

REVIEW

Genomics and genetic breeding in aquatic animals: progress and prospects

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Abstract Genomics focuses on dissection of genome structure and function to provide a molecular basis for understanding the genetic background. In a pivotal step, the expense of whole genome sequencing has been largely eliminated by the rapid updating of sequencing technology, leading to increasing numbers of decoded genomes of aquatic organisms, driving the aquaculture industry into the genomic era. Multiple aquatic areas have been influenced by these findings, such as accelerated generation shift in the seed industry and the process of breeding improved lines. In this article, we have summarized the latest domestic and international progress of aquatic animals in nine aspects, including WGS and fine mapping, construction of high density genetic/physical maps, trait-related marker/genes screening, as well as sex control, genome editing, and other molecular breeding technologies. Finally, the existing problems in this field have been discussed and five future counter measures have been proposed accordingly.

Keywords aquaculture, genomics, genetic breeding, progress

1 Introduction

Genomics is a subject that concentrates on studying genome structure and function. As the first step in genomic structural analysis, whole genome sequencing (WGS) plays a pivotal role in genetic resource exploitation and functional gene discovery. Through WGS the genomic sequences and important functional genes are obtained, which can in turn reveal the evolution of the species, as well as facilitate understanding of the molecular

mechanism of growth, development, reproduction, sex differentiation and environmental adaptation. In recent years, the rapid development of sequencing technology has resulted in a sharp decline of sequencing cost, so WGS has been accomplished in a number of aquatic organisms. This has led to the rapid accumulation of massive genomic resources and the identification of many markers/genes related to important traits. These findings have greatly promoted the study of multiple aquatic areas, e.g., identification of aquatic germplasm, genetic dissection of important traits, molecular sex control, genetic improvement, genome editing and whole genome selection, and thus driven the aquaculture industry into the genomic era. On the one hand, the development of genomic technologies has facilitated the generation shift in the seed industry and breeding process of improved lines. On the other hand, genomic resources and technologies have also provided huge application potential and improved prospects for aquatic breeding, germplasm conservation, disease control and prevention. This article summarizes the latest progress in aquatic animals, including fine mapping of genomes, screening of markers and genes related to important traits, construction of high-density genetic/physical maps, marker assisted sex control, genome editing, and whole genome selection. Finally, the existing problems are discussed and future development in aquatic genome and genetic breeding technology proposed.

2 Recent progress in genome research in aquatic animals

2.1 Whole genome sequencing and fine mapping

WGS in aquatic animals can be dated from the beginning of this century. In 2002, Singaporean scientists deciphered the genome of fugu rubripes, the first animal in aquaculture

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to be sequenced^[1]. Subsequently, domestic and foreign scientists have completed the genomic sequencing and fine mapping of the Atlantic cod, Atlantic salmon, carp, grass carp, half-smooth tongue sole, large yellow croaker, oysters, rainbow trout, tilapia, tuna and other important animals in aquaculture. Among them, the genome of Atlantic cod was decoded by University of Oslo in 2011^[2], three stickleback by Stanford University and the Broad Institute in 2012^[3], zebrafish by the Sanger Institute in 2013^[4], rainbow trout (first trout species sequenced) by French scientists in 2014^[5] and Atlantic salmon by the Norwegian University of Life Sciences in 2016^[6].

In recent years, Chinese scientists have also made great progress, for example, the sequencing of oyster genome by Zhang's group from the Institute of Oceanology, Chinese Academy of Sciences (IOCAS)^[7] and half-smooth tongue sole, the world's first Pleuronectiformes sequenced and the first fish sequenced in China, was completed by Chen's team from the Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (YSFRI, CAFS)^[8]. Soon afterwards, genomes of other fish were published, including carp (Sun's laboratory from Heilongjiang Fisheries Research Institute, CAFS)^[9], large yellow croaker genome (Wu's laboratory from Zhejiang Ocean University)^[10] and grass carp genome (Wang's laboratory, Institute of Hydrobiology, Chinese Academy of Sciences)^[11]. Sequenced aquatic animal genomes and their features are listed in Table 1.

