Marker-aided Genetic Stock Management: Prospects in Philippine Aquatic Biodiversity Conservation and Aquaculture

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Abstract

With the advent of DNA marker-based technologies and applications, genetic stock assessment incorporating molecular marker information has become an important tool in managing resources both for aquaculture and stock enhancement. Local initiatives toward this end have been undertaken by several research and academic agencies particularly those with access to advanced molecular genetic laboratory facilities both in the Philippines and in collaborating foreign institutions. Funds coming from the Philippine Department of Science and Technology and/or international research grants have supported work on commercially valuable species such as tilapia, shrimp, mud crabs, abalone, milkfish and some high value marine fishes with a view of utilizing and in the process, demonstrating the significance of more scientific micro-level assessment of stocks. Information drawn from marker-aided genetic stock evaluation can contribute to a better understanding of the impact of how proper stock management can be more effectively achieved and how this method can gradually translate to improved yields both from culture and fisheries. This paper covers a review of the status of this technology as applied to on-going fish conservation and aquaculture production efforts in the Philippines.

Keywords: DNA markers, genetic stock assessment

Introduction

Being an archipelagic country, the Philippines has vast water resources found inland as well as along an expansive 36,389 km. coastline. Marine areas cover territorial waters, shelf areas, coral reef areas to coastal waters while inland waters consist of swamplands, fishponds, lakes, rivers and reservoirs (Table 1). Hence, aquatic organisms abound which are for the most part, directly extracted and/or produced for human consumption. Current fish production estimates can attest to the richness of such resources. Production from capture fisheries is very diverse, ranging from aquatic plants or seaweeds at 458 metric tons (MT), and fishes at 2,363,221 MT which consist of tuna species (frigate, yellowfin, Eastern little tuna, skipjack), big-eye scad, roundscad, mackerel, anchovies, sardines, squid and slipmouth etc. Meanwhile, farmed species is estimated at 1,840,833 MT for aquatic plants or seaweeds and 767,287 MT for tilapias, carps, prawns, mud crabs, abalone, grouper, seabass, siganids, pompano, oysters, mussels, penaeid shrimps, sea cucumbers, the native catfishes and indigenous species such as the giant trevally, climbing perch, silver therapon, etc.

In 2011, the Philippines ranked seventh among the major fish producers in the world, contributing 2.79% of fish, crustaceans, mollusks and aquatic plants, to global fisheries production (Table 2). Increasing fish production holds a lot of potential in aquaculture since fish breeding and farming technologies are now well established, if not, advanced. As for capture fisheries, the challenge is in protecting the habitats (which serve as breeding grounds), from degradation brought about by anthropogenic activities apart from them being exposed to climatic changes resulting from global warming. Aquatic habitat destruction cause irreversible damage to natural biodiversity of which the Philippines is known for.

Table 1. Aquatic Resources in the Philippines (BFAR, 2012).

MARINE RESOURCES	**Area**
1. Total territorial water area (including EEZ)	2,200,000 sq km
a. Coastal	266,000 sq km
b. Oceanic	1,934,000 sq km
2. Shelf area (depth 200m)	184,600 sq km
3. Coral reef area	27,000 sq km (within the 10-20 fathoms where reef fisheries occur)
4. Coastline (length)	36,289 km
INLAND **RESOURCES**	
1. Swamplands	246,063 ha
a. Freshwater	106,328 ha
b. Brackishwater	139,735 ha
2. Existing fishpond	253,854 ha
a. Freshwater	14,531 ha
b. Brackishwater	239,323 ha
3. Other Inland Resources	250,000 ha
a. Lakes	200,000 ha
b. Rivers	31,000 ha
c. Reservoirs	19,000 ha

Philippine Aquatic Biodiversity

The Philippines is one of several countries in Southeast Asia that has an abundance of diverse terrestrial and aquatic biological organisms. Reference to the Philippines being the center of the center of marine biodiversity is an understatement to say the least. There are aquatic organisms that are successfully bred and farmed in captivity apart from those that thrive in natural waters. Due to overexploitation, illegal extraction and simply an inexcusable disregard for the aquatic environment and its fauna, some species are now considered vulnerable and/or threatened while many are ironically, yet to be discovered and named. Some of the known threatened/vulnerable species are the seahorses (*Hippocampus* spp), sea turtles, abalone, Napoleon wrasse (*Cheilinus undulatus*), sea cucumber (*Holothuria* spp), clams, among others. High value marine species that are often illegally extracted from the wild include the orange-spotted grouper, Napoleon wrasse (which incidentally is also vulnerable), sharks, corals, etc. On the other hand, the Philippines has indigenous species that are known to have commercial aquaculture potential, these are the giant trevally (*Caranx ignobilis*), silver therapon (*Leiopotherapon plumbeus*), climbing perch (*Anabas testudinaeus*), freshwater sardines (*Sardinella tawilis*), to name a few.

