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REVİEW

Advances in Transcriptomic Technologies and Their Applications in Fisheries Science

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Abstract

Transcriptomics is the study of the entire set of RNA transcripts within a cell. It provides insights into the active and latent processes occurring at the subcellular level. Since its inception in the early nineties, it has revolutionized molecular biology and related fields. It has helped uncover the connections between genes and pathways, as well as their roles in physiology, biochemical processes, and disease. Advances in sequencing technologies have further resolved the complexity of cellular functions and provided a snapshot of the transcripts present in a cell at a given time. Over the past decade, transcriptomics has been extensively applied in aquaculture to identify and characterize transcripts associated with immune responses and physiological pathways. This approach allows researchers to study how transcript levels change across organisms, tissues, and cells in response to different stimuli, and thus permits the detection of broadly coordinated mechanisms. More recently, single-cell-based multi-omics techniques have revolutionized biological research. Although their application in fisheries science is still limited; however, it has enormous potential to address many unanswered research problems in this area. In this review, a broad idea about transcriptomics has been given with emphasis on single-cell isolation techniques and the available sequencing domain of single-cell omics. Additionally, we attempt to highlight a few of the important applications of single-cell and multi-cell transcriptomics in fisheries science.

Introduction

Omics refers to the systematic characterization and quantification of complete sets of biological molecules, elucidating their roles in the structure, function, and dynamics of an organism or population (Brooks et al., 2024; Vailati-Riboni et al., 2017). Table 1 summarizes various omics approaches and their applications in aquaculture, while Figure 1 illustrates the key technologies utilized in these studies. The transcriptome encompasses the entire set of mRNA transcripts produced by a specific cell or tissue type (Tang et al., 2009; Zong et al., 2012). The main goal of transcriptome analysis is to identify genes that show differential expression under various conditions. Transcriptome analysis typically involves converting mRNA into complementary DNA (cDNA) followed by sequencing the cDNA library (Gawad et al., 2014; Huang et al., 2015; Navin, 2015). Transcript quantification is achieved through sequence-based or hybridization-based methods like microarrays (Bertone et al., 2004; Clark et al., 2002; Harbers & Carninci, 2005; Reinartz et al., 2002). The objectives of transcriptomic analysis are to decode protein-coding regions of genes, determine gene structures (start and stop codons), and quantify gene expression under various physiological stages and

Table 1. Different omics studies and their applications in aquaculture

Omics Studies	Applications in Aquaculture	References
Proteomics	- Immune response to non-infectious and infectious agents - Host-microbe interaction analysis - Identification of cross-protective immunogens - Host/pathogen stress response	Peng and Immunology (2013)
Transcriptomics	- Immune responses and disease pathways - Reproductive processes and development - Growth regulation - Nutritional factors - Toxicity and stress responses	S. Chandhini and V. J. J. R. i. A. Rejish Kumar (2019)
Genomics	- Vaccine development - Identification of disease resistance and susceptibility traits	Munang'andu et al. (2017)
Metagenomics	- Diagnostics and pathogen identification - Environmental microbiome monitoring - Recirculation system analysis - Mucosal microbiota studies (skin, gut)	Munang'andu et al. (2017)
Nutrigenomics	- Microarray and RNA-seq - Formulation of diets to maintain GALT homeostasis in the gut	Munang'andu et al. (2017)
Epigenetics	- Embryogenesis and reproduction traits - Growth and adaptation traits	Munang'andu et al. (2017)

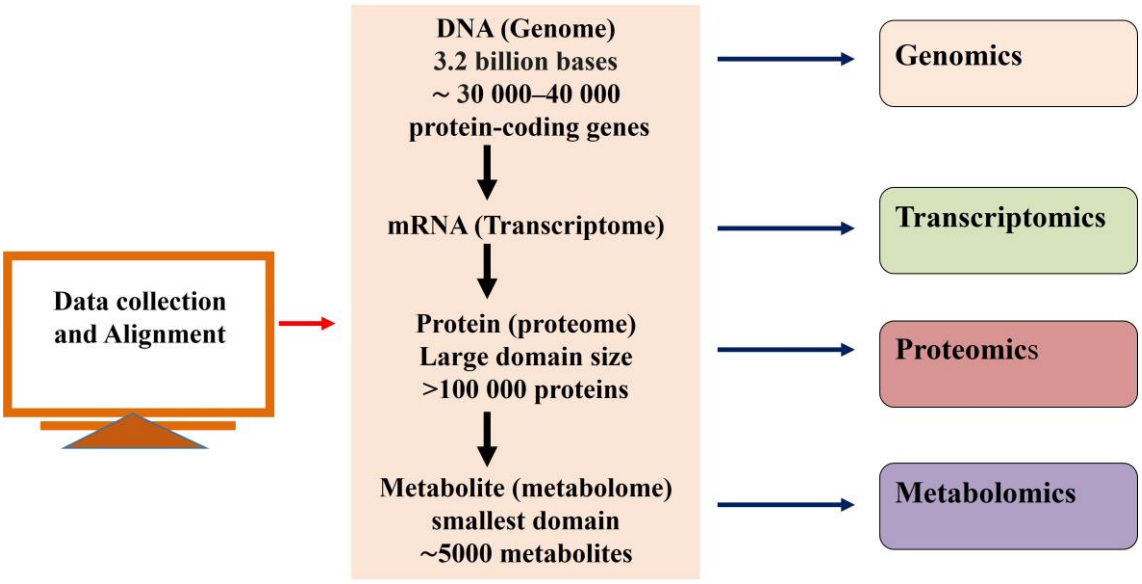

Figure 1. Omic Technologies.

environmental conditions (Li et al., 2003). Single-cell and multicell transcriptomes analyze gene expression at the individual cell level and across multiple cells or tissues, respectively (Table 2). Single-cell genomics has emerged as a transformative method, with increasing applications in aquaculture species. It holds great promise for advancing knowledge in areas such as reproduction, growth, immunity, and stress responses (He et al., 2023). This approach allows for the identification of cell-specific gene expression patterns and regulatory networks critical for understanding complex traits in aquaculture species (Daniels et al., 2023). The Advantages and limitations of transcriptomics are provided in Table 3. Moreover, integrating multi-omics data at the single-cell level offers high-resolution insights into cellular functions and interactions. Combining transcriptomic data with proteomics and epigenomics helps provide a more comprehensive understanding of cellular processes, which is especially useful in complex biological systems like those in aquaculture (Lim et al., 2024). The transcriptome provides valuable information on gene expression variations across different samples and aids in mapping and structurally characterizing functional genes (Costa et al., 2007; Li et al., 2012). This review highlights the advancements in single-cell omics technologies such as Single-cell Genome and Transcriptome sequencing (scG&T-seq), Single-cell Methylome and Transcriptome sequencing (scMT-seq), and Single-cell Triple Omics sequencing (scTrio-seq).

Additionally, it discusses single-cell isolation methods and available sequencing platforms, offering insights into how these approaches can address critical research gaps. While single-cell omics is extensively explored in other fields, its application in fisheries science remains limited despite its enormous potential to address unanswered research challenges.

In this article, we provide a broad overview of transcriptomics, focusing on single-cell isolation techniques and sequencing domains. Additionally, we discuss key applications of single-cell and multi-cell transcriptomics in fisheries science, aiming to emphasize its potential to unravel molecular mechanisms, improve disease management, and enhance genetic and functional understanding in aquatic organisms. The transcriptomic profiles of various aquaculture species in response to different exogenous stimuli are summarized in Table 4.

Table 2. Difference between the single cell and multi-cell transcriptomics

Aspect	Single-Cell Transcriptome Profiling	Complete Cell Transcriptome Profiling
Methodology	RNA is extracted from individual cells using microfluidic capillaries, serial dilution, or flow sorting into multi-well plates, then reverse transcribed into a cDNA library	mRNA or total RNA is reverse transcribed into cDNA using oligo dT primer or hexamers
Cellular Specificity	Reveals cellular specificity and complexity of tissue microenvironments under physiological conditions without relying on specific antibodies	Does not provide deep insights about cellular heterogeneity
Discovery of Cell Types	Enables the discovery of new cell types and states, as well as the tracing of their developmental origins	Difficult to resolve subcellular states due to biological noise
Characterization of Cell States	Offers high-resolution insights into transitions between states and their origins in development and disease	Bulk measurements are inherently limited by averaging, providing less resolution on individual cell states
Integration with Epigenome	By integrating the transcriptome and epigenome at the single-cell level, it decodes the "histone code," elucidating how histone modifications regulate transcription	Unable to determine epigenetic changes
Gene Regulatory Patterns	Provides gene regulatory patterns at the whole genome scale	Gene expression is analyzed for functions associated with growth, immune response, and reproduction
Targeted Analysis	Can target specific cell populations and elucidate signaling pathways and networks for self-renewal, differentiation, and trace cell lineage	Cannot provide information about cell lineage
Isolation Challenges	Single-cell isolation from cell populations is challenging and demands highly efficient and advanced techniques	RNA isolation from tissues is more convenient

Table 3. Comparative Advantages and Limitations of Transcriptomic Techniques

Technique	Advantages	Limitations	References
Bulk RNA-seq	Provides average gene expression across large cell populations Cost-effective for whole-tissue profiling High sequencing depth	Masks cell-to-cell heterogeneity Cannot distinguish rare cell types Limited resolution for complex tissues	S. Chandhini and V. J. Rejish Kumar (2019)
scRNA-seq	Captures gene expression at single-cell resolution Identifies rare and novel cell types Reveals cell-to-cell variability and dynamic processes	Expensive and technically demanding Sensitive to dropout events (missing transcripts) Requires complex data analysis pipelines	Alalwani (2009)
scG&T-seq (Genome and Transcriptome sequencing)	Simultaneous profiling of genome and transcriptome from same cell Useful for linking genomic variation with transcriptional output	Limited throughput Technically challenging library preparation	Macaulay et al. (2015)
scMT-seq (Methylome and Transcriptome sequencing)	Integrates DNA methylation and transcriptome data Provides insights into epigenetic regulation at single-cell level	Labor-intensive Requires high-quality DNA and RNA Limited scalability	Angermueller et al. (2016)
scTrio-seq	Measures genome, DNA methylome, and transcriptome in same cell Provides holistic multi-omics view Helps uncover links between mutations, epigenetic regulation, and gene expression	Complex workflow Low throughput High sequencing cost	Hou et al. (2016)

Table 4. Exploration of multi cell transcriptomics in fishery science

a) Diseases and immunity				
Fish species	Pathogen	Tissue/stage analyzed	Transcriptome profile	References
Oreochromis niloticus	*Vibrio vulnificus*	Larvae	Augmentation of polyunsaturated fatty acids for bacterial growth inhibition and immunomodulation	Pan et al. (2017)
O. niloticus	*Streptococcus agalactiae*	Spleen	2,822 transcripts identified, linked to pathogen attachment, recognition, antioxidant activity, apoptosis, cytoskeletal rearrangement, and immune activation	Zhang et al. (2013); Zhu et al. (2017)
Ctenopharyngodon idella	*Aeromonas hydrophila*	Intestine	235 downregulated and 315 upregulated immune genes identified	Song et al. (2017)
Macrobrachium rosenbergii	*Vibrio parahaemolyticus*	Hepatopancreas	14,569 unigenes with differential expression trends, offering insights into antibacterial mechanisms	Rao et al. (2015)
Cyprinus carpio	*Cyprinid herpesvirus 3*	Kidney	5,000 genes showing expression variation. Downregulation of GST (glutathione S transferase) induces apoptosis, while IL-1β and IL-10 homologs suppress cell-mediated immunity	Neave et al. (2017)
Shrimp	White Spot Syndrome Virus (WSSV)	Hepatopancreas	1,496 differentially expressed unigenes identified. Exploration of viral suppression mechanisms in host defense	Chen et al. (2013)
Fenneropenaeus chinensis	WSSV	Cephalothorax and pleopods	Transcriptome revealed prophenoloxidase activating cascade (proPO) genes	Li et al. (2013)
Eriocheir carinicauda	NG	Eyestalk and Hemocyte	Genes involved in immunity and growth	Chen et al. (2015)
Eriocheir sinensis	*Vibrio alginolyticus, Micrococcus luteus, Pichia pastoris*	Larvae	Several genes characterized in IMD, Toll, MAPK, JAK-STAT pathways linked to signal transduction and immunity	Cui et al. (2013)
Carassius auratus gibelio	Cyprinid herpesvirus	Head Kidney	Immune gene expression profile identified	Liu et al. (2018)
Scophthalmus maximus	Poly I:C	Spleen	Potential mechanisms underlying impaired disease resistance and thermal stress	Yue et al. (2018)
Epinephelus coioides	*Cryptocaryon irritans*	Skin	Highlighted the role of localized immune response in skin against ectoparasitic infections	Hu et al. (2017)
Channa striatus	*Nervous Necrosis Virus (NNV)*	SSN-1 (cell line)	Investigation into the pathogenicity mechanisms	Chen et al. (2017)
Salmo salar	*Salmon alphavirus subtype 3 (SAV-3)*	Infected TO-cells and macrophage-like cells	Identification of antiviral mechanisms in salmonids	Xu et al. (2015)
Litopenaeus vannamei	WSSV	Lymphoid tissue, gills, hepatopancreas, muscle, intestine, hemocytes	Enhancement of immune system through inhibition of NDPK expression	Liu (2017)
Crassostrea virginica	*Perkinsus marinus*	Hemolymph, gill, digestive gland, mantle, adductor muscle	Over 7 TLR-like pattern recognition receptors initiating signal transduction pathways	Tanguy et al. (2004)
Crassostrea virginica	*P. marinus*	Gill	Upregulation of C-lectin in highly infected oysters, downregulation of galectin in all infected oysters	Wang et al. (2010)

Table 4. continued

b) Reproduction and Development			
Species	Tissue/Stage Analyzed	Transcriptome Profile	References
Channa punctatus	Testis (*de novo*)	Identified variation in the expression of testicular genes across distinct reproductive phases	Roy et al. (2017)
Morone saxatilis	Ovary	Impairment in the COP9 signalosome and ubiquitin-26S proteasome linked to embryonic development	Chapman et al. (2014)
Sarotherodon melanotheron heudelotii	Liver and gonad	Analyzed the influence of salinity on reproductive traits and their molecular basis	Avarre et al. (2014)
Thunnus maccoyii	Gonadal	Comparative analysis revealed higher expression of stem cell-associated genes in the ovary and genes involved in hormone synthesis in the testis	Bar et al. (2016)
Amur sturgeon	Testis and ovary	Identified 26 gene families governing reproductive pathways and sex determination mechanisms	Jin et al. (2015)
Crustaceans	Androgenic gland	Androgenic gland hormones regulate testis development, male-specific traits, and reproductive behaviors	Jin et al. (2013)
Macrobrachium nipponense	Life stages from larvae to adult	Expression of insulin-like androgenic gland (IAG) transcripts was lower during developmental stages but increased significantly in adults	Ma et al. (2013)
Procambarus clarkii	Gonad	Genes linked to ovary development (*vitellogenin*, *Dmc1*), testis development, ubiquitin proteasome pathways, and cell cycle regulation were identified	Jiang et al. (2014)
Mitten crab	Testis	Genes involved in spermatogenesis, nuclear protein transformation, and cytoskeletal functions were identified	Zhao et al. (2013)
Scylla olivacea	Testis	SNPs and transcripts revealed a β-crystallin-like gene active during immature stages but suppressed in later stages	Waiho et al. (2017)
Portunus trituberculatus	Gonads	Analyzed genes associated with hormone synthesis, metabolism, and biogenic amine receptors	Meng et al. (2015)
P. trituberculatus	Gonads	Studied genes and mechanisms active during reproductive stage II	Wang et al. (2018)
Macrobrachium rosenbergii	Larval and post-larval stages	Increased expression of transcripts linked to cell cycle, genomic replication, and signaling pathways like Hedgehog and Wnt.	Ventura et al. (2013)
Litopenaeus vannamei	Five continuous developmental stages	Examined histogenesis, muscle formation, dietary shifts, and exoskeleton reconstruction	Wei et al. (2014)
L. vannamei	Whole transcriptome	Identified 5,117 genes differentially expressed across intermolt, premolt, and postmolt stages, influencing hormone regulation and immunity	Gao et al. (2015)
L. vannamei	All developmental stages	Genes governing exoskeleton formation, calcification, and hardening processes were studied	Gao et al. (2015)
Pinctada margaritifera	Gonad	Sex-related genes such as *vitellogenin* and *foxl2* in females and *fem-1* and *dmrt* in males were identified, revealing mechanisms of sexual reversal	Teaniniuraitemoana et al. (2014)
Pinctada fucata	Five larval stages	Investigated transcripts and gene expression changes across five developmental stages	Li et al. (2016)
Hyriopsis cumingii	Multiple tissues (gonad, hepatopancreas, etc.)	Explored mechanisms of sex determination and identified numerous candidate genes involved in the process	Zhang et al. (2016)
Tegillarca granosa	Gonads	A variety of sex-related genes were identified and characterized	Chen et al. (2017)
Danio rerio	Ovary	Pro-male genes (*amh*, *sycp3*, *spata6*, and *sox3*) were upregulated under high-population density conditions compared to low-density groups	Valdivieso (2020)
Ruditapes philippinarum	Foot, gill, and adductor muscle	Transcripts involved in diverse biological processes in mollusks were identified	Mun et al. (2017)

