



翟雪, 张明皓, 黄丹, 等. 单细胞技术在水产动物中的研究: 进展、应用与未来展望 [J]. 水产学报, 2026, 50(5): 059104.
Zhai X, Zhang M H, Huang D, *et al.* Single-cell technologies in aquatic organisms: advances, applications, and future perspectives [J]. *Journal of Fisheries of China*, 2026, 50(5): 059104 (in Chinese).

· 综述 ·

Single-cell technologies in aquatic organisms: advances, applications, and future perspectives

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Abstract: Single-cell sequencing technologies have become powerful tools for dissecting complex biological processes in aquatic organisms by revealing cellular heterogeneity that is obscured in bulk analyses. This review systematically summarizes recent advances in single-cell RNA sequencing, single-nucleus RNA sequencing, and single-cell multi-omics applied to aquatic research, highlighting methodological developments in cell isolation, microfluidic capture, library construction, and bioinformatic analysis. We further outline representative biological applications of these approaches in studies of skeletal development, immune responses, environmental adaptation, reproductive regulation, and specialized cell types. Despite their considerable promise for aquaculture research, single-cell technologies remain challenged by data sparsity, limited genomic resources for non-model species, and incomplete molecular resolution. Looking ahead, the integration of single-cell multi-omics with spatial transcriptomics and artificial intelligence-driven analytical frameworks is expected to enable the construction of fish-specific cell atlases and accelerate precision breeding, thereby supporting the sustainable development of aquaculture.

Key words: aquaculture; single-cell RNA sequencing; single-nucleus RNA sequencing; single-cell multi-omics

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Funding projects: National Natural Science Foundation of China (32373113, 32341061)

Multicellular organisms are highly complex systems composed of hundreds of distinct cell types. Although all cells originate from the same fertilized egg, the progressive accumulation of genetic and epigenetic variations during development leads to substantial heterogeneity among tissues, organs, and even cells of the same type. This heterogeneity is reflected

not only in differential gene expression but also plays a crucial role in determining cell fate and shaping cellular responses to physiological and pathological stimuli. Conventional bulk genomic analyses fail to capture such intercellular variability, whereas advances in single-cell sequencing technologies now enable the resolution of cellular heterogeneity at single-cell resolu-

收稿日期: 2026-01-07 修回日期: 2026-01-28

资助项目: 国家自然科学基金面上项目 (32373113, 32341061)

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ation^[1]. These technologies facilitate: 1) the identification of rare cell subpopulations, 2) the elucidation of gene regulatory networks, and 3) the reconstruction of cellular developmental trajectories, thereby providing new insights for both fundamental research and clinical applications.

Since its introduction in 2009, single-cell RNA sequencing (scRNA-seq) has shown distinct advantages in dissecting cellular heterogeneity and biological complexity^[2]. This technology enables the generation of high-resolution cellular atlases that reveal the composition and functional states of cells under diverse physiological and pathological conditions. Moreover, when integrated with single-cell multi-omics approaches—such as transcriptomics, epigenomics, and proteomics—as well as spatial transcriptomics, scRNA-seq has facilitated a shift from two-dimensional to three-dimensional characterization of biological systems. To date, it has driven significant advances across multiple fields, including developmental biology, tumor microenvironment studies, immune regulation, and infectious disease research.

In contrast, the application of single-cell technologies in aquaculture is still in its early stages. Although initial studies have been performed in economically important species such as grass carp (*Ctenopharyngodon idella*) and Nile tilapia (*Oreochromis niloticus*), the molecular mechanisms underlying key biological processes—including developmental regulation, immune responses, and environmental adaptation—remain poorly understood in aquatic organisms. This review provides a systematic overview of recent advances in single-cell technologies, with an emphasis on their potential applications in developmental biology, disease immunity, and environmental stress research in aquatic species. Our goal is to offer new research perspectives and methodological frameworks that may help address critical challenges in the aquaculture industry.

1 Single-cell sequencing technologies

In 2009, Tang *et al.*^[2] reported the first transcrip-

tion sequencing of individual blastomeres and oocytes, marking the emergence of single-cell sequencing technology. The core workflow of this technology consists of three major steps: cell isolation and capture, library preparation and sequencing, and data analysis. In recent years, continuous innovations in cell sorting, nucleic acid amplification, library construction strategies, and bioinformatic analysis have transformed single-cell sequencing into a powerful tool that drives progress across the life sciences.

The efficient isolation of high-quality single cells remains a major technical challenge in single-cell sequencing. Conventional approaches, such as enzymatic digestion and density gradient centrifugation, often result in substantial cell loss and transcriptional perturbations and show limited effectiveness when applied to cryopreserved tissues. To overcome these limitations, optimized nuclear isolation protocols have been developed^[3], which effectively alleviate these issues. More recently, broad-spectrum cell and nuclear dissociation methods suitable for aquatic fish species have been established^[4], including specialized techniques for processing frozen samples from Antarctic fishes^[5]. These advances provide essential technical support for the application of single-cell technologies in aquaculture research.

Single-cell capture is a critical step in single-cell sequencing workflows. Early techniques, including limiting dilution, micromanipulation, and fluorescence-activated cell sorting (FACS), were constrained by high sample input requirements (>10 000 cells), low throughput (tens to hundreds of cells), and reliance on specific antibodies. The advent of microfluidic technology has effectively overcome these limitations (Fig. 1). By encapsulating individual cells in nanoliter-scale droplets that serve as independent reaction chambers, microfluidic platforms enable 1) low sample consumption (<1 000 cells), 2) high throughput (thousands to tens of thousands of cells), and 3) cost-efficient parallel processing. Consequently, microfluidics-based high-throughput single-cell sequencing has become the standard in the field.

