



COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

Item 6.2 of the Provisional Agenda

Twentieth Regular Session

Rome, 24–28 March 2025

DRAFT GUIDELINES ON GENETIC MANAGEMENT OF STOCKING PROGRAMMES FOR AQUATIC SPECIES

At its Second Session the Intergovernmental Technical Working Group on Aquatic Genetic Resources for Food and Agriculture (Working Group) recommended the development of a number of voluntary guidelines and frameworks, including¹ on stock enhancement of aquatic genetic resources for food and agriculture (AqGR).

In, 2022, FAO started a process towards the preparation of guidelines focusing on genetic management of stocking programmes for aquatic species. In line with the recommendation of the Working Group, the Commission, at its last session, welcomed the ongoing development of such a document and recommended that FAO finalize the guidelines for review by the Working Group at its next session.²

This document contains the final draft *Guidelines on Genetic Management of Stocking Programmes for Aquatic Species*. The draft guidelines fall into five main sections: the introduction; the scope of the guidelines; the objectives of the guidelines; the basic management and genetic principles on which the guidelines are based, and the key considerations for planning, implementing and monitoring stocking programmes. As recommended by the Working Group,³ the Guidelines also address the issue of translocations for stocking purposes.

The draft Guidelines have been reviewed by the Members of the Working Group and have been revised on the basis of their feedback. The Fifth Session of the Working Group recommended their finalization for consideration by the Commission at its Twentieth Session.⁴

¹ CGRFA/WG-AqGR-2/18/Report, paragraph 25.

² CGRFA-19/23/Report, paragraph 116.

³ CGRFA/WG-AqGR-4/24/Report, paragraph 17.

⁴ CGRFA/WG-AqGR-5/24/Report, paragraph 14.

Draft guidelines for genetic management of stocking programmes for aquatic species

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1. Introduction

The Second Session of the Intergovernmental Working Group on Aquatic Genetic Resources for Food and Agriculture (Working Group) called for the development of a range of voluntary guidelines including practical guidelines on genetic management in stock enhancement which include culture-based fisheries and other types of stocking of hatchery reared farmed types into the natural environment. The Fourth Session of the Working Group welcomed and reviewed the outline of these guidelines and recommended that they include translocations of wild relative genetic resources for stocking purposes, in addition to stocking of farmed types.

Guidelines on general issues regarding management of genetic resources in aquaculture and stocking programmes have been published earlier by FAO and address genetic improvement, dissemination of genetically altered organisms, risk analysis, economic considerations, culture-based fisheries, and *in situ* and *ex situ* conservation (FAO 2008).⁵ Furthermore, two important and well cited publications make specific reference to genetic issues with regard to responsible approaches to marine stocking programmes (Lorenzen, Leber and Blankenship, 2010 and Grant *et al.*, 2017), providing respectively, useful criteria for designing biological technical components of stocking programmes and, guidance on a stepwise approach to responsible managing genetic resources in relation to stocking programmes. The guidelines presented here draw from those early guidelines and these key publications, going into further detail and presenting case studies on a variety of ‘stocking’ strategies. These guidelines are intended to be used by international, regional and national agencies (including FAO), aquaculturists, non-governmental organizations, and academic researchers.

Aquatic genetic resources (AqGR) are the basis for long-term survivability and evolution of species including wild stocks and farmed types. AqGR are in many areas endangered through *inter alia* mismanagement, over-fishing, alien invasive species (including their escape from aquaculture) and habitat loss. Stocking programmes have been used to address these issues but frequently have not met desired objectives. Often aquatic organisms that are produced in a hatchery and then released into natural or modified water bodies will not reproduce or may not even survive; use of inappropriate genetic resources can be a contributory factor to such failures. Additionally, the release of hatchery-raised organisms, i.e. farmed types, may present risks to the survivability of closely related species if genetic resource management is not considered, and can also result in significant and long-term genetic changes to the status of conspecific populations. Deliberate translocation of species from one location to another, especially between watersheds or ecoregions, even though they have not generally been genetically changed by domestication, can have some similar consequences. Therefore, a genetic resource management plan for stocking programmes (see below for definitions of the different types of stocking programmes that can be implemented) is required. The genetic management issues considered for stocking programmes must necessarily be different from those in genetic improvement programmes for aquaculture which have different objectives and are influenced by different factors.

‘Stocking’ is a general term that includes three broad rationales, each with slightly differing implications on how genetic resources should be managed, and each with differing levels of risks imposed on con-specifics and related species. Therefore, these guidelines:

- define common terms that are necessary for understanding the different rationales and objectives of ‘stocking’;
- outline the risks and benefits (with a focus on genetics related issues) of stocking farmed types and translocating stocks, and describe how risks and benefits can be analyzed;
- provide guidance on genetic resource management in relation to the different objectives of stocking;
- provide guidance on founder stock selection and broodstock management prior to stocking;

⁵ FAO. 2008. Aquaculture development. Genetic resource management. FAO Technical Guidelines for Responsible Fisheries, No. 5, Suppl. 3. Rome. 125 pp.
<https://openknowledge.fao.org/handle/20.500.14283/i0283e>

- provide guidance on release strategies with regard to genetic implications; and
- call for improved monitoring and reporting on the genetic impacts (good and bad) of stocking programmes.

Genetic resource management is only one of many necessary components of a successful stocking programme. For a broader coverage of the many components see other sources (Bartley and Leber, 2004; Claussen and Philipp; 2022, FAO, 2008; FAO, 2015). However, most of those general sources do not address genetic resource management within stocking programmes in any depth.

1.1. The code of Conduct for Responsible Fisheries

The Code of Conduct for Responsible Fisheries (the Code) was adopted in 1995 and provides justification and guidance for FAO's programme of work, including on stocking and genetic resource management. Given the importance of genetic resource management for a variety of aquaculture objectives, there are several articles of the Code that these guidelines will help implement.

1.1.1. Relevant articles of the Code

2e 7.2.2.d biodiversity of aquatic habitats and ecosystems is conserved and endangered species are protected.

9.1.2 States should promote responsible development and management of aquaculture, including an advance evaluation of the effects of aquaculture development on genetic diversity and ecosystem integrity, based on best available scientific information.

