Genetic Impact Determination of Farmed Fish on Native Fish by mtDNA Markers

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Abstract: Aquaculture is one of the world's fastest-developing and growing food-producing sectors. The subsector's expansion commenced in the 1970's, actuated by move forwards in hatchery technology and pond husbandry. Aquaculture and especially fish farming, however, have been discussed as negative potential effects on environment. The negative potential effects are, direct mortality, loss of biodiversity, tainting of wild species. Moreover these are disease transmission to other species, displacement of wild fish from natural habitat.
Although these negative potential effects, cultural fish represent genetically exogenous populations or crosses between them. Some pauper gen pool of cultured fish population can develop with fertile gen pool of natural fish population or just the opposite of them.
It can be said that cultured fish typically constitute gene pools. We need an observation which brings an urgent focus for conservation between natural populations and spawning populations. In this review it was observed negative genetic impacts of escaped farmed fish population on wild fish population by mtDNA markers.
Keywords: Genetic impact, escaped farmed fish, molecular markers, mtDNA markers

INTRODUCTION

Aquaculture has elaborated rapidly over the decades due to growing in the world population. Especially, the growing trend in aquaculture has expanded enormously since 1970's (Brugère and Ridler, 2004; Tacon, 2003; Duarte, Marba &Holmer, 2007; Flassch and Leborgne, 1994). Aquaculture is not applied only for consumption, but also is applied for recreation, decoration, bio manipulation, the protection of threatened species and research (Welcomme & Bartley, 1998; Utter & Epifanio, 2002). Inevitably, fish is the most important source of protein for many people, especially in the developing locations of the world (Rodwell et al., 2003). In addition to this, it has substantial activity for economy, providing jobs and investment opportunities (Smith, 2007).

The aquaculture activities has positive contributions, although, it has been lead to some problems, such as habitat destruction, blockage of migration routes, and changing of genetic structure of native fish stocks (Ferguson et al, 1995). Many cultured fish species used in aquaculture are usually genetically different from local wild populations. In the case of escape from the aquaculture sites, the breeding of escaped fish with native ones have result in genetic changes in wild populations. The attempt has been focusing on understanding of the process of changes in genetics of wild population using mitochondrial DNA molecular markers. Because, genetic diversity measurement in wild fish populations is significant for understanding and effective management of these populations (Farias, 2001; Knibb, 2000; Okumus and Cifci, 2003; Aquilino et al., 2011; Cawthorn et al., 2011; Lakra et al., 2011; Mecklenburg et al., 2011; Kartavtsev et al., 2009; Ward et al., 2005; Hubert et al., 2008; Nicolas et al., 2012).

Owing to increase the contingency that gene trees reverberate the topology of the species tree which is the true a taxons gene pool, phylogenetic hypotheses comprehended from many dissimilar and independently transmitted loci must be compared. Therefore, mitochondrial DNA (mtDNA) can use specification a phylogenetic analysis based on diversity within the fishes subfamily.

However, high resolution of many methods of DNA analysis, up to the probability to detect individualistic fish, including those aimed at detecting within-population coactions. Based on mtdna, new approaches are developed to resolve the problems that were not solved by means of investigating fish morphology, physiology, and behavior.

ESCAPED FARMED FISH

Escapes of fish from sea-cage or inland water-cage aquaculture have typically been thought of as referring to juvenile and adult fish. Such escapes have been reported for almost all species presently cultured around the world, including Atlantic salmon *Salmosalar*, Atlantic cod *Gadusmorhua*, rainbow trout *Oncorhynchusmykiss*, Arctic charr *Salvelinusalpinus*, halibut *Hippoglossushippoglossus*, sea bream *Sparusaurata*, sea bass *Dicentrachuslabrax*, meager *Angyrosomusregius* and kingfish *Seriolalalandi* (e.g. Soto et al. 2001, Naylor et al. 2005, Gillanders& Joyce 2005, Moe et al. 2007a, Toledo Guedes et al. 2009).