Single-cell RNA sequencing (scRNA-seq) and

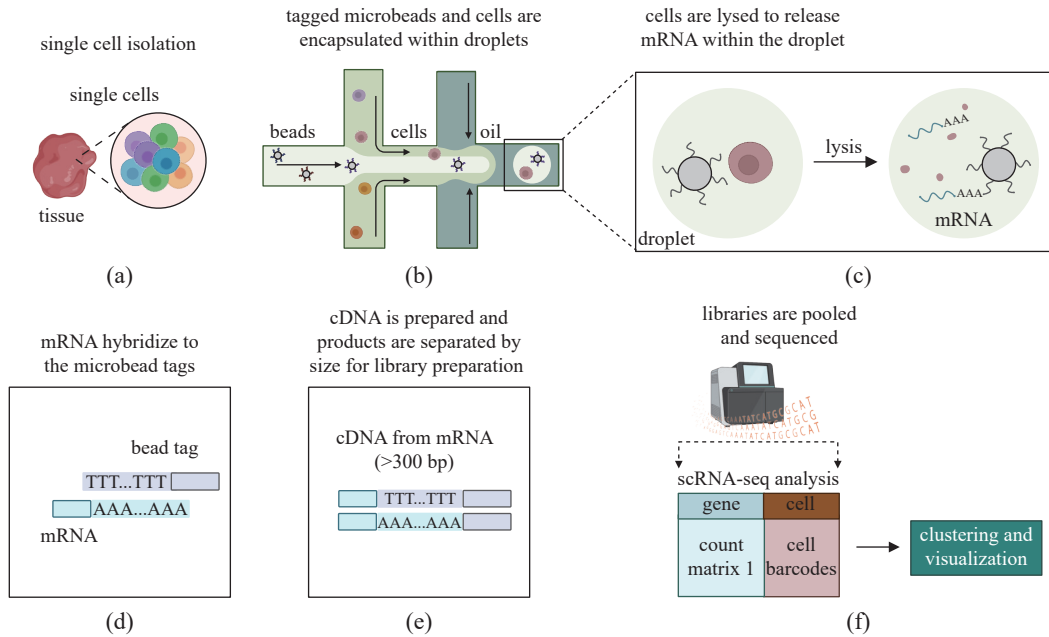


Fig. 1 Workflow of single-cell RNA sequencing (scRNA-seq)

(a) tissue samples are dissociated to obtain a suspension of individual cells. (b) single cells are co-encapsulated with barcoded microbeads in nanoliter droplets using a microfluidic platform. (c) within each droplet, cells are lysed to release mRNA. (d) released mRNA molecules hybridize to oligo (dT)-containing barcoded primers on microbeads. (e) reverse transcription is performed to synthesize cDNA, followed by amplification and size selection for library preparation. (f) libraries from all droplets are pooled and subjected to high-throughput sequencing. Sequencing data are processed to generate gene-cell expression matrices, followed by downstream analyses such as dimensionality reduction, clustering, cell type identification, and functional interpretation. Illustrations were created with BioRender and are licensed for publication.

single-nucleus RNA sequencing (snRNA-seq) are complementary approaches for transcriptional profiling at single-cell resolution^[6]. They differ systematically in sample compatibility, molecular capture bias, and the type of biological information obtained. scRNA-seq relies on intact whole cells to generate sequencing libraries and primarily captures mature cytoplasmic mRNAs, thereby better reflecting the real-time functional states and transcriptional activity of cells. It is widely applied in cell type identification, immune microenvironment characterization, and developmental trajectory reconstruction. However, this method depends heavily on high-quality fresh samples, and enzymatic or mechanical dissociation during tissue processing can induce stress-related transcriptional artifacts and result in substantial loss of sensitive cell types. By contrast, snRNA-seq uses isolated nuclei as input material, capturing nascent unspliced transcripts and nuclear-retained RNAs. This approach is well suited for cryopreserved samples and complex tissues that are difficult to dissociate, and it

retains more complete cellular representation, particularly of fragile cell types. Moreover, it provides deeper insights into transcriptional regulation, including enhancer activity and alternative splicing. Nonetheless, because of its limited coverage of cytoplasmic mature mRNAs, snRNA-seq may systematically underestimate gene expression levels. Overall, scRNA-seq remains the preferred method when accurate functional states of cells are required, whereas snRNA-seq offers unique advantages for studying developmental origins and conducting large-scale retrospective analyses (Tab. 1).

2 Technological advances in Single-cell and Multi-omics sequencing

As a cornerstone of single-cell omics, scRNA-seq enables high-resolution transcriptomic profiling at the individual cell level, providing a powerful tool for dissecting cellular heterogeneity, identifying cell types, and characterizing functional states. Early scRNA-seq protocols were developed using micro-

