ABSTRACTS

SESSION 11: POPULATION GENETICS

THE STATE OF THE WORLD'S AQUATIC GENETIC RESOURCES

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The sustainable management, development, conservation and use of aquatic genetic resources for food and agriculture (AqGR) are crucial for the continued development of aquaculture, and there is an urgent need to preserve and better manage our aquatic genetic diversity to enhance its contribution to food security, nutrition and livelihoods. It is further evident that expanding genetic improvement of key AqGR represents a significant opportunity to enhance the efficiency of aquaculture production globally. FAO's Department of Fisheries and Aquaculture together with its Commission on Genetic Resources for Food and Agriculture (CGRFA) have implemented a process with FAO member countries to enhance our understanding of the status of the world's aquatic genetic resources for food and agriculture. This process, which has taken many years to complete, was focused on species that are cultured, and their wild relatives, that occur within national jurisdictions. A comprehensive questionnaire was completed by 92 countries around the world; these countries represented >96% of global aquaculture production. The report is in its final stages of publication. This presentation will identify some of the key findings from the report and identify some key priority actions that may arise from this process.

The Report highlights that current data on aquaculture production does not fully reflect the broad diversity of AqGR currently in use. Countries reported farming of 694 species/species items including over 250 that have not previously been reported to FAO with their production statistics. Asia farms the most species, North America the fewest. Available information systems often lack the capacity to record information on strains or stocks, or other farmed types. The two most commonly reported species being farmed were common carp (*Cyprinus carpio*) and Nile tilapia (*Oreochromis niloticus*). Over 200 aquatic species were reported to have been exchanged (import and export) with Nile tilapia (*Oreochromis niloticus*) and North African catfish (*Clarias gariepinus*), the most exchanged species globally. Nine of the ten most widely cultured species were farmed in more countries where they are non-native than in countries where they are native.

The Report further recognises capacity building needs for future assessment of AqGR. The importance of the appropriate application of selective breeding and other approaches to genetic improvement is highlighted, as is the significance of both *in situ* and *ex situ* conservation. The Report identifies that some degree of genetic management is applied in 60% of species reported under culture but recognises aquaculture's continuing dependency on wild stocks, emphasizing the interconnectivity between ecosystem conservation, sustainable fisheries and aquaculture development.

The Report includes five detailed Thematic Background Studies which provide more detailed information on important topics that may not be well covered in the national reports including on: i) genome-based biotechnologies in aquaculture, ii) incorporating genetic diversity and indicators into statistics and monitoring, iii) genetic resources for microorganisms of relevance to aquaculture, iv) genetic resources of farmed seaweeds, and v) genetic resources of freshwater aquatic macrophytes.

It is evident that this process, in itself, has enhanced engagement, built capacity and strengthened information systems related to AqGR in many member countries. In partial response to the early findings from this report FAO has developed a Framework of Minimum Requirements for Sustainable Management, Development, Conservation And Use Of Aquatic Genetic Resources which is already being utilised to support cooperation in AqGR management in Southern Africa. Some of the next steps identified include recommendations to assess, explore and develop mechanisms to monitor the status and trends of AqGR, including through the establishment of a global information system and a registry of farmed types and stocks of wild relatives. Furthermore, FAO has been called upon to prepare a Global Plan of Action on AqGR, underlining the importance of sustained funding for the continued development, use and conservation of AqGR.

LACK OF INTROGRESSIVE HYBRIDIZATION BY NORTH AFRICAN CATFISH (*CLARIAS GARIEPINUS*) IN NATIVE VIETNAMESE BIGHEAD CATFISH (*CLARIAS MACROCEPHALUS*) POPULATIONS