Jørstad (et al. 2008) focused on a second form of escaping, fertilized eggs spawned by farmed individuals from sea- cage facilities, or so-called 'escape through

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spawning. This situation caused a redefinition of the term 'escapes from aquaculture'. This new term should include the escapement of fertilized eggs into the wider marine environment (Jensen et al. 2010). Fish escape incidents from sea-cages have been reported for almost all species presently cultured across Europe, including many different ones. The amount of the fugitive fish has an important place on the ecological and genetic effects. According to Naylor (et al., 2005) escapes can have detrimental genetic and ecological effects on populations of wild conspecifics. Numbers about escapes incidents can be find in some researchers studies.

Farm fish escape may have an indirect impact on the genetic composition of wild populations. Ferguson (et al., 2007) claim this situation may appear due to behavioral, ecological and disease interactions with the wild population. He also claims that these reduce the success of wild fish population and increasing genetic drift. Beside this event, a serious problem may occur: diseases. They may come along from aquaculture and could have negative consequences for the long-term persistence of the species in the wild (De Eyto et al. 2007). Escapes from Norwegian salmon farms in 2002, 2003 and 2004 were shown in figure 1.

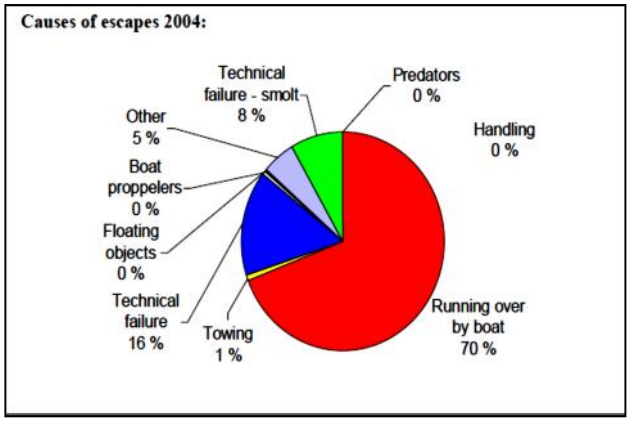

Figure 1. Causes of reported escapes from Norwegian Salmon farms in 2002, 2003 and 2004. Data source: Directorate of Fisheries. Figures from Valland (2005).

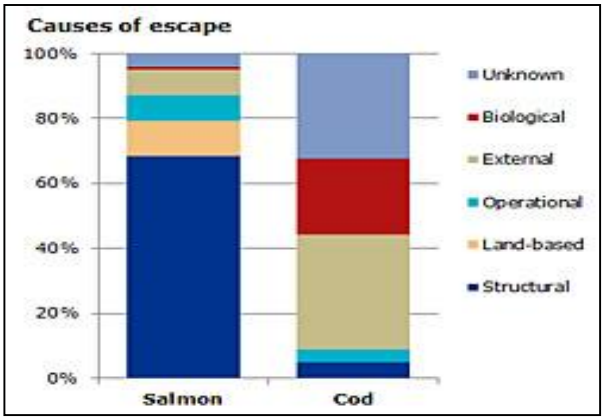

Figure 2. Causes of reported escapes farmed salmon and farmed cod fish in from 2006 to 2009. Data source: http://cdn.phys.org/newman/gfx/news/2013/1-satellite(4).jpg

Aquaculture escapes are seen as a serious threat to wild fish populations. Lack of technical and operational failures in fish farming equipment causes escapes. For instance, 3.93 million Atlantic salmon *Salmosalar*, 0.98 million rainbow trout *Oncorhynchusmykiss* and 1.05 million Atlantic cod *Gadusmorhua* escaped from 2001 to 2009 in Norway (Jensen et al., 2010). Another problem about escapes is in topics of genetic and ecological effects. It is regarded as a problem for the future sustainability of sea-cage aquaculture (Naylor et al. 2005). Naylor described this as a future sustainability of sea-cage aquaculture problem. Scientific Advisory Committee (2009) claimed over 325 million Atlantic salmon are held in sea-cages in Norway. It is known that large numbers of population fish are caged in farms. But wild fish population is lesser then caged fish. Here importance of escapement occurs about ecological and genetic impacts (Jensen et al 2010).