Tab. 1 Comparison between single-cell RNA sequencing (scRNA-seq) and single-nucleus RNA sequencing (snRNA-seq)

feature	single-cell RNA sequencing (scRNA-seq)	single-nucleus RNA sequencing (snRNA-seq)
target	whole cells	isolated nuclei
RNA source	primarily cytoplasmic (mature mRNA)	primarily nuclear (nascent RNA, unspliced RNA)
sample requirements	high; requires fresh or live tissue, challenging for frozen samples	low; suitable for both fresh and frozen archived tissues (e.g., clinical biobanks)
cell capture	potential loss of fragile cell types during tissue dissociation	better preservation of all cell types, especially fragile populations
gene detection	generally detects more genes, reflecting mature transcripts	detects fewer genes, biased toward nuclear transcripts; may underestimate expression levels
information focus	more accurately reflects current functional states and cell identity	better reveals transcriptional regulation (e.g., enhancer activity, splicing) and cell type origins
main applications	cell atlases, immune responses, developmental biology, cancer (fresh samples)	neuroscience, complex/frozen tissues (e.g., brain, adipose, kidney), retrospective clinical studies, biobank analysis

plate-based platforms combined with full-length transcript amplification methods, such as Smart-seq^[7], which allowed detailed analysis of alternative splicing and allele-specific expression but were limited by low throughput. Subsequent high-throughput approaches, including droplet microfluidics-based platforms such as Drop-seq^[8] and the 10x Genomics Chromium system, encapsulated single cells with barcoded beads in water-in-oil emulsions, enabling the simultaneous analysis of tens of thousands of cells. However, these methods primarily sequence the 3' or 5' ends of transcripts and are therefore better suited for large-scale cell classification. More recently, ultra-high-throughput strategies such as single-cell combinatorial fluidic indexing (sci-fi) have been developed based on advances in droplet-based scRNA-seq. These innovations are driven by continuous improvements in single-cell isolation, cellular barcoding strategies, and cDNA library construction efficiency.

Full-length transcript sequencing approaches^[9-11] (e.g., Smart-seq2) are best suited for studies requiring high sensitivity, comprehensive transcript coverage, and accurate detection of alternative splicing and isoform diversity, such as analyses of non-coding RNAs and fine-scale differential expression. However, their low throughput, high cost, and operational complexity limit scalability and make them impractical for large-scale cell atlas projects. UMI-based high-throughput single-cell RNA-seq platforms (e.g., 10x Chromium, Drop-seq, CEL-seq2)^[9-11] are optimal for large-scale cell type identification and cell atlas construction,

offering clear advantages in cell throughput, quantitative accuracy, reproducibility, and cost efficiency. These benefits come at the expense of reduced transcript coverage, as 3'-end-biased strategies limit the resolution of full-length transcripts and complex splicing events. Ultra-high-throughput single-cell approaches (e.g., sci-RNA-seq)^[10] provide a powerful solution for profiling millions of cells, enabling population-level surveys and rapid cell type discovery at unprecedented scale. Nonetheless, current implementations exhibit reduced sensitivity and incomplete recovery of cellular diversity, particularly for low-abundance transcripts, and are therefore best complemented by higher-resolution methods. Droplet-based mid-throughput methods (e.g., Drop-seq, inDrops, Seq-Well)^[9-11] offer a pragmatic compromise between cost and throughput, making them suitable for exploratory studies or projects with limited resources. Their overall performance, particularly in terms of sensitivity, generally remains inferior to plate-based full-length sequencing strategies. Nucleus-based transcriptomic strategies (snRNA-seq)^[9] are especially advantageous for frozen samples, poorly dissociable tissues, and non-model species, where they reduce dissociation-induced biases and improve cell type representation. However, the limited capture of cytoplasmic mature transcripts introduces systematic biases in gene expression quantification and constrains functional state inference. Also, no single-cell RNA-seq technology is universally optimal. Method selection should be guided by the biological question, sample characteristics, and

resource constraints, balancing trade-offs between cellular throughput, transcriptomic depth, and analytical resolution.

Building upon scRNA-seq, single-cell technologies have expanded into the multi-omics domain, enabling systematic dissection of cellular regulatory networks (Fig. 2). At the genomic level, single-cell DNA sequencing (scDNA-seq)^[12] utilizes whole-genome amplification (WGA) strategies-such as multiple displacement amplification (MDA)^[13], multiple anneal-

ing and looping-based amplification cycles (MAL-BAC)^[14], and degenerate oligonucleotide-primed PCR (DOP-PCR)^[15]-to uniformly amplify genomic DNA from individual cells, thereby facilitating the detection of DNA variants, including single-nucleotide variants (SNVs) and copy number variations (CNVs).

In epigenomics, the transposase-based single-cell assay for transposase-accessible chromatin sequencing (scATAC-seq) enables genome-wide mapping of open chromatin regions and identification of key tran-

