	- 15,110 and 12,145 protein-coding genes were identified in female and male abalones, respectively. - 519 and 391 isoforms were genome-widely identified from female and male transcriptome databases, respectively. - Number of isoforms and their alternatively spliced patterns were variable and sex-dependent.	Kim et al. (2017)
RNA-Seq (Illumina HiSeq2000)	*H. discus discus*	Cerebral ganglia, ovary, testis and unfertilized eggs	Preproduction and development (different tissues)	- 234,353 unigenes of which 36 genes were found by screening known gene names related to germ cell development. - ZP12 is an especially useful germ cell-specific marker in immature adults.	Yu et al. (2018)
RNA-Seq (Illumina HiSeq2000)	*H. tuberculata*	Larvae	Preproduction and development	- 2,176,887 SNPs was filtered to select 500 for high throughput genotyping. - 298 SNPs with >90% call success across >1000 genotyped individuals. - 123 SNPs was used to successfully assign parentage in 98.9% of 945 offspring from 40 parents representing 189 mixed families.	Harney et al. (2018)
RNA-Seq (Illumina HiSeq2500)	*H. discus hannai*	Neural ganglia	Preproduction and development (immature and mature female)	- 76,684 transcripts which 28.54% were annotated and classified according to Gene Ontology terms. - There were 256 DEGs between the immature and mature abalone.	Kim et al. (2019)
RNA-Seq (Illumina HiSeq2500)	*H. discus hannai*	Ovaries	Preproduction and development (immature and mature abalone)	- 8779 unigenes were obtained from the ovaries of immature and mature abalone (average length of 379 bp/gene). - 470 DEGs were identified, including 213 and 257 genes down-regulated and up-regulated in mature abalone, respectively.	Kim et al. (2020)
HPLC and RNA-Seq (Illumina HiSeq2000)	*H. discus hannai*	Eggs	Preproduction and development (green and gray eggs)	- 272,310 unigenes were received from 461,162 transcripts with a mean length of 985 bp. - 185 DEGs between green and gray eggs.	Feng et al. (2020)
RNA-Seq (Illumina HiSeq2000)	*H. rufescens*	Mantle tissue	Populations (geographic locations)	- 1.17×10^6 SNPs of which 21,579 could be genotyped for all individuals. - A large number of genes involved in biomineralization, energy metabolism, heat-, disease- or hypoxia-tolerance.	De Wit and Palumbi (2013)
RNA-Seq (Illumina Genome Analyzer II)	*H. midae*	Unknown	Populations (wild and cultured populations)	- 505 putative SNPs from a total of 316 selected contigs. - 174 SNPs were good candidates for linkage map construction.	Blaauw et al. (2013)
454 pyrosequencing	*H. midae*	Muscle, ganglion, hepatopancreas, gonad and gill.	Populations (cultured and wild specimens)	- 11,240 annotated genes and 516 transcription factors that are valuable	Picone et al. (2015)

(*continued on next page*)

Table 2 (*continued*)

Approaches/ Techniques/ Platforms	Species	Tissues	Study field (Stress factors/Comparison parameters)	Key Findings	Reference
				candidates for developmental and reproductive processes.	
RNA-Seq (Illumina Genome Analyzer II)	*H. midae*	Whole abalone soft tissue	Growth	- Significant differences in gene expression between large and small abalone, and between treated and untreated haemocyte cell cultures. - Insulin may be involved in enhanced growth rate	van der Merwe (2010); van der Merwe et al. (2011)
RNA-Seq (Unknown platform)	*H. discus hannai*	Ganglion, tentacle, gill, heart, hepatopancreas, intestine, gonad, mantle and muscle	Growth	- 6 DEGs were identified as associated with faster growth.	Choi et al. (2015)
454 pyrosequencing (454 GS FLX Titanium)	*H. rufescens*	Muscle	Growth	- 44,312 contigs. 1437 DEGs (435 were up-regulated in the high growth rate abalone and 1002 in low growth rate individuals)	Valenzuela-Miranda et al. (2015)
RNA-Seq (Illumina Genome Analyzer II)	*H. midae*	Whole body tissue	Growth and disease	- More than 25 million short reads assembled in 22,761 contigs. - Many gene families involved in immune response.	Franchini et al. (2011)
EST database generated with 454 pyrosequencing	*H. rufescens*	Haemolymph and the gills	Immunology	- Initiator caspase (HrCas8) showed a complete sequence of 2529 bp, with an ORF of 1911 bp, a 50UTR of 201 bp, and a 30UTR of 417 bp. - Effector caspase HrCas3 showed a complete sequence of 1404 bp, with an ORF of 747 bp, a 50UTR of 82 bp, and a 30UTR of 574 bp.	Chávez-Mardones and Gallardo-Escárate (2014)
454 pyrosequencing and ESTs	*H. discus discus*	Digestive tract, gills, hemolymph, gonads, muscles, mantle and hepatopancreas	Immunology (bacterial and viral infections)	- The significant up-regulation of a selenium- dependent glutathione peroxidases (AbSeGPxs) in a time-dependent manner after bacterial and viral infections.	Bathige et al. (2015)
RNA-Seq (Illumina HiSeq2000)	*H. tuberculata*	Larvae and haemolymph of adults	Immunology (temperature and pH variation and *Vibrio harveyi*)	- 328,519 contigs with 41,099 transcripts. - 5690 transcripts in larvae and 10,759 transcripts in adult haemolymph were significantly higher expression stress-exposed abalone than control animals.	Harney et al. (2016)
RNA-Seq (Illumina HiSeq2000)	*H. discus hannai*	Hemocytes, gill, hepatopancreas and mantle of adults	Immunology (*Vibrio parahaemolyticus*)	- 10,575 transcripts exhibited the differential expression.	Nam et al. (2016)
RNA-Seq (Illumina HiSeq2000)	*H. laevigata*	Epipodial tentacle	Immunology (summer mortality)	- Two genes showed significantly higher expression in resilient abalone relative to susceptible abalone prior to the laboratory-induced summer mortality event.	Shiel et al. (2017) Shiel (2017)
RNA-Seq (Illumina HiSeq2000)	*H. diversicolor supertexta*	Mantle tissues	Immunology (Haliotid herpesvirus-1)	- 2.08 × 10^5 unigenes with a mean length of 1486 bp and an N50 of 2455 bp. - A rich immune-related gene set was over-expressed at 60 hpi compared to 0 hpi.	Bai et al. (2019)
cDNA microarrays	*H. diversicolor*	Whole body tissue (small abalone)	Environmental stress (TBT Exposure)	- 2473 unique transcripts. 107 up-regulated genes and 41 down-regulated genes after TBT exposure.	Jia et al. (2011)
SSH	*H. rufescens*	Soft tissue, the digestive tract and digestive glands (juveniles)	Environmental stress (copper)	- 368 different sequences regulated by copper. - 14 potential genes regulated by metal stress	Silva-Aciares et al. (2011)
RNA-Seq (Illumina HiSeq2000)	*H. laevigata*	Tissue samples consisted of epipodial tentacle samples (juveniles), a haemolymph sample (juvenile) and an epipodial tentacle sample (male adult).	Environmental stress (thermal stress)	- A trinity assembly of 222,172 contigs (from 200 bp to 33 kilobases) with annotated 20,702 contigs. - 26 contigs with homology to the HSP70 family of genes with 91 putative single-nucleotide polymorphisms.	Shiel et al. (2015)
RNA-seq (Unknown platform)	*H. discus hannai*	Gills	Environmental stress (thermal stress)	- 3370 and 1351 DEGs were identified between the control and the heat-stress temperature in in heat-sensitive abalone and heat-sensitive abalone, respectively.	Chen et al. (2018)
RNA-Seq (Illumina HiSeq2500)	*H. discus hannai*	Hepatopancreas	Environmental stress (thermal stress)	- 17,852 unigenes with 8532 DEGs in the test group compared with the control group, including 4788 upregulated and 3744 downregulated genes.	Yao et al. (2019)
RNA-Seq (Illumina HiSeq2000)	*H. discus hannai*	Hemocytes, gill, mantle and muscle	Environmental stress (thermal stress)	- 413 proteins were annotated as members of the heat shock protein (HSP)	Kyeong et al. (2020)

(*continued on next page*)

Table 2 (*continued*)

Approaches/ Techniques/ Platforms	Species	Tissues	Study field (Stress factors/Comparison parameters)	Key Findings	Reference
				super families of which 26 HSP genes were differentially expressed under cold and heat stress conditions.	
RNA-Seq (Illumina NovaSeq 6000)	*H. rufescens* and hybrid *H. rufescens* (♀) × *H. corrugata* (♂)	Tissues of juveniles	Environmental stress (thermal stress)	- The trinity assembly generated 690,964 contigs. - The higher levels of genes involved in biomineralization and growth in hybrids indicate that the hybrid abalone is more able to cope with warm temperature.	Tripp-Valdez et al. (2021)
RNA-Seq (Illumina HiSeq2000)	*H. laevigata*	Tissues	Environmental stress (simulated heat wave event)	- 487 DEGs between heat resilient and susceptible abalone throughout the heat stress event.	Shiel et al. (2020)
RNA-Seq (Illumina MiSeq)	*H. fulgens*	Gill and muscle	Environmental stress (temperature, acute hypoxia and hypercapnia)	- A total of 13,481 transcripts (14.17%) were annotated from which 383 are derived from mollusk species and 102 from *Haliotis* species. - Gene networks of gill and muscle conform with different levels of thermal sensitivity. - Warming combined with hypercapnia and hypoxia enhanced mitochondrial capacity.	Tripp-Valdez et al. (2019)
RNA-Seq (Illumina NextSeq 500)	Diploid and triploid *H. discus hannai*	Hepatopancreas	Environmental stress (temperature and hypoxia)	- A total of 316 million clean reads were de novo assembled into 271,039 contigs with 209,974 non-redundant transcripts. - Many DEGs were identified from diploid and triploid abalone in responses to acute heat-stress and hypoxia treatments.	Kim et al. (2021)
RNA-Seq (Illumina HiSeq2000)	*H. diversicolor*	Hemocytes	Environmental stress (hypoxia stress and bacterial challenge)	- 307,395,572 clean reads were generated and assembled into 99,774 unigenes. - 225 unigenes with immunologic function were mapped into immune-related pathways	Sun et al. (2019)
RNA-Seq (Illumina HiSeq2500)	*H. discus hannai*	Hemolymph	Environmental stress (hypoxia)	- 954 DEGs were detected under different degrees of deoxygenation.	Shen et al. (2019)

compare transcriptomes of control abalone and abalone challenged with different pathogens, such as *Vibrio parahaemolyticus* (Silva-Aciares et al., 2013; Nam et al., 2016), *Vibrio harveyi* (Harney et al., 2016) and Haliotid herpesvirus-1 (HaHV-1) (Bai et al., 2019). In general, these studies observed DEGs related to immunity and other physiological processes. The information about DEGs have provided not only a better understanding of abalone immunology and the molecular mechanisms underlying the response of abalone to pathogens, but also candidate molecule markers for selective breeding programs targeting disease-resistant broodstock. For example, comparative analysis of transcriptome changes between *V. parahemolyticus* infected and non-infected *H. discus hannai* revealed 10,575 transcripts exhibiting the differential expression of at least one pair of comparisons (Nam et al., 2016). The functional annotations identified genes involved in immune response, cell adhesion, immune regulation, redox molecules and mitochondrial coding genes in *H. discus hannai* (Nam et al., 2016). This study identified the physiological traits controlling abalone ability to survive against *Vibrio* infection, which is a prerequisite for selective breeding of *Vibrio*-resistant lines.