2.2 High-density genetic linkage maps

High-density genetic linkage mapping has served as an important tool for genome assembly, important economic trait-related QTL mapping and gene screening. The sequencing of aquatic genomes can identify a large number of polymorphic SSR and SNP markers, which provide sufficient resources for construction of high density genetic linkage maps. Over the past five years, high density genetic linkage maps have been constructed for over 20 important aquatic animals, including Japanese flounder^[29,30], half-smooth tongue sole^[8,31], common carp^[32], channel catfish^[33], rainbow trout^[34], turbot^[35,36], Nile tilapia^[37], Atlantic salmon^[38], large yellow croaker^[39,40], silver carp^[41], bighead carp^[42], orange-spotted grouper^[43], European seabass^[44], red drum^[45], bay scallop^[46], Zhikong scallop^[47] and Pacific white shrimp^[48]. These successes have laid a solid foundation for fine mapping of disease resistance-related QTLs or gene cloning. For example, with an SNP genetic linkage map and genome-wide association analysis, Geng et al. located a 620 kb QTL region on the catfish chromosome 7. This QTL contained five immune related genes that might play a role in columnaris disease resistance^[49]. The reported high-density genetic linkage maps of aquatic animals are summarized in Table 2.

2.3 Construction of physical maps

The construction of physical maps for aquatic animals was initiated in 2004 and has so far been successful for over 10 species, including the Asian sea bass^[50], Atlantic salmon^[51], channel catfish^[52,53], common carp^[54], rainbow trout^[55], half-smooth tongue sole^[56], Medaka^[57], threespine sticklebacks^[58], tilapia^[59], Zhikong scallop^[60] and large yellow croaker (the first optical physical map in a non-model animal)^[61]. The reported physical maps of aquatic animals are summarized in Table 3.

2.4 Cloning and analysis of genes related to important traits in aquatic animals

2.4.1 Sex determination genes

For many years, scientists have focused on identifying the key genes in processes such as sex determination and differentiation, and this is also the case in aquatic animals. With the recent advance of high-throughput sequencing technology, remarkable progress has been achieved and many candidate genes in sex determination/differentiation have been identified, such as *DMY*, *Amhy*, *Gsdf*, *Amhr2*, *SdY*, *Sox3*, *Sox9*, *SoxE*, *dmrt1* and *Amh*^[62–66]. Except for a few reports in shrimp, shellfish and other species, most of these genes have been identified from fish, including *DMY* in medaka, *Amhy* in *Odontesthes hatcheri*, *Gsdf* in *Oryzias luzonensis*, *Sox3* in *Oryzias dancena*, *SdY* in *Oncorhynchus mykiss*, *Amhr2* in *Takifugu rubripes*^[65–68]. A further study found that *Amhy*, *Gsdf* and *Amhr2* were non-transcription factors, and all three were involved in the TGF- β signaling pathway^[63], which was suggested to be an important pathway in the sex determination process and also implied the conservation of sex determining mechanism among different fish species.

Chinese scientists have also made great progress in screening sex determination genes. Based on whole genome sequencing, Shao et al. has uncovered the epigenetic mechanism of sex reversal in half-smooth tongue sole^[69]. They found that the Z chromosome-linked gene *dmrt1* was testis specific and indispensable for testis development. *Dmrt1* was further confirmed to be the male determining gene through TALEN technology^[8,70]. Researchers from Southwest University have knocked out the *dmrt1* gene in tilapia with TALENs technology. The knockout mutant showed an obvious phenotype in the testis, including the deformation of the seminiferous duct and the degeneration of germ cells^[71].