Figure 3. Examples of the major structural causes of escape incidents: (A) progressive mooring failure; (B) breakdown and sinking of steel fish farms; and (C) abrasion and tearing of nets. Photo: Jensen et al (2010).

Ecological interactions have genetic effects in cultured and wild fish interbreeding zone. Population interactions are dependent on the state of the wild population in terms of carrying capacity, genetic

population structure and integrity (Weber & Fausch, 2003).

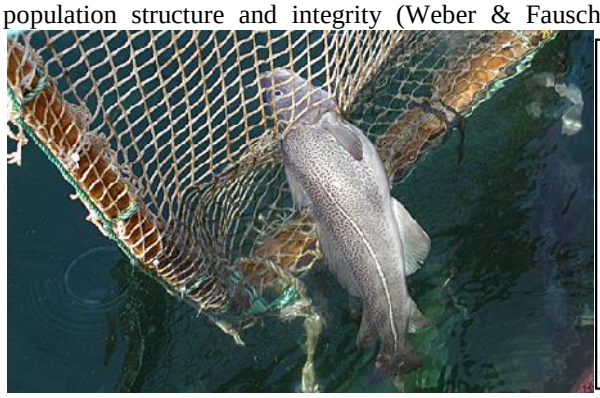

Figure 4. A clear sign of cod curiosity: Instead of swimming around a panel of netting, the fish has tried to swim through an impassable hole. Photo: Project on escape free net cages for cod. (http://cdn.phys.org/newman/gfx/news/2013/1-satellite(7).jpg)

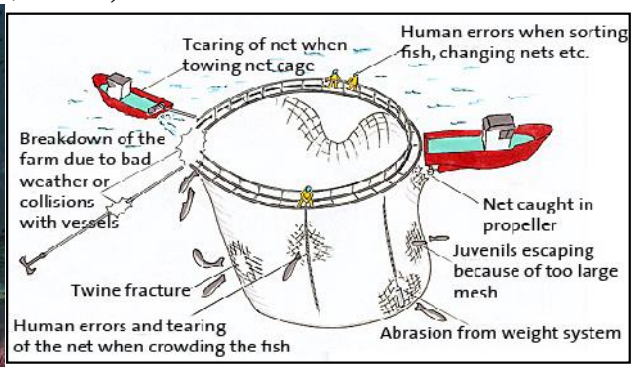

Figure 5. The illustration shows common escape factors with special focus on holes in the nets and sea-cage operations. Credit: Mats A. Heide, Sintef. (http://cdn.phys.org/newman/gfx/news/2013/1-satellite(6).jpg)

Accidental releases from sea cages may happen in aquaculture farms. In these cases of accidental escapes, massive numbers of fish enter the wild. Dimitriou (et al., 2007) claims market requirement for larger fish caused the release of gametes by gilthead sea bream spawners from the cages in the past. Related with this study, Sola (et al., 2007) studied on the impact of these activities on wild gilthead sea bream populations.

MtDNA Molecular Marker Used to Determine Genetics Distortion in Natural Fish Stocks

Mitochondrial DNA (mtDNA) analysis is being increasingly used in recent population and phylogenetic surveys of organisms. Brown (1985) studied on vertebrate species in literature. It was shown that sequence divergence accumulates more rapidly in mitochondrial than in nuclear DNA in his study. This causes to a faster mutation rate in mtDNA that may result from a lack of repair mechanisms during replication (Wilson et al., 1985). According to Briky (et al., 1989) it has smaller effective population size due to the strict maternal inheritance of the haploid mitochondrial genome. However, Meyer (1993) claims different parts of the mitochondrial genome are known to evolve at different rates in his study. Aquacultured Atlantic salmonis genetically different from local wild populations. They usually are being derived from geographically remote populations. (Cross and Challanain, 1991; Youngson et al., 1991).