Apart from the commercially caught and/or farmed species, several expeditions conducted by foreign scientists in collaboration with local researchers have enabled the collection and subsequent identification of new species. Attempts to document aquatic biodiversity in the Philippines began as early as 1907 when a research vessel, the USS Albatross did a two and a half year survey of aquatic resources in the Philippines. The survey encompassed rocky shores, coral reefs, mangroves, estuaries, deep ocean basins as well as freshwater lakes and rivers (Smithsonian National Museum of Natural History, http://vertebrates.si.edu/ fishes/ albatross/ philippines_exp.html).

Another expedition, under the Panglao Marine Biodiversity Project (PANGLAO 2004), was conducted from May to July 2004 in selected coastal areas in Bohol, Central Philippines to measure aquatic species richness. This involved 70 participants (from 16 countries) who worked on the molluscs and crustaceans collected in 150 km^2 of municipal waters covering Panglao, Dauis, Cortes, Tagbilaran and Baclayon. The samples were taken through different methods (intertidal collection, SCUBA collection, traps, tangle netting, dredging and trawling). An estimated 1200 species of decapod crustaceans and 5000-6000 mollusc species were obtained by the investigators (Bouchet *et al.*, 2009).

The most recent expedition conducted in 2011 by researchers from the California Academy of Sciences (CAS) resulted to about 300 new species being identified and several more yet to be taxonomically described and named. It is said to be the largest CAS expedition that was tasked to implement a 1 1/2 month comprehensive survey of terrestrial and marine diversity found in shallow water reefs, deep sea and terrestrial freshwater areas in the Philippines, mainly to look for new species (http://www.calacademy.org./science/ hearst/).

Biodiversity conservation

Food production especially from fisheries and aquaculture, is generally confronted with numerous challenges, from climate change, habitat destruction, overexploitation, inappropriate fishing practices, indiscriminate stock movement

Table 2. 2011 World Fisheries Production of fish, crustaceans, molluscs and aquatic plants (including seaweeds), by the top ten producers (BFAR, 2012).

Major countries	Total		Fish, Crustaceans, and Molluscs			Aquatic Plants (includes seaweeds)		
	MT	% share	Capture	Aquaculture	Total	Capture	Aquaculture	Total
1. China	66,216,938	37.15	15,772,054	38,621,269	54,393,323	274,060	11,549,555	11,823,615
2. Indonesia	13,601,785	7.63	5,707,684	2,718,421	8,426,105	5,479	5,170,201	5,175,680
3. India	8,879,499	4.98	4,301,534	4,573,465	8,874,999	-	4,500	4,500
4. Peru	8,346,483	4.68	8,248,482	92,200	8,340,682	5,801		5,801
5. USA	5,559,907	3.12	5,153,452	396,841	5,550,293	9,614		9,614
6. Vietnam	5,555,000	3.12	2,502,500	2,845,600	5,348,100	-	206,900	206,900
7. Philippines	4,971,799	2.79	2,363,221	767,287	3,130,508	458	1,840,833	1,841,291
8. Japan	4,755,453	2.67	3,761,176	556,761	4,317,937	87,779	349,737	437,516
9. Chile	4,436,484	2.49	3,063,449	954,845	4,018,294	403,496	14,694	418,190
10. Russian Fed	4,391,154	2.46	4,254,864	128,830	4,383,694	6,639	821	7,460

and poor management, alien species introductions, diseases, pollution, etc. (Table 3). Establishing schemes in maintaining and/or conserving biodiversity is a means of securing these resources in the light of such pressing concerns. Hence proper management of aquatic stocks should be a major consideration.