Table 4. continued

c) Growth and nutrition				
Species	Tissue/Stage Analyzed	Nutritional Content	Transcriptome Profiling	References
Epinephelus hybrid	Liver and brain	Pelleted feed	Identified differentially expressed genes like *GnRH1* and *GnRH3*, linked to protein and glycogen synthesis pathways	Sun et al. (2016)
Scophthalmus maximus	Muscle and liver cells	Daily feeding vs. fasting	Growth-related genes and SNPs identified	Robledo et al. (2016)
Trachinotus ovacus	Brain and pituitary	Not available (NA)	Genes related to growth, reproduction, immunity, and microsatellite markers analyzed	Zhenzhen et al. (2014)
Pelteobagrus fulvidraco	Gonad, brain, and pituitary	NA	Examined the role of the brain in sexual dimorphism via sex-related gene expression	Lu et al. (2014)
Rainbow trout	Muscle	NA	Comparative analysis across seasons revealed gene expression linked to growth rates and environmental adaptation	Danzmann et al. (2016)
Epinephelus akaara	Liver	Low, medium, and high carbohydrate diets	High-carbohydrate diets led to reduced growth performance	Yang et al. (2018)
Litopenaeus vannamei	Muscle	Pellet diets (three daily feedings)	Molecular mechanisms involved in feed efficiency elucidated	Dai et al. (2017)
L. vannamei	Developmental stages	NA	Identified genes linked to endoskeleton formation and reconstruction	Gao et al. (2017)
Macrobrachium rosenbergii	Muscle, ovary, and testis	NA	Growth-related genes and growth markers identified	Jung et al. (2011)
Portunus trituberculatus	Muscle, heart, eyestalk, gill, and hepatopancreas	NA	Developed a comprehensive transcript dataset for key tissues, identifying genes linked to muscle growth	Lv et al. (2014)
Pinctada fucata martensii	Adductor muscle, gill, mantle, gonad, and foot	NA	Upregulation of epidermal growth factor receptor in fast-growing groups	Wang et al. (2018)
Rainbow trout	Intestinal mucosa	Mannan oligosaccharide and *Saccharomyces cerevisiae*	Positive effects of prebiotics on fish welfare observed	Gonçalves et al. (2017)
Danio rerio	Muscle	Plant-based diet (powdered commercial feed)	Analyzed SNPs and gene expression variations linked to plant-protein diets	Ulloa et al. (2015)
Atlantic salmon	Liver and distal gut	Soybean meal	Evaluated hepatic response to nutritional stress	De Santis et al. (2015)
Rainbow trout	Whole body	Plant-based diet	Effects of replacing fishmeal and oils with plant-based diets on progeny metabolic performance	Lazzarotto et al. (2016)
Atlantic salmon	Liver	Plant-based diet	Impact on metabolic processes in diploid and triploid organisms assessed	Vera et al. (2017)
Yellow croaker	Epithelial tissue	Taurine	Taurine-regulated gene expression patterns studied	Huang et al. (2018)
Carassius gibelio	Liver	Fish meal and rapeseed meal	Amino acid metabolism, lipid metabolism, and digestive system affected by rapeseed meal diet	Xu et al. (2018)
Danio rerio	Liver	High arachidonic acid diet	Dietary ω-3/n-6 PUFA ratios altered gene expression in adult progeny, potentially through epigenetic regulation	Adam et al. (2018)
Oncorhynchus mykiss	Fry	Plant-based diet	Identified molecular markers for plant-based diet acceptance and utilization	Callet et al. (2018)
Oncorhynchus mykiss	Intestinal and liver	Plant-based diet	Examined diet's effects on metabolism-related gene expression	Lazzarotto et al. (2018)
Oncorhynchus mykiss	Fry	Plant and marine ingredient diets	Molecular markers associated with plant-based diets identified	Leduc et al. (2018)
Gadus morhua	Intestine	Diets supplemented with Camelina oil	Replacing fish oil with vegetable oil altered intestinal transcriptome profile	Morais et al. (2012)
Larimichthys crocea	Muscle	Hydroxyl proline-supplemented diet	Studied molecular mechanisms influencing flesh quality	Wei et al. (2014)

Table 4. continued

d) Toxicology and stress				
Species	Tissue/Stage Analyzed	Causes of Toxicity	Transcriptome Profiling	References
Danio rerio	Embryo	Polychlorinated biphenyl and perfluorooctane sulfonate	Disruption of cell signaling regulation and nervous system development	S. Chandhini and V. J. J. R. i. A. Rejish Kumar (2019)
Gobiocypris rarus	Liver and testis	Tributylin	Alteration in *Gobiocypris* gene expression due to tributylin exposure	Zhang et al. (2016)
Oreochromis niloticus	Liver and testis	Benzo(a)Pyrene (BaP)	Transcriptomic changes and molecular alterations in *Tilapia* due to BaP exposure	Colli-Dula et al. (2018)
Coryphaena hippurus	Liver	Horizon oil toxicity	Toxic effects on genes involved in liver proliferation, cardiac hypertrophy, cholesterol biosynthesis, and renal necrosis	Xu et al. (2017)
Danio rerio	Embryo	Polystyrene particles	Stimulation of the complement system in zebrafish embryos	S. Chandhini and V. J. J. R. i. A. Rejish Kumar (2019)
Danio rerio	Liver	Iron oxide nanoparticles	Elucidation of negative effects induced by iron oxide nanoparticles	S. Chandhini and V. J. J. R. i. A. Rejish Kumar (2019)
Danio rerio	Liver	Carboxymethyl cellulose-stabilized iron sulfide nanoparticles	Mechanistic insights into potential toxic effects of organic matter-stabilized FeS nanoparticles	Zheng et al. (2018)
O. niloticus	Gill	Alkalinity stress	Molecular mechanisms of adaptation to alkaline environments in *O. niloticus*	Zhao et al. (2017)
Glyptosternon maculatum, *Pareuchiloglanis sinensis*, *P. macrotrema*, *Silurus asotus*, *Pelteobagrus fulvidraco*, *Synodontis nigriventris*	Liver	Salinity adaptation	Genetic adaptation to elevated altitude in various fish species	Ma et al. (2016)
P. hypophthalmus	Intestine and swim bladder	Salinity adaptation	Identified genes associated with salinity adaptation in *P. hypophthalmus*	Thanh et al. (2014)
Oncorhynchus mykiss gairdneri	Gill	Heat stress	Mechanisms of physical stress and mortality in fish due to heat stress	Garvin et al. (2015)
Coilia nasus	Liver	Physical stress	Observed genes responsive to physical stress in *Coilia nasus*	Du et al. (2014)
M. nipponense	Hepatopancreas	Hypoxia	Genes associated with oxygen transport, electron transport, ROS generation, and immune response were expressed	Sun et al. (2014)
L. vannamei	Hepatopancreas	Acute ammonia stress	Ammonia exposure suppressed certain immune genes	Lu et al. (2016)
Paratya australiensis	Whole transcriptome	Acid sulfate drainage water	Activated stress response pathway due to short-term exposure to acid sulfate drainage water	Bain et al. (2016)
Oratosquilla oratoria	Hepatopancreas	Chromium hexavalent	Chromium hexavalent toxicity triggered upregulation of genes related to protein digestion and absorption	Zhang et al. (2018)
Tigriopus japonicus and *Pseudodiaptomus annandalei*	Whole organism	Mercury pollution	Mercury interferes with endocrine function, triggering molecular responses specific to each species	Wang et al. (2017)

Table 4. continued

d) Toxicology and stress				
Species	Tissue/Stage Analyzed	Causes of Toxicity	Transcriptome Profiling	References
Paracylipina nana	Whole transcriptome	Xenobiotic degradation	Transcripts associated with xenobiotic degradation and metabolic processes identified	Han et al. (2017)
Sinopotamon henanense	Hepatopancreas	Cadmium	Presence of cadmium and its role as a biological indicator in stress response	Sun et al. (2014)
Mud crabs	Muscle, gills, and hepatopancreas	Oil spills	Oil spill exposure upregulated genes for cellular differentiation and downregulated apoptosis and immunity genes	Vazquez-Miranda et al. (2017)
Crassostrea gigas	Gill	High carbon dioxide and surface temperature levels	Transcriptome profiling of selectively bred Pacific oyster families with varying heat shock tolerance	Lang et al. (2009)
Pinctada fucata	Mantle	Elevated CO2 and temperature	Stress-induced overexpression of biomineralization genes	Nie et al. (2016)
Crassostrea angulate	Gill	Copper	GABA transporter 2 (GAT2) genes are involved in copper accumulation in gills and mantles	Shi et al. (2015)
Perna viridis	Adductor muscle, gills, and hepatopancreas	Copper	Identified numerous genes involved in stress responses and xenobiotic degradation in Perna viridis	Leung et al. (2014)

Transcriptome Profiling Techniques

Transcriptome profiling methods, such as Serial Analysis of Gene Expression (SAGE) (Matsumura et al., 2005; Velculescu et al., 1995) and polony multiplex analysis of gene expression (PMAGE) (Kim et al., 2007), allow researchers to explore gene expression dynamics. For instance, SAGE has been employed to investigate transcriptomic changes during oogenesis (Knoll-Gellida et al., 2006) and sexual dimorphism in zebrafish (Zheng et al., 2013). These studies have provided critical insights into the molecular mechanisms regulating important processes in aquaculture species.

Concept of Single Cell Transcriptomics

Cells are the fundamental units of life, with each cell type performing specialized functions that contribute to the formation of tissues, organs, and organisms. Cellular type and state are influenced by internal regulatory mechanisms and external environmental factors. In multicellular organisms, a single zygote differentiates into a diverse array of cell types, each exhibiting unique gene expression profiles and functions (Lim et al., 2024). Even within seemingly uniform tissues, there exists a remarkable diversity of cells, each reflecting unique aspects of the tissue type and contributing to its overall functionality (Dezem et al., 2024). Traditional methods for transcriptome analysis, such as bulk-tissue or cell-population studies, often provide averaged results, masking the underlying cellular heterogeneity (Wu et al., 2024). This limitation hinders the ability to capture the nuances of individual cellular states, which are crucial for understanding complex biological processes. In contrast, single-cell transcriptomics enables high-resolution molecular analysis at the individual cell level, bridging the gap between bulk-omics techniques and the need to uncover cellular diversity (Figure 1).

The advent of single-cell -omics technologies has revolutionized biology by enabling the unbiased characterization of molecular states in individual cells. These high-throughput approaches provide unprecedented insights into cellular diversity, differentiation, and interactions within tissues and organisms (Hu et al., 2018). Single-cell transcriptomics is particularly valuable for exploring cellular functions, regulatory networks, and molecular behavior, making it an indispensable tool for modern biological research (Gawad et al., 2014).

Prerequisites of Single Cell Transcriptomics

The foundation of single-cell omics research lies in high-quality RNA and DNA extraction from individual cells, free of contamination (Valihrach et al., 2018). Single-cell isolation from tissues is achieved using advanced cell sorting techniques (Baysoy et al., 2023; Hodne & Weltzien, 2015). Emerging technologies, like scDNA-seq and scRNA-seq, offer granular insights into cellular heterogeneity, tumor microenvironments, and clonal evolution (Gawad et al., 2014; Pan & Jia, 2021; Wu et al., 2024). However, analyzing one molecular type (e.g., DNA or RNA) in isolation provides limited understanding, necessitating multi-omics approaches to study complex biomolecular interactions at the cellular level (Sandberg, 2014; Spitzer & Nolan, 2016; Wu et al., 2024; Zenobi, 2013).

Therefore, innovative technologies are essential for simultaneously analyzing multiple molecular types to gain a more complete understanding of cellular processes. Multi-omics approaches are vital for linking

genomic variation to transcript and protein expression dynamics. Gene regulation at the single-cell level involves stochastic gene activation, resulting in variable expression even in uniform cell populations (Craig et al., 2012). Traditional tissue-level analysis often obscures unique molecular features of individual cells, limiting insights into complex regulatory mechanisms.

Advancements in single-cell omics, enabled by innovations like lab-on-a-chip technologies, now allow large-scale transcript screening and integration of genotype and phenotype data, offering deeper insights into cellular functions (Tan et al., 2022). Recent innovations, such as lab-on-a-chip technologies, now allow for large-scale transcript screening within single cells, enabling the direct linking of a cell's genotype to its transcriptomic phenotype (Gao et al., 2021; Xie et al., 2020). Since transcriptomic variations are often a consequence of genomic variations, this integration of genotype and phenotype data provides a deeper understanding of cellular function (Alvarez et al., 2015).

Single Cell Omics Methods

Current single-cell omics technologies have enabled the comprehensive analysis of various molecular layers from individual cells, offering insights into the intricate biology of organisms. Techniques like Single-cell Genome and Transcriptome sequencing (scG&T-seq), Single-cell Methylome and Transcriptome sequencing (scMT-seq), Single-cell Triple Omics sequencing (scTrio-seq), and Single-cell Chromatin Overall Omic-scale Landscape Sequencing (scCOOL-seq) combine different omics approaches to analyze genomic, transcriptomic, epigenomic, and proteomic data from a single cell (Angermueller et al., 2016; Hou et al., 2016; Hu et al., 2016; Macaulay et al., 2015; Stuart & Satija, 2019). These multi-omics methods aim to minimize information loss while preserving the biological diversity and fidelity of each cell, thus offering a holistic view of cellular states and functions.

A key consideration in these methods is the integrity of cellular material during the process. Effective lysis and division of cellular content are crucial to prevent the loss of valuable genomic and transcriptomic data. DR-seq is one such method that allows simultaneous sequencing of genomic DNA and mRNA, minimizing material loss, even when working with very small cell samples (Hu et al., 2018). This approach ensures that both the genome and transcriptome are analyzed concurrently, providing a more complete picture of cellular activity.

Another common approach in single-cell RNA sequencing is using oligo-dT primer-coated magnetic beads to isolate polyadenylated mRNA from DNA (Angermueller et al., 2016; Hou et al., 2016; Hu et al., 2016; Macaulay et al., 2015; Stuart & Satija, 2019). This method is compatible with automated liquid-handling systems, which helps increase throughput. However, the DNA isolated in this way may not provide as uniform coverage of the genome, which can compromise the accuracy of copy number analysis in specific genomic regions (Hu et al., 2016; Stuart & Satija, 2019). This limitation is one of the challenges faced by these methods, as they may not capture certain genomic variations as effectively as whole-genome sequencing. An alternative strategy to overcome this limitation involves pre-amplification of both DNA and RNA, followed by their separation (Dey et al., 2015). This approach allows for the amplification of both genetic materials in parallel, ensuring that sufficient material is available for sequencing without significant bias toward one molecule over the other. In addition to these methods, the splitting strategy divides a single cell into two parts, enabling concurrent analysis of RNA and protein (Darmanis et al., 2016). While this method is more effective for protein and RNA analyses, it is not as efficient for isolating DNA. However, it still proves valuable when high-copy-number molecules, such as RNA and proteins, need to be studied from individual cells. The comparison of single-cell omics techniques is provided in Table 5.

Single Cell New Age Sequencing Techniques for Whole Transcriptome and Genome

Single-cell transcriptomics has been greatly advanced by the pioneering work of Tang et al. (2009), which marked the beginning of single-cell RNA sequencing technology (Tang et al., 2009). This foundational research set the stage for subsequent innovations in the field, including introduction of Quartz-seq, a method that detects the 3’ end of transcripts, allowing for a more focused investigation of gene expression (Hashimshony et al., 2012; Sasagawa et al., 2013). Further improved single-cell RNA sequencing with their Cel-seq technique, which employs barcoding and sample pooling to enable multiplexing of single-cell samples, thus increasing the throughput and scalability of transcriptomic analyses. With advancements in single-cell genomic technologies, Genome and Transcriptome sequencing (G&T-seq) was developed to analyze a single cell's genome and transcriptome simultaneously, representing a major breakthrough in integrating genomic and transcriptomic data (Hu et al., 2018). Complementing these developments, Degenerate Oligonucleotide-Primed PCR (DOP-PCR) was introduced as a crucial tool for amplifying genomic DNA from single cells, enabling more accurate genome mapping and sequencing (Carter, 1993; Cheng et al., 1998). Further advancements followed with the development of Multiple Displacement Amplification (MDA), a technique that ensures uniform DNA amplification across the genome, making it ideal for the genetic analysis of single cells (Dean et al., 2002). To overcome some limitations of MDA, Multiple Annealing and Looping-Based Amplification Cycles (MALBAC) was introduced, improving sequencing precision by

preventing over-amplification and enhancing the accuracy of sequence reads (Zong, 2017; Zong et al., 2012). Additionally, the development of the PicoPLEX® WGA kit introduced a user-friendly, single-tube protocol for amplifying DNA from as little as 6 pg, further streamlining single-cell amplification processes (Han et al., 2014). To integrate the analysis of both the genome and transcriptome, DR-seq was developed, enabling the simultaneous sequencing of DNA and RNA from a single cell and facilitating genotype-phenotype correlation studies (Dey et al., 2015). This was complemented by G&T-seq, which employs separate amplification techniques for the genome (using WGA) and the transcriptome (using SMART-seq2), offering a comprehensive approach to understanding gene expression at the single-cell level (Macaulay et al., 2015). Finally, the potential of these methods was demonstrated in a study that employed single-cell sequencing techniques to explore the heterogeneity of tumor cells (Shapiro et al., 2013). This application highlighted the power of these technologies in understanding complex biological phenomena, such as the molecular mechanisms driving tumorigenesis, by directly linking genomic variations to transcriptional changes within individual cells.