9.3.1 States should conserve genetic diversity and maintain integrity of aquatic communities and ecosystems by appropriate management. In particular, efforts should be undertaken to minimize the harmful effects of introducing non-native species or genetically altered stocks used for aquaculture including culture-based fisheries into waters, especially where there is a significant potential for the spread of such non-native species or genetically altered stocks into waters under the jurisdiction of other States, as well as waters under the jurisdiction of the State of origin. States should, whenever possible, promote steps to minimize adverse genetic, disease and other effects of escaped farmed fish on wild stocks.

9.3.3 States should, in order to minimize risks of disease transfer and other adverse effects on wild and cultured stocks, encourage adoption of appropriate practices in the genetic improvement of broodstocks, the introduction of non-native species, and in the production, sale and transport of eggs, larvae or fry, broodstock or other live materials. States should facilitate the preparation and implementation of appropriate national codes of practice and procedures to this effect.

9.3.5 States should, where appropriate, promote research and, when feasible, the development of culture techniques for endangered species to protect, rehabilitate and enhance their stocks, taking into account the critical need to conserve genetic diversity of endangered species.

1.2. The Global Plan of Action for the Conservation, Sustainable Use and Development of Aquatic Genetic Resources for Food and Agriculture

The *Global Plan of Action for the Conservation, Sustainable Use and Development of Aquatic Genetic Resources for Food and Agriculture* (Global Plan of Action) (FAO, 2022) was adopted by the FAO Council in December 2021. It is a voluntary and non-binding instrument that was developed in consultation with members of the Commission on Genetic Resources for Food and Agriculture and endorsed by the FAO Committee on Fisheries and its subsidiary bodies. The Global Plan of Action has provisions for the enhancement of conservation and sustainable use of cultured and wild relative AqGR and the promotion of their development for aquaculture. It has 21 strategic priorities and nearly 100 recommended actions with the relative importance of the

priorities and the relative value of actions expected to vary nationally and regionally. The following Strategic Priorities have relevance to the genetic management of genetic resources used for stocking.

1.2.1. Relevant strategic priorities and actions

Strategic Priority 2.1: *Identify wild relatives of AqGR most at risk (e.g. through an AqGR information system) and ensure that they are managed sustainably and appropriate conservation measures are implemented where necessary, nationally and regionally.*

Strategic Priority 2.3: *Actively incorporate in situ conservation of AqGR in the development of fisheries management and ecosystem-based management plans, particularly for threatened species.*

Strategic Priority 2.5: *Improve sustainable use of domesticated farmed types through improved management of genetic diversity.*

Strategic Priority 4.3: *Support the responsible introduction, exchange and use of AqGR, including through appropriate risk assessments, adequate policies and their effective implementation.*

From among the recommended actions under these strategic priorities the following actions relate directly to the issues covered in these guidelines and indeed these guidelines are a direct response from FAO to the final recommended actions in this list.

Actions

- *Promote collaboration among fishery managers, aquaculture managers and conservationists (SP: 2.3).*
- *Incorporate conservation into fishery management and stock enhancement objectives where appropriate, considering genetic variability as well as real stock size. (SP:2.3)*
- *Promote use of genetic tools in fishery stock assessment and management. (SP:2.3)*
- *Develop recommendations and guidelines for genetic management of cultured resources for release into the natural environment. (SP:2.5)*

2. Scope of the guidelines

The guidelines primarily cover technical aspects, including associated risk analysis, of genetic resource management in stocking of hatchery raised farmed types, at various stages of their life cycle, into open waters (covering both marine and inland waters). Such stocking programmes are carried out for a range of purposes including fisheries, conservation and ecosystem services. The guidelines also cover the translocation of wild caught genetic resources for the purposes of stocking in a new location.

The guidelines are not intended to directly cover stocking for the purpose of aquaculture, in which ownership of the stocked organisms is retained, nor do they cover inadvertent escapes or recruitment from aquaculture. These latter issues are important and do represent risks to the environment and biodiversity and many of the issues and best practices presented in these guidelines also have relevance to these aquaculture related circumstances. However, in planning these guidelines it was agreed that they should focus on genetic management in relation to the planning and implementation of planned and deliberate releases into natural or modified common waterbodies, for purposes other than aquaculture.

While many of the issues and recommendations within these guidelines have implications for the economics and resourcing of stocking programmes, and economic constraints will limit the capacity of many stakeholders to implement best practices, the issues of economics and resourcing of genetic management programmes is outside the scope of these guidelines.

3. Objectives of the guidelines

The overall objective of the guidelines is to provide practical genetics related guidance and advice to hatchery, fishery and resource managers on how to responsibly and sustainably use aquatic genetic resources for food in stocking programmes for enhancing fisheries, both commercial and recreational, habitat and ecosystem rehabilitation and conservation.

In preparing these guidelines it is acknowledged that national or regional guidelines and even sector specific guidelines, that overlap with these guidelines, may exist. However, these guidelines are intended to be generic in nature, applicable to different geographic locations and national systems and to different sectors of aquatic food production. In this regard they may also have the function to harmonize practices at an international level.

These guidelines are intended to be used by all stakeholders when considering initiating or continuing on-going stocking programmes. They can be incorporated into national strategies for fisheries and ecosystem management and used to inform stocking decisions and policies on a case-by-case basis and should be consulted prior to initiating any stocking.⁶

4. General genetic principles of stocking

In general, 'stocking' involves raising aquatic organisms in a hatchery or other aquaculture facility for eventual release into natural or modified waterbodies. Occasionally, the aquatic organisms for stocking may be sourced from wild stocks and translocated directly, or indirectly via a holding/raising facility, to a new location, for stocking. Organisms are usually released at an early life history stage; however, juveniles and even adult organisms are sometimes released. Organisms that are hatched or spend a part of their life cycle in a hatchery, can adapt to hatchery conditions. There are two types of adaptation that occurs when organisms are domesticated and it is important to understand the difference: behavioural and physiological adaptation by individual organisms that happens following the introduction of the organism to a new environment (e.g. harvesting in the wild and moving it to captive environment) and secondly genetic adaptation which occurs in the population of the organisms over multiple generations held and bred in captivity. However, as noted below, these two forms of adaptation are not mutually exclusive.