Managing stocks for biodiversity conservation and aquaculture

Aquatic stock management is a method of dealing with aquatic organisms that are propagated, maintained and utilized for food or other purposes. Any scheme that is adopted to properly manage stocks is done to minimize their depletion in natural waters and in captivity. Management of aquatic stocks may also be done with an understanding and consideration of the genetic structure of individuals, stocks and/or populations. In this context, proper management is done to reduce not only the depletion of these stocks in terms of numbers but also to minimize their genetic deterioration.

Table 3. Current challenges in fish production.

Fisheries	Aquaculture
Depletion of fishery resources due to overexploitation; illegal fishing practices	Declining production due to poor quality seed stock
Genetic contamination of threatened stocks if stock enhancement is not done properly	Low yield due to diseases, improper nutrition caused by poor management practices
Poor catches due to displacement/predation by exotic species	Poor harvests due to displacement/predation by exotic species
Habitat degradation due to anthropogenic causes	Environmental degradation due to anthropogenic causes
Climate change, vulnerability to disasters	Climate change, vulnerability to disasters
Reduction of value of catches due to improper post harvest protocols	Reduction of value of yield due to improper post harvest protocols
Food safety issues	Food safety issues

Stock management can be through conventional means and/or genetic (DNA marker based) methods. Traditional stock management can be done through (a) restocking and monitoring of tagged organisms, (b) regulating fishing intensity/practices through the use of appropriate fishing gears, declaring seasonal fishing ban, etc. and (c) adoption of proper breeding/farming schemes. On the other hand, genetic stock management is implemented with the use of DNA marker methods as supportive tools in planning and managing breeding and farming operations in aquaculture and/or in stock enhancement.

DNA markers as tools for stock management

In managing stocks used in aquaculture and biodiversity conservation, individual tagging/marking is done to enable ease in determining stock characteristics and monitoring stock quality, movement etc. There are several tags/markers, the most common types that describe or define individual aquatic organisms in a stock or in a population are the following: (a) phenotypic description, and (b) physical tags. A third type, which requires knowledge in molecular genetics methods, is referred to as genetic markers. Phenotypic description of individual aquatic organisms can be useful for describing animals at any age. However, as such, these are not permanent for the observable traits (e.g. size, color, metric parameters etc.) are unstable for these descriptions change in response to environmental changes. Physical tags on the other hand, are physical identifiers that can help monitor/trace individual organisms. These can be anywhere from coded microwire tags, numbered physical implant tags, diet tags, fin clips, etc. The main drawback is that these may be invasive and the retention rate is not 100%. Meanwhile, genetic markers or biochemical traits that are detectable as protein variants are considered useful at any age, less invasive (except for protein analyses using allozyme markers), stable and heritable hence may provide the best alternative to the other tags, given the resource, skill and knowledge of molecular marker analysis procedures, and marker variation assessment. Table 4 shows a summary of the characteristics of all three tag/marker types.

Genetic markers such as DNA-based markers allow us to know the genetic make-up of individuals and genetic structure as well as phylogenetic relationships of stocks/ populations. In aquaculture, examining stock performance would be relevant in planning how farmed fish stocks are to be managed to increase yield or production for improved fishfood sufficiency. Stock performance is assessed through economically important traits (phenotypes) that are essentially the physical expression of genes (genotypes) possessed by individual aquatic organisms. It is observed that the higher the genetic variability or the more genetically diverse stocks are, the more fit and better these are in terms of production or performance traits (growth, disease resistance, survival, etc.).

DNA markers can either be simple protein markers known as allozymes or either of two other types, namely: (a) mitochondrial DNA (mtDNA) markers (e.g mtDNA sequence data, mtDNA restriction fragment length polymorphism or mtDNA-RFLP) that are maternally inherited or, (b) nuclear DNA markers (randomly amplified DNA or RAPD, amplified fragment length polymorphism or AFLP, and microsatellite DNA markers or msDNA) which are biparentally inherited. MtDNA and nuclear DNA markers have often been used recently in view of the numerous advances in DNA marker analysis using polymerase chain reaction (PCR), automated sequencing equipment and web-based analysis software (Romana-Eguia, 2006).

Table 4. Characteristics of the different tag/marker types.