2.4.2 Immune related genes

Disease is among the top factors that lead to huge economic loss in aquaculture, thus it is becoming a

Table 1 Reported genomes of aquatic animals by *de novo* sequencing

Common name	Scientific name	Genome size	ContigN50	ScaffoldN50	Reference
Fugu rubripes	<i>Takifugu rubripes</i>	380 Mb	n/a	n/a	[1]
Puffer fish	<i>Tetraodon nigroviridis</i>	340 Mb	16 kb	984 kb	[12]
Medaka	<i>Oryzias latipes</i>	700 Mb	9.8 kb	1.41 Mb	[13]
Atlantic cod	<i>Gadus morhua</i>	830 Mb	2.8 kb	688 kb	[2]
Channel catfish	<i>Ictalurus punctatus</i>	783 Mb	77.2 kb	7.7 Mb	[14]
Pacific oyster	<i>Crassostrea gigas</i>	559 Mb	19.4 kb	401 kb	[7]
Pearl oyster	<i>Pinctada fucata</i>	1.15 Gb	1.6 kb	14.5 kb	[15]
Threespine stickleback	<i>Gasterosteus aculeatus</i>	463 Mb	83.2 kb	10.8 Mb	[3]
Lamprey	<i>Petromyzon marinus</i>	816 Mb	174 kb	n/a	[16]
Zebrafish	<i>Danio rerio</i>	1.4 Gb	n/a	1.55 Mb	[4]
African coelacanth	<i>Latimeria chalumnae</i>	2.86 Gb	12.7 kb	924 kb	[17]
Pacific bluefin tuna	<i>Thunnus orientalis</i>	800 Mb	7.5 kb	136 kb	[18]
Platyfish	<i>Xiphophorus maculatus</i>	750–950 Mb	22 kb	1.1 Mb	[19]
Soft-shell turtle	<i>Pelodiscus sinensis</i>	2.21 Gb	21.9 kb	3.3 Mb	[20]
Elephant shark	<i>Callorhynchus milii</i>	937 Mb	46.6 kb	4.5 Mb	[21]
Antarctic bullhead notothen	<i>Notothenia coriiceps</i>	637 Mb	11.6 kb	219 kb	[22]
Half-smooth tongue sole	<i>Cynoglossus semilaevis</i>	477 Mb	26.5 kb	867 kb	[8]
	<i>Scartelaos histophorus</i>	806 Mb	8.4 kb	14.3 kb	
	<i>Boleophthalmus pectinirostris</i>	983 Mb	20.2 kb	2.31 Mb	[23]
Four representative mudskippers	<i>Periophthalmodon schlosseri</i>	780 Mb	16.8 kb	39.1 kb	
	<i>Periophthalmus magnuspinnatus</i>	739 Mb	27.6 kb	288.5 kb	
	<i>Cyprinus carpio</i>	1.53 Gb	68.4 kb	1 Mb	[9]
Large yellow croaker	<i>Larimichthys crocea</i>	644 Mb	25.7 kb	498.7 kb	[10]
	<i>Larimichthys crocea</i>	679 Mb	63.11 kb	1.03 Mb	[24]
Tawny puffer	<i>Takifugu flavidus</i>	390 Mb	2.8 kb	305.7 kb	[25]
Grass carp	<i>Ctenopharyngodon idellus</i> (female)	900 Mb	40.8 kb	6.46 Mb	
	<i>Ctenopharyngodon idellus</i> (male)	1.07 Gb	18.25 kb	2.28 Mb	[11]
Killfish	<i>Nothobranchius furzeri</i>	1.24 Gb	n/a	494 kb	[26]
Silver pomfret	<i>Pampus argenteus</i>	550 Mb	499 bp	1.58 kb	[27]
Spotted gar	<i>Lepisosteus oculatus</i>	945 Mb	68.3 kb	6.9 Mb	[28]
Atlantic salmon	<i>Salmo salar</i>	2.97 Gb	57.6 kb	2.97 Mb	[6]

Note: n/a, not available.

key topic for aquaculture. The studies mainly include identification of immune related genes, elucidation of disease resistance mechanisms and improvement of disease resistance in aquatic animals. Recent progress in this area is summarized below.