37 years ago, ribosomal RNA sequences have been used for determination of species diversity (Woese and Fox, 1977). MtDNA molecular weight has become a target in the late 1980s (Avise 1994). In this scope mainly mitochondrial (mt) cytochrome b (Cyt-b), cytochrome oxidase subunit 1 (CO1) and 16S rRNA-targeted genes and species, genus and family separations could be done successfully (Baharum and Nurdalila 2012; Weight et al. 2012; Hebert et al., 2004). According another study, 16S gene is 2.5 less variable than the Cyt-b and CO1 genes. Here, it is seen that discriminatory power is smaller. Suggesting DNA markers for determining species in the Praomyini tribe is a valid approach using the CO1 and Cyt-b genesthan the 16S gene (Nicolas et al. 2012).

Martins et al.(2003) discussed the genetic variation of natural populations of *L. elongates*. Its aim was to present a preliminary assessment of the genetic variableness of six wild populations.

Research based on the nucleotide sequence of a segment of the non-coding control region (D-loop) of the mitochondrial genome. The results were useful for recovery efforts and to the biodiversity maintenance of this fish species.

During analyzing studies of mtDNA in European anchovy *(Engraulisencrusicolus)* a large number of mitotypes exposed two distinct clusters (phylads) (Magoulas et al, 1996). Phylogenetic analysis of *Engraulisencrusicolus*, mtDNA provides a reconstruction of population history in the Mediterranean, which is consistent with the geological information. Some points of phylogenetic analysis are given below.

- Phylad A consists of one common mitotype and many rare secondary mitotypes.
- Phylad B has a complex pattern of mitotype connectedness, high nucleotide diversity, and a large number of homoplasious changes.
- It is suggested that the two phylads evolved in isolation from each other is the result of a secondary contact.

Fish barcoding upgrade is made in many different geographical areas. MtDNA reflects a considerable degree of intra-species diversity (Aquilino et al.., 2011; Cawthorn et al.. 2011; Lakra et al.. 2011; Mecklenburg et al.. 2011; Kartavtsev et al., 2009; Ward et al.. 2005; Hubert et al.. 2008).

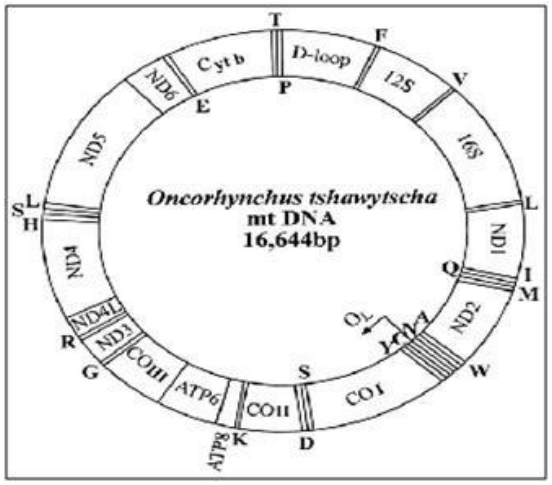

Figure 6. The organization of the mitochondrial genome of *O. tshawytscha.*
Photo: http://www.scielo.cl/fbpe/img/bres/v36n2/fig25.gif