Phenotype/observable traits (size, color, etc)	**Physical tags** (diet tags, coded microwire tags, fin clips)	**Genetic markers** (biochemical traits detectable as protein or DNA variants)
Useful for identifying/ describing animals at any age	Tags could not be used for younger animals	Useful at any age/size
Plastic/ unstable	May be lost as the animal grows (tag retention 85%-90%)	Intrinsic, stable
Heritable but expression of the traits are influenced by external factors	--	Heritable
--	Invasive for some tags	Less invasive especially for PCR-based DNA markers; small tissue can be used

Several studies on marker-assisted selective breeding and/or stock enhancement have utilized mtDNA sequencing data and microsatellite DNA marker information as tools in stock management programs. Both methods require knowledge and skills in DNA extraction; primer development; PCR amplification of target gene marker regions; purification of DNA samples; automated sequencing; sequence data analysis and/or fragment analysis, to process microsatellite marker data; and should next generation sequencing (NGS) is done, bioinformatics or genetic data management (this is mainly for primer development and genomics work).

DNA markers: Uses and Applications

DNA-based markers are used to:

1. ***Discover genes, study their structure and function.*** This is referred to as genomics or genome technology research where gene maps and linkage maps are developed. Such maps are used as reference in:
   a. Marker-assisted selection for genetic improvement based on quantitative trait loci;
   b. Development of effective fish vaccines and delivery technologies;
   c. Monitoring antibiotic resistance;
   d. Diagnosis of aquatic animal diseases;
   e. Understanding the mechanisms and the genes involved in viral disease development and management e.g. determining genes involved in viral infection in shrimps (Alenton *et al.*, Tare *et al.*, this proceedings);
   f. Evaluating success in the development of transgenic fish; and
   g. Discovery of genes useful for biotechnology and pharmaceutical purposes.

2. ***Confirm and/or validate the taxonomic identity of individual organisms.*** Examples of such researches are current efforts on barcoding Philippine lake fauna (Aquilino *et al.*, 2011, Aquino *et al.*, 2011) as well as to determine traceability and mislabeling in commercial fishery products (Maralit *et al.*, 2013)

3. ***Elucidate/reveal cryptic biodiversity in marine and freshwater areas in support to the description of historical studies for marine and freshwater biodiversity.*** This is of utmost importance in countries like the Philippines which is known as to be the center of aquatic (especially) marine biodiversity.

4. ***Identify and discriminate populations, stocks.*** This is a common application of genetic markers particularly if marker-based genetic variation between and within stocks is notably high and significant. There have been several studies conducted for this purpose, mainly to generate genetic databases for aquaculture purposes. Locally, genetic differences in the population structure of farmed tilapias (Macaranas *et al.*, 1986, Macaranas *et al.*, 1995, Romana-Eguia *et al.*, 2004, 2005), wild milkfish stocks (Ravago *et al.*, 2002; Ravago-Gotangco and Juinio-Menez, 2004), farmed and natural tiger shrimp populations (Xu *et al.*, 2001),

among others, have been studied by Philippine scientists under internationally-funded projects. At present, there are several recently completed and on-going DNA marker-based stock assessment/ discrimination studies in the Philippines. Among these are on newly developed tilapia strains (Quilang and Basiao, pers. comm.), tiger shrimp wild stocks which have not been previously characterized, mud crab *Scylla* sp, abalone *Haliotis asinina* stocks and milkfish, albeit using microsatellite markers and mtDNA sequence data information. Unless otherwise stated, these on-going studies are spearheaded by SEAFDEC/AQD, funded by DOST PCAARRD under their National R&D Programs, in collaboration with the Tohoku University. Apart from applications in aquaculture, genetic markers can also be used to discriminate species produced from capture fisheries. An example is a study on distinguishing between juvenile yellowfin and big-eye tunas, both being commercially important fishery products (Pedrosa-Gerasmio *et al*., 2012).

5. ***Monitor changes within and between stocks and determine inbreeding.*** Changes in individual organisms comprising stocks which are either simply domesticated or bred selectively to promote genetic improvement, can be detected not only phenotypically but also at the molecular level using genetic markers. A study on the use of microsatellite markers in determining genetic variability changes in selected and unselected tilapia stocks, have demonstrated this application (Romana-Eguia *et al*., 2005). In the aforementioned study, the difference in the specific growth rate of a mass-selected and a control line of tilapia showed a reduction from 0.034% /day to 0.016% /day after four generations. Meanwhile, a higher increase in the inbreeding coefficient was noted in the mass-selected line (108%) as compared to the increase in the inbreeding coefficient of the control line (64.2%) based on genetic variability in four generations of the stocks using five microsatellite marker loci.