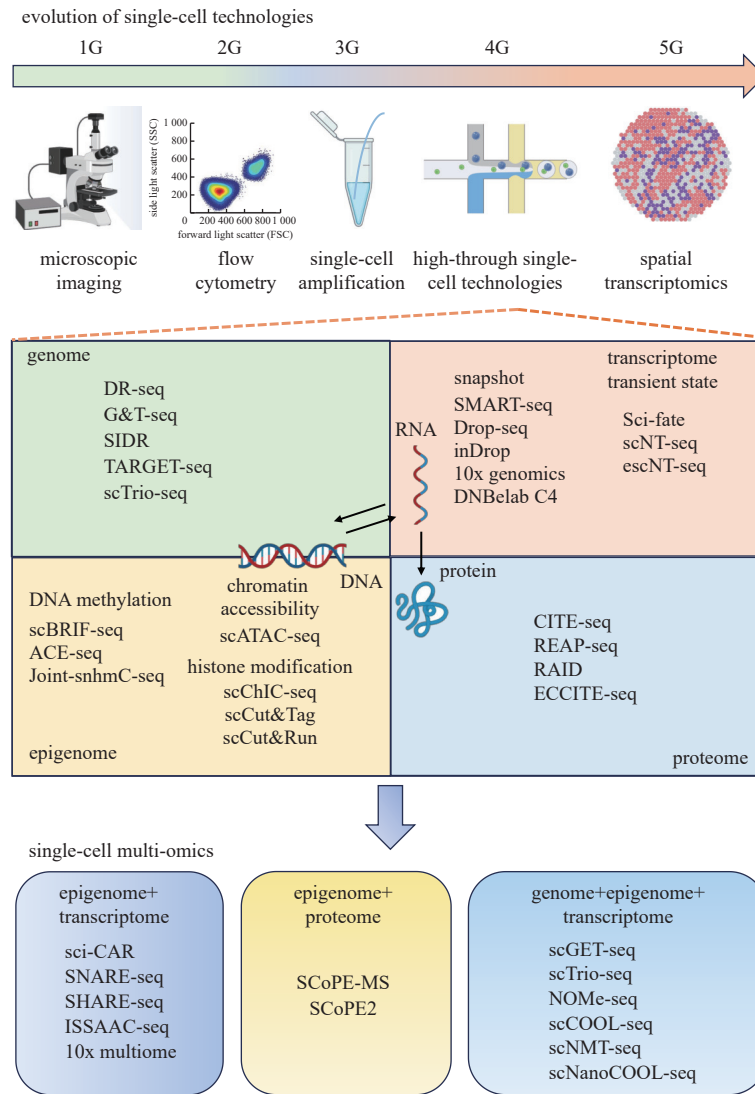


Fig. 2 Overview of single-cell sequencing technologies and their multi-omics integration

Single-cell sequencing has expanded from single-modality transcriptomic analysis to comprehensive multi-omics profiling. Representative technologies across molecular layers include genome sequencing (e.g., DR-seq, G&T-seq), transcriptome profiling (e.g., SMART-seq, Drop-seq, 10x Genomics), DNA methylation analysis (e.g., scBS-seq, ACE-seq), chromatin accessibility assays (e.g., scATAC-seq), histone modification mapping (e.g., scCUT&Tag, scCut&Run), and proteome profiling (e.g., CITE-seq). Integrated multi-omics platforms combine these layers within the same cell-such as epigenome-transcriptome (e.g., SHARE-seq), epigenome-proteome (e.g., SCoPE2), and genome-epigenome-transcriptome (e.g., scNMT-seq)- enabling multidimensional reconstruction of cellular states and regulatory mechanisms.

scriptional regulators at single-cell resolution^[16]. For histone modification profiling, conventional single-cell ChIP-seq has been largely superseded by more sensitive and streamlined approaches, such as single-cell cleavage under targets and tagmentation (scCUT&Tag)^[17-18]. This method employs a Protein A-Tn5 transposase fusion protein directed by specific antibodies to cleave and tag chromatin regions bound by target proteins, enabling high-resolution mapping of histone modifications and transcription factor binding sites.

DNA methylation is a key epigenetic modification, and 5-methylcytosine (5mC) plays a central role in gene regulation, transposon silencing, and chromatin organization. Single-cell bisulfite sequencing (scBS-seq) enables genome-wide analysis of DNA methylation heterogeneity^[19]. During active demethylation, 5mC is oxidized to 5-hydroxymethylcytosine (5hmC), which exerts distinct regulatory functions: promoter methylation by 5mC typically represses gene expression, whereas 5hmC is frequently associated with transcriptional activation. Conventional bisulfite sequencing cannot distinguish between these two modifications, which led to the development of APOBEC-coupled epigenetic sequencing (ACE-seq) for specific 5hmC profiling^[20]. However, these approaches are generally limited to profiling a single modification at a time. Recently, Joint-snhmC-seq^[21] was developed to enable simultaneous quantification of both 5mC and 5hmC at single-cell resolution. To investigate higher-order chromatin architecture, single-cell Hi-C (scHi-C) has also been established to capture chromatin conformation and spatial interactions within individual cells^[22].

The scope of single-cell technologies continues to expand, driving the development of innovative methods tailored to address specific biological questions. For example, in studies of RNA dynamics, techniques such as single-cell thiol (SH)-linked alkylation for the metabolic sequencing of RNA (scSLAM-seq)^[23] combine metabolic labeling with 4-thiouridine (4sU) and high-throughput sequencing to distinguish newly synthesized transcripts from pre-existing RNA molecules^[24-25]. This approach adds a temporal dimen-

sion to transcriptional analysis and provides critical insights into gene expression kinetics.

In the technically challenging field of single-microbe RNA sequencing^[26-28], progress has been driven by improved RNA capture strategies, such as the use of random primers and *in vitro* poly(A) tailing, combined with more effective ribosomal RNA (rRNA) depletion methods (Fig.3). These advancements are gradually overcoming longstanding challenges, including low RNA abundance, the absence of poly(A) tails, and the physical barriers imposed by microbial cell walls.

Building on established single-omics methodologies, single-cell multi-omics technologies have been developed through continuous refinement and innovation. These approaches enable the simultaneous measurement of multiple molecular modalities-such as DNA, RNA, and proteins-within the same individual cell. By integrating multilayered molecular information, single-cell multi-omics transcends the correlation-based insights derived from single-modality analyses and allows direct inference of regulatory interactions and causal relationships across molecular layers. This capability substantially improves the resolution of complex, cell type-specific regulatory mechanisms.

Recent advances in single-cell multi-omics technologies have enabled increasingly robust co-profiling of multiple molecular modalities within the same cell. For example, Cellular Indexing of Transcriptomes and Epitopes by sequencing (CITE-seq) integrates phenotypic information-such as cell surface protein expression-with single-cell transcriptomic data^[29]. Technologies such as sci-CAR^[30], SNARE-seq^[31], SHARE-seq^[32], ISSAAC-seq^[33], 10x Genomics Multiome platform^[30, 32], and Parallel-seq^[34] enable the simultaneous profiling of chromatin accessibility and gene expression in individual cells or nuclei. Furthermore, scGET-seq expands multimodal capability by combining chromatin accessibility profiling with genomic and heterochromatin-associated epigenomic analysis within single cells^[35].