Simultaneous Sequencing of Single Cell Methylome and Transcriptome

DNA methylation plays a critical regulatory role in gene expression, making the study of the relationship between the DNA methylome and transcriptome within the same single cell highly significant. The first method to combine DNA methylome and transcriptome profiling was Single-cell Methylome and Transcriptome Sequencing (scM&T-seq). This approach was adapted from the G&T-seq protocol, allowing for the isolation of both DNA and RNA from a single cell population (Hu et al., 2016). In a parallel technique, genomic DNA is treated with bisulfite followed by sequencing, enabling the simultaneous profiling of the RNA transcriptome and DNA methylome from a single cell population (Angermueller et al., 2016). Subsequently, methods such as scMT-seq and scTrio-seq were introduced, offering an alternative strategy for isolating DNA and RNA from single cells. In these techniques, the cell

Table 5. Comparison of Single-cell Omics Techniques

Technique	Ease of Entry	Quality of Data Output	Cost	Throughput	Typical Applications	References
scRNA-seq (Single-cell RNA sequencing)	Moderate – requires specialized library prep and bioinformatics expertise	High – captures gene expression at single-cell resolution, but dropout events possible	High (due to reagents and sequencing depth)	High (10x Genomics enables thousands of cells per run)	Cell type identification, gene expression heterogeneity, immune profiling	Tang et al. (2009)
scATAC-seq (Assay for Transposase-Accessible Chromatin)	Moderate – similar to scRNA-seq but with additional chromatin accessibility assays	High – maps open chromatin regions and regulatory elements	High	Medium	Epigenetic regulation, enhancer–promoter mapping	Dey et al. (2015)
scG&T-seq (Genome and Transcriptome sequencing)	Difficult – requires separation of genome and transcriptome from same cell	Very High – links genomic variation with transcriptome	Very High	Low	Mutation–expression relationships, cancer genomics	Macaulay et al. (2015)
scMT-seq (Methylome and Transcriptome sequencing)	Difficult – requires high-quality DNA and RNA	High – integrates DNA methylation with transcriptome	Very High	Low	Epigenetic regulation and gene expression	Angermueller et al. (2016)
scTrio-seq (Genome, Methylome, and Transcriptome sequencing)	Very Difficult – complex workflow, advanced equipment	Very High – provides multi-omics from same cell	Extremely High	Very Low	Systems biology, linking mutation, epigenetics, and expression	Hu et al. (2016)
Mass Cytometry (CyTOF)	Moderate – requires antibody panels and cytometry platform	High – measures protein expression at single-cell level	High (antibody labeling + equipment)	High	Immune profiling, signaling pathways	Zhang et al. (2011)

membrane is selectively lysed to release RNA, while the intact nucleus is physically separated from the cell lysate (Hou et al., 2016; Hu et al., 2016). In scTrio-seq, separation of the nucleus and cytosol is achieved through centrifugation, with genomic DNA in the nucleus sequenced using the scRRBS protocol and mRNA amplified via scRNA-seq (Tang et al., 2009). Simultaneous profiling of methylome and transcriptome provides a unique opportunity to directly measure DNA methylation and gene transcription within a single cell population. Moreover, the association of DNA methylation differences with gene transcription variation across single cells can also be deciphered.

Methods to Simultaneously Study Transcriptome and Proteome

Several single-cell proteomic techniques have been developed based on diverse strategies, including fluorescence-activated cell sorting (FACS), western blotting, and the use of metal-tagged or oligonucleotide-labeled antibodies (Sittner et al., 2018). Although the multiplexing capacity of these methods remains limited to detecting tens of proteins per cell, they effectively demonstrate the feasibility of simultaneously assessing protein levels and RNA expression. This capability provides insights into the dynamics of RNA and protein within individual cells.

One approach involves a homogeneous affinity-based proximity extension assay that converts protein abundance into tag-oligo levels, enabling quantification of both transcript and protein levels through qPCR (Darmanis et al., 2016). Another technique, PLAYR (Proximity Ligation Assay for RNA), allows for the simultaneous detection of RNA and protein in the same cell. This method uses isotope-labeled probes that bind and ligate RNA transcripts, enabling the quantification of over 40 different mRNAs and proteins simultaneously. However, further advancements are needed to achieve genome-wide measurements with higher throughput.

High-throughput methods, such as REAP-seq and CITE-seq, have been introduced to integrate cellular protein and transcriptome measurements. These methods use oligonucleotide-labelled antibodies to efficiently combine protein and RNA data into a single-cell readout (Peterson et al., 2017; Stoeckius et al., 2017).

Novel Computational and Algorithmic Methods

Single cell sequencing techniques for whole-genome profiling of DNA and RNA, as well as the successive integrative computational analysis methods, are essential to interpret single cell multi-omics data (Hu et al., 2018). The effectiveness of such analyses largely depends on advancements in bioinformatics tailored to single-cell sequencing datasets. Since different single-cell sequencing protocols exhibit unique technical characteristics, bioinformatic approaches must be specifically designed to address the requirements of each protocol.

The development of computational methods has been driven by the need to analyze the distinct features of various single-cell sequencing techniques (Hu et al., 2018). These tools facilitate the evaluation of sequencing datasets with multiple layers of information across diverse omics types, enabling a comprehensive characterization of the state of individual cells (Sittner et al., 2018). With the advent of advanced bioinformatic algorithms and computational tools, multi-omics data analysis has provided profound insights into complex biological systems, uncovering critical and intricate cellular processes (Huang et al., 2012; Hwang et al., 2018).

Single-cell RNA-seq approaches were shown to identify cell subtypes such as human blood monocytes, dendritic cells, and neurons (Macosko et al., 2015; Villani et al., 2017). Epigenetic modifications, including DNA methylation, are both cell-type specific and developmentally regulated, remaining stable over an organism's lifespan. By integrating epigenome and transcriptome profiling, researchers can overcome the limitations of scRNA-seq, gaining a more comprehensive understanding of cellular identity (Hwang et al., 2018). Multi-omics approaches allow for non-redundant data collection, offering a detailed and inclusive characterization of complex tissues. These methods are particularly valuable for reconstructing cell lineage trajectories, a critical goal in developmental biology. Tracking cell lineage across an organism's developmental timeline enables the study of DNA mutations and epigenetic changes that occur during cell division and are inherited by daughter cells. Such lineage tracing reveals the genetic and transcriptional changes that govern cell proliferation and differentiation. (Sittner et al., 2018). For instance, in cancer biology, understanding the inherently unstable genome of cancer cells is vital for identifying "driver" mutations or copy number variations that contribute to tumorigenesis (Hu et al., 2018).

Single-cell multi-omics data offers high-resolution insights into the relationships between various omics layers, facilitating a deeper understanding of cellular processes. Correlation analysis between different omics datasets is a commonly used strategy to develop regulatory hypotheses connecting multiple data types (Hu et al., 2018). For instance, cytosine methylation, one of the most well-characterized epigenetic modifications, serves as a key regulatory mechanism for numerous critical biological functions (Kubik & Summerer, 2015). Integration of DNA and RNA sequencing techniques, such as DR-seq and G&T-seq, enables the identification of correlations between copy number variations (CNVs) and gene expression levels at the single-cell level. Additionally, scTrio-seq has demonstrated that large-scale CNVs can lead to corresponding changes in the

RNA expression of genes located within the affected genomic regions (Hou et al., 2016).

Computational Approaches

Multi-omics approaches have proven to be highly valuable in understanding the complex relationships between DNA mutations, epigenetic modifications, and their impact on gene expression. These approaches are particularly instrumental in shedding light on intricate biological processes such as dosage compensation and X-chromosome inactivation (Graves, 2016; Livernois et al., 2012). A key aspect of these applications lies in refining computational techniques to integrate diverse omics data and mitigate information loss from sequencing small biological samples. The advancement of bioinformatics and computational strategies has enabled significant progress in single-cell multi-omics technologies, facilitating the recovery of "dropped-out" single-cell measurements and allowing for the indirect inference of additional omics layers from existing datasets (Bock, 2021; Farlik et al., 2015). Moreover, mathematical and statistical models have been developed to quantify multi-dimensional associations (Lane et al., 2017) and frameworks. These methods prove valuable in integrating unpaired single-cell multi-omics data and uncovering regulatory relationships, with GLUE (Graph-Linked Unified Embedding) serving as a key example (Cao & Gao, 2022). As our understanding of biological regulatory interactions deepens, different omics layers can serve as proxies for one another. For example, transcription factor binding or copy number alterations can be inferred indirectly from single-cell methylation data (Farlik et al., 2015; Hou et al., 2016; Li & Li, 2018). With increasing high-throughput data generation in single-cell multi-omics technologies, there has been a growing demand for computational resources to process and analyze this raw data efficiently. Deep learning approaches have been employed to capture intricate relationships between various omics layers, enhancing the accuracy and interpretability of data integration (Athaya et al., 2023). Additionally, raw omics data must undergo various processing steps, such as alignment, filtering, and quality control, to preserve the complex nature of single-cell measurements. Challenges like low signal-to-noise ratios, amplification artifacts, and technical variability must also be addressed during these processes (Bock et al., 2016). Once processed, the data is mapped back to the corresponding single cell and analyzed using mathematical and statistical models to uncover regulatory patterns.

The advent of single-cell multi-omics approaches has opened new opportunities for exploring biological diversity, identifying rare cell types, and understanding their unique features with remarkable precision. By integrating data from different molecular layers, such as DNA, RNA, and proteins, these methods offer comprehensive insights into cellular function and regulation (Hu et al., 2018). Single-cell multi-omics approaches are rapidly gaining attraction in biological research and are increasingly being used to study cellular heterogeneity, trace lineage relationships, discover novel cell types, and explore regulatory interactions among biomolecules (Hu et al., 2018). While initial applications have shown promising results, there is still significant potential for further exploration, and ongoing advancements in multi-omics technologies are expected to continue enhancing their utility and expanding their applications.

The integration of single-cell sequencing techniques for whole-genome profiling of DNA and RNA, along with advanced computational analysis, is essential for interpreting single-cell multi-omics data (Hu et al., 2018). The effectiveness of these analyses is largely contingent on developments in bioinformatics specifically tailored for single-cell sequencing data (Wu & Zhang, 2020). Since each sequencing protocol exhibits unique technical characteristics, bioinformatics tools must be adapted to accurately process and analyze the corresponding data types (Figure 1). The necessity of addressing the unique attributes of various single-cell sequencing methods has led to the creation of numerous computational tools that facilitate the evaluation of sequencing datasets by integrating multiple omics layers. This enables a holistic view of the state of a single cell (Chen et al., 2019). With ongoing advancements in bioinformatic algorithms and computational resources, the integration of multi-omics datasets is uncovering increasingly complex and significant biological insights. Several online databases and tools are available to collect, align, analyze, and interpret the data, contributing to standardized data management (Table 6).

Application of Single Cell-omics in Fishery Science

Advancements in single-cell gene analysis have revolutionized modern biology, providing deep insights into the variability within individual cells in isogenic populations and how these differences impact cellular function (Wong et al., 2005). Although analyzing cells produced in small quantities and streamlining their heterogeneity remains challenging, emerging technologies are facilitating the study of biological systems at the single-cell level. These technologies offer enhanced measurement of parameters, enabling the preservation of cell integrity while examining intracellular biomolecular dynamics. Single-cell omics strategies are particularly valuable for describing normal cell-to-cell variation and can correlate biological function with disease processes (Efremova & Teichmann, 2020). Moreover, these approaches hold promise for identifying crucial pathways for detecting and molecularly characterizing stem cells, cancer stem cells, and tumor-initiating cells, ultimately supporting personalized pharmacological treatments (He et al., 2020).

Single-cell omics offers a way to dissect complex tissues and cellular lineages in a data-driven manner, complementing classical methods such as fluorescent activated cell sorting (FACS) and lineage tracing. Profiling a cell in the desired omics dimension, coupled with bioinformatic analysis, can overcome the limitations of classical approaches, reducing dependence on surface markers. However, a major challenge of current single-cell omics assays is the inability to capture cell-cell contacts and their spatial locations within tissues, a limitation that could potentially be addressed by multi-omics approaches (Bock et al., 2016). In medicine, single-cell data maps can provide crucial insights into biological variations within tumors, helping to identify factors contributing to drug resistance, tumor progression, metastasis, and relapse. These data sets aid in understanding the biological basis of such variations, contributing to more precise therapeutic interventions. Additionally, single-cell omics is a promising tool for studying unculturable bacteria, as single-cell procedures can facilitate integrative analysis by connecting proteomic assemblies to their associated metabolites (Li et al., 2021).

In fishery science, the concept of omics has gained considerable recognition, with numerous reports on various omics studies in aquaculture. These studies encompass a wide range of applications, but the use of single-cell omics remains limited. Most of the existing research is centered around zebrafish, investigating topics such as hematopoietic cell lineage commitment, whole kidney marrow cell expression profiling, blood cell heterogeneity, and immune-cell deficiencies. Single-cell omics has greatly enhanced our understanding of immune cell evolution in vertebrates, enabling researchers to trace clonal histories and identify cell types from various organs of adult zebrafish (Hu et al., 2024). One notable study used single-cell RNA sequencing (scRNA-seq) to analyze immune cell types in zebrafish spleen cells, thereby creating a comprehensive atlas of these cells. This work has significantly improved our understanding of immune cell diversity and the evolutionary dynamics of vertebrate immune systems (Hu et al., 2024). Another study focused on hematopoietic cells undergoing thrombocyte lineage commitment, using computational analysis to map their developmental trajectory from stem cells to mature cells, providing deeper insights into lineage commitment processes (Macaulay et al., 2016). The immune system's role in zebrafish was also explored by generating a zap70 loss-of-function mutation, leading to the complete absence of mature T cells in the mutant zebrafish. Single-cell gene expression profiling, coupled with morphological analysis and RNA sequencing, confirmed the similarity between immune dysfunction in these mutants and that seen in humans and mice with similar mutations (Moore et al., 2016). Despite challenges in distinguishing complex fluorescent protein combinations in transgenic zebrafish lines, transcriptional profiling of single hematopoietic cells has provided valuable insights into the molecular signatures of erythroid, myeloid, B, and T cell lineages. These approaches have contributed to a more refined understanding of zebrafish hematopoiesis (Moore et al., 2016). Furthermore, studies on Myc-induced T-cell acute lymphoblastic leukemia (T-ALL) in zebrafish have demonstrated how single-cell transcriptomics can reveal key features of immune cell differentiation and stem cell gene reactivation (Park, 2022). While zebrafish studies dominate single-cell omics research in fish, there have been significant contributions to understanding immune cell evolution in vertebrates. One such study analyzed immune cells from adult heterozygous fish, revealing a distinct T-cell molecular signature and differential expression of genes involved in T-cell development and signaling. The study also explored V(D)J recombination and identified potential NKT cell populations, offering new insights into immune cell diversification (Yamagata et al., 2006). The use of single-

Table 6. Available online databases for omic studies

Database	Tools	Function
Genomics	Basic Local Alignment Tool (BLAST)	Comparison of nucleotide and amino acid sequences
	Primer BLAST	Primer designing from target template
	Splign	Alignment tool used to preliminary alignment of cDNA-genomic sequences
	GenBank (Bankit and Sequin)	Submission tool for nucleotide and amino acids sequences to the GeneBank sequences database
	Sequence Read Archive Submission	Stores next generation sequencing data
Proteomics	Translate	Translation of nucleotide sequence to amino acid sequence
	Findmod	Prediction of post translational modification and single amino acid substitution
	Mascot	Identification of protein from peptide sequence databases using spectrophotometry data
	ProtParam	Calculation of physiochemical properties of a protein
	ScanProsite	Scan protein sequences against PROSITE collection of motifs
	Protein Data Bank	Database of 3D structures of proteins
High throughput analysis	Koyoto Encyclopedia of Genes and Genomes (KEGG) Pathway	Database collection deals with genome, biological pathways, diseases, drugs and chemical substances.
	Biocarta	Database for gene interaction models.

cell transcriptomics in fish has proven to be a powerful tool for cell-type classification and understanding the evolution of immune cell types (Carmona et al., 2017). Recent advancements in single-cell sequencing, such as ScarTrace, have provided valuable insights into clonal histories and the commitment of embryonic progenitors to specific adult cell types, further elucidating the development of various tissues and organs in zebrafish (Alemany et al., 2018). Despite the limited use of single-cell omics in fish, there is substantial research on other omics strategies in fisheries science, especially multi-omics approaches. These strategies have advanced our understanding of fish immune responses, both innate and humoral, as well as the discovery of new pathogens, development of vaccines, and marker-assisted selection in aquaculture (Brandtzaeg et al., 2008; Costa et al., 2007; Liu et al., 2016; Müller & Kersten, 2003; Munang'andu, 2016). The potential for innovation and further application of these techniques is vast, and ongoing research will continue to expand their use in understanding fish biology and improving aquaculture practices.

In Deciphering the Fish Mucosal Immunity Genes

Similar to other chordates, fish, too possess a network of immune cells and molecules involved in persistent surveillance thus protecting it from infection. High throughput single cell multi-omics strategies along with methodologies for global data acquisition, computational data tools, and advanced mathematical algorithms have made it possible to annotate and decipher the genes responsible for this unique immune response in fishes. Transcriptomics is arguably the most widely used technique in fish immunology, enabling the comprehensive measurement of immune gene expression within a sample (immune-ome). Additionally, single-cell omics facilitates the discovery of novel immune genes, including those unique to fish that lack homologs in other vertebrates. Omics tools provide essential insights for characterizing and deciphering the intricate dynamics of the fish mucosal immune system (Brandtzaeg et al., 2008). For example, whole-genome analysis of gill tissues in European sea bass (Dicentrarchus labrax) detected transcripts encoding cytokines and T-cell-associated transcription factors, suggesting the presence of Th1, Th2, Th17, and Treg cells in this mucosal tissue (Ortiz et al., 2014). These findings not only underline the functional complexity of the teleost mucosal immune system but also establish a connection to mammalian immune mechanisms, demonstrating the utility of teleosts as models for studying mucosal immunity and T-cell biology. T cells are abundant in mucosal tissues of teleost (Abelli et al., 1997; Araki et al., 2005; Picchietti et al., 2011). Multi-omics studies performed in different teleost species have contributed to the identification of T cell components associated with already characterized mammalian T cell subsets (Tc, Th1, Th2, Th17 and Treg) and the four TCR chains (αβ, γδ) (Castro et al., 2011; Yamaguchi et al., 2015). Analysis of the whole genome from gills of Europian sea bass (*Dicentrarchuslabrax*) detected transcripts for cytokines and T-cell associated transcription factors suggesting the presence of Th1/Th2/Th17/Treg cells in this tissue (Ortiz et al., 2014).