Molecular responses of abalone to combinations of biotic and abiotic stressors are beginning to reveal in the complex interactions at the interface between host, pathogen and environment within aquaculture settings (Sun et al., 2019). Changes in the transcriptome of *H. diversicolor* haemocytes after exposure to bacterial challenge with or without hypoxia revealed that hypoxia stress delays the rapid immune response of abalone, and also demonstrated that immune defense genes may also participate in hypoxia-induced immunosuppression (Sun et al., 2019). Further transcriptomics investigations on combined effects of biotic and abiotic stressors, ideally paired with single-stress controls, would provide a broader understanding of abalone-pathogen-environment interactions. This is a research priority of increasing importance as climate change is predicted to cause concomitant expansions of low-oxygen zones and promote pathogen growth and dispersal.

Developmental biology has been an important field for transcriptomic investigations in abalone. Transcriptomics has been employed to identify genes and molecular pathways expressed throughout development that may underlie crucial processes including sexual maturation and reproduction (Valenzuela-Muñoz et al., 2014; Botwright et al., 2019; Kim et al., 2019), larval development and metamorphosis (Williams et al., 2009; Huang et al., 2012) and germ cell development (Yu et al., 2018). Knowledge of molecular processes underlying abalone development are fundamental for development of new methods to control maturation and spawning, improve survival and quality of larvae, and to support in identification of sex and production of mono-sex lines for aquaculture. As a showcase, Yu et al. (2018) conducted transcriptome analysis of *H. discus discus* for gene discovery in the brain (cerebral ganglia), ovary, testis and unfertilized eggs to study the molecular mechanisms governing gonadal development in abalone. The study identified 36 genes linked to germ cell development. Two of these genes, *vasa* and *ZP12*, were confirmed to be involved in germ cell development and could be used as cell-specific markers in immature adult abalone for future maternal mRNA knockdown experiments by RNA interference to induce sterility in Pacific abalone (Yu et al., 2018).

The inherently slow growth rate of abalone is a major remaining obstacle in abalone aquaculture. Hence, understanding physiological

traits that determine growth rates is important to improve productivity and profitability of abalone aquaculture. RNA-Seq techniques have been employed by researchers to identify differential growth-related gene expression in different abalone species (van der Merwe et al., 2011; Choi et al., 2015; Valenzuela-Miranda et al., 2015). These expressed genes provide valuable data towards understanding the molecular underpinnings of growth regulation in abalone, and may be used as molecular markers in selective breeding programs. For example, Choi et al. (2015) used RNA-Seq to identify genes associated with fast growing *H. discus hannai* siblings of various sizes reared in the same environmental conditions. There were six DEGs associated with fast growth of the *H. discus hannai*, including five up-regulated genes and one down-regulated gene. Adding known growth-stimulating factors is another promising avenue for aquaculture improvement. For this purpose, transcriptome analysis and qPCR of fast- and slow-growing *H. midae* abalone and *in vitro* primary haemocyte cultures treated with different growth-stimulating factors revealed significant differences in gene expression between the two groups in both abalone and haemocytes (van der Merwe et al., 2011). Among the expressed genes, the up-regulation of genes involved in the insulin signaling pathway implicates insulin in enhancing growth rates of *H. midae*. Further investigations to identify the role of insulin will provide a knowledge base for applying this molecule in abalone growth enhancement in aquaculture.

Other key aspects of abalone biology that have been investigated with transcriptomics include differences between wild and farmed abalone (Picone et al., 2015), patterns of gene flow and local adaptation (De Wit and Palumbi, 2013) and characterization of selenium-dependent glutathione peroxidase in immune responses of abalone to bacterial and viral stresses (Bathige et al., 2015). These studies demonstrate broad applications of transcriptomics in abalone research on a spectrum from fundamental to applied aquatic research.

4. Abalone proteomics

Proteomics is the global analysis of proteomes, which are the entire complements of proteins expressed in a biological system at any given time (Liebler, 2001). Proteomics investigations in marine sciences have increased in recent years. Marine proteomics studies focus on responses of marine organisms to environmental stress (reviewed by Tomanek, 2014), marine microbiology, seafood safety, aquaculture, natural products, marine algae and plants and marine invertebrates (reviewed by Slattery et al., 2012). Proteomic studies in abalone started two decades ago, and cover a wide range of biological questions from stress responses to biomineralization, embryonic development, effects of supplemented diets and mechanisms of heterosis, among many others. The majority of these studies used mass spectrometry (MS)-based platforms, while a few studies employed tradition gel-based proteomic approaches (Table 3).

Studies on stress responses account for a large proportion of proteomics investigations for abalone (Table 3). Since proteins are constantly influenced by environmental conditions, proteomes of abalone are dynamic, which could provide insight into the molecular phenotypes that represent the functional adaptations to environmental change (Tomanek, 2014). Many of these studies compared the proteome of controlled abalone with abalone exposed to abiotic stressors, such as endocrine disrupting chemicals (EDCs) (Zhou et al., 2010a; Liu et al., 2011) and temperature (Calder, 2014; Kang et al., 2019). Exposure of abalone at different developmental stages to EDCs, which may lead to failure of metamorphosis revealed many differentially expressed proteins from various functional categories, such as energy metabolism, cell signaling, formation of cytoskeleton and cilium, immune and stress responses (Zhou et al., 2010a; Liu et al., 2011). For example, Kang et al. (2019) observed a notable discrepancy in protein expression between abalone exposed to prolonged high temperatures (26 °C) and those exposed to fluctuating temperatures (20–26 °C). Most upregulated proteins are structural, whereas most downregulated proteins are metabolic enzymes in abalone exposed to fluctuating temperatures compared with those at an acclimation temperature (26 °C). Overall, these comparative proteomics studies have shown which cellular processes in abalone are critical to adapt to environmental stress. Such information is important in setting environmental tolerance ranges and provides perspectives for future studies in development of resilient abalone. For biotic stress, there is currently only one proteomic study for abalone conducted by Beltran (2015), who identified and quantified 118 non-redundant unique haemocyte proteins from *H. midae* where 16 proteins were found to be significantly regulated after *Vibrio anguillarum* challenge. Analysis of the mechanistic and functional roles of these proteins provide insights into the molecular pathways involved in innate immunity and the pathophysiology of disease in abalone (Beltran, 2015).

Reliable and reproducible spawning in abalone, which results in high quality of larvae for grow-out is one of the keys for the success in abalone farming. To this end, proteomics offers opportunities for identification of key proteins involved in development and reproduction, which are prerequisites for selective breeding programmes. The proteomic analyses of Taiwanese and Japanese populations of *H. diversicolor* and their hybrids revealed 46 differentially expressed proteins between these populations, which were involved in major biological processes, including energy metabolism, stress responses and muscle contraction and regulation (Di et al., 2013). Furthermore, the hybrids exhibited additivity or overdominance in 73.9% of the 46 identified proteins (Di et al., 2013). The additivity in this study indicates intermediate expression levels between those of the two parental lines while the overdominance refers to expression levels higher than those of either parental line (Di et al., 2013). Similarly, proteomics was used to compare muscle and eggs between hybrid abalone and parental lines of *H. gigantea* and *H. discus hannai* (Di et al., 2015a; Di et al., 2015b). In eggs, a total of 20 differential protein level spots in hybrid and parental abalone eggs were identified from 112 protein gel spots from the eggs (Di et al., 2015a). In muscle tissue, 136 differential protein level spots were identified between hybrid offspring and parental lines (Di et al., 2015b). Hybrid offspring with additive or over-dominance accounted for 68.4% of these protein spots. These results suggest that proteomics approaches can be used to provide insights into the molecular mechanism of heterosis and functional prediction of abalone interspecific hybridization for selective breeding. Other authors applied proteomics to identify proteins involved in sexual maturity, fertilization and reproduction and protein expression following spawning (Mendoza-Porras et al., 2014; Mendoza-Porras et al., 2017). These findings have contributed to a better understanding of abalone reproduction and development of new spawning practices that provide more control and less stressful induction, as well as improved recovery of post-spawning broodstock.

There are many other aspects of abalone research investigated by proteomics approaches, such as effects of supplemented diets (Dias, 2016; Nel et al., 2017), formation of the shell (Marie et al., 2010; Le Roy et al., 2012; Mann et al., 2018) and differences between abalone populations (Di et al., 2016). These primary proteomics studies provide a good background for future proteomics investigations in abalone and other marine organisms. However, the high cost of high-throughput proteomics analyses (approximately US$ 300–400 per sample for LC-MS platforms) are also obstacles for large-scale projects (Nguyen and Alfaro, 2020). As a consequence, most comparative proteomics studies in abalone have limited numbers of tissues, samples, sampling time points and treatments which limits our ability to understand the whole biological process. Thus, there is a need for cost-effective high-throughput proteomics workflows to make proteomics more accessible for aquatic research and large-scale studies.

5. Abalone metabolomics

Metabolomics, the study of intermediates and end products of

Table 3
Proteomics investigations for abalone (*Haliotis* spp.)