Individual and groups of an organism can undergo physiological and behavioural adaptation to the captive environment following introduction to that environment, which can impact on their potential to survive and thrive in the natural environment after release. For example, a dependency on artificial formulated feeds may impact the potential of the organism to adapt to foraging for natural sources food; absence of predators in the captive environment may lead to inadequate predator avoidance behaviour once released. Whilst these adaptations are not bringing about genetic change in the individual organisms these physiological and behavioural adaptations of course may have a genetic basis with some genotypes better able to survive or thrive in the captive environment than others and where there is this genetic basis, it is then likely that these genotypes that enable better adaptation will be passed on to the next generation. These initial genetic changes can be seen within the first generation held in captivity as maladapted genotypes are lost through mortality in the captive environment, but such genetic differences accumulate over generations in captivity to generate more profound genetic changes as a result of this process, termed domestication selection (a form of natural selection that takes place in the captive environment), over time.

Thus, of greatest relevance to these guidelines is the understanding that over multiple generations in captivity all the individual organisms in the captive bred population are likely to have undergone a genetic adaptation to the captive environment through domestication selection. It can be debated

⁶ See footnote 3

whether domestication selection is as a result of conscious process by humans or is an inadvertent consequence of cultivation by humans (Gepts, 2004).

Genetic changes can begin to occur even prior to domestication in a captive environment, for example through pre-domestication as humans' took deliberate care of wild stands of seaweed (Valero et al, 2017) although these genetic changes are likely to be more profound in the captive environment. The hatchery or aquaculture environment can impart genetic changes to a group of organisms through various processes; the changes may be deliberate or inadvertent (accidental) (see Table 1). Inadvertent genetic changes may result as a consequence of genetic drift and unconscious selection over generations in captivity, representing domestication selection. High mortality in the hatchery may lead to loss of genetic variation changing the frequency of alleles through genetic drift. This loss of variation may also include loss of genes important for adaptation once released, i.e. related to fitness. With several generations of hatchery breeding there is the chance of inadvertent domestication selection that could render the group less fit once released. Clearly there will be interaction between genetic and non-genetic effects, such as where the genetic adaptation over generations is essentially changing the capacity for behavioural and physiological adaptation of individuals in a population.

In understanding risks and developing strategies for genetic management in the stocking of farmed types it is important to understand the distinction between behavioural and physiological adaptation that happens within the individual organisms and do not represent genetic change within individuals, with the adaptation that can occur within a population which accumulates over generations in captivity which can have a significant genetic component as outlined above and influences the genetic make-up of all individuals in subsequent generations.

Table 1: Summary of the mechanisms that can result in changes to genetic variation in domesticated farmed types.

Hatchery mechanism	Genetic mechanism	Mitigation
Poor husbandry leading to low survival	Founder effect Genetic drift	Improve growing conditions to reduce mortality, i.e. a non-genetic solution
Low number of broodstock	Founder effect Inbreeding depression Genetic drift	Increase number of unrelated broodstock and use single pair mating to equalize ratios of males to females among parents
Mating of close relatives	Inbreeding depression	Record via tagging or physical separation genetic relatedness and increase number of unrelated broodstock in the breeding population
Mating of incompatible ⁷ broodstock	Outbreeding depression	Choose unrelated broodstock from same area/stock
Broodstock with low genetic variation	Founder effect	Increase number of genetically diverse broodstock
Inappropriate hatchery conditions for farmed types for release	Selection for maladaptive traits in the hatchery	Use more natural hatchery conditions, e.g. natural light cycles, substrate and food. Reduce number of generations bred in hatchery.
Collecting small numbers of organisms from the wild for hatchery rearing and release	Founder effect	Collect large numbers of individuals from the wild and breed and retain progeny from as many as is feasible in order to maximise effective population size (see Section 5.5).

⁷ Incompatible in this context means genetic incompatibility such as mating adults with very different genotypes that influence fitness, e.g. mating of adults with genetically determined differences in migration time could lead to offspring not migrating at the appropriate time. Improper species identification in broodstock could also lead to non-viable offspring.

Where stocking of individuals from the wild is occurring via translocation without being held in captivity for any period of time and without breeding in captivity the genetic principles are less complex. Genetic differences between stocks harvested from different locations do exist. In some cases, genetic differences are established and verified through genetic analysis and can thus be considered directly when reviewing the genetic implications of translocation and stocking.

FAO's information system AquaGRIS⁸ is being populated with information on genetic status of farmed types and genetic stocks which can be used to identify where genetic differences may exist between aquacultured farmed types and wild stock and between different genetic stocks in the wild which can inform stocking decisions.

4.1. Rationale and objectives for stocking

There are three general rationales or reasons for 'stocking' considered in these guidelines: i) to create or improve fisheries; ii) to help conserve aquatic species; iii) to create or improve ecosystem services (e.g. through ecosystem restoration by recovering key species). The rationales can be addressed by four general objectives of stocking covered in these guidelines defined as follows (adapted from Bell et al, 2008):⁹

- **Stock establishment** – *The release of farmed types or translocated wild stocks into natural or modified aquatic habitats where they did not previously exist for the purpose of establishing a new population of self-sustaining organisms.*
- **Restocking**—*The release of farmed types or translocated wild stocks into wild population(s) to restore severely depleted spawning biomass to a level where it can once again provide regular, substantial yields.* This may also involve re-establishing a commercial species where it is locally extinct due to overfishing, or release of juveniles reared in “conservation hatcheries” to help restore endangered or threatened species, including rehabilitation of their ecosystems.
- **Stock enhancement**—*The release of farmed types or translocated wild stocks into wild population(s) to augment the natural supply of juveniles and optimize harvests by overcoming recruitment limitation.* Note that recruitment limitation is common for many coastal species with pelagic larvae in open ecosystems, even when spawning biomass is at the desired level.
- **Ranching** (also known as ‘sea ranching’ or ‘put and take’ fisheries)—*The release of farmed types or translocated wild stocks into unenclosed marine and inland aquatic environments for harvest at a larger size in “put, grow, and take” operations.* Note that the released animals are not expected to contribute to spawning biomass, although this can occur when the size at harvest exceeds the size at first maturity, or when not all the released animals are harvested.