The mucosal microenvironment is a critical site for immune regulation, where T cells like Th17 and Treg play vital roles in maintaining tolerance and immunity against microbial symbionts (Nutsch & Hsieh, 2012). For example, in Japanese flounder, immersion vaccination with *Vibrio anguillarum* leads to increased expression of CD4-1, CD4-2, and CD8α in the gills (Kato et al., 2013). Similarly, in the interbranchial lymphoid tissue (ILT) of salmon challenged with infectious salmon anemia virus, elevated levels of CD3ζ transcripts suggest a role in ILT antiviral immunity (Aas et al., 2014). Additionally, gill T cells contribute to responses against amoebic gill disease, as indicated by the upregulation of IL-4/13, which is potentially associated with the Th2 pathway (Benedicenti et al., 2015).

However, immune responses in other tissues show variability. For instance, studies on skin immunity against parasitic infections in rainbow trout and Baltic salmon (*Salmo salar*) revealed contrasting results: trout exhibited a decrease in $CD8^+$ cells and a Th1/Th2 switch, while salmon showed an increase in $CD8^+$ T cells in the skin (Chettri et al., 2014). On the other hand, IgM^+ and IgT/Z^+ B cells are widely distributed across the gut, gills, skin, and olfactory organs in fish (Tacchi et al., 2014; Xu et al., 2016). This distribution highlights the compartmentalization of IgT/Z-mediated mucosal immunity and IgM-mediated systemic immunity (Xu et al., 2016; Zhang et al., 2010).

Techniques such as oligo-microarrays enable gene expression analysis at the tissue, cell population, or single-cell level, making them valuable tools for studying immune responses (Miller & Maclean, 2008). Although limited, transcriptomic studies have explored immune gene expression changes in fish intestine, gills, skin, and olfactory organs. For instance, an intranasal vaccination study demonstrated significant alterations (>2-fold) in the expression of ~8,000 genes, including those linked to innate and adaptive immunity, four days post-vaccination with live attenuated infectious hematopoietic necrosis virus (Tacchi et al., 2014).

Transcriptomic approaches have shed light on microbial pathogenesis, immune evasion, host immune responses, and their kinetics (Tacchi et al., 2014). At a single-cell resolution, omics techniques offer precise insights into immune gene loss or expansion, single-nucleotide polymorphisms, and gene expression dynamics under various conditions. Multi-omics integration further enables comprehensive analysis of these datasets (Castro et al., 2013).

System biology approaches, leveraging omics data, enhance our understanding of complex biological processes at fish mucosal surfaces. This knowledge can

be translated into improved aquaculture practices that prioritize mucosal health. Microarrays developed in the late 1990s were based on expressed sequence tags (ESTs) generated from cDNA libraries enriched with infection-responsive genes (O'Farrell et al., 2002; Taggart et al., 2008). Such arrays have been developed for several fish species, including catfish (Li & Waldbieser, 2006), carp (Gracey, 2008), rainbow trout (Li & Waldbieser, 2006), rainbow trout (Koskinen et al., 2004), and halibut (Douglas, 2006). Additionally, RNA sequencing-based digital gene expression (DGE) generates short sequences close to restriction enzyme sites in the 3' end of cDNAs, which are sequenced using Illumina platforms (Hrdlickova et al., 2017).

In Diagnosis and Discovery of Novel Diseases

Metagenomics, a culture-independent diagnostic approach, eliminates the need for prior knowledge of the nucleic acid sequences to be analyzed, distinguishing it from conventional PCR, which requires specific primers designed based on known sequences (Bibby, 2013).

This advantage enables metagenomics to detect a wide array of pathogens, including those previously unidentified. In particular, metagenomic analysis excels in the detection of viral pathogens, as it can generate a comprehensive set of variable proteins that constitute complete virions, facilitating comparative phylogenetic analyses with viruses in public databases (Khan et al., 2019). As a proactive diagnostic tool, viral metagenomics has the unique ability to identify emerging pathogens before they lead to disease outbreaks. This is in contrast with traditional diagnostic approaches, which typically identify etiological agents only after outbreaks have occurred (Maljkovic Berry et al., 2020).

For example, infectious pancreatic necrosis (IPN) was initially described as acute infectious enteritis in salmonids in the 1940s (M'gonigle, 1941), but its causative agent was not identified as IPN virus until two decades later (Wolf et al., 1960). Similar delays were observed with other diseases, such as viral hemorrhagic septicemia (VHS) and infectious hematopoietic necrosis virus (IHNV), where etiological agents were characterized years after initial reports of disease outbreaks (Jensen, 1965).

The application of metagenomics has dramatically accelerated the discovery of fish viruses. For instance, circoviruses have been identified in common bream (Amin & Trasti, 1988; Boucher et al., 1995; Castric et al., 1997; Wingfield et al., 1969) and European eel (Fichtner et al., 2013), while poxvirus (Reuter et al., 2015) and seadornavirus (Reuter et al., 2013) were discovered in freshwater carp. Over 20 novel fish pathogenic viruses were identified using metagenomics within a span of four years, a number significantly exceeding discoveries made through conventional diagnostics over the past five decades (Munang'andu et al., 2014). While metagenomics provides a broad, population-level view of microbial and viral diversity, it often lacks resolution on how individual pathogens interact with host cells. This gap can be bridged by single-cell omics (Tigistu-Sahle et al., 2023). Single-cell omics holds promise for unraveling viral tropism in fish, providing insights into the pathogenesis of both existing and emerging viruses that are difficult to culture (Sudhagar et al., 2018). By investigating host-virus interactions at a single-cell resolution, these advanced tools can reveal critical mechanisms of viral infection and replication. Knowledge gained from such studies has the potential to inform the development of innovative control measures, thereby enhancing disease management strategies in aquaculture.

Marker-assisted Selection of Growth and Disease Resistance Traits

Marker-assisted selection (MAS) is a powerful tool employed in aquaculture to improve production by selecting individuals with optimal growth traits and disease resistance for breeding. Traditional breeding programs typically rely on natural selection, which involves several generations of breeding to identify individuals with positive traits. This process is time-consuming and can significantly delay genetic improvements. To accelerate the identification of desirable genetic traits, MAS techniques have been introduced, utilizing molecular markers such as single nucleotide polymorphisms (SNPs), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), and quantitative trait loci (QTL) mapping (Li et al., 2003; López et al., 2015). QTL analysis is among the most widely applied techniques in aquaculture for identifying genetic factors associated with desirable traits in fish and crustacean species. QTLs represent chromosomal regions, which may contain one or more genes, that contribute to the variation of a particular trait (Geldermann, 1975). Mapped QTLs are invaluable in selective breeding programs due to their high heritability, which facilitates the rapid improvement of growth performance and disease resistance in aquaculture species. The combination of AFLP markers with RFLP and RAPD techniques has proven useful in overcoming limitations of individual methods, offering a simpler, more efficient approach. AFLP markers are dominant and do not require detailed molecular information, making them suitable for large-scale QTL analysis, including studies on disease resistance in Atlantic salmon, such as resistance to infectious salmon anemia (Moen et al., 2004).

In addition, RFLP-based approaches have been used to identify QTLs associated with disease resistance in other species, such as the eastern oyster (*Crassostrea virginica*), where disease resistance to MSX and dermo was mapped (Guo et al., 2008). The detection of SNPs, facilitated by the increasing availability of expressed

sequence tags (ESTs), has further advanced MAS in aquaculture. For example, SNPs have been identified in various species, including channel catfish (He et al., 2003), and Atlantic salmon (Hayes et al., 2007; Smith et al., 2005), to identify disease resistance loci. In yellowtail, two QTLs, Squ2 (BDR-1) and Squ20 (BDR-2), were identified for resistance to *Benedenia seriolae*, a parasitic infection (Xu et al., 2008).

SNPs have also been linked to disease resistance in several species. For instance, SNPs in the major histocompatibility complex (MHC) class I and II genes have been associated with resistance to infectious salmon anemia (ISA) in Atlantic salmon (Holborn, 2019). MHC class IIB gene polymorphisms have been found to be associated with resistance or susceptibility to *Vibrio anguillarum* in different families of Japanese flounder (Xu et al., 2008). Similarly, SNPs in the RIG-I gene of grass carp (*Ctenopharyngodon idella*) have been linked to resistance to reovirus infection (Wan et al., 2013), while SNPs in the interferon regulatory factor 2 (IRF-2) gene of freshwater mussels (*Hyriopsis cummingii*) have been associated with resistance to *Aeromonas hydrophila* (Wan et al., 2013).

An Omics Approach Free from *In-vitro* Culture Associated Modulation of Expressome

While studying host-pathogen interaction in fish or any other model, in usual practice, the pathogen needs to be isolated from the host and cultured *in-vitro* for a significant amount of time to achieve good growth. Conditions of culturing *in vitro*, even if carried out in the presence of simulated host environment (culture in close to body temperature of host or culture in presence of host cell-line etc), are always markedly different from the conditions of pathogen growing inside the host. The different environment of *in-vitro* culture is capable of modulating the expressome of the pathogen and so always an altered omics profile is achieved (Daniels et al., 2023). In single-cell omics method, on the contrary, the isolated pathogen cell could be directly subjected to omics analysis as soon as it is removed from host without any need of culturing it *in vitro* in a simulated host-environment. This would omit addition of background signal by non-natural (*in-vitro*) culture condition and a much more real snapshot of pathogens omics profile would be achieved (Krekhno, 2023).

Similarly, host cells can be directly isolated from the infection site and analyzed using single-cell omics, eliminating the need for culturing, thus saving valuable time. A further advantage of single-cell omics is that the same animal can be used repeatedly in time-course experiments to study disease progression (Hu et al., 2018) . Since the cells are needed in extremely small quantities, down to the level of a single cell, sacrificing the animal(s) may not be necessary (Hu et al., 2018).

Single Cell Omics for Studying the Rare Cell Types of Fish

Many cell-types in different organisms are found in extremely low number (such as somatic stem cells and certain immune cells in case of mammals). These kinds of cell populations though found in small number, tend to play important roles in the biology of the organism. However, for the conventional omics protocols, their isolation inadequate number is extremely cumbersome and poses a prime obstacle (Sehgal & Chaturvedi, 2022). Conversely, single cell omics protocols for studying such cell-population in fish or other organisms may be relatively easier and efficient (Clarke et al., 2021). Additionally, in the same rare population of cells if experimental replicates give different results, then, it may provide an indication of occurrence of novel subpopulations in that group. Three different cell populations, T cells, a novel type of NK-like cells and a smaller population of myeloid-like cells were identified in the spleen of zebra fish using the Single-cell T- cell receptor locus reconstruction (Carmona et al., 2017).

For Study of Viruses or Other Pathogens with Low *In-vitro* Infectivity

In-vitro culture-based infection models though may have certain limitations, on the other hand, they also have some advantages as well. Ease of handling, less time-required in experimental monitoring, easy protocol modulation and minimal loss to the biodiversity are among few of them. Researchers have used GFP-expressing viral pathogens for examining and tracking the infection in the *in-vitro* infection model (Jha et al., 2013). Additionally, the extent of infection in this system may be examined by studying the pathogen-associated expression markers or by simply visualizing the cytopathic effects or foci (in case of many viral pathogens). Traditional omics approaches have been extensively used in association with *in-vitro* culture-based infection models in fish and higher animals. However, in cases when the infectivity of the pathogen is low, these traditional approaches usually fail in studying the expressome of the infected cells (Fondi & Liò, 2015). With the use of proper cell isolation protocol combined with single cell omics, the expressome of infected cells could be precisely studied in fish and other cell-lines (or primary cells) (Hu et al., 2018; Hwang et al., 2018). Though the isolation of GFP-expressing cells may be easy, extensive protocol standardization may be required for isolation of infected cells based on cytopathic effects.

As a Preferred Method for Studying Rare Fish-species and Conservation of Biodiversity

With the changing environment and increasing anthropogenic activity in natural habitats, many fish varieties are at the verge of extinction (Hunter, 2007).

Studying these varieties is an important step towards their conservation. However, extensive use of these varieties for research purpose also poses threat to their diversity. As discussed earlier, due to the minimal amount of cell/tissue requirement, single cell omics could be a method of choice to study such cases. Because of minimal cell/tissue requirement, the fish needs not be necessarily sacrificed and even the time course experiments could be carried out without much harm to the animal. Similarly, for studying diverse and internal tissue-expressome of a rare fish species, sacrificing a single animal (if essentially required) may be good enough and scientifically acceptable (in terms of replicates) in the interest of biodiversity conservation.

Disease Resistance and Susceptibility Traits

Excessive use of antibiotics to reduce disease burden has led to the development of antibiotic-resistant strains of many infectious agents. Such microorganisms are seriously affecting our environment and pose a grave problem that needs to be addressed (Ahmed et al., 2024; Muteeb et al., 2023; Serwecińska, 2020). The field of genomics has witnessed significant advancements in identifying disease resistance traits across various cultured aquatic organisms. Techniques such as single nucleotide polymorphism (SNP) analysis, microsatellite loci (MTLs), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), and quantitative trait loci (QTL) mapping have been extensively employed to uncover genetic markers associated with disease resistance and susceptibility. These genomic tools have proven invaluable in selective breeding programs, enabling precise identification of individuals with desirable traits. SNP analysis, for example, has been widely used to pinpoint genetic variations linked to disease resistance, offering a high-resolution approach for marker-assisted selection. Similarly, AFLP and RAPD are useful in identifying polymorphic markers, while RFLP has been instrumental in mapping genes related to disease resilience in species such as oysters and salmonids.

QTL mapping remains one of the most comprehensive approaches, allowing researchers to link specific chromosomal regions to phenotypic traits such as growth performance and pathogen resistance. This technique has been applied to various commercial fish and crustacean species, accelerating the development of genetically superior stocks with enhanced resistance to diseases (Munang'andu & Evensen, 2017; Peace et al., 2019). To combat fish viral diseases, QTL resistance traits have been identified for grass carp reovirus (GCRV) infection in grass carp (Huang et al., 2015), nervous necrosis virus (NNV) in seabass (Liu et al., 2016), viral hemorrhagic septicemia (VHS) in turbot (Rodríguez-Ramilo et al., 2014) and rainbow trout (Verrier et al., 2013), besides infectious salmon anaemia (ISAV) virus in Atlantic salmon, lymphocytic disease virus in Japanese flounder (Fuji et al., 2006) and infectious pancreatic necrosis virus (IPNV) in Atlantic salmon (Gheyas et al., 2010). The introduction of QTL-based breeding for IPNV resistance in Norwegian Atlantic salmon significantly reduced disease incidence by over 80% between 2008 and 2015 (Moen et al., 2015). Bacterial diseases for which QTL resistance traits have been detected include Coldwater disease in rainbow trout (Wiens et al., 2013), *Aeromonas hydrophila* in rohu (*Labeo rohita*) (Robinson et al., 2014), *Vibrio anguillarum* in Japanese flounder (Shao et al., 2015), *Flavobacterium psychrophilum* in rainbow trout (Vallejo et al., 2014) and pastuerellosis in gilthead seabream (Massault et al., 2011).

Developmental Biology

RNA-Seq has been successfully employed to compare the transcriptome profiles of four early developmental stages (1-, 16-, 512-cell stage, and 50% epiboly) in zebrafish on a global scale (Vesterlund et al., 2011). In this study, 177 genes were detected as developmentally regulated. Whereas a majority of gene transcripts were present at a steady level, a major switch in gene regulation and transcription activity took place between the 512-cell and 50% epiboly stages (Vesterlund et al., 2011). The application of RNA-Seq extends to understanding the role of vitamin D in zebrafish embryogenesis. Treatment with $1\alpha,25(OH)_2D_3$, an active vitamin D_3 metabolite, significantly altered the expression of genes involved in transcriptional regulation, hormone signaling, and metabolic pathways (Craig et al., 2012). This underscores the importance of the vitamin D receptor (VDR) in orchestrating multiple developmental processes, linking external metabolites to internal gene regulatory networks. In the context of retinal development, RNA-Seq identified Id2a as a key regulator that balances proliferation and differentiation by modulating the Notch signaling pathway (Uribe et al., 2012). This connection highlights how transcriptome studies can elucidate specific pathways critical for organogenesis. Recent advancements using single-cell RNA-Seq have provided finer resolution into the transcriptomic profiles of zebrafish primordial germ cells (PGCs) at 6, 11, and 24 hours post-fertilization (hpf). Early stages (6–11 hpf) are characterized by active migration and division, while the 24 hpf stage exhibits high translational activity. Interestingly, genes essential for PGC migration were downregulated between 11 and 24 hpf, contrary to earlier reports (Mich et al., 2009). This finding connects the cellular behavior of PGCs with stage-specific transcriptional regulation, offering new insights into germ cell development.