Proteomics	Species	Tissues	Study field (Stress factors/Comparison parameters)	Key findings	References
HPLC	*H. asinina*	Calcified layers of the shell	Shell formation	- 14 proteins from distinct calcified layers of the shell of which 12 are novel proteins.	Marie et al. (2010)
nano-LC-MS/MS	*H. tuberculata*	Mantle	Shell formation	- Identification of 2 carbonic anhydrases: htCA1 and htCA2. - htCA1 is secreted but is not incorporated in the organic matrix of the abalone shell and that htCA2 is transmembrane.	Le Roy et al. (2012)
LC-MS	*H. laevigata*	Nacre and prismatic layer	Shell formation (biomineralization)	- 297 proteins from the nacreous shell layer and 350 proteins from the prismatic shell layer.	Mann et al. (2018)
2D gel electrophoresis and MALDI–TOF-MS	*H. diversicolor supertexta*	Hepatopancreas	Environmental stress (bisphenol A and diallyl phthalate)	- 24 spots significantly increased or decreased at protein expression level by 2D gel electrophoresis. - 8 protein spots were identified by MALDI–TOF-MS.	Zhou et al. (2010a)
2-DE separation and MALDI–TOF/TOF MS	*H. diversicolor supertexta*	Embryo	Environmental stress (nonylphenols and bisphenol A)	- Two chemical altered various functional proteins in the abalone larvae with slight differences between each chemical and affected various physiological functions.	Liu et al. (2011)
2D PAGE analysis and iTRAQ	*H. midae*	Hemolymph	Environmental stress (thermal stress)	- 11 proteins with significant heat-induced differential expression were identified.	Calder (2014)
Quantification iTRAQ coupled with LC-MS/MS	*H. discus hannai*	Foot tissues	Environmental stress (thermal stress)	- 317 proteins were quantified on the basis of 913 unique peptides. - 317 proteins were quantified on the basis of 913 unique peptides. - 40 proteins with significantly changed expression in control treatment (20 °C) compared to the acclimation temperature (26 °C) treatment.	Kang et al. (2019)
Quantification iTRAQ coupled with LC-MS/MS	*H. midae*	Haemocytes	Environmental stress (*Vibrio anguillarum*)	- 118 non-redundant unique haemocyte proteins were identified and quantified in response to bacterial-challenge, with 16 found to be significantly regulated relative to the unchallenged and mock-infected controls.	Beltran (2015)
Quantification iTRAQ coupled with LC-MS/MS	*H. midae*	Haemocytes	Environmental stress (*Vibrio anguillarum*)	- 118 non-redundant, unique haemocyte proteins were identified and quantified, with 16 proteins significantly regulated.	Beltran and Coyne (2020)
LC–MS/MS	*H. laevigata*	Female and male gonads	Reproduction and development	- 162 and 110 proteins were identified in females and males respectively with 40 proteins common to both sexes. - For proteins involved in sexual maturation, sperm and egg structure, motility, acrosomal reaction and fertilization, 23 were identified only in females, 18 only in males and 6 were common.	Mendoza-Porras et al. (2014)
2-DE and mass spectrometry (MS)	Hybrid abalone and parental lines *H. gigantea* Reeve and *H. discus hannai* Ino	Muscle	Reproduction and development (heterosis)	- 136 DEP spots involved in major biological processes, including energy metabolism and stress response.	Di et al. (2015b)
2-DE	*H. discus hannai* and *H. gigantea*	Eggs	Reproduction and development (heterosis)	- 112 protein gel spots were identified; of these, 59 were abalone proteins. - Identification of many DEP spots between different parental lines.	Di et al. (2015a)
2D-DIGE and MRM-MS	*H. laevigata*	Female gonad	Reproduction and development	- A number of reproductive proteins were identified.	Mendoza-Porras et al. (2017)
2-DE and MS/MS	*H. diversicolor*	Larval stages	Reproduction and development	- 150 2-DE gel spots were identified. - DEP spots showed upregulation of 15 proteins and downregulation of 28 proteins as abalone developed from trochophore to veliger larvae. - Compared with trochophore larvae, veliger larvae had 55 proteins upregulated and 49 proteins downregulated.	Di et al. (2017)
Quantification iTRAQ coupled with LC-MS/MS	*H. midae*	Haemocytes	Feed and nutrition (probiotics)	- 128 haemocyte proteins were identified.	Dias (2016)
LC-MS	*H. midae*	Hepatopancreas, crop–stomach contents and gut-lining samples	Feed and nutrition (formulated feed)	- Prominent bacterial proteins were identified in the abalone digestive tract: glucan 1,4-α-maltotetraohydrolase and β-peptidyl aminopeptidase, and malate dehydrogenase, succinyl- CoA ligase and pectate lyase.	Nel et al. (2017)
Quantification iTRAQ coupled with LC-MS/MS	*H. discus hannai*	Muscle, gill, visceral mass and mantle	Growth	- 1904 proteins identified with 125 PEPs in large specimens as compared to small specimens.	Huang et al. (2017)
2DE and MALDI-TOF/TOF analyses	Hybridization between *H. diversicolor* Reeve	Foot muscle	Populations (hybrid and parental populations)	- 46 gel spots were identified and a total of 15 spots matched with abalone proteins (a 33.6%	Di et al. (2013)

(*continued on next page*)

Table 3 (*continued*)

Proteomics	Species	Tissues	Study field (Stress factors/Comparison parameters)	Key findings	References
	Japan and Taiwan populations			identification rate). - Hybrid exhibiting additivity or overdominance accounted for 73.9% of these 46 identified proteins.	
2-DE	*H. diversicolor*	Foot muscle	Populations (three geographical populations)	- 254 spots showed differential expression among the three populations and 85 protein spots percentage volumes varied more than twofold.	Di et al. (2016)

Abbreviations: 2D PAGE: Two-dimensional polyacrylamide gel electrophoresis, 2D-DIGE: Two-dimensional difference gel electrophoresis, 2D/2-DE: Two-dimensional gel electrophoresis, DEP: differentially expressed protein, HPLC: High performance liquid chromatography, LC-MS/MS: Liquid chromatography with tandem mass spectrometry, MALDI-TOF/TOF: Matrix-assisted laser desorption/ionization (MALDI) time-of-flight/time-of-flight (TOF/TOF), MALDI–TOF-MS: Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS), MRM-MS: Multiple reaction monitoring (MRM)- mass spectrometry (MS).

cellular regulatory processes (metabolites). Metabolomics has been employed in many studies to characterize the metabolic profiling of abalone under different conditions. This approach has already shown to have diverse applications with regards to types of samples (e.g., tissues, organs), species and analytical platforms (Table 4). Abalone metabolomics studies have used a wide range of metabolomics platforms, including nuclear magnetic resonance (NMR), gas chromatography–mass spectrometry (GC–MS), gas chromatography time-of-flight mass spectrometry (GC-TOF-MS), high performance liquid chromatography (HPLC), liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) and liquid chromatography with tandem mass spectrometry (LC-MS-MS). Among these, NMR-based metabolomics is the most common approach used in studies of abalone. Interestingly, multiplatform approaches have been used in a few studies (Venter et al., 2018a; Venter et al., 2018b; Venter et al., 2018c). As the goal of metabolomics is typically to obtain as many metabolites as possible, implementation of analytical multiplatform approaches broadens the metabolite coverage and also allows technically complementary validation of the metabolites (Sussulini, 2017).

The majority of metabolomics studies in abalone have focused on responses of abalone to different stressors, such as environmental stress (e.g., temperature, food limitation, hypoxia, toxins) and pathogen infections (Table 4). These studies have successfully identified metabolic pathways underlying responses of abalone to different stress factors and candidate metabolite biomarkers associated with specific stress responses. Environmental stressors typically cause a disturbance in energy metabolism and osmotic balance of abalone, and lead to increased levels of anaerobic end-products (Zhou et al., 2010b; Zhou et al., 2015; Lu et al., 2016; Tripp-Valdez et al., 2017; Venter et al., 2018a; Venter et al., 2018b). Other effects include amino acid metabolism, lipid metabolism, inflammatory responses and oxidative stress (Zhou et al., 2010b; Zhou et al., 2015; Lu et al., 2017a). The combination of environmental stressors, such as hypoxia and hypercapnia, were reported to cause greater metabolic perturbations than the effect of a single stress factor (Rosenblum et al., 2005; Tripp-Valdez et al., 2017). Similar to environmental stressors, *V. parahaemolyticus* infections caused disturbances in energy metabolism, nucleotide metabolism and osmotic balance, oxidative stress, immune stress and neurotoxicity in different tissues of abalone (Lu et al., 2017b). Overall, these observations are similar to those observed for other molluscan species, such as oysters or mussels, experiencing environmental stress and pathogen infections (Young et al., 2017; Nguyen et al., 2018a; Nguyen et al., 2018c; Alfaro et al., 2019). These results suggest that sharing of similar functional processes is a common finding across molluscan species, along with species-specific differences of response.

Metabolomics has also been applied to other aspects of abalone biology such as growth, muscle colouration and lipid metabolism. Venter et al. (2018c) used metabolomics to investigate variations between fast and slow growing individuals, while Koyama et al. (2020) examined gluconeogenesis and glycogen metabolism during development. Characterization of opines in abalone adductor muscle (Venter et al., 2017), carotenoid-based orange colouration of muscle (Wei et al., 2019), and effects of feed supplemented with probiotics and proline are further examples of potential applications of metabolomics to abalone biology (Grandiosa et al., 2018; Venter et al., 2019). Lipidomics is a branch of metabolomics that focus on pathways and networks of all cellular lipids in biological systems, such as cells, tissues or organisms. UPLC-ESI-Q-TOF-MS-based lipidomics has been applied to evaluate the lipid profiles in different tissues of Japanese abalone (*H. discus hannai*) (Zhang et al., 2018).

Metabolomics approaches have led to the discovery of many candidate biomarkers for stress and health of abalone. For example, glucose-to-homarine ratio in foot muscle could be a potential marker for differentiating the Rickettsiales-like prokaryote (WS-RLP)-infected animals from healthy abalone and abalone suffering from food limitations (Rosenblum et al., 2005). Glutamate and glutamine have the potential to be biomarkers in phthalate compound pollution in abalone (*H. diversicolor supertexta*) (Zhou et al., 2015). Levels of aromatic amino acids, lysine and glutamate in the gills as well as pantothenate in the hepatopancreas could potentially be used as biomarkers of tributyltin (TBT) and triphenyltin (TPT) exposure in *H. diversicolor* (Lu et al., 2017a). However, most of these studies have used discovery-based approaches, and metabolomic applications targeted at specific metabolites (targeted metabolomics) and validation of these candidate biomarkers remain largely unexplored.