Whilst it is important to define these specific stocking related terms here, these guidelines do not include a more general glossary of terms but users are referred to FAO's thesaurus of terms used for the description and genetic resources.¹⁰

Stocking programmes have been used in recreational and commercial fisheries to stabilize or increase yields (e.g. see Box 1, Box 10 and Box 13), in conservation to support the survival or genetic health

⁸ www.fao.org/fishery/aquagris/en

⁹ Restocking, stock enhancement and ranching adapted from Bell *et al.*, 2008. Here we have replaced ‘cultured individuals’ from Bell et al. with ‘farmed types or translocated wild stocks’ and added *stock establishment* as a rationale.

¹⁰ [link to be added prior to publication](#)

and biomass of a wild population of rare or endangered species or species of special cultural significance that may or may not be harvested (e.g. **Error! Reference source not found.**, Box 2 and Box 9), and in providing ecosystem services to aquatic habitats (e.g. **Error! Reference source not found.** and Box 12).

Box 1

A case study on stocking for conservation – the Rio Grande silvery minnow

Aquatic biodiversity is threatened by habitat loss and degradation, unsustainable fishing, climate change and the introduction of alien invasive species. Freshwater fishes are the most endangered group of vertebrates used by humans (Ricciardi and Rasmussen, 1999) and the IUCN Red List (IUCN, 2022) contains over 2 200 marine species that are either critically endangered, endangered or vulnerable. Stocking of farmed types has been used in both marine and freshwater habitats to help prevent the loss of valuable AqGR. The Dexter National Fish Hatchery in New Mexico, along with hatcheries in Albuquerque and the Interstate Stream Commission, (www.ose.state.nm.us/LLSMR/index.php) has, since 2003, been using hatcheries to conserve a number of freshwater fish species. One such species is the Rio Grande silvery minnow (*Hybognathus amarus*).

Native from Southwest USA to the Gulf of Mexico, the silvery minnow experiences large fluctuations in population size due to habitat loss and fragmentation, water diversion, drought, and introduction of invasive species and the fish was listed on the USA Endangered Species Act in 1994. The silvery minnow populations in the wild are now managed with the support of an extensive hatchery breeding programme that has released over 1.1 million fish throughout the current range of the species. Eggs are the most sensitive life-history stage of the silver minnow; therefore, the supportive breeding programme collects fertilized eggs throughout the minnow's current range and spawning season. The programme also collects wild caught young-of-the-year fish. The goals of the restocking programme are to produce enough minnows to supplement the wild populations and thus to reduce chance of extinction, to maximize genetic diversity of captive stocks such that it is reflective of the wild populations and to maintain broodstock from wild-caught eggs in refugia at multiple locations (Osborne et al 2020).

Monitoring revealed that minnows from wild caught eggs had higher genetic diversity than minnows produced in the hatcheries, therefore these fish were prioritized for stocking, broodstock development and maintenance. Restocking of silvery minnows is only done when less than half of the sample sites within a habitat area are occupied or if catch per unit effort falls below 1.0 fish/m²; stocking is reduced when natural recruitment is strong. Effective population size and genetic diversity in wild populations did not correlate with fish density until hatchery supplementation became well established. Twenty years of monitoring revealed that genetic diversity is maintained in the wild stocks in spite of the fact that the minnow has been eliminated from the Rio Grande multiple times, i.e. 'supportive breeding and supplementation buffers the population against loss of genetic diversity following population bottlenecks' (Osborne et al 2020). In addition to collecting wild-caught eggs and young-of-year minnows, the hatcheries try to create natural rearing conditions for the fish. This reduces the chance for domestication selection to occur and allows mate selection during spawning, feeding, predator avoidance, and other behavioural traits that have an impact on fitness, to occur more naturally (Osborne *et al.*, 2020).

Acknowledgements to Wade Wilson, Dexter National Fish Hatchery



Hatchery and resource managers need to work together to establish the rationale of the stocking programme, e.g. for fisheries, conservation, or for provision of ecosystem services, and then choose the most appropriate objective, e.g. stock establishment, restocking, stock enhancement or ranching, in order to fully understand the genetic implications of the practice and thus to fully consider the appropriate genetic management steps that need to be taken (see Box 1).

While the focus of these guidelines is on the implications of stocking on the genetic status of conspecifics in the environment it is strongly recommended that a fuller environmental impact assessment should be conducted prior to stocking, particularly in the case of stocking for stock establishment and ranching purposes. It is important to determine potential impacts on habitat and other species in the ecosystem prior to conducting such stocking practices. Assessment of genetic risks and implications is just one component of such an environmental impact assessment.

It is also possible that the recipient population may be maintained by a combination of natural breeding and hatchery supplementation. It is important to establish an understanding of the baseline situation (in terms of the stock status and its genetic properties which can be garnered respectively from fisheries management reports and research publications and/or information systems on genetic resources (such as AquaGRIS) and conduct monitoring and evaluation of the stocking programme to know if stocking is effective and to evaluate whether it remains necessary (refer to Section 5.2 below for further information on baseline data and information systems).

In addition to genetic resource management, all stocking programmes need an overall management plan for the organisms that are released and the recipient ecosystem, fishery or wild stock (see example in

Figure 1) that clearly articulates the objectives of the programme and targets the most effective mechanisms to achieve those objectives. For example, for stock enhancement it needs to be understood, if fishing regulations are in place, that it is necessary to allow the released farmed types of translocated wild stock to grow and recruit into the fishery. For restocking threatened or endangered species it is necessary to ensure that the causes of the species decline have been adequately addressed prior to initiating the restocking. Similarly for ecosystem services it needs to be determined if the causes of the decline in original ecosystem services have been addressed? Failure to address initial conditions can result in the stocking programme failing to achieve its objectives.

Box 1

Examples of the need for management plans to ensure that stocking programmes can achieve their objective

Management plans are necessary to optimize the chances of successful stocking programmes and addressing the root causes of a declining population is essential to the success of stocking programmes.