At the protein level, Single-Cell Proteomics by Mass Spectrometry (SCoPE-MS)^[36] and its improved version SCoPE2^[37] enable the quantification of thou-

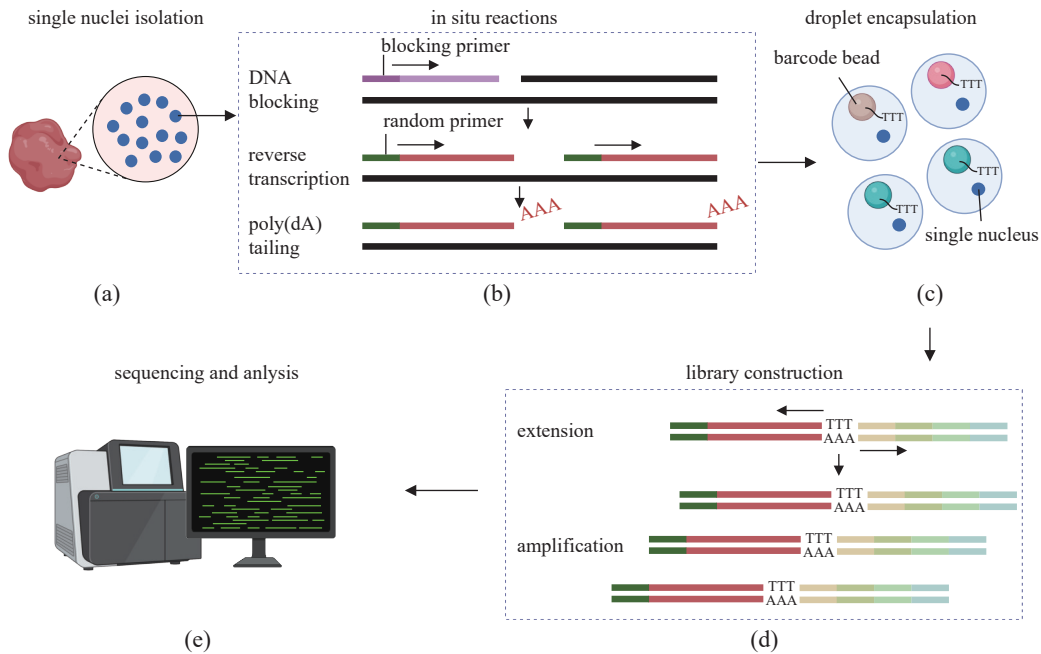


Fig. 3 Workflow of single-cell RNA sequencing (scRNA-seq) based on random primer^[28]

(a) tissue samples are dissociated to obtain a suspension of individual nuclei. (b) isolated single nuclei undergo three in situ reactions: single-stranded DNA blocking, cDNA synthesis using random primers, and poly(dA) tailing at the 3' end of cDNA. (c) using a microfluidic platform, single nuclei, barcoded beads, and reagent mixtures are efficiently encapsulated into microdroplets. (d) (e) through an extension reaction, the cDNA within each microdroplet is uniquely barcoded. The microdroplets are broken, and the barcoded cDNA is amplified and sequenced. Illustrations were created with BioRender and are licensed for publication.

sands of proteins and their post-translational modifications in individual cells. In parallel, methodologies such as single-cell Chromatin Overall Omic-scale Landscape sequencing (scCOOL-seq)^[38] and single-cell Nucleosome, Methylation, and Transcriptome sequencing (scNMT-seq)^[39] permit the concurrent detection of genomic, DNA methylation, chromatin state, and transcriptomic information from the same cell. More recently, scNanoCOOL-seq has integrated scCOOL-seq with third-generation sequencing platforms to provide a unified strategy for simultaneous profiling of the genome, DNA methylome, chromatin accessibility, and transcriptome at single-cell resolution^[40]. This method also allows the detection of structural variations and allele-specific epigenetic features at single-molecule precision^[40].

3 Data analysis: Extracting biological meaning from high-volume datasets

Single-cell data analysis is primarily conducted using the R and Python programming languages. Rep-

resentative analysis frameworks include Seurat and Scater in R, and Scanpy in Python, which offer comprehensive pipelines and extensive toolboxes for data processing, visualization, and downstream analysis.

Data preprocessing in scRNA-seq involves generating a count matrix—either molecular counts when unique molecular identifiers (UMIs) are used or read counts otherwise. The workflow typically includes: 1) quality control of raw sequencing data, 2) demultiplexing reads based on cell barcodes, 3) alignment to a reference genome, and 4) gene quantification using tools such as Cell Ranger or zUMIs.

Quality control (QC) of single-cell RNA sequencing data is essential to ensure that each barcode corresponds to a viable cell. QC commonly relies on three metrics: the total number of detected transcripts per cell, the number of detected genes, and the proportion of mitochondrial gene expression. Barcodes with low transcript or gene counts and high mitochondrial content typically represent ruptured or dying cells, whereas those with abnormally high counts may indicate doublets or multiplets. In addition, contamination

from ambient RNA—often originating from lysed cells—should be corrected using tools such as SoupX, as it can introduce bias into downstream analyses. Because QC thresholds often require adjustment based on clustering outcomes, the process is typically iterative.

Normalization of the single-cell count matrix aims to reduce technical variation, such as differences in sequencing depth, and ensure comparability of gene expression across cells. Commonly used normalization methods include counts per million (CPM), LogNormalize, and SCTransform, which adjust molecular counts to facilitate more accurate downstream analyses.