Chinese scientists have made great contribution in this field. Dozens of immune related genes have been identified and characterized from fish (common carp, grass carp, grouper, half-smooth tongue sole, Japanese flounder, large yellow croaker, sea bass, silver carp and turbot), crustacean (shrimp and crab) and other aquatic animals. Among them, *STAT2*, *GRIM-19*, *hepcidin*, *ferroportin1*, *transferrin*

receptor in turbot, *akirin1*, *HEPN* and *CIQ* in Japanese flounder, and *ghCIq* and interferon regulatory factor 1 in half-smooth tongue sole have been identified and their structures, expression patterns and immune functions further analyzed^[72–74]. Beside genes, a series of micro-RNAs associated with disease resistance have been identified in half-smooth tongue sole^[75]. Chinese scientists have also made great progress in this field. Sun's team from IOCAS has cloned and analyzed the turbot chemokine and half-smooth tongue sole nuclear transcription factor and Hu et al. from the Ocean University of China have cloned and carried out functional analysis

Table 2 Reported high-density genetic linkage maps of aquatic animals

Common name	Maker type	No. of markers	Map size/cM	Linkage groups (No.)	Average interval/cM	Reference
Half-smooth tongue sole	SSR	1024	1667.3	21	1.7	[31]
Half-smooth tongue sole	SNP	12142	n/a	21	0.326	[8]
Japanese flounder	SSR	841	1733.8	24	2.22	[29]
Japanese flounder	SNP	13362	3497.29	24	0.47	[30]
Common carp	SSR	732	3278	50	4.48	[32]
Channel catfish	SNP	54342	3505.4	29	0.22	[33]
Rainbow trout	SSR SNP	2226	2900	29	1.3	[34]
Large yellow croaker	SSR	289	1430.8	24	5.4	[40]
Large yellow croaker	SNP	10150	5451.3	24	0.54	[39]
Silver carp	SSR	703	1561.1	24	2.2	[41]
Bighead carp	SSR	659	1917.3	24	2.9	[42]
orange-spotted grouper	SNP	4608	1581.7	24	0.34	[43]
Turbot	SSR	438	1402.7	24	3.7	[35]
Turbot	SNP	6647	2262.09	22	0.395	[36]
European sea bass	SNP	6706	4816	24	0.72	[44]
Red drum	SSR	486	1815.3	24	3.96	[45]
Bay scallop	SSR	161	849	16	5.9	[46]
Zhikong scallop	n/a	3806	1543	19	0.41	[47]
Pacific white shrimp	SNP	6146	4271.43	44	0.7	[48]

Note: n/a, not available.

Table 3 Physical maps of aquatic animals

Common name	Scientific name	No. of BACs	No. of BACs for assembly	No. of contigs	No. of clones per contig	Average size of contig/kb	Reference
Medaka	<i>Oryzias latipes</i>	64512	41882	n/a	n/a	n/a	[56]
Threespine stickleback	<i>Gasterosteus aculeatus</i>	102178	71211	8397	8.4	n/a	[58]
Tilapia	<i>Oreochromis niloticus</i>	40704	35245	3621	9	389.9	[59]
Atlantic salmon	<i>Salmo salar</i>	200640	185938	4354	34	592	[51]
Channel catfish	<i>Ictalurus punctatus</i>	40416	34580	3307	9.25	292	[52]
Rainbow trout	<i>Oncorhynchus mykiss</i>	192096	154439	4173	34.75	482	[57]
Asian seabass	<i>Lates calcarifer</i>	38208	35265	2865	11	232	[50]
Common carp	<i>Cyprinus carpio</i>	89088	72158	3696	18.26	476	[54]
Half-smooth tongue sole	<i>Cynoglossus semilaevis</i>	33575	30294	1485	20	537	[55]
Zhikong scallop	<i>Chlamys farreri</i>	63641	37046	3696	10	490	[60]

Note: n/a, not available.