Evolution Biology

In lake whitefish (*Coregonus clupeaformis* spp., *Salmonidae*), RNA-seq analysis revealed distinct expression patterns between dwarf and normal

morphotypes (Jenkins et al., 2010). Normal whitefish showed overexpression of genes involved in protein synthesis, while dwarf whitefish exhibited higher expression of genes related to immunity, DNA replication and repair, and energy metabolism. A positive correlation between RNA-seq results and earlier microarray data further validated these findings.

Similarly, transcriptomic studies have uncovered genetic bases for phenotypic variation in other species. In Siscowet and lean lake trout (*Salvelinus namaycush*), RNA-seq revealed differential gene expression patterns linked to their ecological and morphological differences (Goetz et al., 2010), Likewise, differences in pigment patterns between marine and freshwater sticklebacks (*Gasterosteus aculeatus*) (Greenwood et al., 2012), and adaptations of Mexican tetra (*Astyanax mexicanus*) to cave environments (Gross et al., 2013), have been elucidated through RNA-seq. Further, RNA-seq has shed light on evolutionary homologous structures, such as the relationship between the zebrafish swimbladder and the mammalian lung, providing insights into conserved genetic pathways and functional evolution (Zhang et al., 2019). These studies demonstrate the power of RNA-seq in linking gene expression with phenotypic and ecological adaptations, offering valuable insights into the processes of divergence and speciation across diverse taxa.

Affordability of Single Cell–omics

Single-cell genomics is far more expensive than conventional metagenomics, transcriptomics or genomics. It requires skilled personnel besides the state-of-the-art facilities that include expensive equipment like the flow cytometers, robotic liquid handlers, clean rooms and workflows to minimize contamination (Xu & Zhao, 2018). Highly expensive microfluidic set-ups may exclude some technical hurdles, but they need to be custom-designed which adds to the cost of equipment. Measurement of as numerous cellular parameters is also a major constraint if lower-throughput flow cytometry is attempted (Darnell & Mooney, 2017). High-throughput next-generation sequencing further adds to the cost (Xu & Zhao, 2018). Moreover, the requirement of advanced computational facilities along with technical expertise is another bottleneck due to which this technique is not accessible to most researchers as there are only a few centralized facilities around the world. To address these challenges, several strategies are being explored. Low-cost single-cell RNA-seq protocols, such as Drop-seq and Seq-Well, have been developed to reduce reagent and equipment expenses while maintaining reliability (Salomon et al., 2019). Collaborative research models and shared core facilities allow institutions to pool resources, thereby lowering individual project costs.(Landry & Amara, 1998)m These strategies may gradually make single-cell omics more accessible to a wider community of researchers, including those working in applied fields such as fisheries science.

Conclusion

In conclusion, single-cell omics technologies have emerged as a transformative tool for exploring biological diversity, unraveling cellular functions, and understanding lineage dynamics at an unprecedented resolution. By enabling the integration of genomic, epigenomic, transcriptomic, and proteomic data at the single-cell level, these methodologies provide an exceptional opportunity to investigate the uniqueness of individual cells and their roles in complex biological systems. The ability to identify rare cell types, trace developmental trajectories, and delineate cellular heterogeneity opens up vast possibilities across multiple disciplines, including developmental biology, disease research, and applied sciences like aquaculture. The application of single-cell multi-omics in aquaculture research has already shown promise, with studies demonstrating its potential to uncover key regulatory mechanisms, identify stress and disease markers, and enhance selective breeding programs. However, the field is still in its infancy, and significant challenges remain in making these technologies more accessible, affordable, and adaptable to real-world applications. The high cost of instrumentation, data complexity, and the need for specialized computational tools have limited the scalability of these methods, particularly in applied industries like aquaculture.

Future advancements in single-cell multi-omics technologies are expected to address these challenges by reducing costs, increasing throughput, and enhancing the integration of diverse data types. Innovations such as microfluidic devices, machine learning-based data analysis, and improved multi-modal platforms will likely accelerate the adoption of single-cell omics in both research and practical settings. Moreover, interdisciplinary collaborations will play a pivotal role in translating these high-resolution insights into actionable strategies, such as developing disease-resistant aquaculture species, optimizing nutrition, and improving environmental sustainability in aquaculture practices. Although the journey toward widespread and affordable applications is still in its early stages, the potential of single-cell omics to revolutionize biological research and applied sciences is undeniable. As this area of biology continues to flourish, it will undoubtedly unlock new horizons, offering profound implications for understanding cellular mechanisms and addressing real-world challenges. With continued innovation and investment, single-cell multi-omics is poised to become a cornerstone of modern biology and a catalyst for transformative advancements in aquaculture and beyond.

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Author Contribution

All the authors have contributed equally to this review paper.

Conflict of Interest

The authors declare that there is no conflict of interest.

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References

Aas, I. B., Austbø, L., König, M., Syed, M., Falk, K., Hordvik, I., & Koppang, E. O. (2014). Transcriptional characterization of the T cell population within the salmonid interbranchial lymphoid tissue. *The Journal of Immunology*, *193*(7), 3463-3469.

Abelli, L., Picchietti, S., Romano, N., Mastrolia, L., & Scapigliati, G. (1997). Immunohistochemistry of gut-associated lymphoid tissue of the sea bassDicentrarchus labrax (L.). *Fish & shellfish immunology*, *7*(4), 235-245.

Adam, A.-C., Skjaerven, K. H., Whatmore, P., Moren, M., & Lie, K. K. J. P. O. (2018). Parental high dietary arachidonic acid levels modulated the hepatic transcriptome of adult zebrafish (Danio rerio) progeny. *13*(8), e0201278.

Ahmed, S. K., Hussein, S., Qurbani, K., Ibrahim, R. H., Fareeq, A., Mahmood, K. A., & Mohamed, M. G. (2024). Antimicrobial resistance: impacts, challenges, and future prospects. *Journal of Medicine, Surgery, and Public Health*, *2*, 100081.

Alalwani, M. S. (2009). Cathelicidin regulates homeostasis of innate immune responses.

Alemany, A., Florescu, M., Baron, C. S., Peterson-Maduro, J., & Van Oudenaarden, A. (2018). Whole-organism clone tracing using single-cell sequencing. *nature*, *556*(7699), 108-112.

Alvarez, M., Schrey, A. W., & Richards, C. L. (2015). Ten years of transcriptomics in wild populations: what have we learned about their ecology and evolution? *Molecular ecology*, *24*(4), 710-725.

Amin, A., & Trasti, J. (1988). Endomyocarditis in Atlantic salmon in Norwegian seafarms. A case report.

Angermueller, C., Clark, S. J., Lee, H. J., Macaulay, I. C., Teng, M. J., Hu, T. X., Krueger, F., Smallwood, S. A., Ponting, C. P., & Voet, T. (2016). Parallel single-cell sequencing links transcriptional and epigenetic heterogeneity. *Nature methods*, *13*(3), 229-232.

Araki, K., Suetake, H., Kikuchi, K., & Suzuki, Y. (2005). Characterization and expression analysis of CD3ε and CD3γ/δ in fugu, Takifugu rubripes. *Immunogenetics*, *57*, 158-163.

Athaya, T., Ripan, R. C., Li, X., & Hu, H. (2023). Multimodal deep learning approaches for single-cell multi-omics data integration. *Briefings in Bioinformatics*, *24*(5), bbad313.

Avarre, J.-C., Guinand, B., Dugué, R., Cosson, J., Legendre, M., Panfili, J., & Durand, J.-D. J. P. (2014). Plasticity of gene expression according to salinity in the testis of broodstock and F1 black-chinned tilapia, Sarotherodon melanotheron heudelotii. *2*, e702.

Bain, P. A., Gregg, A. L., & Kumar, A. J. B. g. (2016). De novo assembly and analysis of changes in the protein-coding transcriptome of the freshwater shrimp Paratya australiensis (Decapoda: Atyidae) in response to acid sulfate drainage water. *17*, 1-18.

Bar, I., Cummins, S., & Elizur, A. J. B. g. (2016). Transcriptome analysis reveals differentially expressed genes associated with germ cell and gonad development in the Southern bluefin tuna (Thunnus maccoyii). *17*, 1-22.

Baysoy, A., Bai, Z., Satija, R., & Fan, R. (2023). The technological landscape and applications of single-cell multi-omics. *Nature Reviews Molecular Cell Biology*, *24*(10), 695-713.

Benedicenti, O., Collins, C., Wang, T., McCarthy, U., & Secombes, C. J. (2015). Which Th pathway is involved during late stage amoebic gill disease? *Fish & shellfish immunology*, *46*(2), 417-425.

Bertone, P., Stolc, V., Royce, T. E., Rozowsky, J. S., Urban, A. E., Zhu, X., Rinn, J. L., Tongprasit, W., Samanta, M., & Weissman, S. (2004). Global identification of human transcribed sequences with genome tiling arrays. *science*, *306*(5705), 2242-2246.

Bibby, K. (2013). Metagenomic identification of viral pathogens. *Trends in biotechnology*, *31*(5), 275-279.

Bock, A. (2021). *RNA-seq Analysis of Tigriopus Californicus under Multiple Dietary Conditions*. University of California, San Diego.

Bock, C., Farlik, M., & Sheffield, N. C. (2016). Multi-omics of single cells: strategies and applications. *Trends in biotechnology*, *34*(8), 605-608.

Boucher, P., Castric, J., & Laurencin, F. B. (1995). Observation of virus-like particles in rainbow trout Oncorhynchus mykiss infected with sleeping disease virulent material.

Brandtzaeg, P., Kiyono, H., Pabst, R., & Russell, M. (2008). Terminology: nomenclature of mucosa-associated lymphoid tissue. *Mucosal immunology*, *1*(1), 31-37.

Brooks, T. G., Lahens, N. F., Mrčela, A., & Grant, G. R. (2024). Challenges and best practices in omics benchmarking. *Nature Reviews Genetics*, *25*(5), 326-339.

Callet, T., Dupont-Nivet, M., Cluzeaud, M., Jaffrezic, F., Laloë, D., Kerneis, T., Labbé, L., Quillet, E., Geurden, I., & Mazurais, D. J. P. O. (2018). Detection of new pathways involved in the acceptance and the utilisation of a plant-based diet in isogenic lines of rainbow trout fry. *13*(7), e0201462.

Cao, Z.-J., & Gao, G. (2022). Multi-omics single-cell data integration and regulatory inference with graph-linked embedding. *Nature biotechnology*, *40*(10), 1458-1466.

Carmona, S. J., Teichmann, S. A., Ferreira, L., Macaulay, I. C., Stubbington, M. J., Cvejic, A., & Gfeller, D. (2017). Single-cell transcriptome analysis of fish immune cells provides insight into the evolution of vertebrate immune cell types. *Genome research*, *27*(3), 451-461.

Carter, N. (1993). Gene mapping and PCR applications with flow-sorted chromosomes. In *Flow Cytometry: New Developments* (pp. 327-342). Springer.

Castric, J., Baudin Laurencin, F., Bremont, M., Jeffroy, J., Ven, A. l., & Bearzotti, M. (1997). Isolation of the virus responsible for sleeping disease in experimentally infected rainbow trout (Oncorhynchus mykiss).

Castro, R., Bernard, D., Lefranc, M., Six, A., Benmansour, A., & Boudinot, P. (2011). T cell diversity and TcR repertoires in teleost fish. *Fish & shellfish immunology*, *31*(5), 644-654.

Castro, R., Jouneau, L., Pham, H.-P., Bouchez, O., Giudicelli, V., Lefranc, M.-P., Quillet, E., Benmansour, A., Cazals, F., & Six, A. (2013). Teleost fish mount complex clonal IgM and IgT responses in spleen upon systemic viral infection. *PLoS pathogens*, *9*(1), e1003098.

Chandhini, S., & Rejish Kumar, V. J. (2019). Transcriptomics in aquaculture: current status and applications. *Reviews in aquaculture*, *11*(4), 1379-1397.

Chandhini, S., & Rejish Kumar, V. J. J. R. i. A. (2019). Transcriptomics in aquaculture: current status and applications. *11*(4), 1379-1397.

Chapman, R. W., Reading, B. J., & Sullivan, C. V. J. P. O. (2014). Ovary transcriptome profiling via artificial intelligence reveals a transcriptomic fingerprint predicting egg quality in striped bass, Morone saxatilis. *9*(5), e96818.

Chen, G., Ning, B., & Shi, T. (2019). Single-cell RNA-seq technologies and related computational data analysis. *Frontiers in genetics*, *10*, 317.

Chen, K., Li, E., Li, T., Xu, C., Wang, X., Lin, H., Qin, J. G., & Chen, L. J. P. O. (2015). Transcriptome and molecular pathway analysis of the hepatopancreas in the Pacific white shrimp Litopenaeus vannamei under chronic low-salinity stress. *10*(7), e0131503.

Chen, X., Wang, J., Yue, W., Liu, J., Wang, C. J. F., & Immunology, S. (2017). Hepatopancreas transcriptome analysis of Chinese mitten crab (Eriocheir sinensis) with white hepatopancreas syndrome. *70*, 302-307.

Chen, X., Zeng, D., Chen, X., Xie, D., Zhao, Y., Yang, C., Li, Y., Ma, N., Li, M., & Yang, Q. J. P. o. (2013). Transcriptome analysis of Litopenaeus vannamei in response to white spot syndrome virus infection. *8*(8), e73218.

Cheng, Y., Poulos, N. E., Lung, M. L., Hampton, G., Ou, B., Lerman, M. I., & Stanbridge, E. J. (1998). Functional evidence for a nasopharyngeal carcinoma tumor suppressor gene that maps at chromosome 3p21. 3. *Proceedings of the National Academy of Sciences*, *95*(6), 3042-3047.

Clark, T. A., Sugnet, C. W., & Ares Jr, M. (2002). Genomewide analysis of mRNA processing in yeast using splicing-specific microarrays. *science*, *296*(5569), 907-910.

Clarke, Z. A., Andrews, T. S., Atif, J., Pouyabahar, D., Innes, B. T., MacParland, S. A., & Bader, G. D. (2021). Tutorial: guidelines for annotating single-cell transcriptomic maps using automated and manual methods. *Nature protocols*, *16*(6), 2749-2764.

Colli-Dula, R. C., Fang, X., Moraga-Amador, D., Albornoz-Abud, N., Zamora-Bustillos, R., Conesa, A., Zapata-Perez, O., Moreno, D., & Hernandez-Nuñez, E. J. A. t. (2018). Transcriptome analysis reveals novel insights into the response of low-dose benzo (a) pyrene exposure in male tilapia. *201*, 162-173.

Costa, J., Adams, A., Bron, J., Thompson, K., Starkey, W., & Richards, R. (2007). Identification of B-cell epitopes on the betanodavirus capsid protein. *Journal of Fish Diseases*, *30*(7), 419-426.

Craig, T.A., Zhang, Y., McNulty, M.S., Middha, S., Ketha, H., Singh, R.J., Magis, A.T., Funk, C., Price, N. D., & Ekker, S.C. (2012). Research resource: whole transcriptome RNA sequencing detects multiple 1α, 25-dihydroxyvitamin D3-sensitive metabolic pathways in developing zebrafish. *Molecular endocrinology*, *26*(9), 1630-1642.

Cui, Z., Li, X., Liu, Y., Song, C., Hui, M., Shi, G., Luo, D., & Li, Y. J. P. o. (2013). Transcriptome profiling analysis on whole bodies of microbial challenged Eriocheir sinensis larvae for immune gene identification and SNP development. *8*(12), e82156.

Dai, P., Luan, S., Lu, X., Luo, K., & Kong, J. J. S. R. (2017). Comparative transcriptome analysis of the Pacific White Shrimp (Litopenaeus vannamei) muscle reveals the molecular basis of residual feed intake. *7*(1), 10483.

Daniels, R. R., Taylor, R. S., Robledo, D., & Macqueen, D. J. (2023). Single cell genomics as a transformative approach for aquaculture research and innovation. *Reviews in aquaculture*, *15*(4), 1618-1637.

Danzmann, R. G., Kocmarek, A. L., Norman, J. D., Rexroad, C. E., & Palti, Y. J. B. G. (2016). Transcriptome profiling in fast versus slow-growing rainbow trout across seasonal gradients. *17*, 1-18.

Darmanis, S., Gallant, C. J., Marinescu, V. D., Niklasson, M., Segerman, A., Flamourakis, G., Fredriksson, S., Assarsson, E., Lundberg, M., & Nelander, S. (2016). Simultaneous multiplexed measurement of RNA and proteins in single cells. *Cell reports*, *14*(2), 380-389.

Darnell, M., & Mooney, D. J. (2017). Leveraging advances in biology to design biomaterials. *Nature materials*, *16*(12), 1178-1185.

De Santis, C., Taylor, J. F., Martinez-Rubio, L., Boltana, S., & Tocher, D. R. J. P. o. (2015). Influence of development and dietary phospholipid content and composition on intestinal transcriptome of Atlantic salmon (Salmo salar). *10*(10), e0140964.

Dean, F. B., Hosono, S., Fang, L., Wu, X., Faruqi, A. F., Bray-Ward, P., Sun, Z., Zong, Q., Du, Y., & Du, J. (2002). Comprehensive human genome amplification using multiple displacement amplification. *Proceedings of the National Academy of Sciences*, *99*(8), 5261-5266.

Dey, S. S., Kester, L., Spanjaard, B., Bienko, M., & Van Oudenaarden, A. (2015). Integrated genome and transcriptome sequencing of the same cell. *Nature biotechnology*, *33*(3), 285-289.