Data correction and integration aim to further reduce technical artifacts and biological confounders. Batch effects can be corrected using tools such as Harmony (R), canonical correlation analysis (CCA; R), scVI (Python), FastMNN (Python), or BBKNN (Python). In addition, doublet detection and removal can be performed with packages such as DoubletFinder (R) and Scrublet (Python). The choice of correction strategy depends on the analytical objective and the characteristics of the dataset.

Cell type identification generally consists of three major steps: dimensionality reduction, clustering analysis, and cell annotation. Dimensionality reduction methods such as principal component analysis (PCA), t-distributed stochastic neighbor embedding (t-SNE), and uniform manifold approximation and projection (UMAP) are first applied to visualize high-dimensional gene expression data. Next, clustering algorithms—often based on statistical models such as the negative binomial distribution—are used to identify cell subpopulations and detect low-quality cells (e.g., those with excessively high proportions of mitochondrial gene expression). Differential expression analysis is then conducted to identify cluster-specific marker genes, and cell types are annotated by integrating prior biological knowledge, including canonical cell type-specific marker genes.

In non-model organisms, the absence of well-annotated marker gene databases and comprehensive reference resources makes cell type identification par-

ticularly challenging. Several strategies are commonly employed to address this limitation. First, orthologous gene mapping or conserved marker genes can be used to transfer annotations from model organisms through cross-species inference. Second, anatomical context and cellular morphology derived from experimental evidence can provide additional validation. Furthermore, integrative computational approaches, such as homology-based mapping and co-expression network analysis, can improve annotation accuracy. With the continued expansion of single-cell atlases across diverse species and ongoing advances in computational methods, the accuracy and interpretability of cell type annotation in non-model organisms are steadily improving.

In multicellular organisms, cells continuously adapt to internal and external cues by transitioning between functional states through processes such as differentiation, proliferation, and migration. For instance, immune cells rapidly shift to an activated state upon stimulation, characterized by increased cytokine production, enhanced proliferation, and changes in cell surface marker expression. Single-cell RNA velocity analysis estimates the instantaneous rate of gene expression change by quantifying the ratio of unspliced to spliced mRNA, thereby inferring the directionality of cell state transitions and predicting lineage trajectories. Compared with pseudotemporal trajectory inference methods, RNA velocity offers a more dynamic representation of cell fate decisions. Commonly used tools for RNA velocity analysis include Velocity, scVelo, and DeepVelo, the latter of which applies neural ordinary differential equations to model transcriptomic velocity fields.

RNA velocity analysis^[41] plays a critical role in elucidating dynamic cellular processes across diverse research contexts, including tracking immune cell activation during immune responses, investigating remodeling mechanisms in the tumor microenvironment, inferring stem cell differentiation trajectories, and characterizing transdifferentiation in neural cells. This approach provides valuable insights into disease mechanisms and supports the development of thera-

peutic strategies^[42].

Monocle2^[43] is an R-based trajectory inference tool that employs a reversed graph embedding framework combined with unsupervised learning. It first selects highly variable genes to construct a feature space and then applies the DDRTree algorithm for nonlinear dimensionality reduction while preserving the topological structure of the data. A trajectory tree is subsequently constructed using shortest-path algorithms to assign pseudotemporal coordinates to individual cells. Differential expression analysis is then performed to identify dynamically regulated genes and critical branching points along the trajectory. Monocle2 has been widely applied in studies of stem cell differentiation, immune cell activation, tumor heterogeneity, and other biological processes, establishing it as a foundational computational method for investigating cell fate transitions.

Gene Co-expression-Based Analysis: This strategy infers putative regulatory relationships between transcription factors (TFs) and their target genes by calculating gene expression correlations (e.g., Pearson or Spearman correlation coefficients) or by constructing gene co-expression networks such as WGCNA. However, co-expression relationships can be confounded by indirect regulation or shared upstream factors, which often leads to a high false-positive rate and limits the ability to distinguish direct from indirect interactions.

Multi-omics Data Integration Strategy: To improve inference accuracy, multiple layers of omics data—such as chromatin accessibility (ATAC-seq), transcription factor binding profiles (ChIP-seq), protein-protein interactions (PPIs), and epigenetic modifications—can be integrated. For example, incorporating TF motif enrichment within open chromatin regions enables more reliable prediction of direct regulatory targets, while protein interaction data help identify higher-order regulatory modules and functional complexes. Nevertheless, this strategy is challenged by the heterogeneity, variable resolution, and noise inherent in different data types, which necessitate advanced computational algorithms and standard-

ized processing pipelines.

Recent methods such as SCENIC+ and Pando integrate both gene expression and chromatin accessibility data at single-cell multi-omics resolution, thereby enhancing the accuracy and biological interpretability of inferred gene regulatory networks.

Cell communication analysis systematically deciphers intercellular signaling networks mediated by ligand-receptor interactions, cytokines, adhesion molecules, and other signaling factors. It reveals how distinct cell types coordinate physiological processes—such as immune responses, development and differentiation, and tissue homeostasis—as well as pathological processes including tumorigenesis and inflammation within tissue microenvironments. This analytical approach aims to elucidate the directionality, strength, and functional consequences of cell-cell communication at both the molecular and cellular population levels, offering key insights into the dynamic regulation of microenvironmental interactions.

Commonly used tools for cell communication analysis include CellPhoneDB, CellChat, NicheNet, iTALK, CellTalker, SingleCellSignalR, and InterCellDB. Although these tools differ in algorithmic frameworks and analytical features, they share the common objective of inferring and visualizing intercellular communication events from single-cell transcriptomic data and interpreting their biological significance.