of interferon regulatory factors 5, 8, and 9 in Japanese flounder^[76]. Teams from Nanjing Normal University have cloned Toll-like receptors, antimicrobial peptides, immunoglobulin, lectin and other important immune related genes^[77]. A research group at the Institute of Hydrobiology have systematically studied interferon

(*IFN*) genes and their regulatory mechanism in fish and produced a draft *IFN* regulatory network illustrating the role of key genes in the antiviral pathway^[78,79]. Wang's group from Jimei University has systematically identified and characterized components of the TLR and Ran/Rab/Rac small G protein signaling pathways in

large yellow croaker^[80–83]. Chen's group from the Third Institute of Oceanography, State Oceanic Administration has also characterized a series of immune-related genes in this fish, such as toll-like receptor, chemokine^[84,85]. These studies have laid an important foundation for revealing the mechanism of immune regulation and exploring the molecular approaches to improve disease resistance in aquatic animals.

2.4.3 Growth- and reproduction-related genes

Growth is one of the most important economic traits in aquatic organisms, and a number of genes have been found to have important roles in growth and development, such as growth hormone (*GH*), GH releasing hormone (*GHRH*), GH binding protein, GH receptor, insulin-like growth factor, and leptin. In recent years, several GH-inhibiting hormones and their receptors have been cloned from grouper (*Epinephelus coioides*), e.g., two feeding-related receptor genes *npy8br* and *npy2r*, cysteamine and neuropeptide Y. Cysteamine was found to promote pituitary *GH* expression while neuropeptide Y plays a role in GH secretion^[86]. In half-smooth tongue sole, the growth-related genes *GH*, *GHRH*, and pituitary adenylate cyclase activating polypeptide were also cloned and studied. It was found that the expression level of *GH* in pituitary was much higher in females than in male, and this may be an important reason why female tongue sole grows faster than the males^[87–90].

Aquatic animal reproduction is regulated by the neuroendocrine axis, and several hormones including gonadotropin-releasing hormone, gonadotropin-releasing hormone, follicle stimulating hormone, and luteinizing hormone, are involved in reproductive regulation. In recent years, kisspeptin, which can tune gonadal differentiation and other signaling pathways, is considered to be a key regulator in vertebrate reproduction. Cloning and characterization of tilapia *kiss* gene revealed that it was indirectly regulated by thyroid hormone T3. The grouper melatonin receptor gene *MT1* was cloned and its influence on *kiss2* expression was reported. In contrast to *kiss* genes, gonadotropin inhibitory hormone is generally recognized as a negative regulator in the reproductive axis^[91]. In addition to these findings, genes related to other reproductive processes have also investigated and recently, researchers have cloned and characterized *tesk1* and *neur13* in half-smooth tongue sole, suggesting their roles in spermatogenesis^[92,93].

2.5 Screening and application of molecular markers related to important traits in aquatic animals

2.5.1 Sex specific markers

Many aquatic animals exhibit significant sexual dimorphism

in growth, a particularly frequent phenomenon in fish. For example, female flatfish grows 2–4 times faster than the males, while in yellow catfish and tilapia, the growth of males was 30%–100% faster than that of females. Thus, it is very important to screen sex-specific markers and develop sex control technology, in order to enhance aquaculture productivity. However, due to the low degree of sex chromosome differentiation, it is difficult to distinguish sex chromosomes at the morphological level, which hinders the direct selection of specific molecular markers from sex chromosome. Although many studies have focused on screening of fish sex-specific markers, so far male specific DNA fragments or AFLP have only been reported for rainbow trout, Atlantic salmon, African catfish, yellow catfish, rock bream, and tilapia^[94]. In comparison, the number of female specific markers (AFLP and SSR) identified in fish were much lower, for example in half-smooth tongue sole and spotted halibut. Through the comparison of female and male tongue sole genomes, female-specific SSR markers have been identified and a technique for genetic sex identification has been established^[94].