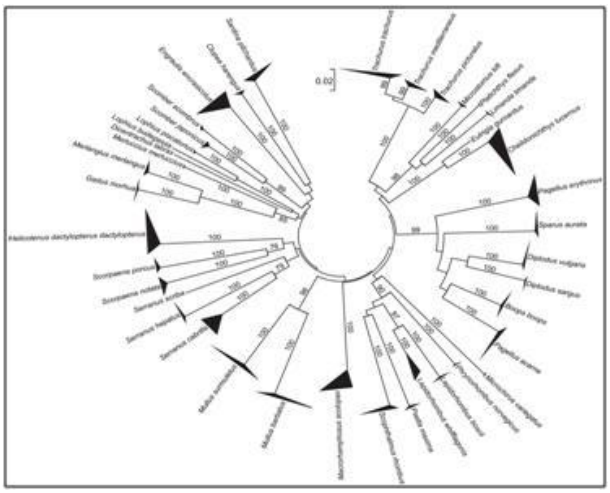

Figure 7. Phylogenetic analysis (cyt *b*). Neighbour Joining tree for partial sequences of the mitochondrial cytochrome b gene of fishes from European seas.
Photo:http://www.plosone.org/article/fetchObject.action?uri=info:doi/10.1371/journal.pone.0012620.g004&representation=PNG_I

Selective pressure and levels of inbreeding aquaculture, natural and non-artificial environment varies in the same. For this reason, the performance in terms of growth and survival of natural and cultural bream lines which might have different growing conditions. When there are not good genetic programs, regional fish farming enterprises insufficient selectivity studies and outnumbered in the breeding populations were trying to do with and is an unplanned selectivity intensive inbreeding has led to the emergence of farming. This situation has put pressure on the genetic diversity of wild populations to extinction even been reported to cause (Knibb, 2000).

Levels of selection pressures and inbreeding may vary in different natural and artificial environments. Therefore, wild and captive sea bream strains may take issue for growth and survival performance in immurement. Choice of the best existing strains could equal the genetic gains made by years of within-strain selection using inferior strains. Hence, strain testing should proceed within strain selection (Gjedrem, 1998). It seem that performance in captivity can be assessed only by direct experimental trials and cannot be predicted a priori by indirect measures of genetic variation, including allozyme, mitochondrial and microsatellite polymorphism (Bentsen and Gjerde 1994).

Bernatchez (et al., 1991) used mtDNA restriction analysis to assess phylogenetic patterns 21 taxa of the subfamily *Coregoninae* in his study. The genus *Prosopium* formed a very distinct group differing by 10 % (squence divergence estimate) from other species. *Coregonus* and *Stenodus* species were closely related, diverging by sequence divergence estimates of less than 5-6 %. In the other study, they show us that the brown trout *Salmotrutta L.* presents an elaborate pattern of morphological and life-history degree of diversity. This prevents the understanding of the evolutionary history of the species; it has led to significant taxonomic confusion.

The phylogenetic kinships among morphologically and relating to geography remote brown trout populations from side to side western Europe, they determined the DNA sequence variation in segments of the mitochondrial control area of activity for 151 individuals representing 24 populations (Bernatchez et al., 1992).

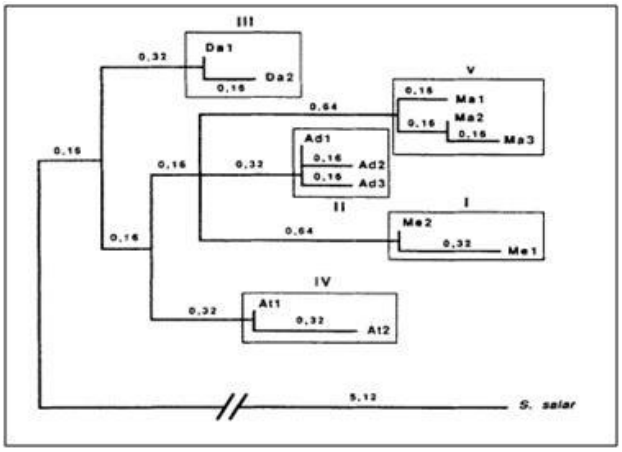

Figure 8. Sequences for two segments of the mtDNA control region, type Atl, from *S.trutta*. (Bernatchez et al., 1992).