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Abstract

The widespread farming of catfish (genus *Clarias*) hybrids between bighead catfish (*C. macrocephalus, Cm*), and the introduced North African Catfish (*C. gariepinus, Cg*) in the Mekong Delta, where flooding is common, has raised a concern of introgression of *Cg* into the native catfish due to backcrossing escaped hybrids with wild *Cm*. This study employed a novel PCR-RFLP analyses of a mitochondrial (*Cytochrome C oxidase subunit I, COI*) and two nuclear (*Rhodopsin* and *Tropomyosin*) markers and six microsatellite loci to differentiate hybrid individuals from their parental species, and evaluate whether introgression has occurred in wild and cultured *Cm* populations in the region. Results of marker screening showed that sizes and frequencies of microsatellite alleles differed between the two parental species. Four loci exhibited fixed species-specific differences in allele frequency (monomorphic in *Cg* and highly polymorphic in *Cm*). Levels of sequence variation varied at the three genes examined, ranging from 3 to 8% (sequence length 652 bp for *COI*, 802 bp for *Rhodopsin*, and 925 bp for *Tropomyosin*). Based on variable sites identified that were not shared between the two species, 3 restriction enzymes (*SpeI, XcmI,* and *PflMI*) were selected to digest PCR products at species-specific sites within the *COI, Rhodopsin,* and *Tropomyosin* genes, respectively. Results of cytonuclear PCR-RFLP analyses of restriction fragment profiles confirmed that *Cm* is the maternal lineage of cultured hybrids, while nuclear genes (*Rhodopsin* and *Tropomyosin*), and microsatellite loci revealed that hybrids possessed admixed multi-locus genotypes relative to the two parental species. Analyses of six microsatellite loci and PCR-RFLP of two nuclear genes in 473 individuals collected from 11 wild and three cultured populations revealed one F1 hybrid in the wild but no evidence of widespread introgression into native bighead catfish. These results differ from findings from Thailand where intensive catfish culture and deliberate species hybridization is practiced.

Keywords: cytonuclear marker, introgression, hybrid identification, Clarias, PCR-RFLP, microsatellite

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BROODSTOCK CONTRIBUTION AND GENETIC DIVERSITY FROM WEANING TO MARKETABLE SIZE IN A COMMERCIAL COHORT OF DUSKY KOB

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Abstract

Dusky kob (*Argyrosomus japonicus*), is a large Sciaenid finfish that has been identified as an emergent aquaculture species in South Africa due to its high market value. Current production practices are based on mass spawning of unimproved, wild-caught, broodstock. With increasing global competitiveness and market demand, producers are considering genetic improvement of the species through selective breeding. However, broodstock founder populations are relatively small and well-kept pedigree records, to manage the rate of inbreeding, is largely lacking. The mass spawning behaviour of dusky kob further presents a major challenge to the implementation of selective breeding programmes, because single pair matings cannot easily be conducted to record individual family relationships. Applications of DNA parentage analysis to mass spawning species have shown that limited and unequal parental contributions are prevalent, which in turn increases the risk of inbreeding and excessive loss of genetic variation in closed selective breeding programmes. Furthermore, these earlier studies mainly focused on early developmental stages; however, considering that broodstock candidates are generally selected at later stages, it is important to investigate whether grow-out rearing practices (including grading and culling) will further impact on initial levels of genetic diversity and relatedness. During this study, 14 microsatellite markers were used to assess offspring genetic diversity and family compositions throughout the production cycle of three temporal F_1 dusky kob cohorts from a single spawning event. Overall, the F_1 animals presented statistically significant differentiation from the wild, due to a substantial loss in allelic content. Parentage analyses indicated that effectively only 58% of broodstock contributed to the offspring and that family sizes were highly skewed. Two families each had a starting contribution of less than 5%, though neither was eliminated following removal of the smallest animals by culling. Culling did, however, contribute to a significant increase in genetic relatedness in the oldest (*i.e.* market-sized) cohort analysed, and a single family represented 88% of the resulting stock. Results show that culling practices do have the potential to create an increased bias in the representation of families, further complicating the selection of unrelated broodstock candidates in selective breeding programmes.

Keywords: Dusky Kob; Genetics Diversity; Broodstock; Parentage Analysis; Culling; Selective breeding

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USE OF GENOTYPING-BY-SEQUENCING FOR STUDYING THE GENETIC CHARACTERISTICS OF A POPULATION

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Understanding the genetic characteristics of populations is important to help inform management decisions for breeding, conservation and harvest populations. Molecular markers are a resource for making discoveries about a population. Methods have been developed to estimate relatedness between individuals and between populations, to calculate effective population sizes and other measures of genetic structure. These methods assume that co-dominant genotypes from positions across the genome have been obtained for a set of individuals from the population(s). The characterisation of the population benefits from using many molecular markers. Genotyping-by-sequencing (GBS), or other sequencing protocols that subsample the genome, offers a cost-effective approach to assaying many markers, especially if applied at low-depth. One drawback, however, is that alleles, rather than genotypes, are sampled and so it is unclear whether both alleles (for a diploid) have been sampled when all reads give the same base at a position. We investigate methods which calculate measures of population structure by modelling the allele sampling process in GBS, thereby allowing GBS to be used for these studies.