2.5.2 Disease resistance-related markers

In the breeding process, disease is a leading factor that seriously affects aquatic animals and limits sustainable development of aquaculture. Current treatments have several disadvantages including antibiotic residues, environmental pollution, and health risk to consumers. Screening disease resistance-related markers and breeding disease resistant lines would be a promising solution to overcome these problems in aquatic animals and has become a worldwide hot spot. Recently, great progress has been achieved in this field. Norwegian scientists initiated the study of resistance-related *MHC* genes in Atlantic salmon^[95]. Rodriguez-Ramilo et al. found an anti-ciliate related SSR marker, Sma-USC256, in turbot^[96] and Dutta et al. found a RAPD-SCAR marker related to white spot disease (WSD) resistance in *Penaeus monodon*, and subsequently identified another 71bp SSR marker associated with WSD^[97,98]. In rainbow trout, Campbell et al. isolated SNP markers related to bacterial cold water disease and infectious hematopoietic necrosis virus^[99]. Also, Japanese scientists have screened Lymphocystis disease resistance SSR markers, which have been used for vaccine development.

Chinese scientists also carried on the screening of *MHC* genes and SSR markers related to *Vibrio anguillarum* in Japanese flounder, common carp and half-smooth tongue sole. For example, Wang et al. identified two SSR markers associated with *V. anguillarum* disease in Japanese flounder using the established families^[100] and Nie et al. found three EST-SSR markers (MM959, MM4765, MM8364) associated with *V. anguillarum* in *Meretrix meretrix*^[101].

3 Recent progress in aquatic animals breeding research

3.1 Sex control and monosexual breeding in aquatic animals

Many fish exhibit obvious sexual dimorphism in growth and reproduction. For example, in yellow catfish, tilapia and snakehead, male individuals grow 30%–200% faster than the females, while in many marine fish, females can grow 30%–300% faster than males^[102]. Half-smooth tongue sole is the best example of sexual difference in growth, with females being nearly 2–4 times larger than males^[103]. Hence, the study of sex control has important potential applications in fish culture and breeding. In this study, sex specific molecular marker selection is the most important technique for the final realization of sex control. Over the past five years, Songlin Chen from YSFRI directed and accomplished the Special Fund for Agro-scientific Research in the Public Interest (Project: Research on fish sex control and monosexual fry breeding techniques). Artificial gynogenesis and marker-assisted sex control technique were investigated in this project for several important fish in China, including half-smooth tongue sole, Japanese flounder, tilapia, large yellow croaker, grouper, sturgeon, and carp. In yellow catfish, with the identification male-specific markers, an all-male breeding technique has been developed and all-male fry have been obtained. Using female-specific SRR markers in half-smooth tongue sole, the explanation and mechanism accounting for low female ratio (20%) in cultured populations was elucidated. Based on these findings, high female breeding was established and applied. The book “Fish Sex Control and Cell Engineering Breeding” was a landmark publication resulting from this project, being, the first academic monograph in this field^[102].

The main types of sex determination in fish involve genetic or genetic-environmental mechanisms. Temperature can affect sex differentiation and gonadal development. In 59 fish species, sex differentiation was found to be regulated by temperature, most of which showed sex reversal (genetic female to phenotypic male) at high temperature, such as Japanese flounder and half-smooth tongue sole. In 2011, Navarro-Martín et al. found that DNA methylation is important in temperature dependent regulation of sex differentiation in European seabass^[104]. More recently, through whole genome methylation analysis in half-smooth tongue sole, Chen and his colleagues have found that in sex-reversed fish (pseudomale), 38% of the W chromosome genes are methylated. Moreover, they found the methylation pattern of the sex determination gene *dmrt1* can be inherited by the next generation, so offspring of pseudomales are more prone to becoming pseudomales^[8,69]. This explains the increasing proportion of phenotypic males in cultured populations,

which has laid the foundation for studying sex control in half-smooth tongue sole.