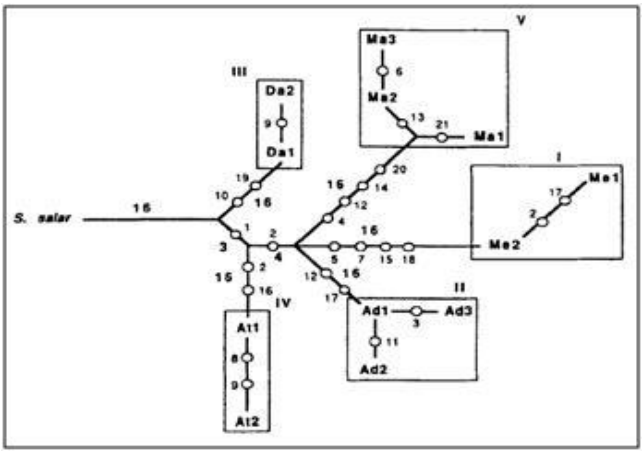

Figure 9. Variable nucelotide positions of 12 genotypes resolved among 151 *S. trutta*mt DNAs sequenced. Nucelotide at each position is given for genotype Atl. For other genotypes, nucleotides are given when different from ATl, while identity is indicated by dashes. Asterisks indicate deletions (or insertions) (Bernatchez et al., 1992).

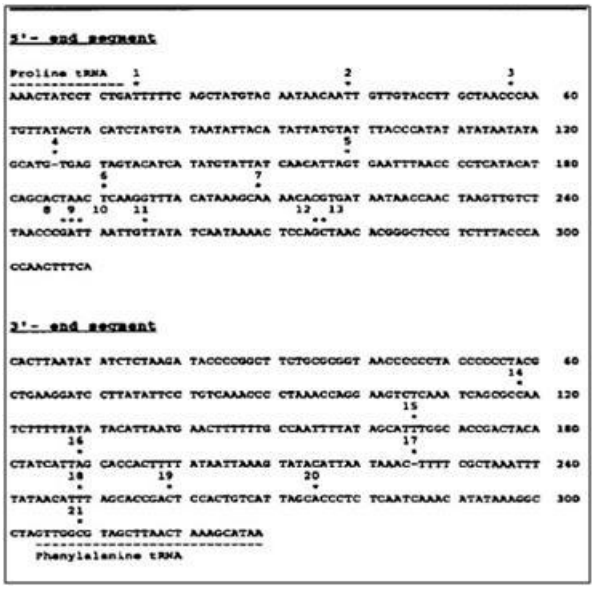

Figure 10. Unconshained branch-length phencF gram clustering 12 genotypes observed among 151 *S.trutta* mtDNA sequences. Branch lengths are given on the tree. *Salmosalar* was used as an outgroup taxon (Bernatchez et al., 1992).

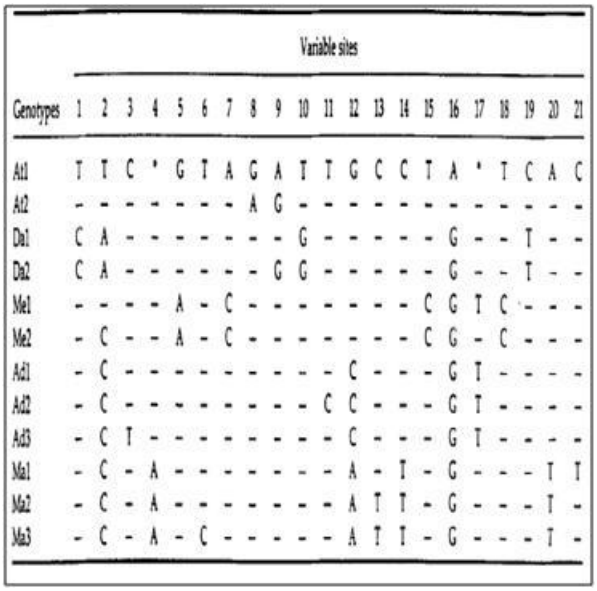

Figure 11. Majority-rule consensus network resulting from the 16 equally most parsimonious trees found by the MIX program.