CellPhoneDB^[44] is a widely used tool whose major strength lies in its curated repository of ligand-receptor interactions derived from multiple authoritative databases, including UniProt, Ensembl, PDB, and IUPHAR. Its reference database contains 978 proteins (501 secreted and 585 membrane proteins) involved in 1 396 documented interactions, and uniquely accounts for the subunit composition of heteromeric complexes, thereby improving representation of receptor and ligand isoform diversity. In addition, CellPhoneDB allows users to incorporate custom interaction pairs, providing flexibility for application across diverse biological contexts.

CellChat^[45] is an interactive cell communication

analysis framework based on network theory and probabilistic modeling. It integrates single-cell expression data with known ligand-receptor-cofactor interaction networks to infer communication probabilities and reconstruct signaling networks. Its key features include: 1) a built-in interaction database browser for efficient exploration of molecular communication links, 2) multiple visualization modules to display cell-cell communication patterns, pathway activity, and key ligand-receptor interactions, and 3) support for functional enrichment analysis linking inferred signaling events to downstream biological processes, enabling multiscale biological interpretation.

NicheNet^[46] extends beyond traditional ligand-receptor identification by integrating gene expression data with prior knowledge of intracellular signaling and gene regulatory networks. It not only predicts ligand-receptor pairs but also infers downstream target gene regulation, thereby generating causal hypotheses that link intercellular signaling to transcriptional responses. This capability makes NicheNet particularly valuable for investigating how extracellular cues shape cell fate decisions and functional states.

In summary, these computational tools offer complementary strengths in database design,

algorithmic modeling, and functional interpretability. Tool selection should be guided by research objectives—for example, the need for subunit-level precision, interest in downstream regulatory mechanisms, or preference for interactive visualization. Moreover, combining multiple tools can improve robustness through cross-validation and provide deeper mechanistic insights into cell-cell communication.

4 Single-cell technologies to systematically decipher molecular mechanisms in aquatic animals

This review summarizes key applications of scRNA-seq and snRNA-seq in elucidating molecular mechanisms across diverse aquatic species (Fig. 4). Major findings include.

Skeletal Development: In zebrafish, scRNA-seq revealed that intermuscular bones (IBs) originate from mesenchymal stem cells (MSCs). Functional validation identified *runx2b* as a key transcriptional regulator, whose knockout abolished IB formation by depleting osteoblast-lineage cells^[47]. Similarly, *bmp6* deficiency disrupted IB development by simultaneously suppressing osteogenesis and promoting osteo-

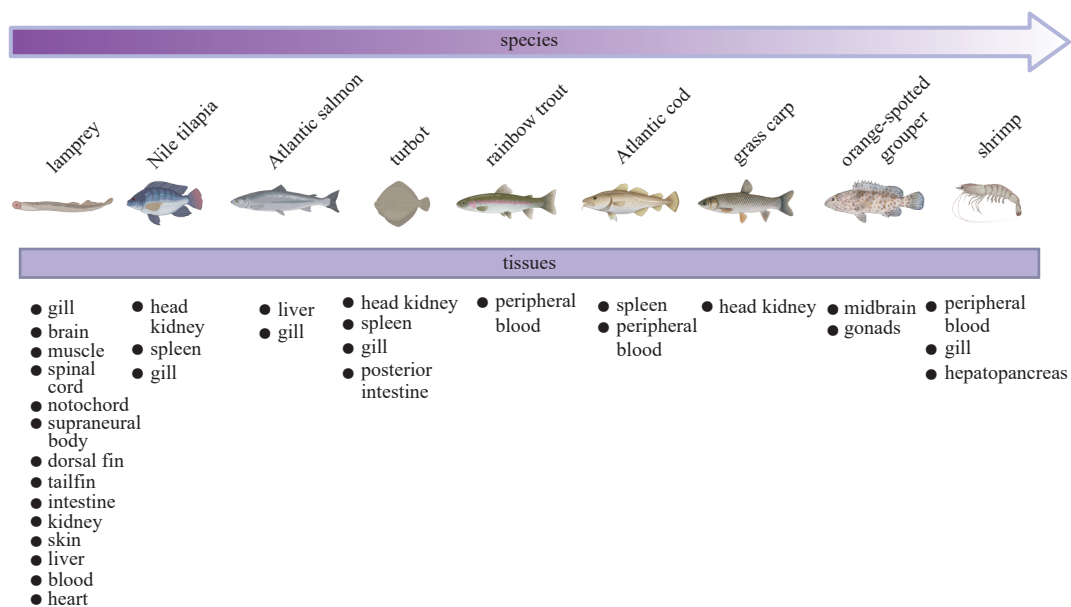


Fig. 4 Cell atlases of representative aquatic animals

Icons represent major aquaculture or aquatic species and tissue types that have been profiled using single-cell sequencing technologies. Illustrations were created with BioRender and are licensed for publication.

clastogenesis^[48].

Immune Response: scRNA-seq has uncovered the cellular heterogeneity of immune systems in teleosts (e.g., tilapia, turbot, gilthead sea bream)^[49-62] and crustaceans (e.g., Pacific white shrimp)^[63-64]. These studies have identified novel T cell subsets, elucidated mechanisms of immune cell migration^[65], and characterized pathogen-specific hemocyte responses to WSSV and *Aeromonas veronii*. This study performed single-cell transcriptomic analysis of 113 356 immune cells from Nile tilapia infected with *Streptococcus agalactiae*, revealing the dynamic responses of neutrophils, macrophages, T cells, and B cells across distinct infection stages. The results showed that neutrophils undergo early transcriptional reprogramming centered on inflammatory and IFN γ signaling pathways, and identified a cross-species conserved IFN γ -driven differentiation trajectory toward il8+ neutrophils^[62].