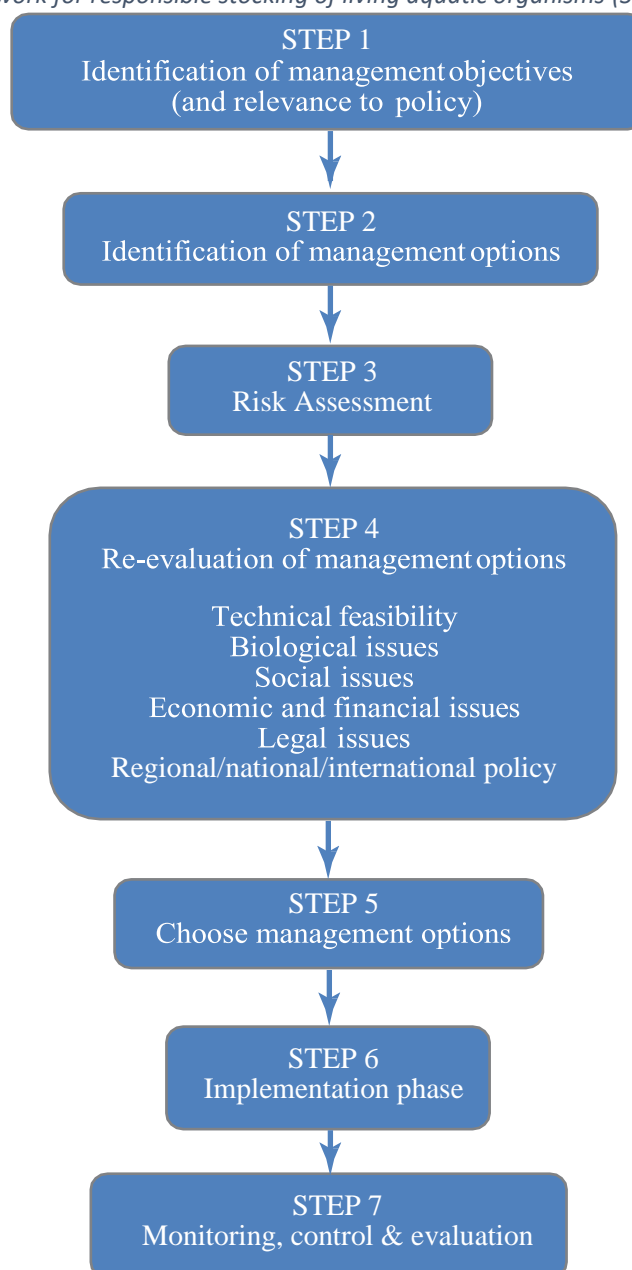
Japan is a global leader in release of hatchery produced farmed types to support wild populations. Stocking of red sea bream (*Pagrus major*) is one of the world's largest stocking programme and is well-managed and well-studied. Genetic monitoring of the red sea bream stocking programme in Japan revealed that the stocking programme reduced the overall genetic diversity of the wild population, even though genetic management had been applied to the farmed type stocked (Kitada *et al.*, 2019). This effect lessened as the wild population increased in size. The study concluded that, despite the programme of stocking occurring over decades, improving the seaweed communities where juvenile red sea bream fed, and reductions in fishing effort were the primary factors associated with the wild population recovery.

Due to declining natural populations in the Caspian Sea, a strong restocking program exists in Iran for five sturgeon species (*Acipenser spp*). Although a moratorium on commercial sturgeon fishing was established by the five Caspian Sea littoral states in 2011, poaching and illegal fishing continue. In spite of good information on the genetic structure of hatchery and wild populations of sturgeon in the Caspian (Pourkazemi, 2006), populations continue to decline, partially as a result of the long generation time of sturgeon, but also because of lack of coordinated enforcement of commercial fishing regulations (Professor Mohammad Pourkazemi, Fisheries and Aquaculture Genetics, Iranian Fishery Science Research Institute, Tehran, Iran, pers comm.). The recovery of sturgeon populations in the Caspian Sea will depend on joint management and enforcement of fishing regulations by the five littoral states.

The management plan must include clear objectives of the programme, take fishing regulations into account, monitor the organisms released and any associated fishery, and evaluate how well the programme meets its stated objectives (

Figure 1). Specific genetic considerations can be added to the general plan below (see Box 2, Box 3 and Box 11).

Figure 1 Steps in a Framework for responsible stocking of living aquatic organisms (Source: FAO, 2015)



Box 2

Restocking of minor and major carps in India a case study of conservation of indigenous species in India

The ICAR-National Bureau of Fish Genetic Resources (ICAR-NBFGR) addresses researchable issues on fish genetic resources in India. In association with Kerala Agriculture University, ICAR-NBFGR developed broodstock and captive breeding technology to restock two endemic species in the rivers of western Ghats, *Horabagrus brachysoma* and *Labeo dussumieri*, from 1999-2004. These efforts resulted in the reappearance of the two species in capture fisheries within the area of their native distribution: *H. brachysoma* landings increased from 1.8 percent to 11 percent and *L. dussumieri* from 0.68 percent to 3.9 percent of the total landings from the Vembanad Lake and their adjacent rivers (Padmakumar *et al.*, 2011). Similarly, Renjithkumar *et al.* (2017) recorded revival of *L. dussumieri* in the river Pampa, where the catch had been drastically reduced in 1990; stocking interventions increased catches 10-fold increasing yields by 60–70 tons from 2007 to 2015.

This helped revise the IUCN status of the species raising its category to “Least concern” from “endangered”.

A conceptual framework on conservation aquaculture of indigenous species was put forward by ICAR-NBFGR through a consultation process enabling prioritization of fish species for conservation and aquaculture, captured in documented guidelines. This framework formed the basis of a project initiated under a consortium research program on agrobiodiversity, to breed and propagate wild-sourced germplasm for use in aquaculture and stocking programmes.

Under this programme, since 2015, wild germplasm of over 5000 individual fish representing 44 species have been made available. All of these fish are PIT (passive integrated transponder) tagged, and used for research on breeding, evaluation of aquaculture potential, and conservation. For example, breeding populations of *Clarias dussumieri*, *L. dussumieri* and *H. brachysoma* have been created and seed are available for restocking. *C. dussumieri*, known to have declined in nature, and *Hemibagrus punctatus* (listed as extinct in 1998), have been bred in captivity up to the F2 generation and are being conserved in captivity enabling restocking in the wild. Breeding of wild sourced Indian major and minor carps is conducted, incorporating the appropriate genetic management, e.g. by minimizing transfers amount regions, and breeding a minimum of 30 breeding pairs, produced, in 2022, 1.6 million seed (2022) for stocking in Ganga River system.