Several metabolomics investigations for abalone have also focused on gender- and tissue-specific metabolic responses (Table 4). The most commonly used tissue for abalone metabolomics studies is the foot muscle, followed by adductor muscle, digestive gland (hepatopancreas), hemolymph, gills and epipodial tissue (Table 4). While many studies have focused on a single tissue, others have compared metabolite profiles of different tissues and observed tissue-specific responses (Table 4). A few authors also compared metabolic responses of male and female abalone (Lu et al., 2017a; Lu et al., 2017b). For example, Lu et al. (2017b) found that metabolic responses in female abalones (*H. diversicolor*) to *V. parahaemolyticus* exposure was more clearly observed than those in male abalone (Lu et al., 2017a). Similarly, metabolic differences between male and female have been observed in hepatopancreas and gill of *H. diversicolor* to organotin compounds, suggesting gender-specific metabolic responses to environmental stressors (Lu et al., 2017a). These findings in abalone are similar to those of other marine invertebrates. For example, sex was reported to have significant impact on the metabolome of gill and hepatopancreas of *Mytilus* mussels (*Mytilus galloprovincialis* and *Mytilus edulis*) (Ji et al., 2013; Ellis et al., 2014). However, sex-based difference was not observed in haemolymph of the mussel *Perna canaliculus* exposed to *Vibrio* sp. (Nguyen et al., 2018b). These studies suggest that sex-based differences among abalone of metabolite profiles in abalone and other molluscs may vary among tissue types, depending on the stimulus. Hence, understanding the tissue-specific and gender-related metabolic

Table 4
Metabolomics (and lipidomics) investigations for abalone (*Haliotis* spp.)

Metabolomics	*Species*	Tissues	Study field (Stress factors/ Comparison parameters)	Key Findings	Reference
^{1}H NMR	*H. rufescens*	Foot	Environmental stress (thermal stress)	- Thermal stress caused decreased levels of amino acids and carbohydrates and elevated taurine, glycine- betaine, and homarine.	Rosenblum et al. (2006)
LC-MS/MS	*H. discus hannai*	Hepatopancreas	Environmental stress (thermal stress)	- A total of 1815 and 1314 differential metabolites were identified from the 10 °C and 30 °C acclimated groups, respectively.	Xu et al. (2020)
^{1}H NMR	*H. rufescens*	DG and foot	Environmental stress (thermal stress and food limitation and the Rickettsiales-like prokaryote [WS-RLP] infection)	- Increases and decreases of metabolites in foot and digestive gland after exposure to these stress factors. - Levels of homarine increased in the digestive gland of both food-limited and WS-RLP-infected animals. - Homarine levels only increased in the foot muscle of infected abalone	Rosenblum et al. (2005)
^{1}H NMR	*H. diversicolor*	Foot and gills	Environmental stress (thermal and hypoxic stresses)	- Thermal and hypoxic stresses disturb energy metabolism and osmotic regulation. - The double stresses induce gender-, time- and tissue-specific responses. - The gill is more easily affected than muscle by thermal and hypoxic stresses.	Lu et al. (2016)
^{1}H NMR	*H. fulgens*	Gill and hepatopancreas	Environmental stress (thermal stress, hypoxia and hypercapnia)	- Warming under combined hypoxia and hypercapnia elicited a severe change in accumulation of amino acids, osmolytes and anaerobic end products at intermediate temperatures, followed by declining concentrations at warmer temperatures.	Tripp-Valdez et al. (2017)
NMR, GC-TOF, GC–MSD, LC-MS/MS and LC-QTOF	*H. midae*	AM, foot, epipodial tissue, haemolymph, gills.	Environmental stress (hypoxia)	- Functional hypoxia caused increased levels of anaerobic end-products: lactate, alanopine, tauropine, succinate and alanine and elevation in arginine levels.	Venter et al. (2018a)
A multiplatform approach: NMR, LC–MS, GC–MS.	*H. midae*	AM, foot, epipodial tissue, haemolymph, gills.	Environmental stress (hypoxia)	- Hypoxia caused increased levels of anaerobic end-products and elevation of lactate, succinate and arginine.	Venter et al. (2018b)
UHPLC-QTOF-MS/MS	*H. discus hannai (DD)* and the hybrid *H. discus hannai* ♀ × *H. fulgens* ♂	Hemolymph and hepatopancreas	Environmental stress (hypoxia)	- Distinct metabolic shifts occur during the transition between normoxia and hypoxia in two abalones. - L-glutamate, 2-hydroxy-butanoic acid and 2-methyl-3-hydroxybutyric acid as potential biomarkers for hypoxia and reoxygenation response in abalone.	Shen et al. (2021)
HPLC	*H. diversicolor supertexta*	Hemolymph	Environmental stress (tributyltin [TBT])	- TBT caused increases of alanine, glutamate, acetate, pyruvate, succinate and decreases of lactate.	Zhou et al. (2010b)
^{1}H NMR	*H. diversicolor*	Hepatopancreas and gill tissues	Environmental stress (tributyltin/triphenyltin)	- Obvious gender-, tissue- and compound-specific responses were found. - Tributyltin/triphenyltin disturbed energy metabolism and osmotic regulation.	Lu et al. (2017a)
^{1}H NMR	*H. diversicolor supertexta*	Hemolymph	Environmental stress (dibutyl phthalate [DBP])	- DBP caused increases in the levels of intracellular metabolites (betaine, dimethylglycine, homarine, glutamine and lactate) and tricarboxylic acid cycle intermediates.	Zhou et al. (2015)
^{1}H NMR	*H. rufescens*	Foot, DG and hemolymph	Immunology (Rickettsiales-like prokaryote)	- Representative metabolites from several different classes involved in the disease process have been identified.	Viant et al. (2003)
^{1}H NMR	*H. diversicolor*	Hepatopancreas and gills	Immunology (*Vibrio parahaemolyticus*)	- Abalone responded to *V. parahaemolyticus* in sex- and tissue-specific manner. - *V. parahemolyticus* infection caused the disturbance in energy metabolism, nucleotide metabolism and osmotic balance, and also induced oxidative stress, immune stress and neurotoxic effect in different tissues.	Lu et al. (2017b)
GC–MS	*H. iris*	Foot	Immunology (*Vibrio splendidus*)	- *V. splendidus* caused changes of metabolites involved in oxidative stress.	Grandiosa et al. (2020)
^{1}H NMR	*H. rubra* × *H. laevigata*	DG	Feed and nutrition (starvation)	- There were significant differences in metabolite profiles between fed and starved abalone. - *N*-dimethylglycine is a robust marker for short-term starvation in abalone	Sheedy et al. (2016)
GC–MS	*H. iris*	Foot muscle	Feed and nutrition (multi-strain probiotics)	- There were 17 unique metabolites (amino acids, fatty acids and TCA cycle related compounds) different between probiotic-fed abalone and controlled abalone.	Grandiosa et al. (2018)

(*continued on next page*)

Table 4 (*continued*)

Metabolomics	*Species*	Tissues	Study field (Stress factors/ Comparison parameters)	Key Findings	Reference
LC-MS/MS	*H. midae*	AM	Feed and nutrition (proline-enriched abalone feed)	- A total of 29 metabolite were different between proline-enriched abalone and controlled abalone.	Venter et al. (2019)
Untargeted GC-TOF; semi-targeted LC-QTOF and targeted LC-MS/MS	*H. midae*	AM	Growth (slow and fast growth abalone)	- Many significantly different metabolites were identified between fast and slow growing abalone. - Faster growing individuals utilise energy pathways and reserves in such a way that they promote protein synthesis.	Venter et al. (2018c)
LC/MS	*H. discus hannai*	Embryo and larvae	Larval development	- Glucose-6-phosphatase, glucose-1-phosphate and UDP-glucose, the intermediates of glycogenesis and glycogenolysis, showed changes in the levels over the development of the abalone larvae. - Glucose and glycogen are required for proper energy balance in developing abalone and especially impact survival during settling.	Koyama et al. (2020)
GC–MS	*H. midae*	Foot	Others	- Establishing an untargeted GC–MS method for analyzing firstly a standard compound mixture consisting of 10 compounds.	Venter et al. (2016)
LC-QTOF	*H. midae*	AM	Others	- The presence of alanopine, lysopine, strombine and tauropine were confirmed in abalone mus- cle tissue	Venter et al. (2017)
UPLC-ESI-Q-TOF-MS-based lipidomic	*H. discus hannai*	Foot, viscera and gonads of male and female	Others	- 34 species from ten lipid classes were annotated by MS-DIAL to obtain all fragment ions for precursors. - Glycerophospholipids (GPLs) enriched in unsaturated fatty acids were the major components, which accounted for 52–57% of total lipids.	Zhang et al. (2018)
GC-TOF-MS	*H. gigantea*	AM and foot	Others	- Cholesterol was the most significantly different metabolite between abalones with orange muscles against those with common white muscles. - The accumulation of carotenoids was also related to fatty acid contents.	Wei et al. (2019)
LC-MS	*H. discus hannai*	Cerebral ganglia	Others	- The circadian physiological adaptation mechanism of abalone was revealed by metabolomics. - The changes of 11 metabolites were observed with the alternation of day and night.	Gao et al. (2020)

Abbreviations: AM: adductor muscle, DG: Digestive gland, ^{1}H NMR: Proton nuclear magnetic resonance, GC-MS: Gas chromatography–mass spectrometry, GC-MSD: Gas chromatography–mass spectrometry with single quadrupole, GC-TOF: Gas chromatography– time-of-flight, GC-TOF-MS: Gas chromatography– time-of-flight – mass spectrometry, HPLC: High performance liquid chromatography, LC-MS: Liquid chromatography–mass spectrometry, LC-MS/MS: Liquid chromatography with tandem mass spectrometry, LC-QTOF: Qualitative liquid chromatography quadrupole time of flight, LC-TOF: Liquid chromatography-time-of-flight, UPLC-ESI-Q-TOF-MS: Ultraperformance liquid chromatography coupled with electrospray ionization tandem quadrupole time-of-flight mass spectrometry.

differences of abalone in response to environmental stresses and diseases is an important constraint when designing experiments and when interpreting metabolomics data.

Since metabolites are downstream low-weight molecules of gene expression and cellular processes, metabolomics techniques could be used to complement other approaches, such as flow cytometry (Nguyen, 2020), imaging and histology (Calvo et al., 2015) and other omics approaches (e.g., genomics, transcriptomics and proteomics) (Pinu et al., 2019). Many abalone studies have found a correlation between metabolomics data and data obtained from other techniques, such as histopathology (Rosenblum et al., 2005; Rosenblum et al., 2006; Zhou et al., 2010b), enzyme activity assays (Zhou et al., 2010b), flow cytometry (Grandiosa et al., 2018; Grandiosa et al., 2020) and immunofluorescence staining (Koyama et al., 2020). However, no integration between metabolomics and other omics has been reported for abalone and remains as an opportunity for future studies.