Keywords: Genomics, genotyping-by-sequencing, population structure

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APPARENT GLOBAL LOSS OF GENETIC DIVERSITY IN *LITOPENAEUS VANNAMEI* AND OPTIONS TO RESTORE LOST DIVERSITY

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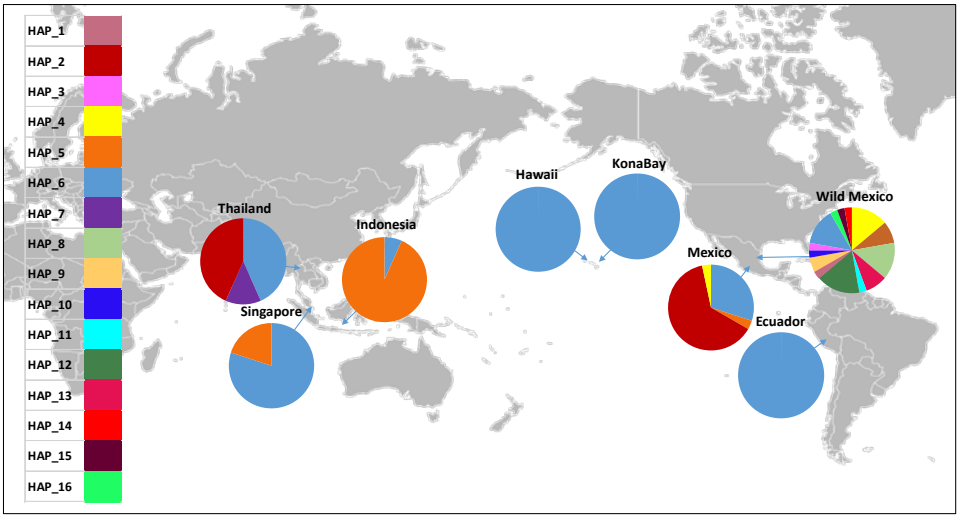
Abstract

The whiteleg shrimp comprises 91% of global aquacultured shrimp production and is important for national economies. The extreme fecundity of shrimp, where few broodstock can produce the next generation, coupled with mass selection, can lead to small effective population (N_e) sizes in captively-bred lines, raising the concern of loss of diversity, globally. Here, for the first time, we made a contemporaneous assessment of the genetic diversity, using DNA microsatellite and mtDNA variation, in hatchery *L. vannamei* stocks that originated from around the Pacific rim and compared stocks with each other and with wild Mexican samples. All hatchery-bred lines had much reduced microsatellite allele counts and mtDNA haplotype numbers, compared with those in wild Mexican samples; N_e sizes were low, as few as two and usually less than 20, suggesting that the contractions in N_e are happening faster than those observed for terrestrial farmed species such as dairy cattle. Without proper pedigree management, most variation within global hatchery stocks of white leg shrimp may soon be exhausted. On a positive note, each hatchery line tended to differ in their types of alleles or haplotypes that they retained, so variation approaching that of the wild may be restored, in part, by crossing lines. This “restoration” may require substantial inter group and international cooperation.

Figure 1. The distribution of the proportions of sixteen mtDNA CO1 haplotypes in seven hatchery lines and a wild sample.



Keywords: *diversity, Microsatellite, mtDNA, shrimp, N_e*

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POPULATION GENOMICS OF COHO SALMON FROM CHILE AND NORTH AMERICA.