6. ***Compare between wild and hatchery stocks.*** An example of this is an on-going study on several wild and hatchery stocks of milkfish collected from local sources as well as from countries (Indonesia and hopefully Taiwan) where most of the commercially imported milkfish seed stock are obtained. This current undertaking has to date identified nine working microsatellite markers that will be used for genetic characterization. The ultimate aim is to enable the identification of sources of good quality milkfish broodstock and genetically improve the Philippine milkfish using marker-aided methods.

7. ***Identify specific markers or quantitative trait loci (QTLs) correlated with fitness and/or quantitative traits for use in marker-assisted selective breeding.*** This is possible if linkage map information is available to allow the determination of QTLs. Marker-assisted selection has been

Table 5. QTL research conducted in different farmed species (Liu, 2007).

Common name	Species Name	Traits	References
Salmonids			
Atlantic salmon	*Salmo salar*	Body weight, condition factor, disease resistance, sex	Reid *et al.*, 2005; Moen *et al.*, 2004; 2004c; Grimholt *et al.*, 2003; Artieri *et al.*,, 2006
Rainbow trout	*Oncorhynchus mykiss*	Albinism, condition factor, disease resistance, growth rate, killer cell-like activity, meristic traits, pyloric caecae number, precocious maturation, spawning date, upper thermal tolerance	Danzmann *et al.*, 1999; Palti *et al.*, 1999; 2001; Ozaki *et al.*, 2001; Perry *et al.*, 2001; 2005; Robison *et al.*, 2001; Martyniuk *et al.*, 2003; Nichols et al., 2003a; O'Malley *et al.*, 2003; Somorjai *et al.*,, 2003; Khoo *et al.*, 2004; Nichols *et al.*, 2004; Zimmerman *et al.*, 2004; 2005; Moen *et al.*, 2004b; Reid *et al.*, 2005; Rodriguez *et al.*, 2005
Coho salmon	*Oncorhynchus kisutch*	Flesh color	Arenada *et al.*, 2005
Artic char	*Salvelinus alpinus*	Temperature tolerance, growth rate, condition factor	Somoraj *et al.*, 2003; Tao and Boulding 2003; Reid *et al.*, 2005
Tilapia			
Tilapias	*Oreochromis spp.*	Body and peritoneum coloration, cold tolerance, disease resistance, growth rate, immune response prolactin expression level, survival, sex determination, sex ratio, stress response	Shirak *et al.*, 2000; 2002; 2006; Streelman and Kocher 2002; Palti 2002; Cnaani *et al.*, 2003; 2004a, 2004b, 2004c; Lee *et al.*, 2003; 2004; 2005; Moen *et al.*, 2004a
Carp			
Common carp	*Cyprinus carpio*	Cold tolerance	Sun and Liang 2004
Molluscs			
Eastern oyster	*Crassostrea virginica*	Disease resistance	Yu *et al.*, 2006
Shrimp			
Kuruma prawn	*Penaeus japonicas*	Body weight, total length and carapace length	Li *et al.*, 2006
Others			
Zebrafish	*Danio rerio*	Behavioral and morphological differentiation	Wright *et al.*, 2006

done on several commercial aquaculture species (refer to Table 5). It has yet to be done on local species.

8. ***Assess success of genetic manipulation methods such as polyploidy induction, gynogenesis and transgenesis.*** DNA markers can be used to distinguish the genetic make-up of manipulated stocks against normal, non-manipulated or genetically unmodified stocks. There are no local initiatives towards this end for development of genetically modified aquatic organisms is not encouraged in the Philippines.

9. ***Monitor the fate of stocks after deliberate or accidental release in the wild.*** This is important in conservation and stock management research. DNA markers can be utilized to trace the impact of stock introductions/ enhancements in sites where natural populations are noted as depleted. Recently, a study co-funded by the Japan Society for the Promotion of Science with SEAFDEC/AQD on the genetic impact of reseeding on natural abalone stocks in the Sagay Marine Reserve, Philippines using molecular marker profiles, was completed in collaboration with the Tohoku University.

Conclusion

Several research initiatives that show the application of DNA marker technology in the Philippines was presented. In most instances, micro-level stock analysis through DNA marker applications were undertaken to provide an effective means of monitoring and managing stocks both for conservation and production purposes. Local scientific undertakings are now possible with trained human resources using laboratory facilities established in national and international research and academic institutions, through research linkages.

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