Overall, these metabolomics studies demonstrate the great potential of this approach for abalone and aquaculture research. The published metabolomics papers have significantly improved our knowledge of molecular mechanisms underlying the different biological processes of abalone. The available metabolic-related knowledge and candidate metabolite biomarkers in abalone represent a good basis for further development of metabolomics, which could be applied for health assessment, disease diagnosis or selective breeding for the traits of interest. Despite great promise, metabolomics applications for marine organisms face some challenges, such as the identification of metabolites, the presence of unknown metabolites, validation of biomarkers, inter-laboratory variations (Beale et al., 2018; Nguyen et al., 2019; Nguyen, 2020). For abalone and other molluscs, there is another obstacle for predicting metabolic pathways due to the unavailability of public databases (e.g., KEGG) for molluscan species. The use of other species for pathway analysis may not accurately reflect the pathways encoded in non-model systems. Hence, it is crucial for future studies to develop a public database for a molluscan model for pathway interpretation.

6. Integrated omics for abalone

Since each omics has its advantages and limitations, the integration of multi-dimensional omics would no doubt provide a more comprehensive investigation of biological pathways. The emergence of different omics techniques (e.g., genomics, transcriptomics, proteomics, metabolomics) in all aspects of life science has led to the growing application of integrated multiple omics. While the majority of these multi-omics

studies only integrate two omics, examples of successful multi-omics integration with at least three different omics platforms have been recently reviewed by Misra et al. (2019).

Successful implementation of datasets from at least two omics in abalone studies is very rare. The first multiple omics investigation for abalone was reported by Palmer et al. (2013) who used transcriptomic (RNA-Seq) and proteomic techniques (MS) to characterize the testis transcriptome and sperm proteome of red abalone (*H. rufescens*). This integrated approach revealed an abundant and rapidly evolving abalone sperm protein. Similarly, Kim et al. (2019) combined RNA-Seq for transcriptome and LC-MS/MS analysis for peptidome of neural ganglia to study sexual maturation in female Pacific abalone (*H. discus hannai*). The results identified 256 DEGs from neural ganglia transcriptomes of immature and mature abalone. The predicted peptide database of abalone ganglia transcriptome unigenes was then used to support analyses of peptides which resulted in identification of 42 neuropeptide precursors. Among these, 29 precursors were validated by peptidomic analyses and 18 neuropeptide families were revealed as different between immature and mature abalone *via* label-free quantification methods. The common feature of these two studies is the use of proteomics techniques to validate and confirm genes of interest. However, no correlation analysis has been used between genes and proteins to date for abalone.

The increasing importance of abalone aquaculture and reducing cost in omics applications will no doubt lead to more multiple integrated omics investigations for abalone as well as other aquaculture species. Such approaches will unlock the full utility of different omics resources which proves critical to overcome poorly understood biological impediments that affect production of abalone (e.g., slow growth rate/stunting, disease, reproductive failure). The integration of multiple omics will also improve the accuracy of biomarkers through different expression levels (e.g., gene, protein, or metabolite) that are critical for future development of breeding selective programmes for important commercial traits (e.g., fast growth rate, disease resistance, heat resistance).

Although integrated multiple-omics approaches offer multiple benefits, differences in nomenclature among omics data types make the integration of these multi-dimensional omics data and their interpretation challenging. These challenges exist in all steps of the workflow from experimental design to data processing, data analysis and biological interpretation (Misra et al., 2019; Pinu et al., 2019). Despite significant efforts underway to explore the capabilities of different omics platforms, no single approach currently exists for optimised workflows (Misra et al., 2019). Omics approaches are relatively expensive, and integrated multi-omics can be even more expensive. For example, the cost for a robust multi-omics study on a human research population could be up to US$ 500,000 (Pinu et al., 2019). Compared to the research funds often available for human research, budgets for marine studies or aquaculture are limited, which is often the central hinderance for multi-omics studies.

7. Conclusions

Applications of omics in abalone research have grown rapidly during the last few years, and have contributed to diverse aspects of abalone biology, immunology and physiology. This knowledge is crucial for the development of management strategies in abalone fisheries and aquaculture. In addition, omics studies have aimed to identify novel biomarker profiles that are characteristic of disease, growth and stress conditions in abalone. Such biomarkers could be used in future studies for health assessment, environmental monitoring, selective breeding programmes and assessment of the efficacy of antibiotic treatments, among others. Despite these efforts, omics research is relatively limited for abalone compared with other commercially important vertebrate and invertebrate species. Since omics is a growing area of research, and many omics approaches (e.g., proteomics and metabolomics) are new in aquaculture, the applications of these techniques are facing a number of technical and financial challenges, especially for truly integrated multi-omics studies. Even with these obstacles in mind, there is no doubt that omics will continue to expand in aquaculture and marine science, leading to novel discoveries and accurate molecular biomarkers, which can be used for management strategies in both aquaculture and fisheries.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This project was funded by the New Zealand Ministry of Business, Innovation and Employment's Aquatic Health Strategies to Maximise Productivity and Security programme (CAWX1707) and Cawthron Shellfish Aquaculture programme (CAWX1801) and a New Zealand Pāua Industry Council grant. We are thankful to the Aquaculture Biotechnology Research Group for ongoing support. This work was also funded by a Vienna Research and Technology Grant VRG14-021 and an ERC Starting Grant 'EvoLucin' and a grant from the Tasmanian Government Abalone Industry Reinvestment Fund (2020/43).

References

Abid, F., Zahid, M.A., Abedin, Z.U., Nizami, S.B., Abid, M.J., Kazmi, S.Z.H., Khan, S.U., Hasan, H., Ali, M., Gul, A., 2018. Chapter 3 - Omics approaches in marine biotechnology: The treasure of ocean for human betterments. In: Barh, D., Azevedo, V. (Eds.), Omics Technologies and Bio-Engineering. Academic Press, pp. 47–61.

Aizat, W.M., Goh, H.-H., Baharum, S.N., 2018. Omics Applications for Systems Biology. Springer.

Alfaro, A., Nguyen, V.T., Mellow, D., 2019. A metabolomics approach to assess the effect of storage conditions on metabolic processes of New Zealand surf clam (*Crassula aequilatera*). Aquaculture. 498, 315–321. https://doi.org/10.1016/j.aquaculture.2018.08.065.

Bai, C.-M., Zhang, S.-M., Li, Y.-N., Xin, L.-S., Rosani, U., Wang, C.-M., 2019. Dual transcriptomic analysis reveals a delayed antiviral response of *Haliotis diversicolor supertexta* against Haliotid Herpesvirus-1. Viruses. 11, 383. https://doi.org/10.3390/v11040383.

Bathige, S.D.N.K., Umasuthan, N., Godahewa, G.I., Thulasitha, W.S., Whang, I., Won, S. H., Kim, C., Lee, J., 2015. Two variants of selenium-dependent glutathione peroxidase from the disk abalone *Haliotis discus discus*: molecular characterization and immune responses to bacterial and viral stresses. Fish Shellfish Immunol. 45, 648–655. https://doi.org/10.1016/j.fsi.2015.05.028.

Beale, D.J., Pinu, F.R., Kouremenos, K.A., Poojary, M.M., Narayana, V.K., Boughton, B. A., Kanojia, K., Dayalan, S., Jones, O.A., Dias, D.A., 2018. Review of recent developments in GC–MS approaches to metabolomics-based research. Metabolomics. 14, 1–31.

Beltran, C.G.G., 2015. A Proteomic Investigation of the Immune Response of the South African Abalone, *Haliotis midae*. University of Cape Town, PhD, Cape Town, South Africa (201 pp).

Beltran, C.G.G., Coyne, V.E., 2020. iTRAQ-based quantitative proteomic profiling of the immune response of the south African abalone, *Haliotis midae*. Fish Shellfish Immunol. 99, 130–143. https://doi.org/10.1016/j.fsi.2020.02.007.

Blaauw, S., Du Plessis, J., Roodt-Wilding, R., 2013. Transcriptome-wide single nucleotide polymorphisms (SNPs) for abalone (*Haliotis midae*): validation and application using GoldenGate medium-throughput genotyping assays. Int. J. Mol. Sci. 14, 19341–19360.

Botwright, N.A., Zhao, M., Wang, T., McWilliam, S., Colgrave, M.L., Hlinka, O., Li, S., Suwansa-ard, S., Subramanian, S., McPherson, L., King, H., Reverter, A., Cook, M.T., McGrath, A., Elliott, N.G., Cummins, S.F., 2019. Greenlip abalone (*Haliotis laevigata*) genome and protein analysis provides insights into maturation and spawning. G3 9, 3067–3078.

Calder, B., 2014. A Proteomic Investigation of the Heat Stress Response of the South African Abalone *Haliotis midae*. University of Cape Town, PhD, Cape Town, South Africa (202 pp).

Calvo, N., Beltrán-Debón, R., Rodríguez-Gallego, E., Hernández-Aguilera, A., Guirro, M., Mariné-Casadó, R., Millá, L., Alegret, J.M., Sabench, F., del Castillo, D., 2015. Liver fat deposition and mitochondrial dysfunction in morbid obesity: An approach combining metabolomics with liver imaging and histology. World J Gastroenterol: WJG 21, 7529.

Chang, P.H., Kuo, S.T., Lai, S.H., Yang, H.S., Ting, Y.Y., Hsu, C.L., Chen, H.C., 2005. Herpes-like virus infection causing mortality of cultured abalone *Haliotis diversicolor* supertexta in Taiwan. Dis. Aquat. Org. 65, 23–27.

Chávez-Mardones, J., Gallardo-Escárate, C., 2014. Immune response of apoptosis-related cysteine peptidases from the red abalone *Haliotis rufescens* (HrCas8 and HrCas3): molecular characterization and transcription expression. Fish Shellfish Immunol. 39, 90–98.

Chen, N., Huang, Z., Lu, C., Shen, Y., Luo, X., Ke, C., You, W., 2018. Different transcriptomic responses to thermal stress in heat-tolerant and heat-sensitive Pacific abalones indicated by cardiac performance. Front. Physiol. 9. https://doi.org/10.3389/fphys.2018.01895.

Choi, M.-J., Kim, G.-D., Kim, J.-M., Lim, H., 2015. Differentially-expressed genes associated with faster growth of the Pacific abalone, *Haliotis discus hannai*. Int. J. Mol. Sci. 16, 27520–27534.

Corbeil, S., 2020. Abalone Viral Ganglioneuritis. Pathogens. 9, 720.

De Wit, P., Palumbi, S.R., 2013. Transcriptome-wide polymorphisms of red abalone (*Haliotis rufescens*) reveal patterns of gene flow and local adaptation. Mol. Ecol. 22, 2884–2897.

Di, G., You, W., Yu, J., Wang, D., Ke, C., 2013. Genetic changes in muscle protein following hybridization between *Haliotis diversicolor reeve* Japan and Taiwan populations revealed using a proteomic approach. Proteomics. 13, 845–859.