The network was rooted using *S.salar* as an outgroup taxon. Open circles indicate mutational events and small character numbers refer to the variable nucleotide positions (Figure 8). Bold character numbers refer to the numbers of trees out of 16 in which particular groupings were observed (Bernatchez et al., 1992).

MtDNA and rDNA give different results in experimental tests. MtDNA phylogeny of cichlid fish is presented for the most taxonomically. Test results show that 16S rDNA data establish with confidence relationships among major lineages of cichlids, with a general pattern congruent with previous morphological studies and less inclusive molecular phylogenies based on nuclear genes. Based on a large number of South American genera, the Neotropical cichlids are defined as a monophyletic assemblage and shown to harbor significantly higher levels of genetic variation than their African counterparts (Farias 1999). Skaala et al. (1990) claims farmed fish are genetically distinct from those of the native populations in the rivers.

In order to resolve divergences at many taxonomic levels, the mitochondrial cytochrome b (cyt-b) gene is widely used. Farias focuses mainly on the utility of cyt-

b as a molecular marker for inferring phylogenetic relationship at various levels within the fish family *Cichlidae*. A total of 78 taxa were used representing all the major groups in the family *Cichlidae* (72 taxa) and other families from the suborders *Labroidei* and *Percoidei*. Relative rate tests detected significantly long branches for some taxa (LB taxa) which were composed mainly by dwarf Neotropical cichlids. An improvement of the phylogenetic signal, as shown by the four-cluster likelihood mapping analysis, and higher bootstrap values were obtained by excluding LB taxa. Despite some limitations of cyt-b as a phylogenetic marker, this gene either alone or in combination with other data sets yields a tree that is in agreement with the well-established phylogeny of cichlid fish (Farias 2001).

MtDNA Cytochrome *b* (cyt-*b*) has been considered one of more useful genes for phylogenetic work, and is probably better-known mitochondrial gene with respect to structure and function of its protein product (Esposti et al., 1993).

DISCUSSION

Genetic enhancement and modification may be desirable for some wild populations, and there may be specialized application, such as artificial urban fishing environmental, where genetically modified wild fish may be of great benefit. Unique distinctive and representative populations would be identified, protected and preserved. Some areas and populations would be designed for genetic improvement to meet recreational goal or to restore or enhance genetically damaged populations. Some areas should be designed for the intentional mixing, deposition and propagation of many genotypes to form large diversified living gene banks for future utilization in fisheries and aquaculture.

Moreover, based on the all countries experience of dealing with the escapes problem, we recommend a range of measures for other countries to introduce effective anti-escape measures. We outline these principles below in 5 steps, and discuss how some of these measures may also be improved;

A. Obligatory reporting of all escape occurrences, involving: (1) a delineation of the marine-net cage technology interested (2) the number of escaped fish and their size; (3) classification of the functional influencing factor or environmental stipulations at the time of escape; and (4) an appraised cause of escape.
B. A determined mechanism to assemble, analyze and learn from the compulsory reporting. This knowledge must then be efficiently disseminated to equipment providers and fish farmers so improvements can be made.
C. Escapes determined by farmers are often inaccurate, we advise to use technical equipment to find out the causes of ‘large-scale’ escape incidents. Learning from each large-scale escape event would assist recommendations for the design and properties of sea-cage systems and help improve technical standards.
D. A technical standard table for sea-cage aquaculture equipment should be improved with an independent mechanism. These standards may upgrade aquaculture system.
E. Daily operations in fish farming such as correct anchoring, mooring, connecting net-cages to floaters have potential risks for escaping. Therefore, these key processes should be identified, and mandatory training of staff who undertakes these processes would likely reduce human errors that lead to escapes.

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