3.2 Molecular breeding technology in aquatic animals

Developments in molecular biology technology have driven the breeding of aquatic animals into a molecular breeding era. In the early stages, molecular breeding relied mainly on transgenic techniques and molecular marker assisted breeding. Over the past 10 years, the study of transgenic fish has mainly focused on edible and ecological safety. Auburn University succeeded in regulation of primordial germ cell migration through transgenic techniques. In this system, exogenous drugs can be used as gene expression switches to control the fish gonadal development by regulating exogenous genes, *dead end* and *nanos*^[105]. In November 2015, the US Food and Drug Administration approved the marketing of transgenic salmon with the *GH* gene. This is the first edible transgenic aquatic animal in the world and has significantly promoted the industrialization process of transgenic aquatic animals.

In recent years, only a few Chinese groups have focused on transgenic fish due to the concern about the ecological safety. Therefore, the current work is mainly involved in transgenic fish sterility and ecological safety assessment. The successful production of sterile Yellow River carp expressing the grass carp *GH* gene laid a foundation to promote the breeding of transgenic fish. As an alternative approach, the genomic sequencing and fine mapping of oysters, half-smooth tongue sole, common carp, large yellow croaker and grass carp, have provided abundant genetic resources for genome-wide selective breeding^[7–11]. This technique employs genome-wide SNP markers to estimate the genomic breeding value, aiding the selection of individuals with high genetic effect and breeding value. Such genome-wide selective breeding is accurate, rapid and efficient, and is now considered the most promising animal breeding technology in the world and attracts much attention from international research teams^[106–108]. During the 12th Five-Year Plan, China has launched genome-wide selective breeding programs for large yellow croaker, half-smooth tongue sole, shrimp, oysters and scallop, and these programs have made considerable progress. The preliminary technique platform was established for scallop, oysters, half-smooth tongue sole, large yellow croaker and Japanese flounder. With the application in practical breeding, a US team has developed a 250 K SNP chip for channel catfish^[109] and a Chinese team has explored using a high throughput SNP chip for common carp^[110].

3.3 Breeding of fine lines

China has attached great importance to genetic improvement and cultivation of fine lines of aquatic animals. Over

the past 30 years, with the support of the National Basic Research Program of China, National High-tech R&D Program of China, and National Key Technology R&D Program, research for fine line breeding has been extensively conducted for aquatic animals, including fish, shrimp, shellfish, crabs, sea cucumbers and sea urchins. Various technologies, such as crossbreeding, population or family selection, artificial gynogenesis, molecular marker assisted selection, and BLUP technology, have been used in this process, which aimed to screen the individuals with desired traits, e.g., fast growth, disease resistance and stress resistance. So far, more than 100 new lines of aquatic animals have been developed, including common carp, bream, tilapia, silver crucian carp, Japanese flounder, turbot, large yellow croaker, Chinese shrimp, abalone, oysters, pearl shell and clams. These successes have not only facilitated the breeding process for fine lines of aquatic animals, but have also promoted the rapid development of the aquaculture industry.

3.4 Gene editing technology

Gene editing is a newly developed gene targeting technology, which refers to precise knockout or knockin (introducing an exogenous gene) at any fixed gene locus. Now CRISPR/Cas9 and TALEN are the most frequently used gene editing technologies. Although gene editing has been widely applied in model organisms^[111,112], the reports for aquatic animals are very limited with no report for marine fish. In Atlantic salmon, juvenile fish with tyrosinase and SLC45A2 mutations were obtained by CRISPR/Cas9 technology^[113]. In China, a research team from Southwest University established CRISPR/Cas9 and TALEN technology in tilapia, and knocked out a number of genes related to sex determination/differentiation. These mutants provided powerful resources for studying the mechanism of sex determination/differentiation^[114]. Gene editing has been initiated in common carp, in order to obtain a mutant with fewer intermuscular bones. In comparison, gene editing of marine fish has been more difficult from a technical perspective, e.g., the fragile egg membrane. In China, YSFRI scientists have applied CRISPR/Cas9 and TALEN in marine flatfish and obtained a half-smooth tongue sole with *dmrt1* mutation. The gonad of the mutant showed an ovarian-like structure although it was a genetic male, further suggesting *dmrt1* is the male determining gene in half-smooth tongue sole^[70].