Di, G., Luo, X., Huang, M., Chen, J., Kong, X., Miao, X., Ke, C., 2015a. Proteomic profiling of eggs from a hybrid abalone and its parental lines: *Haliotis discus hannai* Ino and *Haliotis gigantea*. Anim. Genet. 46, 646–654.

Di, G., Luo, X., You, W., Zhao, J., Kong, X., Ke, C., 2015b. Proteomic analysis of muscle between hybrid abalone and parental lines *Haliotis gigantea* reeve and *Haliotis discus hannai* Ino. Heredity. 114, 564–574.

Di, G., Miao, X., Ke, C., Kong, X., Li, H., You, W., 2016. Protein changes in abalone foot muscle from three geographical populations of *Haliotis diversicolor* based on proteomic approach. Ecol. Evol. 6, 3645–3657.

Di, G., Kong, X., Miao, X., Zhang, Y., Huang, M., Gu, Y., You, W., Zhang, J., Ke, C., 2017. Proteomic analysis of trochophore and veliger larvae development in the small abalone *Haliotis diversicolor*. BMC Genomics 18, 809. https://doi.org/10.1186/s12864-017-4203-7.

Dias, V.L., 2016. Investigation of the Effect of a Probiotic-Supplemented Diet on the Haemocyte Proteome of the Abalone *Haliotis midae*. University of Cape Town, PhD, Cape Tow, South Africa (205 pp).

Ellis, R.P., Spicer, J.I., Byrne, J.J., Sommer, U., Viant, M.R., White, D.A., Widdicombe, S., 2014. 1H NMR metabolomics reveals contrasting response by male and female mussels exposed to reduced seawater pH, increased temperature, and a pathogen. Environ. Sci. Technol. 48, 7044–7052.

Estes, J.A., Lindberg, D.R., Wray, C., 2005. Evolution of large body size in abalones (Haliotis): patterns and implications. Paleobiology. 31, 591–606.

FAO, 2017. The Report Analyses the Market Situation Over the Year 2016. http://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/902597/ (accessed 14 April 2020).

FAO, 2021. Global Aquaculture Production 1950–2019 (Online Query). http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en.

Feng, Y., Yuan, B., Jiang, X., Liu, X., Li, Z., Sun, G., Xu, X., 2020. Comparative transcriptome analysis of abalone Haliotis discus hannai with green and gray egg colors. J. Oceanol. Limnol. 1–12.

Franchini, P., Van der Merwe, M., Roodt-Wilding, R., 2011. Transcriptome characterization of the South African abalone *Haliotis midae* using sequencing-by-synthesis. BMC Res. Notes 4, 59.

Gan, H.M., Tan, M.H., Austin, C.M., Sherman, C.D.H., Wong, Y.T., Strugnell, J., Gervis, M., McPherson, L., Miller, A.D., 2019. Best foot forward: nanopore long reads, hybrid meta-assembly, and haplotig purging optimizes the first genome assembly for the southern hemisphere blacklip abalone (*Haliotis rubra*). Front. Genet. 10 https://doi.org/10.3389/fgene.2019.00889.

Gao, X., Luo, X., You, W., Ke, C., 2020. Circadian movement behaviours and metabolism differences of the Pacific abalone *Haliotis discus hannai*. J. Photochem. Photobiol. B Biol. 211, 111994. https://doi.org/10.1016/j.jphotobiol.2020.111994.

Garalde, D.R., Snell, E.A., Jachimowicz, D., Sipos, B., Lloyd, J.H., Bruce, M., Pantic, N., Admassu, T., James, P., Warland, A., 2018. Highly parallel direct RNA sequencing on an array of nanopores. Nat. Methods 15, 201.

Geiger, D.L., 2000. Distribution and biogeography of the *Haliotidae* (Gastropoda: Vetigastropoda) world-wide. Boll. Malacologico. 35, 57–120.

Grandiosa, R., Mérien, F., Young, T., Van Nguyen, T., Gutierrez, N., Kitundu, E., Alfaro, A.C., 2018. Multi-strain probiotics enhance immune responsiveness and alters metabolic profiles in the New Zealand black-footed abalone (*Haliotis iris*). Fish Shellfish Immunol. 82, 330–338. https://doi.org/10.1016/j.fsi.2018.08.034.

Grandiosa, R., Young, T., Nguyen, V.T., Mérien, F., Alfaro, A.C., 2020. Immune response in probiotic-fed New Zealand black-footed abalone (*Haliotis iris*) under *Vibrio splendidus* challenge. Fish Shellfish Immunol. 104, 633–639. https://doi.org/10.1016/j.fsi.2020.06.007.

Guo, Z., Ding, Y., Han, L., Hou, X., 2019. Characterization of the complete mitochondrial genome of Pacific abalone *Haliotis discus hannai*. Mitochondrial DNA Part B. 4, 717–718. https://doi.org/10.1080/23802359.2018.1495125.

Hannon, C., Officer, R.A., Le Dorven, J., 2013. Review of the technical challenges facing aquaculture of the European abalone Haliotis tuberculata in Ireland. Aquac. Int. 21, 243–254. https://doi.org/10.1007/s10499-012-9584-7.

Harney, E., Dubief, B., Boudry, P., Basuyaux, O., Schilhabel, M.B., Huchette, S., Paillard, C., Nunes, F.L., 2016. De novo assembly and annotation of the European abalone *Haliotis tuberculata* transcriptome. Mar. Genomics 28, 11–16.

Harney, E., Lachambre, S., Roussel, S., Huchette, S., Enez, F., Morvezen, R., Haffray, P., Boudry, P., 2018. Transcriptome based SNP discovery and validation for parentage assignment in hatchery progeny of the European abalone *Haliotis tuberculata*. Aquaculture. 491, 105–113. https://doi.org/10.1016/j.aquaculture.2018.03.006.

Hooper, C., Hardy-Smith, P., Handlinger, J., 2007. Ganglioneuritis causing high mortalities in farmed Australian abalone (*Haliotis laevigata* and *Haliotis rubra*). Aust. Vet. J. 85, 188–193.

Huang, Z.-X., Chen, Z.-S., Ke, C.-H., Zhao, J., You, W.-W., Zhang, J., Dong, W.-T., Chen, J., 2012. Pyrosequencing of *Haliotis diversicolor* transcriptomes: insights into early developmental molluscan gene expression. PLoS One 7, e51279.

Huang, J., You, W., Luo, X., Ke, C., 2017. iTRAQ-based identification of proteins related to muscle growth in the pacific abalone, *Haliotis discus hannai*. Int. J. Mol. Sci. 18, 2237.

Ji, C., Wu, H., Wei, L., Zhao, J., Yu, J., 2013. Proteomic and metabolomic analysis reveal gender-specific responses of mussel *Mytilus galloprovincialis* to 2, 2′, 4, 4′-tetrabromodiphenyl ether (BDE 47). Aquat. Toxicol. 140, 449–457.

Jia, X., Zou, Z., Wang, G., Wang, S., Wang, Y., Zhang, Z., 2011. Gene expression profiling in respond to TBT exposure in small abalone *Haliotis diversicolor*. Fish Shellfish Immunol. 31, 557–563. https://doi.org/10.1016/j.fsi.2011.07.004.

Kang, H.Y., Lee, Y.-J., Song, W.-Y., Kim, T.-I., Lee, W.-C., Kim, T.Y., Kang, C.-K., 2019. Physiological responses of the abalone *Haliotis discus hannai* to daily and seasonal temperature variations. Sci. Rep. 9, 8019. https://doi.org/10.1038/s41598-019-44526-3.

Kim, M., Rhee, J.-S., Kim, T., Lee, J., Choi, A.-Y., Choi, B.-S., Choi, I.-Y., Sohn, Y., 2017. Alternative splicing profile and sex-preferential gene expression in the female and male Pacific abalone *Haliotis discus hannai*. Genes. 8, 99.

Kim, M., Markkandan, K., Han, N.-Y., Park, J.-M., Lee, J.S., Lee, H., Sohn, Y.C., 2019. Neural ganglia transcriptome and peptidome associated with sexual maturation in female pacific abalone (*Haliotis discus hannai*). Genes. 10, 268.

Kim, M.A., Kim, T.H., Lee, S., Nam, B.-H., Lee, J.S., Jang, W., Sohn, Y.C., 2020. Ovarian transcriptome profiles associated with sexual maturation in Pacific abalone (*Haliotis discus hannai*). Genes Genomics 1–10. https://doi.org/10.1007/s13258-020-00983-z.

Kim, C.-H., Kim, E.J., Seo, C., Park, C.J., Nam, Y.K., 2021. Transcriptome expression profiles between diploid and triploid Pacific abalone (Haliotis discus hannai) juveniles in response to acute heat-stress and hypoxia treatments. Mar. Genomics 57, 100820. https://doi.org/10.1016/j.margen.2020.100820.

Koyama, M., Furukawa, F., Koga, Y., Funayama, S., Furukawa, S., Baba, O., Lin, C.-C., Hwang, P.-P., Moriyama, S., Okumura, S.-I., 2020. Gluconeogenesis and glycogen metabolism during development of Pacific abalone, *Haliotis discus hannai*. Am. J. Phys. Regul. Integr. Comp. Phys. 318, R619–R633. https://doi.org/10.1152/ajpregu.00211.2019.

Kyeong, D., Kim, J., Shin, Y., Subramaniyam, S., Kang, B.-C., Shin, E.-H., Park, E.H., Noh, E.S., Kim, Y.-O., Park, J.Y., 2020. Expression of heat shock proteins in thermally challenged Pacific abalone *Haliotis discus hannai*. Genes. 11, 22. https://doi.org/10.3390/genes11010022.

Le Roy, N., Marie, B., Gaume, B., Guichard, N., Delgado, S., Zanella-Cléon, I., Becchi, M., Auzoux-Bordenave, S., Sire, J.-Y., Marin, F., 2012. Identification of two carbonic anhydrases in the mantle of the european abalone *Haliotis tuberculata* (Gastropoda, Haliotidae): phylogenetic implications. J. Exp. Zool. B Mol. Dev. Evol. 318, 353–367.

Liebler, D.C., 2001. Introduction to Proteomics: Tools for the New Biology, 1st Illustrated Edition Ed. Humana Press.

Liu, Y., Tam, N.F.Y., Guan, Y., Yasojima, M., Zhou, J., Gao, B., 2011. Acute toxicity of nonylphenols and bisphenol A to the embryonic development of the abalone *Haliotis diversicolor supertexta*. Ecotoxicology. 20, 1233–1245. https://doi.org/10.1007/s10646-011-0672-7.