Environmental Adaptation and Toxicology: scRNA-seq has revealed cellular and molecular adaptations to extreme environments, such as host-symbiont interactions in deep-sea tubeworms and methane seep mussels. In toxicological studies, single-cell analyses have pinpointed gill fibroblasts and macrophages as primary targets of micro- and nanoplastic exposure and mapped cell type-specific intestinal responses to methylmercury in fish^[66-68].

Reproductive Biology: Single-cell analyses in mollusks, including Pacific oyster and scallop, have generated comprehensive gonadal cell atlases that illuminate germ cell developmental trajectories and niche interactions within the reproductive microenvironment^[69-73].

Specialized Tissues and Cell Types: Single-cell atlases of specialized tissues, such as oyster gills, have identified previously unrecognized copper-accumulating cell subtypes. In sea cucumber body walls, single-cell profiling has pinpointed pigment-producing cell types, including melanocytes and quinocytes.

Collectively, these studies demonstrate the power of single-cell technologies to dissect developmental, immunological, adaptive, and reproductive mechanisms at single-cell resolution, providing critical molecular insights and valuable resources for both

aquaculture innovation and evolutionary developmental biology.

5 Limitations and challenges of Single-cell sequencing in aquatic animals

Despite the rapid adoption of single-cell sequencing technologies in aquatic research, several technical and conceptual challenges remain. A primary limitation lies in the difficulty of obtaining high-quality single-cell suspensions from many aquatic tissues, as enzymatic or mechanical dissociation can induce stress-related transcriptional artifacts and result in selective loss of fragile or rare cell types. These challenges are further compounded by the ectothermic nature of aquatic animals, whose cells often exhibit heightened sensitivity to temperature and handling conditions. In addition, incomplete or fragmented reference genomes for many aquaculture species constrain accurate read mapping, transcript annotation, and downstream integrative analyses.

Practical issues associated with field-based sampling, including limited access to laboratory facilities and delays between sample collection and library preparation, also pose significant obstacles, particularly for scRNA-seq approaches that rely on fresh viable cells. Although snRNA-seq partially alleviates several of these constraints, it introduces its own limitations, such as reduced coverage of cytoplasmic transcripts and systematic biases in gene expression quantification. Finally, the high cost of large-scale single-cell experiments and the lack of standardized analytical pipelines tailored to non-model aquatic species remain barriers to broader adoption. Addressing these challenges will require continued methodological innovation, improved genomic resources, and the development of best-practice guidelines specifically optimized for aquatic organisms.

6 Future outlook: Revolutionizing aquaculture with Single-cell omics technologies

This review concludes by outlining the major challenges and future directions of single-cell genom-

ics in aquaculture research. The first priority is to enhance data completeness. Although current technologies enable robust cell type classification, data sparsity remains a major limitation, hindering a comprehensive understanding of cellular function and stress responses. Incorporating advanced techniques such as full-length transcriptome sequencing will be essential to capture more complete molecular profiles. Meanwhile, initiatives such as the Fish Cell Atlas are expected to generate and integrate extensive single-cell datasets into publicly accessible databases, thereby facilitating in-depth studies of cell types, gene regulatory networks, gene functions, and cellular interactions under both physiological and stress conditions.

A second key direction is the expansion of single-cell multi-omics capabilities. Future research should focus on simultaneously profiling multiple molecular layers-including DNA-protein interactions and chromatin architecture-to elucidate the regulatory relationships linking epigenetic modifications to gene expression. A third priority is the integration of single-cell multi-omics with spatial transcriptomics. Overcoming the technical challenges associated with tissue dissociation while preserving spatial context will enable precise localization of cell types and mechanistic dissection of cellular interactions within complex aquatic tissues.

Ultimately, the future of aquatic genomics will rely on the convergence of spatial and single-cell technologies. High-resolution spatial data generated through in situ labeling methods (e.g., Slide-tags)^[74] combined with high-throughput sequencing will be analyzed using AI-driven multimodal algorithms to resolve cellular function with unprecedented precision. Gene regulatory models (e.g., Geneformer)^[75] trained on large-scale aquatic genomic datasets will enable prediction of gene expression states and simulation of virtual genetic perturbations. These advances will accelerate precision breeding by guiding CRISPR-based editing of key regulatory elements to improve economically important traits, including stress tolerance, disease resistance, and growth performance,

thereby driving intelligent and sustainable innovation in aquaculture.

Declaration of Competing Interest

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

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单细胞技术在水产动物中的研究: 进展、应用与未来展望

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摘要: 单细胞测序技术通过揭示传统整体分析所掩盖的细胞异质性, 已成为解析水产生物复杂生命过程的重要工具。本文系统综述了单细胞 RNA 测序、单细胞核 RNA 测序及单细胞多组学技术在水产生物研究中的关键进展, 总结了细胞分离、微流控捕获、文库构建及生物信息学分析等方法的发展, 并概括了其在骨骼发育、免疫应答、环境适应、生殖调控和特化细胞类型研究中的代表性应用。尽管单细胞技术在水产养殖研究中展现出巨大潜力, 但仍面临数据稀疏、非模式物种基因组资源不足及分子分辨率有限等挑战。未来, 通过整合单细胞多组学、空间转录组学和人工智能分析, 有望构建鱼类细胞图谱并推动精准育种研究, 为水产养殖的可持续发展提供关键支撑。

关键词: 水产; 单细胞测序; 单细胞核测序; 单细胞多组学技术

中图分类号: S 917.4

文献标志码: A