4 Conclusions and prospects

Aquatic genomic and molecular breeding is one of the most active areas in contemporary fishery research, and it is also the direction with the most development potential. With the increasing numbers of deciphered aquatic genomes, international research on aquatic biotechnology

will turn to functional genomics and its application. We proposed that the following five aspects would be main research focuses in the next few years and also the fields where breakthroughs are most likely to take place.

4.1 Genetic dissection of important economic traits in aquaculture animals will become the focus of basic research

At present, some sex determination genes in fish have been identified and the mechanism has been understood to a certain extent. Whereas in most fish and other aquatic animals, major genes related to economic traits have not been identified, and the understandings of regulatory mechanisms for sex determination, disease resistance, stress resistance, environmental adaptation, reproduction and development are quite limited. Whole genome sequencing in many important aquatic animals has provided a massive genetic resource to identify trait-related genes and clarify their regulatory mechanisms.

4.2 Molecular marker assisted sex control technology will remain important in aquaculture

Obvious sexual dimorphism during growth increases the importance of sex control technology in aquatic animals. During the period of the 12th Five-Year Plan, with the support of the Special Fund for Agro-Scientific Research in the Public Interest (Project: Research on fish sex control and monosexual fry breeding techniques), the study of sex specific marker screening, artificial gynogenesis and sex control technology has been carried out in eight important cultured fish of China. The project's achievements have provided valuable experience for developing sex control technology for aquatic animals. With the increasing number of aquatic genomes completed, the screening of sex-specific markers has become more feasible. It is speculated that genetic sex identification and molecular marker assisted sex control will become a new trend in aquatic sex control field.

4.3 Genome wide selective breeding will become a new tendency in aquatic breeding

Based on whole genome SNP markers, genome-wide selective breeding could facilitate the simultaneous selection of multiple traits, e.g., high yield, disease resistance, stress resistance and high quality. This method is especially suitable for low heritable traits such as disease and stress resistance, and therefore shows great potential for productive application with prospects for broader use in agricultural breeding. It has been successfully used for producing improved lines in a number of crops and livestock. During the 12th Five-Year Plan, with the support of the National High-Tech R&D Program of China, Chinese scientists have successfully applied this technique to scallop (two new lines were approved, Haidajinbei of

Yesso scallop and Haifeng 12 of Bay scallop), half-smooth tongue sole and large yellow croaker^[115,116], consolidating that the advanced technology is suitable for aquatic animals. With more aquatic genomes being sequenced, it is predicted that the genome-wide selective breeding will become an effective approach, which widely used in breeding of aquatic animals.

4.4 Gene editing will become a new tool for genetic improvement in aquatic animals

In life sciences, gene editing is the greatest technological development in genetic manipulation of this century, and is also one of the most promising technologies for breeding. With the development and optimization of gene knockout and knockin (TALEN and CRISPR/Cas9) technologies, gene function can be revealed and effectively utilized. Although there are still difficulties for its application in marine aquaculture animals, the recent success of TALEN in half-smooth tongue sole has demonstrated its potential for use for marine animals.

4.5 Multi-omics will become the new research method

With the rapid development of genomic research, the combination of genome, transcriptome, proteome, metabolome and epigenome will become the new research methods to explore and dissect the mysteries and principles of life. To reveal the mechanisms underlying economic traits, such as high yield, disease resistance, stress resistance, and high quality, a combination of static genomic data, with dynamic proteomic, transcriptomic, epigenetic and metabolomic data are required. This multi-omics approach has been rarely reported for aquatic animals and the accomplishment of more aquatic genome sequencing and other *-omes-* would benefit multi-omics study. It can be predicted that multi-omics will have an important role in elucidating the molecular mechanism of important economic traits leading to the establishment of multi-omics breeding technology in the near future.

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