Long, A., Bunning, B., Borro, M., Sampath, V., Nadeau, K.C., 2019. The future of omics for clinical practice. Ann. Allergy Asthma Immunol. 123, 535–536.

Lu, J., Shi, Y., Wang, S., Chen, H., Cai, S., Feng, J., 2016. NMR-based metabolomic analysis of *Haliotis diversicolor* exposed to thermal and hypoxic stresses. Sci. Total Environ. 545, 280–288.

Lu, J., Feng, J., Cai, S., Chen, Z., 2017a. Metabolomic responses of *Haliotis diversicolor* to organotin compounds. Chemosphere. 168, 860–869.

Lu, J., Shi, Y., Cai, S., Feng, J., 2017b. Metabolic responses of *Haliotis diversicolor* to *Vibrio parahaemolyticus* infection. Fish Shellfish Immunol. 60, 265–274.

Mann, K., Cerveau, N., Gummich, M., Fritz, M., Mann, M., Jackson, D.J., 2018. In-depth proteomic analyses of *Haliotis laevigata* (greenlip abalone) nacre and prismatic organic shell matrix. Proteome Sci. 16, 11. https://doi.org/10.1186/s12953-018-0139-3.

Marie, B., Marie, A., Jackson, D.J., Dubost, L., Degnan, B.M., Milet, C., Marin, F., 2010. Proteomic analysis of the organic matrix of the abalone *Haliotis asinina* calcified shell. Proteome Sci. 8, 54.

Masonbrink, R.E., Purcell, C.M., Boles, S.E., Whitehead, A., Hyde, J.R., Seetharam, A.S., Severin, A.J., 2019. An annotated genome for *Haliotis rufescens* (red abalone) and resequenced green, pink, pinto, black, and white abalone specie. Genome Biol. Evol. 11, 431–438.

Masoomi Dezfooli, S., Gutierrez-Maddox, N., Alfaro, A.C., Seyfoddin, A., 2021. Development of a microencapsulated probiotic delivery system for New Zealand black-footed abalone (*Haliotis iris*). Pharm. Dev. Technol. 1–13. https://doi.org/10.1080/10837450.2021.1876090.

Maynard, B.T., Kerr, L.J., McKiernan, J.M., Jansen, E.S., Hanna, P.J., 2005. Mitochondrial DNA sequence and gene organization in the Australian blacklip abalone *Haliotis rubra* (leach). Mar. Biotechnol. 7, 645–658.

Mendoza-Porras, O., Botwright, N.A., McWilliam, S.M., Cook, M.T., Harris, J.O., Wijffels, G., Colgrave, M.L., 2014. Exploiting genomic data to identify proteins involved in abalone reproduction. J. Proteome 108, 337–353. https://doi.org/10.1016/j.jprot.2014.06.001.

Mendoza-Porras, O., Botwright, N.A., Reverter, A., Cook, M.T., Harris, J.O., Wijffels, G., Colgrave, M.L., 2017. Identification of differentially expressed reproductive and metabolic proteins in the female abalone (*Haliotis laevigata*) gonad following