Dezem, F. S., Morosini, N. S., Arjumand, W., DuBose, H., & Plummer, J. (2024). Spatially Resolved Single-Cell Omics: Methods, Challenges, and Future Perspectives. *Annual Review of Biomedical Data Science*, *7*.

Douglas, S. E. (2006). Microarray studies of gene expression in fish. *OMICS: A Journal of Integrative Biology*, *10*(4), 474-489.

Du, F., Xu, G., Nie, Z., Xu, P., & Gu, R. J. B. g. (2014). Transcriptome analysis gene expression in the liver of Coilia nasus during the stress response. *15*, 1-11.

Efremova, M., & Teichmann, S. A. (2020). Computational methods for single-cell omics across modalities. *Nature methods*, *17*(1), 14-17.

Farlik, M., Sheffield, N. C., Nuzzo, A., Datlinger, P., Schönegger, A., Klughammer, J., & Bock, C. (2015). Single-cell DNA methylome sequencing and bioinformatic inference of epigenomic cell-state dynamics. *Cell reports*, *10*(8), 1386-1397.

Fichtner, D., Philipps, A., Groth, M., Schmidt-Posthaus, H., Granzow, H., Dauber, M., Platzer, M., Bergmann, S. M., Schrudde, D., & Sauerbrei, A. (2013). Characterization of a novel picornavirus isolate from a diseased European

eel (Anguilla anguilla). *Journal of Virology*, *87*(19), 10895-10899.

Fondi, M., & Liò, P. (2015). Multi-omics and metabolic modelling pipelines: challenges and tools for systems microbiology. *Microbiological Research*, *171*, 52-64.

Fuji, K., Kobayashi, K., Hasegawa, O., Coimbra, M. R. M., Sakamoto, T., & Okamoto, N. (2006). Identification of a single major genetic locus controlling the resistance to lymphocystis disease in Japanese flounder (Paralichthys olivaceus). *Aquaculture*, *254*(1-4), 203-210.

Gao, L., He, C., Bao, X., Tian, M., & Ma, Z. J. P. O. (2017). Transcriptome analysis of the sea cucumber (Apostichopus japonicus) with variation in individual growth. *12*(7), e0181471.

Gao, Y., Li, L., Amos, C. I., & Li, W. (2021). Analysis of alternative polyadenylation from single-cell RNA-seq using scDaPars reveals cell subpopulations invisible to gene expression. *Genome research*, *31*(10), 1856-1866.

Gao, Y., Zhang, X., Wei, J., Sun, X., Yuan, J., Li, F., & Xiang, J. J. P. o. (2015). Whole transcriptome analysis provides insights into molecular mechanisms for molting in Litopenaeus vannamei. *10*(12), e0144350.

Garvin, M. R., Thorgaard, G. H., Narum, S. R. J. G. b., & evolution. (2015). Differential expression of genes that control respiration contribute to thermal adaptation in redband trout (Oncorhynchus mykiss gairdneri). *7*(6), 1404-1414.

Gawad, C., Koh, W., & Quake, S. R. (2014). Dissecting the clonal origins of childhood acute lymphoblastic leukemia by single-cell genomics. *Proceedings of the National Academy of Sciences*, *111*(50), 17947-17952.

Geldermann, H. (1975). Investigations on inheritance of quantitative characters in animals by gene markers I. Methods. *Theoretical and Applied Genetics*, *46*(7), 319-330.

Gheyas, A., Houston, R., Mota-Velasco, J., Guy, D., Tinch, A., Haley, C., & Woolliams, J. (2010). Segregation of infectious pancreatic necrosis resistance QTL in the early life cycle of Atlantic Salmon (Salmo salar). *Animal genetics*, *41*(5), 531-536.

Goetz, F., Rosauer, D., Sitar, S., Goetz, G., Simchick, C., Roberts, S., Johnson, R., Murphy, C., Bronte, C. R., & Mackenzie, S. (2010). A genetic basis for the phenotypic differentiation between siscowet and lean lake trout (Salvelinus namaycush). *Molecular ecology*, *19*, 176-196.

Gonçalves, A. T., Valenzuela-Muñoz, V., Gallardo-Escárate, C. J. F., & immunology, s. (2017). Intestinal transcriptome modulation by functional diets in rainbow trout: a high-throughput sequencing appraisal to highlight GALT immunomodulation. *64*, 325-338.

Gracey, A. (2008). The Gillichthys mirabilis Cooper array: a platform to investigate the molecular basis of phenotypic plasticity. *Journal of Fish Biology*, *72*(9), 2118-2132.

Graves, J. A. M. (2016). Evolution of vertebrate sex chromosomes and dosage compensation. *Nature Reviews Genetics*, *17*(1), 33-46.

Greenwood, A. K., Cech, J. N., & Peichel, C. L. (2012). Molecular and developmental contributions to divergent pigment patterns in marine and freshwater sticklebacks. *Evolution & development*, *14*(4), 351-362.

Gross, J. B., Furterer, A., Carlson, B. M., & Stahl, B. A. (2013). An integrated transcriptome-wide analysis of cave and surface dwelling Astyanax mexicanus. *PLoS One*, *8*(2), e55659.

Guo, X., Wang, Y., Wang, L., & Lee, J.-H. (2008). Oysters. In *Genome mapping and genomics in fishes and aquatic animals* (pp. 163-175). Springer.

Han, J., Won, E.-J., Kang, H.-M., Lee, M.-C., Jeong, C.-B., Kim, H.-S., Hwang, D.-S., & Lee, J.-S. J. M. P. B. (2017). Marine copepod cytochrome P450 genes and their applications for molecular ecotoxicological studies in response to oil pollution. *124*(2), 953-961.

Han, L., Zi, X., Garmire, L. X., Wu, Y., Weissman, S. M., Pan, X., & Fan, R. (2014). Co-detection and sequencing of genes and transcripts from the same single cells facilitated by a microfluidics platform. *Scientific reports*, *4*(1), 6485.

Harbers, M., & Carninci, P. (2005). Tag-based approaches for transcriptome research and genome annotation. *Nature methods*, *2*(7), 495-502.

Hashimshony, T., Wagner, F., Sher, N., & Yanai, I. (2012). CEL-Seq: single-cell RNA-Seq by multiplexed linear amplification. *Cell reports*, *2*(3), 666-673.

Hayes, B., Laerdahl, J., Lien, S., Moen, T., Berg, P., Hindar, K., Davidson, W., Koop, B., Adzhubei, A., & Høyheim, B. (2007). An extensive resource of single nucleotide polymorphism markers associated with Atlantic salmon (Salmo salar) expressed sequences. *Aquaculture*, *265*(1-4), 82-90.

He, C., Chen, L., Simmons, M., Li, P., Kim, S., & Liu, Z. (2003). Putative SNP discovery in interspecific hybrids of catfish by comparative EST analysis. *Animal genetics*, *34*(6), 445-448.

He, L., Zhao, C., Xiao, Q., Zhao, J., Liu, H., Jiang, J., & Cao, Q. (2023). Profiling the Physiological Roles in Fish Primary Cell Culture. *Biology*, *12*(12), 1454.

He, X., Memczak, S., Qu, J., Belmonte, J. C. I., & Liu, G.-H. (2020). Single-cell omics in ageing: a young and growing field. *Nature Metabolism*, *2*(4), 293-302.

Hodne, K., & Weltzien, F.-A. (2015). Single-cell isolation and gene analysis: pitfalls and possibilities. *International Journal of Molecular Sciences*, *16*(11), 26832-26849.

Holborn, M. (2019). *Resistance to pathogens in North American Atlantic salmon: Genome wide association analyses for bacterial kidney disease, salmon lice, and infectious salmon anemia* University of Guelph].

Hou, Y., Guo, H., Cao, C., Li, X., Hu, B., Zhu, P., Wu, X., Wen, L., Tang, F., & Huang, Y. (2016). Single-cell triple omics sequencing reveals genetic, epigenetic, and transcriptomic heterogeneity in hepatocellular carcinomas. *Cell research*, *26*(3), 304-319.

Hrdlickova, R., Toloue, M., & Tian, B. (2017). RNA-Seq methods for transcriptome analysis. *Wiley Interdisciplinary Reviews: RNA*, *8*(1), e1364.

Hu, C., Zhang, N., Hong, Y., Tie, R., Fan, D., Lin, A., Chen, Y., Xiang, L.-x., & Shao, J.-z. (2024). Single-cell RNA sequencing unveils the hidden powers of zebrafish kidney for generating both hematopoiesis and adaptive antiviral immunity. *Elife*, *13*, RP92424.

Hu, Y., An, Q., Sheu, K., Trejo, B., Fan, S., & Guo, Y. (2018). Single cell multi-omics technology: methodology and application. *Frontiers in cell and developmental biology*, *6*, 28.

Hu, Y., Huang, K., An, Q., Du, G., Hu, G., Xue, J., Zhu, X., Wang, C.-Y., Xue, Z., & Fan, G. (2016). Simultaneous profiling of transcriptome and DNA methylome from a single cell. *Genome biology*, *17*, 1-11.

Hu, Y., Li, A., Xu, Y., Jiang, B., Lu, G., Luo, X. J. F., & immunology, s. (2017). Transcriptomic variation of locally-infected skin of Epinephelus coioides reveals the mucosal

immune mechanism against Cryptocaryon irritans. *66*, 398-410.

Huang, C., Li, Y., Hu, S., Chi, J., Lin, G., Lin, C., Gong, H.-Y., Chen, J., Chen, R., & Chang, S. (2012). Differential expression patterns of growth-related microRNAs in the skeletal muscle of Nile tilapia (Oreochromis niloticus). *Journal of animal science*, *90*(12), 4266-4279.

Huang, R., Sun, J., Luo, Q., He, L., Liao, L., Li, Y., Guo, F., Zhu, Z., & Wang, Y. (2015). Genetic variations of body weight and GCRV resistance in a random mating population of grass carp. *Oncotarget*, *6*(34), 35433.

Huang, W., Li, H., Cheng, C., Ren, C., Chen, T., Jiang, X., Cheng, K., Luo, P., & Hu, C. J. P. o. (2018). Analysis of the transcriptome data in Litopenaeus vannamei reveals the immune basis and predicts the hub regulation-genes in response to high-pH stress. *13*(12), e0207771.

Hunter, P. (2007). The human impact on biological diversity: How species adapt to urban challenges sheds light on evolution and provides clues about conservation. *EMBO reports*, *8*(4), 316-318.

Hwang, B., Lee, J. H., & Bang, D. (2018). Single-cell RNA sequencing technologies and bioinformatics pipelines. *Experimental & Molecular Medicine*, *50*(8), 1-14.

Jenkins, L., Brea, J., Smith, N. J., Hudson, B. D., Reilly, G., Bryant, N. J., Castro, M., Loza, M.-I., & Milligan, G. (2010). Identification of novel species-selective agonists of the G-protein-coupled receptor GPR35 that promote recruitment of β-arrestin-2 and activate Gα13. *Biochemical Journal*, *432*(3), 451-459.

Jensen, M. H. (1965). Research on the virus of Egtved disease. *Annals of the New York Academy of Sciences*, *126*(1), 422-426.

Jha, H. C., Upadhyay, S. K., AJ, M. P., Lu, J., Cai, Q., Saha, A., & Robertson, E. S. (2013). H2AX phosphorylation is important for LANA-mediated Kaposi's sarcoma-associated herpesvirus episome persistence. *Journal of Virology*, *87*(9), 5255-5269.

Jiang, H., Xing, Z., Lu, W., Qian, Z., Yu, H., & Li, J. J. P. o. (2014). Transcriptome analysis of red swamp crawfish Procambarus clarkii reveals genes involved in gonadal development. *9*(8), e105122.

Jin, S., Fu, H., Zhou, Q., Sun, S., Jiang, S., Xiong, Y., Gong, Y., Qiao, H., & Zhang, W. J. P. o. (2013). Transcriptome analysis of androgenic gland for discovery of novel genes from the oriental river prawn, Macrobrachium nipponense, using Illumina Hiseq 2000. *8*(10), e76840.

Jin, S., Zhang, Y., Dong, X., Xi, Q., Song, D., Fu, H., Sun, D. J. G., & Research, M. (2015). Comparative transcriptome analysis of testes and ovaries for the discovery of novel genes from Amur sturgeon (Acipenser schrenckii). *14*(4), 18913-18927.

Jung, H., Lyons, R. E., Dinh, H., Hurwood, D. A., McWilliam, S., & Mather, P. B. J. P. o. (2011). Transcriptomics of a giant freshwater prawn (Macrobrachium rosenbergii): de novo assembly, annotation and marker discovery. *6*(12), e27938.

Kato, G., Takano, T., Sakai, T., Matsuyama, T., & Nakayasu, C. (2013). Vibrio anguillarum bacterin uptake via the gills of Japanese flounder and subsequent immune responses. *Fish & shellfish immunology*, *35*(5), 1591-1597.

Khan, M. M., Ernst, O., Manes, N. P., Oyler, B. L., Fraser, I. D., Goodlett, D. R., & Nita-Lazar, A. (2019). Multi-omics strategies uncover host–pathogen interactions. *ACS infectious diseases*, *5*(4), 493-505.

Kim, J. B., Porreca, G. J., Song, L., Greenway, S. C., Gorham, J. M., Church, G. M., Seidman, C. E., & Seidman, J. (2007). Polony multiplex analysis of gene expression (PMAGE) in mouse hypertrophic cardiomyopathy. *science*, *316*(5830), 1481-1484.

Knoll-Gellida, A., André, M., Gattegno, T., Forgue, J., Admon, A., & Babin, P. J. (2006). Molecular phenotype of zebrafish ovarian follicle by serial analysis of gene expression and proteomic profiling, and comparison with the transcriptomes of other animals. *BMC genomics*, *7*, 1-28.

Koskinen, H., Krasnov, A., Rexroad, C., Gorodilov, Y., Afanasyev, S., & Mölsä, H. (2004). The 14-3-3 proteins in the teleost fish rainbow trout (Oncorhynchus mykiss). *Journal of experimental biology*, *207*(19), 3361-3368.

Krekhno, Z. (2023). *Multi-omics investigation of host-pathogen interactions during pathogenic Escherichia coli infection* University of British Columbia].

Kubik, G., & Summerer, D. (2015). Deciphering epigenetic cytosine modifications by direct molecular recognition. *ACS chemical biology*, *10*(7), 1580-1589.

Landry, R., & Amara, N. (1998). The impact of transaction costs on the institutional structuration of collaborative academic research. *Research policy*, *27*(9), 901-913.

Lane, K., Van Valen, D., DeFelice, M. M., Macklin, D. N., Kudo, T., Jaimovich, A., Carr, A., Meyer, T., Pe'er, D., & Boutet, S. C. (2017). Measuring signaling and RNA-seq in the same cell links gene expression to dynamic patterns of NF-κB activation. *Cell systems*, *4*(4), 458-469. e455.

Lang, R. P., Bayne, C. J., Camara, M. D., Cunningham, C., Jenny, M. J., & Langdon, C. J. J. M. b. (2009). Transcriptome profiling of selectively bred Pacific oyster Crassostrea gigas families that differ in tolerance of heat shock. *11*, 650-668.

Lazzarotto, V., Corraze, G., Larroquet, L., Mazurais, D., & Médale, F. J. B. J. o. N. (2016). Does broodstock nutritional history affect the response of progeny to different first-feeding diets? A whole-body transcriptomic study of rainbow trout alevins. *115*(12), 2079-2092.

Lazzarotto, V., Médale, F., Larroquet, L., & Corraze, G. J. P. O. (2018). Long-term dietary replacement of fishmeal and fish oil in diets for rainbow trout (Oncorhynchus mykiss): Effects on growth, whole body fatty acids and intestinal and hepatic gene expression. *13*(1), e0190730.

Leduc, A., Zatylny-Gaudin, C., Robert, M., Corre, E., Corguille, G. L., Castel, H., Lefevre-Scelles, A., Fournier, V., Gisbert, E., & Andree, K. B. J. B. g. (2018). Dietary aquaculture by-product hydrolysates: impact on the transcriptomic response of the intestinal mucosa of European seabass (Dicentrarchus labrax) fed low fish meal diets. *19*, 1-20.

Leung, P. T., Ip, J. C., Mak, S. S., Qiu, J. W., Lam, P. K., Wong, C. K., Chan, L. L., & Leung, K. M. J. B. g. (2014). De novo transcriptome analysis of Perna viridis highlights tissue-specific patterns for environmental studies. *15*, 1-14.

Li, G., Ruan, X., Auerbach, R. K., Sandhu, K. S., Zheng, M., Wang, P., Poh, H. M., Goh, Y., Lim, J., & Zhang, J. (2012). Extensive promoter-centered chromatin interactions provide a topological basis for transcription regulation. *Cell*, *148*(1), 84-98.

Li, R. W., & Waldbieser, G. C. (2006). Production and utilization of a high-density oligonucleotide microarray in channel catfish, Ictalurus punctatus. *BMC genomics*, *7*, 1-7.

Li, S., Zhang, X., Sun, Z., Li, F., & Xiang, J. J. P. o. (2013). Transcriptome analysis on Chinese shrimp

Fenneropenaeus chinensis during WSSV acute infection. *8*(3), e58627.

Li, S., Liu, C., Huang, J., Liu, Y., Zhang, S., Zheng, G., Xie, L., & Zhang, R. J. S. r. (2016). Transcriptome and biomineralization responses of the pearl oyster Pinctada fucata to elevated CO2 and temperature. *6*(1), 18943.

Li, W. V., & Li, J. J. (2018). An accurate and robust imputation method scImpute for single-cell RNA-seq data. *Nature Communications*, *9*(1), 997.