Through planning and the engagement of government, academia, fishers and fish farmers, genetic resource management can produce broodstock that can be used to conserve native species *ex situ* and to produce genetically appropriate seed for restocking in the wild, as well as for aquaculture.

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5. Guidelines for Genetic resource management

Genetic resource management in stocking programmes involves understanding the genetic resources of the recipient or new population, the population to be released from the hatchery (or translocated from another stock), and then managing these groups and their interaction to accomplish the objectives of the programme within an acceptable level of risk. A first step is deciding on what kind of stocking intervention is needed, i.e. the rationale, is understanding what genetic information is known or can be assumed with regard to the stocked farmed type (or translocated wild stock) and the receiving population, and then performing risk/benefit analysis.

5.1. Genetics and the benefits of stocking

Genetic diversity in aquaculture, i.e. aquatic genetic resources (AqGR) is the basis for a species/population’s survival and adaptation and therefore allows for them to persist and evolve. The information encoded in genes underpins all aspects of a species’ life-history cycle, including growth, fecundity, immune responses to pathogens and behaviour. The genetic integrity of a wild population and its adaptive potential can be influenced by activities at several stages in a stocking programme in beneficial and detrimental ways.

Stocking and the subsequent addition of genetic diversity to a target population could increase the fitness and long-term viability of a population and this may be an objective of some stocking programmes. Furthermore, genetic diversity will improve the capacity of a population to adapt to changes in the environment, such as through climate change or changes in water quality. It is also possible for targeted genetic improvement of stocked organisms to address a given environmental hazard, e.g. temperature tolerance, to cope with changing water temperatures, resistance to a disease prevalent in the environment, or fishing requirements, e.g., specific migration time.

Conversely, if the objective is to have no genetic impact on the wild stocks it may be possible to release a group of organisms that cannot breed. Genetic manipulations can be employed to produce

sterile organisms which can be released but that cannot reproduce and would thus reduce or even eliminate the risk of mixing genes from the hatchery stock and stocks in the wild.

Genetic analysis can identify genetic markers for monitoring and evaluation that may enable identification of released organisms in order to assist in tracking their survival and recruitment to a fishery or population to be rebuilt or established for conservation purposes. Genetic markers can be tracked over generations without repeated marking, which is not available with other physical marking practices such as fin clips or coded wire tags.

Initially the design of a stocking programme may not anticipate or take into full account how the genotype of a farmed type (or translocated wild stock) will perform once released, or how a specific genetic resource management regime will achieve management objectives. Thus, long term and ongoing monitoring of the stocking programme and its genetic impacts is essential so that hatchery production, stocking programme and genetic management protocols can be optimized.

Genetic resource management requires substantial amounts of information throughout the programme, starting with baseline data.

5.2. The importance of baseline information

Baseline information on the genetic resource, i.e., on the farmed type or translocated wild stock to be stocked and on the wild relatives in the receiving environment, is fundamental for the meaningful management and analysis of risk, as well as for monitoring and evaluation of the impact of the stocking programme (Box 3). The types of baseline genetic information that can be used as bases for monitoring programmes are summarized in

Table 2. It should be noted that baseline data should be collected from adequate numbers of individuals to be fully representative of the status of stock and populations. Access to baseline data is greatly facilitated by the use of standardized information systems such as AquaGRIS.¹¹

Box 3

The importance of baseline genetic data for planning genetic resource management plan for conservation and aquaculture development in Zambia

Species of tilapia provide fishery and aquaculture opportunities in Zambia. However, the spread of the non-indigenous Nile tilapia, *Oreochromis niloticus*, imported for aquaculture in the Kariba dam and elsewhere in the Zambezi River drainage, has raised concerns that it is displacing indigenous tilapias, especially *O. andersonii* and *O. macrochir*. *O. andersonii* is the preferred species for aquaculture in Zambia.

The World Bank in collaboration with the Government of Zambia conducted an assessment of the genetic resources of the main tilapia species of Zambia in order to support the expansion of tilapia farming, minimize the risk to native fish and enhance understanding and value of local biodiversity.

The genetic variability of natural and captive populations of three Zambian tilapia species, *O. andersonii*, *O. macrochir* and *O. niloticus*, was assessed using Single Nucleotide Polymorphisms (SNP) markers. The assessment revealed geographic distribution of pure species of *O. andersonii* and *O. macrochir* and locations where hybrid introgression with *O. niloticus* had occurred. Tilapia populations downstream of Victoria Falls were introgressed with *O. niloticus*, whereas those upstream remained pure for both *O. andersonii* and *O. macrochir*. The same species were also found to remain pure in the separate watershed of Lake Ithezi Tezhi. Recommendations were thus made to ban the use of *O. niloticus* for aquaculture purposes in these two watersheds, not only in Zambia but also in neighboring countries that share these river basins, the Democratic Republic of Congo, Angola and Namibia. Thus, a reserve of natural wild types of *O. andersonii* and *O. macrochir* were identified for management and conservation.

Captive populations of tilapia were also assessed and several farms were shown to be raising

¹¹ www.fao.org/aquatic-genetic-resources/activities/aquagris/en/

introgressed farmed types, while several were raising pure species. Fish from the Kalimba Fish Farm near Lusaka were shown to be pure and also to have higher levels of genetic diversity than natural populations with no evidence of inbreeding having occurred. This could be due to the use of several hundred brood fish from various sources to form the founding stock. The Kalimba farmed type was established with a broodstock of 500 female and 100 male *O. andersonii*, which when bred produced an estimated effective population size (N_e) in excess of 300. An N_e of 500 or more is recommended to long term prevention of inbreeding. Recommendations were made to maintain this level of diversity through breeding a minimum of 1000 fish of equal sex ratio in each generation of captive rearing. Such recommendations can be implemented without the need for genetic markers for pedigree assignment, although genetic markers can be used to further optimize the maintenance of N_e . Here also genetic markers were used identify a reserve of pure *O. andersonii* was identified and recommendations for management to maintain species purity and high N_e were made.

The reserves of pure species in the wild can serve as backup for the expansion of aquaculture or restocking of water bodies in the case of population declines; the genetic management of the farmed types in facilities such as the Kalimba Farm can also act as a reserve for restocking natural populations if needed.