artificial induction of spawning. Comp. Biochem. Physiol. Part D 24, 127–138. https://doi.org/10.1016/j.cbd.2016.04.005.
Misra, B.B., Langefeld, C., Olivier, M., Cox, L.A., 2019. Integrated omics: tools, advances and future approaches. J. Mol. Endocrinol. 62, R21–R45.
Munasinghe, H., Kang, H.S., Lee, J., 2006. Analysis of digestive gland expressed sequence tag library from the disk abalone, *Haliotis discus discus*. J. World Aquacult. Soc. 37, 96–106.
Nam, B.-H., Jung, M., Subramaniyam, S., Yoo, S.-I., Markkandan, K., Moon, J.-Y., Kim, Y.-O., Kim, D.-G., An, C.M., Shin, Y., 2016. Transcriptome analysis revealed changes of multiple genes involved in *Haliotis discus hannai* innate immunity during *Vibrio parahemolyticus* infection. PLoS One 11, e0153474.
Nam, B.-H., Kwak, W., Kim, Y.-O., Kim, D.-G., Kong, H.J., Kim, W.-J., Kang, J.-H., Park, J. Y., An, C.M., Moon, J.-Y., Park, C.J., Yu, J.W., Yoon, J., Seo, M., Kim, K., Kim, D.K., Lee, S., Sung, S., Lee, C., Shin, Y., Jung, M., Kang, B.-C., Shin, G.-H., Ka, S., Caetano-Anolles, K., Cho, S., Kim, H., 2017. Genome sequence of pacific abalone (*Haliotis discus hannai*): the first draft genome in family Haliotidae. GigaScience. 6, 1–8.
Nel, A., Pletschke, B.I., Britz, P.J., 2017. The effect of low-level kelp supplementation on digestive enzyme activity levels in cultured abalone *Haliotis midae* fed formulated feeds. Afr. J. Mar. Sci. 39, 175–182.
Nguyen, V.T., 2020. Metabolomics Applications in Immunological Studies of Marine Molluscs. Auckland University of Technology, Auckland, New Zealand. https://doi.org/10.13140/RG.2.2.10327.21929. PhD Thesis. (329 pp).
Nguyen, V.T., Alfaro, A., 2020. Applications of omics to investigate responses of bivalve haemocytes to pathogen infections and environmental stress. Aquaculture. 518, 734488. https://doi.org/10.1016/j.aquaculture.2019.734488.
Nguyen, V.T., Alfaro, A., Merien, F., Lulijwa, R., Young, T., 2018a. Copper-induced immunomodulation in mussel (*Perna canaliculus*) haemocytes. Metallomics. 10, 965–978. https://doi.org/10.1039/c8mt00092a.
Nguyen, V.T., Alfaro, A., Merien, F., Young, T., Grandiosa, R., 2018b. Metabolic and immunological responses of male and female New Zealand GreenshellTM mussels (*Perna canaliculus*) during *Vibrio* sp. infection. J. Invertebr. Pathol. 157, 80–89. https://doi.org/10.1016/j.jip.2018.08.008.
Nguyen, V.T., Alfaro, A., Young, T., Merien, F., 2018c. Tissue-specific immune responses to *Vibrio* sp. infection in mussels (*Perna canaliculus*): a metabolomics approach. Aquaculture. 500, 118–125. https://doi.org/10.1016/j.aquaculture.2018.09.061.
Nguyen, V.T., Alfaro, A., Merien, F., 2019. Omics approaches to investigate host-pathogen interactions in mass mortality outbreaks of *Crassostrea gigas*. Rev. Aquac. 11, 1308–1324. https://doi.org/10.1111/raq.12294.
Palmer, M.R., McDowall, M.H., Stewart, L., Ouaddi, A., MacCoss, M.J., Swanson, W.J., 2013. Mass spectrometry and next-generation sequencing reveal an abundant and rapidly evolving abalone sperm protein. Mol. Reprod. Dev. 80, 460–465.
Picone, B., Rhode, C., Roodt-Wilding, R., 2015. Transcriptome profiles of wild and cultured south African abalone, *Haliotis midae*. Mar. Genomics 20, 3–6.
Picone, B., Rhode, C., Roodt-Wilding, R., 2017. Identification and characterization of miRNAs transcriptome in the south African abalone, *Haliotis midae*. Mar. Genomics 31, 9–12.
Pinu, F.R., Beale, D.J., Paten, A.M., Kouremenos, K., Swarup, S., Schirra, H.J., Wishart, D., 2019. Systems biology and multi-omics integration: viewpoints from the metabolomics research community. Metabolites. 9, 76.
Ponder, W.F., Lindberg, D.R., Ponder, J.M., 2019. Biology and Evolution of the mollusca (Taylor & Francis Group).
Robinson, N.A., Hall, N.E., Ross, E.M., Cooke, I.R., Shiel, B.P., Robinson, A.J., Strugnell, J.M., 2016. The complete mitochondrial genome of Haliotis laevigata (Gastropoda: Haliotidae) using MiSeq and HiSeq sequencing. Mitochondrial DNA Part A. 27, 437–438.
Roodt-Wilding, R., 2007. Abalone ranching: a review on genetic considerations. Aquac. Res. 38, 1229–1241.
Rosenblum, E., Viant, M., Braid, B., Moore, J., Friedman, C., Tjeerdema, R., 2005. Characterizing the metabolic actions of natural stresses in the California red abalone, *Haliotis rufescens* using 1 H NMR metabolomics. Metabolomics. 1, 199–209.
Rosenblum, E.S., Tjeerdema, R.S., Viant, M.R., 2006. Effects of temperature on host–pathogen– drug interactions in red abalone, *Haliotis rufescens*, determined by 1H NMR metabolomics. Environ. Sci. Technol. 40, 7077–7084.
Sawabe, T., Inoue, S., Fukui, Y., Yoshie, K., Nishihara, Y., Miura, H., 2007. Mass mortality of Japanese abalone *Haliotis discus hannai* caused by *Vibrio harveyi* infection. Microbes Environ. 22, 300–308.
Sheedy, J.R., Lachambre, S., Gardner, D.K., Day, R.W., 2016. 1H-NMR metabolite profiling of abalone digestive gland in response to short-term starvation. Aquac. Int. 24, 503–521. https://doi.org/10.1007/s10499-015-9941-4.
Shen, Y., Huang, Z., Liu, G., Ke, C., You, W., 2019. Hemolymph and transcriptome analysis to understand innate immune responses to hypoxia in Pacific abalone. Comp. Biochem. Physiol. Part D 30, 102–112. https://doi.org/10.1016/j.cbd.2019.02.001.
Shen, Y., Zhang, Y., Xiao, Q., Gan, Y., Wang, Y., Pang, G., Huang, Z., Yu, F., Luo, X., Ke, C., You, W., 2021. Distinct metabolic shifts occur during the transition between normoxia and hypoxia in the hybrid and its maternal abalone. Sci. Total Environ. 794, 148698. https://doi.org/10.1016/j.scitotenv.2021.148698.
Shiel, B., 2017. Stress Transcriptomics: Understanding the Genetic Basis of Summer Mortality in Abalone. La Trobe University, PhD, Victoria, Australia (197 pp).
Shiel, B.P., Hall, N.E., Cooke, I.R., Robinson, N.A., Strugnell, J.M., 2015. De novo characterisation of the greenlip abalone transcriptome (*Haliotis laevigata*) with a focus on the heat shock protein 70 (HSP70) family. Mar. Biotechnol. 17, 23–32.
Shiel, B.P., Hall, N.E., Cooke, I.R., Robinson, N.A., Strugnell, J.M., 2017. Epipodial tentacle gene expression and predetermined resilience to summer mortality in the commercially important greenlip abalone, *Haliotis laevigata*. Mar. Biotechnol. 19, 191–205.
Shiel, B.P., Cooke, I.R., Hall, N.E., Robinson, N.A., Strugnell, J.M., 2020. Gene expression differences between abalone that are susceptible and resilient to a simulated heat wave event. Aquaculture. 526, 735317. https://doi.org/10.1016/j.aquaculture.2020.735317.
Silva-Aciares, F., Zapata, M., Tournois, J., Moraga, D., Riquelme, C., 2011. Identification of genes expressed in juvenile *Haliotis rufescens* in response to different copper concentrations in the north of Chile under controlled conditions. Mar. Pollut. Bull. 62, 2671–2680. https://doi.org/10.1016/j.marpolbul.2011.09.023.
Silva-Aciares, F., Moraga, D., Auffret, M., Tanguy, A., Riquelme, C., 2013. Transcriptomic and cellular response to bacterial challenge (pathogenic *Vibrio parahaemolyticus*) in farmed juvenile *Haliotis rufescens* fed with or without probiotic diet. J. Invertebr. Pathol. 113, 163–176.
Slattery, M., Ankisetty, S., Corrales, J., Marsh-Hunkin, K.E., Gochfeld, D.J., Willett, K.L., Rimoldi, J.M., 2012. Marine proteomics: a critical assessment of an emerging technology. J. Nat. Prod. 75, 1833–1877.
Sun, Y., Zhang, X., Wang, Y., Day, R., Yang, H., Zhang, Z., 2019. Immunity-related genes and signaling pathways under hypoxic stresses in *Haliotis diversicolor* a transcriptome analysis. Sci. Rep. 9, 1–15.
Sussulini, A., 2017. Metabolomics: From Fundamentals to Clinical Applications. Springer.
ten Hacken, E., Clement, K., Li, S., Hernández-Sánchez, M., Redd, R., Wang, S., Ruff, D., Gruber, M., Baranowski, K., Jacob, J., Flynn, J., Jones, K.W., Neuberg, D., Livak, K. J., Pinello, L., Wu, C.J., 2020. High throughput single-cell detection of multiplex CRISPR-edited gene modifications. Genome Biol. 21, 266. https://doi.org/10.1186/s13059-020-02174-1.
Tomanek, L., 2014. Proteomics to study adaptations in marine organisms to environmental stress. J. Proteome 105, 92–106.
Tripp-Valdez, M.A., Bock, C., Lucassen, M., Lluch-Cota, S.E., Sicard, M.T., Lannig, G., Pörtner, H.O., 2017. Metabolic response and thermal tolerance of green abalone juveniles (*Haliotis fulgens*: Gastropoda) under acute hypoxia and hypercapnia. J. Exp. Mar. Biol. Ecol. 497, 11–18. https://doi.org/10.1016/j.jembe.2017.09.002.
Tripp-Valdez, M.A., Harms, L., Pörtner, H.O., Sicard, M.T., Lucassen, M., 2019. De novo transcriptome assembly and gene expression profile of *thermally* challenged green abalone (*Haliotis* fulgens: Gastropoda) under acute hypoxia and hypercapnia. Mar. Genomics 45, 48–56.
Tripp-Valdez, M.A., Cicala, F., Galindo-Sánchez, C.E., Chacón-Ponce, K.D., López-Landavery, E., Díaz, F., Re-Araujo, D., Lafarga-De la Cruz, F., 2021. Growth performance and transcriptomic response of warm-acclimated hybrid abalone Haliotis rufescens (♀) × H. corrugata (♂). Mar. Biotechnol. 23, 62–76. https://doi.org/10.1007/s10126-020-10002-7.
Vailati-Riboni, M., Palombo, V., Loor, J.J., 2017. What are omics sciences? In: Ametaj, B. N. (Ed.), Periparturient Diseases of Dairy Cows: A Systems Biology Approach. Springer International Publishing, Cham, pp. 1–7.
Valenzuela-Miranda, D., Del Río-Portilla, M.A., Gallardo-Escárate, C., 2015. Characterization of the growth-related transcriptome in California red abalone (*Haliotis rufescens*) through RNA-Seq analysis. Mar. Genomics 24, 199–202.
Valenzuela-Muñoz, V., Bueno-Ibarra, M.A., Escárate, C.G., 2014. Characterization of the transcriptomes of *Haliotis rufescens* reproductive tissues. Aquac. Res. 45, 1026–1040.
van der Merwe, M., 2010. Growth-Related Gene Expression in *Haliotis midae*. University of Stellenbosch, PhD, Stellenbosch, South Africa (212 pp).
van der Merwe, M., Franchini, P., Roodt-Wilding, R., 2011. Differential growth-related gene expression in abalone (*Haliotis midae*). Mar. Biotechnol. 13, 1125–1139.
Venter, L., van Rensburg, P.J., Vosloo, A., Lindeque, J.Z., 2016. Untargeted metabolite profiling of abalone using gas chromatography mass spectrometry. Food Anal. Methods 9, 1254–1261.
Venter, L., van Rensburg, P.J.J., Vosloo, A., Lindeque, J.Z., 2017. From untargeted LC-QTOF analysis to characterisation of opines in abalone adductor muscle: theory meets practice. J. Chromatogr. B 1071, 44–48.
Venter, L., Loots, D.T., Mienie, L.J., Jansen van Rensburg, P.J., Mason, S., Vosloo, A., Lindeque, J.Z., 2018a. The cross-tissue metabolic response of abalone (*Haliotis midae*) to functional hypoxia. Biol. Open 7. https://doi.org/10.1242/bio.031070 (bio031070).
Venter, L., Mienie, L.J., van Rensburg, P.J.J., Mason, S., Vosloo, A., Lindeque, J.Z., 2018b. Uncovering the metabolic response of abalone (*Haliotis midae*) to environmental hypoxia through metabolomics. Metabolomics. 14, 49.
Venter, L., Vosloo, A., Loots, D.T., Mienie, L.J., Jansen van Rensburg, P.J., Lindeque, J.Z., 2018c. Characterising the metabolic differences related to growth variation in farmed *Haliotis midae*. Aquaculture. 493, 144–152. https://doi.org/10.1016/j.aquaculture.2018.04.052.
Venter, L., Mienie, L.J., Vosloo, A., Loots, D.T., Jansen van Rensburg, P., Lindeque, J.Z., 2019. Effect of proline-enriched abalone feed on selected metabolite levels of slow-growing adult *Haliotis midae*. Aquac. Res. 50, 1057–1067.
Viant, M.R., Rosenblum, E.S., Tjeerdema, R.S., 2003. NMR-based metabolomics: a powerful approach for characterizing the effects of environmental stressors on organism health. Environ. Sci. Technol. 37, 4982–4989. https://doi.org/10.1021/es034281x.
Wei, X., Chen, N., Tang, B., Luo, X., You, W., Ke, C., 2019. Untargeted metabolomic analysis of the carotenoid-based orange coloration in *Haliotis gigantea* using GC-TOF-MS. Sci. Rep. 9, 1–13.
Williams, E.A., Degnan, B.M., Gunter, H., Jackson, D.J., Woodcroft, B.J., Degnan, S.M., 2009. Widespread transcriptional changes pre-empt the critical pelagic–benthic transition in the vetigastropod *Haliotis asinina*. Mol. Ecol. 18, 1006–1025.
Wu, F., Zhang, G., 2016. Pacific abalone farming in China: recent innovations and challenges. J. Shellfish Res. 35, 703–710 (708).
Xin, Y., Ren, J., Liu, X., 2011. Mitogenome of the small abalone *Haliotis diversicolor* reeve and phylogenetic analysis within Gastropoda. Mar. Genomics 4, 253–262.

Xu, F., Gao, T., Liu, X., 2020. Metabolomics adaptation of juvenile pacific abalone *Haliotis discus hannai* to heat stress. Sci. Rep. 10 https://doi.org/10.1038/s41598-020-63122-4.

Yang, E.C., Nam, B.-H., Noh, S.-J., Kim, Y.-O., Kim, D.-G., Jee, Y.J., Park, J.-H., Noh, J.H., Yoon, H.S., 2015. Complete mitochondrial genome of Pacific abalone (*Haliotis discus hannai*) from Korea. Mitochondrial DNA 26, 917–918.

Yao, T., Ye, L., Lu, J., Jiang, J., Wang, R., Chen, H., Lin, Y., Wang, J., 2019. Physiological response of pacific abalone (*Haliotis discus hannai*) hepatopancreas to heat stress. J. Shellfish Res. 38, 417–428.

Young, T., Kesarcodi-Watson, A., Alfaro, A.C., Merien, F., Nguyen, T.V., Mae, H., Le, D. V., Villas-Bôas, S., 2017. Differential expression of novel metabolic and immunological biomarkers in oysters challenged with a virulent strain of OsHV-1. Dev. Comp. Immunol. 73, 229–245. https://doi.org/10.1016/j.dci.2017.03.025.

Yu, L., Xu, D., Ye, H., Yue, H., Ooka, S., Kondo, H., Yazawa, R., Takeuchi, Y., 2018. Gonadal transcriptome analysis of pacific abalone *Haliotis discus discus*: identification of genes involved in germ cell development. Mar. Biotechnol. 20, 467–480.

Zhang, Y.-Y., Qin, L., Liu, Y.-X., Zhou, D.-Y., Xu, X.-B., Du, M., Zhu, B.-W., Thornton, M., 2018. Evaluation of lipid profile in different tissues of Japanese abalone *Haliotis discus hannai* Ino with UPLC-ESI-Q-TOF-MS-based lipidomic study. Food Chem. 265, 49–56. https://doi.org/10.1016/j.foodchem.2018.05.077.

Zhou, J., Cai, Z.-H., Li, L., Gao, Y.-F., Hutchinson, T.H., 2010a. A proteomics based approach to assessing the toxicity of bisphenol A and diallyl phthalate to the abalone (*Haliotis diversicolor supertexta*). Chemosphere. 79, 595–604.

Zhou, J., Zhu, X.-S., Cai, Z.-H., 2010b. Tributyltin toxicity in abalone (*Haliotis diversicolor supertexta*) assessed by antioxidant enzyme activity, metabolic response, and histopathology. J. Hazard. Mater. 183, 428–433. https://doi.org/10.1016/j.jhazmat.2010.07.042.

Zhou, J., Chen, B., Cai, Z., 2015. Metabolomics-based approach for assessing the toxicity mechanisms of dibutyl phthalate to abalone (*Haliotis diversicolor supertexta*). Environ. Sci. Pollut. Res. 22, 5092–5099.