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Abstract

Domesticated salmon species can provide key insights on the role of genetic variation in adaptive processes. Comparisons between strains and their source populations provide a powerful means for the detection of signatures of selection across the genome, which in turn can be used to identify genes underlying quantitative traits that have influenced evolution within and between populations. Many domesticated strains of Coho salmon are descended from a few source populations in the Pacific Northwest (USA) and have experienced intense natural and artificial selection for more than 20 generations. To date, genomic information relevant for the assessment of evolutionary processes in Coho salmon populations has not been available for this species. By relying on synteny between species of the genera *Onchorhynchus*, a reference genome was developed and used for SNP discovery. Genetic variability at the whole genome level was then surveyed using a SNP array of 220k SNPs. Further, we developed a comprehensive linkage map based on both haploid and diploid families. The linkage map comprised about 3K cM, based on recombination in females. An analysis on more than 1000 samples from domesticated and source populations showed that North American populations clustered separately from the Chilean populations (K=3). Most divergence could be ascribed to genetic drift. However, the functional analysis of highly divergent SNPs shows that 296 markers linked to coding regions appear to have been be under directional selection (FDR<0.05). The ontology of these markers showed that processes such as immune and inflammatory response, transcriptional factors, and membrane transport and adhesion (i.e. Neural cadherin) were involved. All these loci conform to expected outcomes on the basis of the breeding objectives and domestication in the production environment, in particular growth rate and disease resistance (actin-cytosqueleton). This latter result supports experimental results that show increasing levels of resistance to *Piscirickettsia salmonis,* a major pathogen in Chilean populations of Coho salmon. Overall, the results show that important differences exist between commercial populations, which are likely explained by domestication at the production level and artificial selection for specific traits.

Keywords: Coho Salmon, SNP array population genomics.

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POPULATION GENOMICS AND ADAPTIVE EVOLUTION STUDIES OF CHINESE SEA BASS (*LATEOLABRAX MACULATUS*)

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Abstract

The marine species usually show high dispersal capabilities accompanied by high levels of gene flow. In the other hand, many physical barriers distribute along the continental margin seas and may prevent dispersals and increase population divergence. The divergent populations also developed distinct adaptive characters to the specified local environment in their habitat. These complexities along the continental margin generate serious challenges to population genetic studies of marine species. Chinese sea bass *Lateolabrax maculatus* distributes broad latitudinal gradient spanning from the tropical to the mid-temperate zones in the continental margin seas of the Northwest Pacific Ocean. In the past decades, aquaculture of Chinese sea bass has developed fast in south China, but largely rely on germplasm from north China, which creates great uncertainty on wild sea bass conservation and sustainable aquaculture. It's eager to perform comprehensive investigation of population components and structures of Chinese sea bass along the coast of China. In this case, we performed whole genome sequencing and draft genome assembly of *L. maculatus*. The scaffolds were then integrated into 24 pseudo-chromosomes based on Asian sea bass genome. We collected high throughput SNP genotypes of sea bass populations along the Chinese coast employing whole genome resequencing as well as double digest RAD-Seq pipelines. Genetic divergence among these populations was evaluated and population structure was established. The results suggested that geographically distant populations in the Bohai Gulf and the Beibu Gulf retained significant genetic divergence, which are connected by a series of intermediate populations in between. We also investigated the potential genetic basis of local adaptation correlating with population differentiation of *L. maculatus*. The sea surface temperature is a significantly differentiated environmental factor for the distribution of *L. maculatus*. The correlation of water temperature and genetic variations in extensively distributed populations was investigated with Bayesian-based approaches. Overall, our genome scale analysis provided insight into population divergence and local adaptation of the highly dispersed Chinese sea bass in the continental margin seas.

Key words: Chinese sea bass, Genome sequencing, Genetic divergence, Local adaptation, ddRAD

HIGH RATE OF DE NOVO MUTATIONS IN A BIVALVE SPECIES

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Abstract

Marine bivalves (i.e., clams, mussels and oysters) represent an increasingly important segment of the global aquaculture industry. Domestication of shellfish species is in the early stages, with few organized breeding programmes and a heavy reliance on wild seed. Consequently, the development and use of genomic markers may significantly assist shellfish aquaculture breeding and production. However, molecular genetic markers in bivalves typically exhibit unusual patterns of segregation, which result in deviations from Mendelian expectations, and could potentially limit their use for parental assignment, mapping of quantitative trait loci and genomic prediction. Previous studies have suggested that segregation distortions originate at the larval stage and are related to the linkage of markers to deleterious mutations. This high genetic load has been suggested to be linked to a high mutation rate derived from the high fecundity of bivalve species. However, there is no direct evidence of a high incidence of *de novo* mutations in bivalves. The aims of this research were to (i) evaluate whether *de novo* mutations are frequent in bivalves and (ii) if so, determine their origin: if they arise in the germline or post-zygotically. Five Greenshell™ mussel (*Perna canaliculus*) full sibling families were each raised in two replicates, and the parents and pools of their gametes, and pools of their offspring at different larval stages (5 stages in total) were sequenced using RAD-Seq. The results provide preliminary evidence of a high incidence of *de novo* mutations in (i) male and female gametes and (ii) all larval stages sampled. The screening of the sequencing data, which comprised 0.1% of the mussel genome, revealed that the average number of single base de novo mutations was higher in female than male gametes (222 vs. 28.5, respectively). A significant fraction (~4%) of these *de novo* alleles of germline origin were also present in the first larval stage sampled (i.e., the trochophora). In addition mutations were identified across the different pools of developmental stages sampled, ranging from 254 to 871 across families. These findings have significant implications for genetic analysis and selective breeding, and may be related to the high genetic load observed in bivalves.