Acknowledgement : Dr. Jean-François Agnès. (information derived from unpublished Report to the World Bank, Genetic characterization of natural populations of Zambian Tilapia species *Oreochromis andersonii* and *O. macrochir*. December 2019.

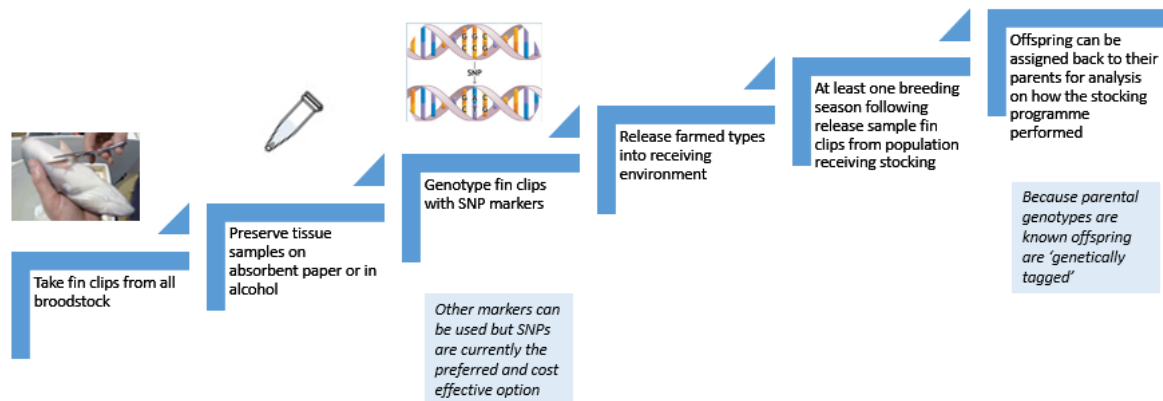
Table 2: Baseline genetic information required for both stocked population (farmed type) and wild relative or receiving population(s)

Baseline data	Comments
Genetic variation	A general measure of genetic variability within and between stocks and farmed types of a species. When quantified, can identify problems such as genetic bottlenecks, accumulation of inbreeding, genetic drift, and planned and unplanned introgression of species and any introgression already occurred between wild and stocked species. Can be used to assess the suitability of farmed types (or wild stock for translocation) by comparing genetic variation with that of original populations and receiving populations.
Frequency of rare alleles	Loss or significant changes in the frequency of rare alleles, detected using genetic markers, can be used as indicators of genetic change which in turn can be indicative of genetic impacts of stock management in both wild stocks and farmed types. Allele frequencies can be targeted in the population of farmed types to be released in order to maintain known frequencies of rare alleles in the wild.
Occurrence and frequency of genetic markers	Genetic markers such as microsatellites DNA and SNP markers can be used across the genome for the purposes of monitoring and evaluation of genetic change in both domesticated farmed types and wild stocks.
Genetic distance	A measure to assess genetic similarity (i.e. relatedness) between genetic isolates/stocks/populations such as between hatchery farmed type and a receiving population. Population differentiation can be measured through a fixation index (F_{ST}). Genetic distance or $t F_{ST}$ targeted may be low or high depending on the objectives of the stocking programme.
Effective population size (N_e)	As a quantification of the number of breeding stock contributing progeny to the next generation of breeding stock, a useful measure of the likelihood of genetic change, including loss of genetic variation, from one generation to the next and estimated or actual accumulation of inbreeding. Helps assessments of the breeding programmes, the farmed type to be released and risk analysis. Can be difficult to estimate without good data on breeding and stock sizes.
Relatedness among broodstock.	Genetic information regarding the relatedness among the broodstock to be used for produce seed to be stocked can help identify the best crossings to avoid inbreeding (e.g. Fonseca et al, 2017) leading to a more genetically diverse population of fingerlings to be released.

DNA based marker methods for parentage assignment, such as parentage-based tagging (PBT) used for salmonids in North America (Beacham *et al.*, 2021), is a powerful tool that can provide valuable

information on the genetic resources of farmed types in the hatchery and once released (see Figure 2). With the rise of genomics there are opportunities to identify and use hundreds of genetic markers, primarily SNPs, to assess genetic diversity, hatchery protocols for genetic resource management, success of farmed types in the wild or fishery, and hybridization and introgression of farmed types with wild relatives. However, it should be acknowledged that whilst the cost of DNA analytics has dropped dramatically in recent years, it still may not be a cost effective option in many countries especially for species for which SNP panels have yet to be developed. In such cases microsatellite DNA analysis maybe more cost effective, even though they may have lower powers or resolution.

Figure 2 Illustration of how parent based tagging is utilized for the monitoring of genetic impacts of stocking programmes in salmonids (Houde, 2020)



5.3. Risk analysis

Risk analysis to reduce the chance of adverse impacts is an essential component of genetic resource management. *Risk analysis is the formal process of identifying, assessing, and managing factors that may jeopardize the achievement of a goal.* Sonesson et al, 2023 provide useful detail of the conduct of risk analysis in relation to genetic resource management and improvement with some of the key points related to stocking noted here below.

Risk analysis involves:

- 1) Identification of potential harms. A harm is a perturbation resulting in negative impacts to a population;
- 2) Identify hazards that might lead to harms. A hazard is an agent or process that has the potential to produce harm. For these guidelines, hazard is the release of farmed types or the translocation of wild stocks into aquatic ecosystems based on the rationale and objectives outlined above;
- 3) Define and assess the likelihood of exposure to the hazard, $P(E)$;
- 4) Quantify the likelihood of harm given that exposure has occurred, $P(H|E)$; and
- 5) Multiply the resulting probabilities to yield a quantitative estimate of risk.

Risk is the likelihood of harm resulting from exposure to the hazard combined with the consequence of that harm. Risk, R , is the product of the probability of exposure, $P(E)$, and the conditional probability of harm given that exposure has occurred, $P(H|E)$; i.e., $R = P(E) \times P(H|E)$. It may be necessary – based on current knowledge of population genetics, population dynamics, aquatic ecology, and empirical experience with farmed types – to classify levels of concern regarding likely ecological or genetic impacts posed by farmed types into qualitative categories ranging from negligible to extreme (Table 3). Principles of risk analysis have been applied to aquaculture and specifically to the production of farmed types (Hallerman, 2008).