Li, Y., Byrne, K., Miggiano, E., Whan, V., Moore, S., Keys, S., Crocos, P., Preston, N., & Lehnert, S. (2003). Genetic mapping of the kuruma prawn Penaeus japonicus using AFLP markers. *Aquaculture*, *219*(1-4), 143-156.

Li, Y., Ma, L., Wu, D., & Chen, G. (2021). Advances in bulk and single-cell multi-omics approaches for systems biology and precision medicine. *Briefings in Bioinformatics*, *22*(5), bbab024.

Lim, J., Park, C., Kim, M., Kim, H., Kim, J., & Lee, D.-S. (2024). Advances in single-cell omics and multiomics for high-resolution molecular profiling. *Experimental & Molecular Medicine*, *56*(3), 515-526.

Liu, H., Fu, B., Pang, M., Feng, X., Wang, X., Yu, X., & Tong, J. (2016). QTL fine mapping and identification of candidate genes for growth-related traits in bighead carp (Hypophthalmichehys nobilis). *Aquaculture*, *465*, 134-143.

Liu, W., Liu, X., Wu, C., Jiang, L. J. F. p., & biochemistry. (2018). Transcriptome analysis demonstrates that long noncoding RNA is involved in the hypoxic response in Larimichthys crocea. *44*, 1333-1347.

Liu, Z. J. (2017). *Bioinformatics in aquaculture: principles and methods*. John Wiley & Sons.

Livernois, A., Graves, J., & Waters, P. (2012). The origin and evolution of vertebrate sex chromosomes and dosage compensation. *Heredity*, *108*(1), 50-58.

López, M. E., Neira, R., & Yáñez, J. M. (2015). Applications in the search for genomic selection signatures in fish. *Frontiers in genetics*, *5*, 458.

Lu, J., Luan, P., Zhang, X., Xue, S., Peng, L., Mahbooband, S., & Sun, X. J. P. g. (2014). Gonadal transcriptomic analysis of yellow catfish (Pelteobagrus fulvidraco): identification of sex-related genes and genetic markers. *46*(21), 798-807.

Lu, X., Kong, J., Luan, S., Dai, P., Meng, X., Cao, B., & Luo, K. J. P. o. (2016). Transcriptome analysis of the hepatopancreas in the Pacific white shrimp (Litopenaeus vannamei) under acute ammonia stress. *11*(10), e0164396.

Lv, J., Liu, P., Gao, B., Wang, Y., Wang, Z., Chen, P., & Li, J. J. P. o. (2014). Transcriptome analysis of the Portunus trituberculatus: de novo assembly, growth-related gene identification and marker discovery. *9*(4), e94055.

M'gonigle, R. (1941). Acute catarrhal enteritis of salmonid fingerlings. *Transactions of the American Fisheries Society*, *70*(1), 297-303.

Ma, D., Ma, A., Huang, Z., Wang, G., Wang, T., Xia, D., & Ma, B. J. P. O. (2016). Transcriptome analysis for identification of genes related to gonad differentiation, growth, immune response and marker discovery in the turbot (Scophthalmus maximus). *11*(2), e0149414.

Ma, K.-Y., Lin, J.-Y., Guo, S.-Z., Chen, Y., Li, J.-L., Qiu, G.-F. J. G., & Endocrinology, C. (2013). Molecular characterization and expression analysis of an insulin-like gene from the androgenic gland of the oriental river prawn, Macrobrachium nipponense. *185*, 90-96.

Macaulay, I. C., Haerty, W., Kumar, P., Li, Y. I., Hu, T. X., Teng, M. J., Goolam, M., Saurat, N., Coupland, P., & Shirley, L. M. (2015). G&T-seq: parallel sequencing of single-cell genomes and transcriptomes. *Nature methods*, *12*(6), 519-522.

Macaulay, I. C., Svensson, V., Labalette, C., Ferreira, L., Hamey, F., Voet, T., Teichmann, S. A., & Cvejic, A. (2016). Single-cell RNA-sequencing reveals a continuous spectrum of differentiation in hematopoietic cells. *Cell reports*, *14*(4), 966-977.

Macosko, E. Z., Basu, A., Satija, R., Nemesh, J., Shekhar, K., Goldman, M., Tirosh, I., Bialas, A. R., Kamitaki, N., & Martersteck, E. M. (2015). Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell*, *161*(5), 1202-1214.

Maljkovic Berry, I., Melendrez, M. C., Bishop-Lilly, K. A., Rutvisuttinunt, W., Pollett, S., Talundzic, E., Morton, L., & Jarman, R. G. (2020). Next generation sequencing and bioinformatics methodologies for infectious disease research and public health: approaches, applications, and considerations for development of laboratory capacity. *The Journal of infectious diseases*, *221*(Supplement_3), S292-S307.

Massault, C., Franch, R., Haley, C., de Koning, D. J., Bovenhuis, H., Pellizzari, C., Patarnello, T., & Bargelloni, L. (2011). Quantitative trait loci for resistance to fish pasteurellosis in gilthead sea bream (Sparus aurata). *Animal genetics*, *42*(2), 191-203.

Matsumura, H., Ito, A., Saitoh, H., Winter, P., Kahl, G., Reuter, M., Krüger, D. H., & Terauchi, R. (2005). SuperSAGE. *Cellular microbiology*, *7*(1), 11-18.

Meng, X.-l., Liu, P., Jia, F.-l., Li, J., & Gao, B.-Q. J. P. O. (2015). De novo transcriptome analysis of Portunus trituberculatus ovary and testis by RNA-Seq: identification of genes involved in gonadal development. *10*(6), e0128659.

Mich, J. K., Blaser, H., Thomas, N. A., Firestone, A. J., Yelon, D., Raz, E., & Chen, J. K. (2009). Germ cell migration in zebrafish is cyclopamine-sensitive but Smoothened-independent. *Developmental biology*, *328*(2), 342-354.

Miller, K., & Maclean, N. (2008). Teleost microarrays: development in a broad phylogenetic range reflecting diverse applications. *J Fish Biol*, *72*(9), 2039-2050.

Moen, T., Fjalestad, K. T., Munck, H., & Gomez-Raya, L. (2004). A multistage testing strategy for detection of quantitative trait loci affecting disease resistance in Atlantic salmon. *Genetics*, *167*(2), 851-858.

Moen, T., Torgersen, J., Santi, N., Davidson, W. S., Baranski, M., Ødegård, J., Kjøglum, S., Velle, B., Kent, M., & Lubieniecki, K. P. (2015). Epithelial cadherin determines resistance to infectious pancreatic necrosis virus in Atlantic salmon. *Genetics*, *200*(4), 1313-1326.

Moore, J. C., Mulligan, T. S., Yordán, N. T., Castranova, D., Pham, V. N., Tang, Q., Lobbardi, R., Anselmo, A., Liwski, R. S., & Berman, J. N. (2016). T cell immune deficiency in zap70 mutant zebrafish. *Molecular and cellular biology*.

Morais, S., Edvardsen, R. B., Tocher, D. R., Bell, J. G. J. C. B., Biochemistry, P. P. B., & Biology, M. (2012). Transcriptomic analyses of intestinal gene expression of juvenile Atlantic cod (Gadus morhua) fed diets with Camelina oil as replacement for fish oil. *161*(3), 283-293.

Müller, M., & Kersten, S. (2003). Nutrigenomics: goals and strategies. *Nature Reviews Genetics*, *4*(4), 315-322.

Mun, S., Kim, Y.-J., Markkandan, K., Shin, W., Oh, S., Woo, J., Yoo, J., An, H., Han, K. J. G. B., & Evolution. (2017). The

whole-genome and transcriptome of the Manila clam (Ruditapes philippinarum). *9*(6), 1487-1498.

Munang'andu, H. M., Mutoloki, S., & Evensen, Ø. (2014). Non-replicating vaccines. *Fish Vaccination*, 22-32.

Munang'andu, H. M. (2016). Environmental viral metagenomics analyses in aquaculture: Applications in epidemiology and disease control. *Frontiers in microbiology*, *7*, 1986.

Munang'andu, H. M., & Evensen, Ø. (2017). Current advances in functional genomics in aquaculture. *Applications of RNA-Seq and Omics Strategies-from Microorganisms to Human Health*, 173-212.

Munang'andu, H. M., Evensen, Ø. J. A. o. R.-S., & Health, O. S.-f. M. t. H. (2017). Current advances in functional genomics in aquaculture. 173-212.

Muteeb, G., Rehman, M. T., Shahwan, M., & Aatif, M. (2023). Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. *Pharmaceuticals*, *16*(11), 1615.

Navin, N. E. (2015). The first five years of single-cell cancer genomics and beyond. *Genome research*, *25*(10), 1499-1507.

Neave, M. J., Sunarto, A., & McColl, K. A. J. S. r. (2017). Transcriptomic analysis of common carp anterior kidney during cyprinid herpesvirus 3 infection: immunoglobulin repertoire and homologue functional divergence. *7*(1), 41531.

Nie, H., Jiang, L., Huo, Z., Liu, L., Yang, F., Yan, X. J. F., & Immunology, S. (2016). Transcriptomic responses to low temperature stress in the Manila clam, Ruditapes philippinarum. *55*, 358-366.

Nutsch, K. M., & Hsieh, C.-S. (2012). T cell tolerance and immunity to commensal bacteria. *Current opinion in immunology*, *24*(4), 385-391.

O'Farrell, C., Vaghefi, N., Cantonnet, M., Buteau, B., Boudinot, P., & Benmansour, A. (2002). Survey of transcript expression in rainbow trout leukocytes reveals a major contribution of interferon-responsive genes in the early response to a rhabdovirus infection. *Journal of Virology*, *76*(16), 8040-8049.

Ortiz, N. N., Gerdol, M., Stocchi, V., Marozzi, C., Randelli, E., Bernini, C., Buonocore, F., Picchietti, S., Papeschi, C., & Sood, N. (2014). T cell transcripts and T cell activities in the gills of the teleost fish sea bass (Dicentrarchus labrax). *Developmental & Comparative Immunology*, *47*(2), 309-318.

Pan, C.-Y., Liu, Y.-H., Gong, H.-Y., Chen, J.-Y. J. F., & Immunology, S. (2017). Transcriptome analysis of the effect of polyunsaturated fatty acids against Vibrio vulnificus infection in Oreochromis niloticus. *62*, 153-163.

Pan, D., & Jia, D. (2021). Application of single-cell multi-omics in dissecting cancer cell plasticity and tumor heterogeneity. *Frontiers in Molecular Biosciences*, *8*, 757024.

Park, G. (2022). *Acute Lymphoblastic Leukemia in Zebrafish: New Models, New Subtypes, and a New Method to Study Leukemia Biology* The University of Oklahoma Health Sciences Center].

Peace, C. P., Bianco, L., Troggio, M., Van de Weg, E., Howard, N. P., Cornille, A., Durel, C.-E., Myles, S., Migicovsky, Z., & Schaffer, R. J. (2019). Apple whole genome sequences: recent advances and new prospects. *Horticulture Research*, *6*.

Peng, X.-X. J. D., & Immunology, C. (2013). Proteomics and its applications to aquaculture in China: infection, immunity, and interaction of aquaculture hosts with pathogens. *39*(1-2), 63-71.

Peterson, V. M., Zhang, K. X., Kumar, N., Wong, J., Li, L., Wilson, D. C., Moore, R., McClanahan, T. K., Sadekova, S., & Klappenbach, J. A. (2017). Multiplexed quantification of proteins and transcripts in single cells. *Nature biotechnology*, *35*(10), 936-939.

Picchietti, S., Guerra, L., Bertoni, F., Randelli, E., Belardinelli, M. C., Buonocore, F., Fausto, A., Rombout, J., Scapigliati, G., & Abelli, L. (2011). Intestinal T cells of Dicentrarchus labrax (L.): gene expression and functional studies. *Fish & shellfish immunology*, *30*(2), 609-617.

Rao, R., Bing Zhu, Y., Alinejad, T., Tiruvayipati, S., Lin Thong, K., Wang, J., & Bhassu, S. J. G. P. (2015). RNA-seq analysis of Macrobrachium rosenbergii hepatopancreas in response to Vibrio parahaemolyticus infection. *7*, 1-16.

Reinartz, J., Bruyns, E., Lin, J.-Z., Burcham, T., Brenner, S., Bowen, B., Kramer, M., & Woychik, R. (2002). Massively parallel signature sequencing (MPSS) as a tool for in-depth quantitative gene expression profiling in all organisms. *Briefings in Functional Genomics*, *1*(1), 95-104.

Reuter, G., Boros, A., Delwart, E., & Pankovics, P. (2013). Novel seadornavirus (family Reoviridae) related to Banna virus in Europe. *Archives of virology*, *158*, 2163-2167.

Reuter, G., Pankovics, P., Delwart, E., & Boros, Á. (2015). A novel posavirus-related single-stranded RNA virus from fish (Cyprinus carpio). *Archives of virology*, *160*, 565-568.

Robinson, N., Baranski, M., Mahapatra, K. D., Saha, J. N., Das, S., Mishra, J., Das, P., Kent, M., Arnyasi, M., & Sahoo, P. K. (2014). A linkage map of transcribed single nucleotide polymorphisms in rohu (Labeo rohita) and QTL associated with resistance to Aeromonas hydrophila. *BMC genomics*, *15*, 1-23.

Robledo, D., Fernández, C., Hermida, M., Sciara, A., Álvarez-Dios, J. A., Cabaleiro, S., Caamaño, R., Martínez, P., & Bouza, C. J. I. J. o. M. S. (2016). Integrative transcriptome, genome and quantitative trait loci resources identify single nucleotide polymorphisms in candidate genes for growth traits in turbot. *17*(2), 243.

Rodríguez-Ramilo, S. T., De La Herrán, R., Ruiz-Rejón, C., Hermida, M., Fernández, C., Pereiro, P., Figueras, A., Bouza, C., Toro, M. A., & Martínez, P. (2014). Identification of quantitative trait loci associated with resistance to viral haemorrhagic septicaemia (VHS) in turbot (Scophthalmus maximus): a comparison between bacterium, parasite and virus diseases. *Marine biotechnology*, *16*, 265-276.

Roy, A., Basak, R., & Rai, U. J. P. O. (2017). De novo sequencing and comparative analysis of testicular transcriptome from different reproductive phases in freshwater spotted snakehead Channa punctatus. *12*(3), e0173178.

Salomon, R., Kaczorowski, D., Valdes-Mora, F., Nordon, R. E., Neild, A., Farbehi, N., Bartonicek, N., & Gallego-Ortega, D. (2019). Droplet-based single cell RNAseq tools: a practical guide. *Lab on a Chip*, *19*(10), 1706-1727.

Sandberg, R. (2014). Entering the era of single-cell transcriptomics in biology and medicine. *Nature methods*, *11*(1), 22-24.

Sasagawa, Y., Nikaido, I., Hayashi, T., Danno, H., Uno, K. D., Imai, T., & Ueda, H. R. (2013). Quartz-Seq: a highly reproducible and sensitive single-cell RNA sequencing

method, reveals non-genetic gene-expression heterogeneity. *Genome biology*, *14*, 1-17.

Sehgal, P., & Chaturvedi, P. (2022). Omic Technologies and Cold Adaptations. *Survival Strategies in Cold-adapted Microorganisms*, 253-284.

Serwecińska, L. (2020). Antimicrobials and antibiotic-resistant bacteria: a risk to the environment and to public health. *Water*, *12*(12), 3313.

Shao, C., Niu, Y., Rastas, P., Liu, Y., Xie, Z., Li, H., Wang, L., Jiang, Y., Tai, S., & Tian, Y. (2015). Genome-wide SNP identification for the construction of a high-resolution genetic map of Japanese flounder (Paralichthys olivaceus): applications to QTL mapping of Vibrio anguillarum disease resistance and comparative genomic analysis. *DNA research*, *22*(2), 161-170.

Shapiro, E., Biezuner, T., & Linnarsson, S. (2013). Single-cell sequencing-based technologies will revolutionize whole-organism science. *Nature Reviews Genetics*, *14*(9), 618-630.

Shi, B., Huang, Z., Xiang, X., Huang, M., Wang, W.-X., & Ke, C. J. S. R. (2015). Transcriptome analysis of the key role of GAT2 gene in the hyper-accumulation of copper in the oyster Crassostrea angulata. *5*(1), 17751.

Sittner, A., Mechaly, A., Vitner, E., Aftalion, M., Levy, Y., Levy, H., Mamroud, E., & Fisher, M. (2018). Improved production of monoclonal antibodies against the LcrV antigen of Yersinia pestis using FACS-aided hybridoma selection. *Journal of Biological Methods*, *5*(4).

Smith, C. T., Elfstrom, C. M., Seeb, L. W., & Seeb, J. E. (2005). Use of sequence data from rainbow trout and Atlantic salmon for SNP detection in Pacific salmon. *Molecular ecology*, *14*(13), 4193-4203.

Song, X., Hu, X., Sun, B., Bo, Y., Wu, K., Xiao, L., & Gong, C. J. S. r. (2017). A transcriptome analysis focusing on inflammation-related genes of grass carp intestines following infection with Aeromonas hydrophila. *7*(1), 40777.

Spitzer, M. H., & Nolan, G. P. (2016). Mass cytometry: single cells, many features. *Cell*, *165*(4), 780-791.

Stoeckius, M., Hafemeister, C., Stephenson, W., Houck-Loomis, B., Chattopadhyay, P. K., Swerdlow, H., Satija, R., & Smibert, P. (2017). Simultaneous epitope and transcriptome measurement in single cells. *Nature methods*, *14*(9), 865-868.