Keywords: Mussel, RAD sequencing, de novo mutation, segregation distortion

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GROWTH AND MICROSATELLITE DNA MARKER ANALYSIS OF PHILIPPINE DONKEY'S EAR ABALONE *HALIOTIS ASININA* STOCKS FOR USE IN BROODSTOCK DEVELOPMENT

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Abstract

In spite the availability of established technologies for abalone aquaculture, steady growth of the Philippine abalone industry is limited by the lack of information on sources of good quality breeders and seedstock. This study aimed to generate a preliminary database on possible sources of genetically diverse *Haliotis asinina* stocks for broodstock development. One hatchery-bred stock (HB) and nine wild-bred stocks – Masbate (MS), Palawan (PA), Pangasinan (PN), Cebu (CE), Sagay (SY), Zamboanga del Sur (ZS), Agusan del Norte (AN), Surigao del Sur (SS) and Dinagat Island (DI) were obtained and analyzed using six novel short tandem repeat markers. Seven (MS, PA, PN, HB, CE, SY and ZS) of these founder stocks were used to produce F_1 batches (mainly families) that were later assessed for genetic variability and growth performance. Mean expected heterozygosities (*He*) in the founder stocks ranged from 0.76 to 0.90 where the highest was the PA or Palawan stock and the lowest was noted to be ZS from Zamboanga del Sur followed by SY from Sagay at 0.79. An analysis of molecular variance (AMOVA) denoted significant but low genetic differentiation among the stocks (F_{ST} = 0.075; p = 0.000) where 92.52% of the variation was explained by within population differences. Meanwhile, *He* in the F_1 stocks ranged from 0.72 (SY stock) to 0.89 (PA stock). An AMOVA likewise showed that genetic variability was explained more by intra-stock differences at 89.59%. These genetic diversity indices shall be discussed together with the results of the growth evaluation to enable the identification of local stocks most suitable for breeding and farming.

Keywords: abalone, microsatellite markers, broodstock development, genetic variability

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DEVELOPMENT AND APPLICATION OF GENOMIC TOOLS FOR CLEANERFISH FARMING

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Cleaner fish are a popular biological control solution to manage sealice infestations in commercial Atlantic salmon (*Salmo salar)* farming. To mitigate the impact on wild cleaner fish stocks there is a move towards the captive breeding and domestication of two cleaner fish species; ballan wrasse (*Labrus bergylta*) and lumpfish (*Cyclopterus lumpus)*. Being a new industry, there is demand for genetic and genomic tools to inform stock management and ultimately help work towards closed lifecycle production where selective improvement of farmed stocks would be possible. For both species genomic information is extremely limited, with very few genetic markers currently described. We report here on the results of RAD (restriction enzyme associated DNA) based genome studies that have been used to generate SNPs and microsatellite markers in ballan wrasse and lumpsuckers. We will present how these have subsequently been rationalised down to targeted panels to be employed in addressing specific production questions. In the case of ballan wrasse, a combination of 11 SNPs and 12 microsatellite markers have been used to enhance our understanding of wild population structuring and thus rationalise the source of founder broodstocks. The same microsatellite panel has also been used to assess parental contribution in relation to spawning behaviour, providing information for effective haremic management and the optimisation of broodstock productivity. This panel has also been used to mitigate the risk of inbreeding and potential loss of genetic diversity by identifying potentially related individuals in both the wild sourced production broodstock as well as in F1 broodstock that are being reared for future use. In lumpfish, a 16-marker microsatellite panel has been applied to compare the genetic diversity of wild fish with that of typical aquaculture production batches to provide insights into the potential for future broodstock selection practices. Due to its relatively short generation time, there is also potential to rapidly select for traits of commercial interest. Ongoing work to refine SNP markers to aid such advances will be discussed.

Keywords: cleanerfish, industry, sustainable, SNPs, Microsatellite

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