Stuart, T., & Satija, R. (2019). Integrative single-cell analysis. *Nature Reviews Genetics*, *20*(5), 257-272.

Sudhagar, A., Kumar, G., & El-Matbouli, M. (2018). Transcriptome analysis based on RNA-Seq in understanding pathogenic mechanisms of diseases and the immune system of fish: a comprehensive review. *International Journal of Molecular Sciences*, *19*(1), 245.

Sun, S., Ge, X., Xuan, F., Zhu, J., Yu, N. J. E. T., & Pharmacology. (2014). Nitrite-induced hepatotoxicity in bluntsnout bream (Megalobrama amblycephala): the mechanistic insight from transcriptome to physiology analysis. *37*(1), 55-65.

Sun, Y., Guo, C.-Y., Wang, D.-D., Li, X. F., Xiao, L., Zhang, X., You, X., Shi, Q., Hu, G.-J., & Fang, C. J. B. g. (2016). Transcriptome analysis reveals the molecular mechanisms underlying growth superiority in a novel grouper hybrid (Epinephelus fuscogutatus♀× E. lanceolatus♂). *17*, 1-10.

Tacchi, L., Musharrafieh, R., Larragoite, E. T., Crossey, K., Erhardt, E. B., Martin, S. A., LaPatra, S. E., & Salinas, I. (2014). Nasal immunity is an ancient arm of the mucosal immune system of vertebrates. *Nature Communications*, *5*(1), 5205.

Taggart, J. B., Bron, J. E., Martin, S. A., Seear, P. J., Høyheim, B., Talbot, R., Carmichael, S. N., Villeneuve, L. A., Sweeney, G. E., & Houlihan, D. F. (2008). A description of the origins, design and performance of the TRAITS–SGP Atlantic salmon Salmo salar L. cDNA microarray. *Journal of Fish Biology*, *72*(9), 2071-2094.

Tan, C., Li, Q., Yang, X., Chen, J., Zhang, Q., & Deng, X. (2022). Lactococcus lactis' effect on the intestinal microbiota of Streptococcus agalactiae-infected zebrafish (Danio rerio). *Microbiology Spectrum*, *10*(5), e01128-01122.

Tang, F., Barbacioru, C., Wang, Y., Nordman, E., Lee, C., Xu, N., Wang, X., Bodeau, J., Tuch, B. B., & Siddiqui, A. (2009). mRNA-Seq whole-transcriptome analysis of a single cell. *Nature methods*, *6*(5), 377-382.

Tanguy, A., Guo, X., & Ford, S. E. J. G. (2004). Discovery of genes expressed in response to Perkinsus marinus challenge in Eastern (Crassostrea virginica) and Pacific (C. gigas) oysters. *338*(1), 121-131.

Teaniniuraitemoana, V., Huvet, A., Levy, P., Klopp, C., Lhuillier, E., Gaertner-Mazouni, N., Gueguen, Y., & Le Moullac, G. J. B. g. (2014). Gonad transcriptome analysis of pearl oyster Pinctada margaritifera: identification of potential sex differentiation and sex determining genes. *15*, 1-20.

Thanh, N. M., Jung, H., Lyons, R. E., Chand, V., Tuan, N. V., Thu, V. T. M., Mather, P. J. C. B., Genomics, P. P. D., & Proteomics. (2014). A transcriptomic analysis of striped catfish (Pangasianodon hypophthalmus) in response to salinity adaptation: De novo assembly, gene annotation and marker discovery. *10*, 52-63.

Tigistu-Sahle, F., Mekuria, Z. H., Satoskar, A. R., Sales, G. F., Gebreyes, W. A., & Oliveira, C. J. (2023). Challenges and opportunities of molecular epidemiology: Using omics to address complex One Health issues in tropical settings. *Frontiers in Tropical Diseases*, *4*, 1151336.

Ulloa, P. E., Rincón, G., Islas-Trejo, A., Araneda, C., Iturra, P., Neira, R., & Medrano, J. F. J. M. B. (2015). RNA sequencing to study gene expression and SNP variations associated with growth in zebrafish fed a plant protein-based diet. *17*, 353-363.

Uribe, R. A., Kwon, T., Marcotte, E. M., & Gross, J. M. (2012). Id2a functions to limit Notch pathway activity and thereby influence the transition from proliferation to differentiation of retinoblasts during zebrafish retinogenesis. *Developmental biology*, *371*(2), 280-292.

Vailati-Riboni, M., Palombo, V., & Loor, J. J. (2017). What are omics sciences? *Periparturient diseases of dairy cows: a systems biology approach*, 1-7.

Valdivieso, A. (2020). Environmental effects during gonadal development in fish: role of epigenetics.

Valihrach, L., Androvic, P., & Kubista, M. (2018). Platforms for single-cell collection and analysis. *International Journal of Molecular Sciences*, *19*(3), 807.

Vallejo, R. L., Palti, Y., Liu, S., Evenhuis, J. P., Gao, G., Rexroad, C. E., & Wiens, G. D. (2014). Detection of QTL in rainbow trout affecting survival when challenged with Flavobacterium psychrophilum. *Marine biotechnology*, *16*, 349-360.

Vazquez-Miranda, H., Thoma, B., Wong, J., Felder, D., Crandall, K. A., & Bracken-Grissom, H. J. P. P. (2017). Transcriptomic effects of dispersed oil in a non-model decapod crustacean. *5*.

Velculescu, V. E., Zhang, L., Vogelstein, B., & Kinzler, K. W. (1995). Serial analysis of gene expression. *science*, *270*(5235), 484-487.

Ventura, T., Manor, R., Aflalo, E. D., Chalifa-Caspi, V., Weil, S., Sharabi, O., & Sagi, A. J. P. O. (2013). Post-embryonic transcriptomes of the prawn Macrobrachium rosenbergii: multigenic succession through metamorphosis. *8*(1), e55322.

Vera, L., Metochis, C., Taylor, J., Clarkson, M., Skjaerven, K. H., Migaud, H., & Tocher, D. R. J. B. g. (2017). Early nutritional programming affects liver transcriptome in diploid and triploid Atlantic salmon, Salmo salar. *18*, 1-15.

Verrier, E. R., Dorson, M., Mauger, S., Torhy, C., Ciobotaru, C., Hervet, C., Dechamp, N., Genet, C., Boudinot, P., & Quillet, E. (2013). Resistance to a rhabdovirus (VHSV) in rainbow trout: identification of a major QTL related to innate mechanisms. *PLoS One*, *8*(2), e55302.

Vesterlund, L., Jiao, H., Unneberg, P., Hovatta, O., & Kere, J. (2011). The zebrafish transcriptome during early development. *BMC developmental biology*, *11*, 1-18.

Villani, A.-C., Satija, R., Reynolds, G., Sarkizova, S., Shekhar, K., Fletcher, J., Griesbeck, M., Butler, A., Zheng, S., & Lazo, S. (2017). Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. *science*, *356*(6335), eaah4573.

Waiho, K., Fazhan, H., Shahreza, M. S., Moh, J. H. Z., Noorbaiduri, S., Wong, L. L., Sinnasamy, S., & Ikhwanuddin, M. J. P. O. (2017). Transcriptome analysis and differential gene expression on the testis of orange mud crab, Scylla olivacea, during sexual maturation. *12*(1), e0171095.

Wan, Q., Su, J., Chen, X., Yang, C., Chen, L., Yan, N., & Zhang, Y. (2013). Genomic sequence comparison, promoter activity, SNP detection of RIG-I gene and association with resistance/susceptibility to grass carp reovirus in grass carp (Ctenopharyngodon idella). *Developmental & Comparative Immunology*, *39*(4), 333-342.

Wang, M., Jeong, C.-B., Li, Y., & Lee, J.-S. J. A. T. (2017). Different transcriptomic responses of two marine copepods, Tigriopus japonicus and Pseudodiaptomus annandalei, to a low dose of mercury chloride (HgCl2). *187*, 124-131.

Wang, Q., Hao, R., Zhao, X., Huang, R., Zheng, Z., Deng, Y., Chen, W., Du, X. J. B., Biotechnology,, & Biochemistry. (2018). Identification of EGFR in pearl oyster (Pinctada fucata martensii) and correlation analysis of its expression and growth traits. *82*(7), 1073-1080.

Wang, S., Peatman, E., Liu, H., Bushek, D., Ford, S. E., Kucuktas, H., Quilang, J., Li, P., Wallace, R., Wang, Y. J. F., & Immunology, S. (2010). Microarray analysis of gene expression in eastern oyster (Crassostrea virginica) reveals a novel combination of antimicrobial and oxidative stress host responses after dermo (Perkinsus marinus) challenge. *29*(6), 921-929.

Wei, J., Zhang, X., Yu, Y., Huang, H., Li, F., & Xiang, J. J. P. o. (2014). Comparative transcriptomic characterization of the early development in Pacific white shrimp Litopenaeus vannamei. *9*(9), e106201.

Wiens, G. D., Vallejo, R. L., Leeds, T. D., Palti, Y., Hadidi, S., Liu, S., Evenhuis, J. P., Welch, T. J., & Rexroad III, C. E. (2013). Assessment of genetic correlation between bacterial cold water disease resistance and spleen index in a domesticated population of rainbow trout: identification of QTL on chromosome Omy19. *PLoS One*, *8*(10), e75749.

Wingfield, W., Fryer, J., & Pilcher, K. (1969). Properties of the sockeye salmon virus (Oregon strain). *Proceedings of the Society for Experimental Biology and Medicine*, *130*(4), 1055-1059.

Wolf, K., Snieszko, S., Dunbar, C., & Pyle, E. (1960). Virus nature of infectious pancreatic necrosis in trout. *Proceedings of the Society for Experimental Biology and Medicine*, *104*(1), 105-108.

Wong, A. H., Gottesman, I. I., & Petronis, A. (2005). Phenotypic differences in genetically identical organisms: the epigenetic perspective. *Human molecular genetics*, *14*(suppl_1), R11-R18.

Wu, X., Yang, X., Dai, Y., Zhao, Z., Zhu, J., Guo, H., & Yang, R. (2024). Single-cell sequencing to multi-omics: technologies and applications. *Biomarker Research*, *12*(1), 110.

Wu, Y., & Zhang, K. (2020). Tools for the analysis of high-dimensional single-cell RNA sequencing data. *Nature Reviews Nephrology*, *16*(7), 408-421.

Xie, X., Shi, Q., Wu, P., Zhang, X., Kambara, H., Su, J., Yu, H., Park, S.-Y., Guo, R., & Ren, Q. (2020). Single-cell transcriptome profiling reveals neutrophil heterogeneity in homeostasis and infection. *Nature immunology*, *21*(9), 1119-1133.

Xu, C., Li, E., Suo, Y., Su, Y., Lu, M., Zhao, Q., Qin, J. G., Chen, L. J. F., & immunology, s. (2018). Histological and transcriptomic responses of two immune organs, the spleen and head kidney, in Nile tilapia (Oreochromis niloticus) to long-term hypersaline stress. *76*, 48-57.

Xu, T.-j., Chen, S.-l., Ji, X.-s., & Tian, Y.-s. (2008). MHC polymorphism and disease resistance to Vibrio anguillarum in 12 selective Japanese flounder (Paralichthys olivaceus) families. *Fish & shellfish immunology*, *25*(3), 213-221.

Xu, Y., Li, X., Deng, Y., Lu, Q., Yang, Y., Pan, J., Ge, J., & Xu, Z. J. A. r. (2017). Comparative transcriptome sequencing of the hepatopancreas reveals differentially expressed genes in the precocious juvenile Chinese mitten crab, Eriocheir sinensis (Crustacea: Decapoda). *48*(7), 3645-3656.

Xu, Y., & Zhao, F. (2018). Single-cell metagenomics: challenges and applications. *Protein & cell*, *9*(5), 501-510.

Xu, Z., Gan, L., Li, T., Xu, C., Chen, K., Wang, X., Qin, J. G., Chen, L., & Li, E. J. P. O. (2015). Transcriptome profiling and molecular pathway analysis of genes in association with salinity adaptation in Nile tilapia Oreochromis niloticus. *10*(8), e0136506.

Xu, Z., Takizawa, F., Parra, D., Gómez, D., von Gersdorff Jørgensen, L., LaPatra, S. E., & Sunyer, J. O. (2016). Mucosal immunoglobulins at respiratory surfaces mark an ancient association that predates the emergence of tetrapods. *Nature Communications*, *7*(1), 10728.

Yamagata, T., Benoist, C., & Mathis, D. (2006). A shared gene-expression signature in innate-like lymphocytes. *Immunological reviews*, *210*(1), 52-66.

Yamaguchi, T., Takizawa, F., Fischer, U., & Dijkstra, J. M. (2015). Along the axis between type 1 and type 2 immunity; principles conserved in evolution from fish to mammals. *Biology*, *4*(4), 814-859.

Yang, Y., Fu, Q., Wang, X., Liu, Y., Zeng, Q., Li, Y., Gao, S., Bao, L., Liu, S., & Gao, D. J. P. G. (2018). Comparative transcriptome analysis of the swimbladder reveals

expression signatures in response to low oxygen stress in channel catfish, Ictalurus punctatus. *50*(8), 636-647.

Yue, C., Li, Q., & Yu, H. J. M. b. (2018). Gonad transcriptome analysis of the Pacific oyster Crassostrea gigas identifies potential genes regulating the sex determination and differentiation process. *20*, 206-219.

Zenobi, R. (2013). Single-cell metabolomics: analytical and biological perspectives. *science*, *342*(6163), 1243259.

Zhang, A., Liu, S., Zhu, J., Gu, Z., Zhou, Z., Zhang, G., & Lu, K. J. I. J. o. F. S. (2016). Transcriptome analysis of the freshwater pearl mussel, Hyriopsis cumingii (Lea) using illumina paired-end sequencing to identify genes and markers. *15*(4), 1425-1440.

Zhang, D., Liu, J., Qi, T., Ge, B., Wang, Z., Jiang, S., Liu, Q., Zhang, H., Ding, G., Tang, B. J. J. o. a., & chemistry, f. (2018). Transcriptome analysis of hepatopancreas from the Cr (VI)-stimulated mantis shrimp (Oratosquilla oratoria) by illumina paired-end sequencing: Assembly, annotation, and expression analysis. *66*(11), 2598-2606.

Zhang, R., Zhang, L.-l., Ye, X., Tian, Y.-y., Sun, C.-f., Lu, M.-x., & Bai, J.-j. J. M. b. r. (2013). Transcriptome profiling and digital gene expression analysis of Nile tilapia (Oreochromis niloticus) infected by Streptococcus agalactiae. *40*, 5657-5668.

Zhang, W., Wan, H., Jiang, H., Zhao, Y., Zhang, X., Hu, S., & Wang, Q. (2011). A transcriptome analysis of mitten crab testes (Eriocheir sinensis). *Genetics and Molecular Biology*, *34*, 136-141.

Zhang, X., Li, X., Li, R., Zhang, Y., Li, Y., & Li, S. (2019). Transcriptomic profile of early zebrafish PGCs by single cell sequencing. *PLoS One*, *14*(8), e0220364.

Zhang, Y.-A., Salinas, I., Li, J., Parra, D., Bjork, S., Xu, Z., LaPatra, S. E., Bartholomew, J., & Sunyer, J. O. (2010). IgT, a primitive immunoglobulin class specialized in mucosal immunity. *Nature immunology*, *11*(9), 827-835.

Zhao, L., Zhang, Y., Ji, P., Zhang, X., Zhao, Z., Hou, G., Huo, L., Liu, G., Li, C., & Xu, P. J. P. o. (2013). A dense genetic linkage map for common carp and its integration with a BAC-based physical map. *8*(5), e63928.

Zhao, X., Duan, X., Wang, Z., Zhang, W., Li, Y., Jin, C., Xiong, J., Li, C. J. F., & Immunology, S. (2017). Comparative transcriptome analysis of Sinonovacula constricta in gills and hepatopancreas in response to Vibrio parahaemolyticus infection. *67*, 523-535.

Zheng, M., Lu, J., & Zhao, D. J. S. o. t. t. e. (2018). Effects of starch-coating of magnetite nanoparticles on cellular uptake, toxicity and gene expression profiles in adult zebrafish. *622*, 930-941.

Zheng, W., Xu, H., Lam, S. H., Luo, H., Karuturi, R. K. M., & Gong, Z. (2013). Transcriptomic analyses of sexual dimorphism of the zebrafish liver and the effect of sex hormones. *PLoS One*, *8*(1), e53562.

Zhenzhen, X., Ling, X., Dengdong, W., Chao, F., Qiongyu, L., Zihao, L., Xiaochun, L., Yong, Z., Shuisheng, L., & Haoran, L. J. P. o. (2014). Transcriptome analysis of the Trachinotus ovatus: identification of reproduction, growth and immune-related genes and microsatellite markers. *9*(10), e109419.

Zhu, J., Fu, Q., Ao, Q., Tan, Y., Luo, Y., Jiang, H., Li, C., Gan, X. J. F., & immunology, s. (2017). Transcriptomic profiling analysis of tilapia (Oreochromis niloticus) following Streptococcus agalactiae challenge. *62*, 202-212.

Zong, C. (2017). Multiple Annealing and Looping-Based Amplification Cycles (MALBAC) for the Analysis of DNA Copy Number Variation. *Genomic Mosaicism in Neurons and Other Cell Types*, 133-142.

Zong, C., Lu, S., Chapman, A. R., & Xie, X. S. (2012). Genome-wide detection of single-nucleotide and copy-number variations of a single human cell. *science*, *338*(6114), 1622-1626.