As Sonesson *et al.*, 2023 state, “The scope of existing case studies regarding the impacts of introduced aquaculture species and farmed types on AqGR is narrow, limiting our ability to predict and manage the likelihood of harms being realized from the distribution and production of introduced species and

developed farmed types. However, even with limited predictive ability, risk analysis provides approaches for managing risks to acceptable levels.”

5.3.1. Harm identification

Whilst these guidelines are concerned specifically with risks related to genetic resources, these risks should be considered in the broader context of risk analysis covering habitat, ecosystem and community impacts. The degree of harm will be a function of the degree of differentiation between the gene pools of the farmed types or translocated wild stocks and the recipient population, the relative proportion of spawners from the respective groups and the natural selective pressure imposed by the receiving ecosystem. Stocking a non-native species, non-endemic stock, or fertile farmed types poses the risk of interbreeding with reproductively compatible species in the receiving environment and the risk of introgression of genetically distinct stocks or species. Genetic harm may arise from loss of adaptive genes, introgression of new maladapted genes, genetic drift through reduced population size and, in extreme cases, loss of local populations (Box 4). Generally harms can only be identified if there is monitoring of the impacts of harm but often such monitoring is lacking and harm can go undetected leading to uninformed decisions being made with regard to risk management (Box 5).

Box 4

Some examples of genetic harm caused by the introduction of non-native species for aquaculture

Sonesson *et al.* (2023) give some examples of documented impacts of the introduction of non-native species for aquaculture. Perhaps the species most studied in the context of interbreeding of non-native or conspecific farmed types with natural populations are the salmonids (Ferguson *et al.*, 2007; Hindar *et al.*, 1991) where spatial heterogeneity of natural selection has resulted in adaptive genetic divergence of populations. The introduction of cultured farmed types, either through deliberate release or escapes from aquaculture, and subsequent interbreeding with local populations of wild relatives can act to homogenize genetic variation. The migratory life history of salmonids contributes to their susceptibility to harm. Studies identified a complete range of effects with introductions bringing about total displacement of the wild stock, complete introgression was seen in some cases and in others no impact at all was detected. McGinnity *et al.*, 2003 provided evidence that interbreeding of farmed with wild Atlantic salmon (*Salmo salar*) resulted in lowered fitness of the wild stocks which accumulated over time. Similarly, significant losses of fitness were observed after just two generations of captive breeding in anadromous rainbow trout (*Oncorhynchus mykiss*) and this reduced fitness was also noted in their wild-born descendants (Araki *et al.*, 2007, 2009).

This type of work is not however limited to salmonids, Segovia-Viadero *et al.* (2016) analysed the risk that hatchery reared sea urchins (*Paracentrotus lividus*) could pose to wild stocks. The study demonstrated genetic bottlenecks were occurring in the hatchery resulting in hatchery reared stock that were less genetically diverse and had become somewhat differentiated from their wild relatives. The authors recommended caution over developing stock enhancement programs given the risk of impacts of the release of hatchery reared stocks into the environment.

Models have been conceived that enable prediction of the impacts of introductions of maladapted genotypes into locally adapted gene pools (Tufto 2001, 2010) that can be applied to estimate the relative likelihoods of impacts (both positive and negative) on the receiving populations (Garant *et al.*, 2007) but actual empirical studies of impacts are lacking. Sonesson *et al.*, 2023 also present examples of escape or release of interspecific hybrids into the natural environment which, especially if fertile, poses risk of harm due to introgressive hybridization into wild stocks. For example, in Thailand hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) are widely farmed and when escaping from farms are able to interbreed with native populations of *C. macrocephalus*, causing introgressive hybridization (Senanan *et al.* 2004) and thus loss of species purity in important wild stocks. Mwanja and Kaufman (1995) present another example with tilapia, using

genetic markers to demonstrate hybridization in both directions among *O. niloticus* and *O. esculentus* in satellite lakes of Lake Victoria, concluding that no pure stocks of *O. esculentus* remain in these systems. As a third example Sattar *et al.* (2005) looked at the genetic basis of stocks of Indian major carp in Bangladeshi hatcheries. They demonstrated that 8.3 percent of farmed silver carp (*Hypophthalmichthys molotrix*) broodstock exhibited bighead carp (*Aristichthys nobilis*) alleles, and 23.3 percent of farmed bighead carp exhibited silver carp alleles), compromising the integrity of the broodstock and their performance in aquaculture and creating the risk of these introgressed genotypes entering the natural environment thus also compromising genetic diversity in wild stocks.

Box 5

Harm or no harm? – a search for evidence and a call for greater monitoring

Although these guidelines address the potential harm to wild AqGR from introgression of ‘hatchery’ farmed types (or even translocated wild stocks), and there is good theoretical evidence for this harm, there is a scarcity of documented harms to wild populations arising from introgression, especially outside of studies on salmonids. There is good evidence for introgression between wild and escaped domesticated salmon resulting in genetic alternation and loss of fitness of wild salmon in North America and Europe (Bradbury *et al* 2020; Fleming *et al*, 2000 and McGinnity, 2003) but it is harder to find examples arising from deliberate stocking programmes, especially for non-salmonids.

These guidelines do not recommend releasing highly domesticated organisms including developed strains and varieties; these are likely maladapted to life in the wild and are best suited for grow-out in contained aquaculture facilities. Nevertheless, escaped organisms such as Atlantic salmon have the potential to harm wild populations through competition for food and breeding, but if they interbreed with wild fish, the question is: are F1 crossbreds or hybrids and any resulting outbreeding depression really causing harm? Outbreeding has the clear potential to cause harm through reduction in fitness and may erode possible local adaptations. Documented evidence of such harm is not apparent, although in theory it should be. This lack of evidence fuels skepticism on responsible stocking practices and their implications for genetic resource management. This lack of knowledge and the application of a precautionary approach may force hatchery managers to undergo complicated and expensive breeding and monitoring programmes with the objective to minimize genetic impacts where, in reality, the risk of adverse impacts may be low. Therefore, a recommendation inherent in these guidelines is to encourage further research and to enhance monitoring to assess the real impacts of stocking programmes and help provide the evidence needed to support the application of precautionary approaches and point the way to responsible and cost-effective management of genetic resources for stocking.

5.3.2. Genetic risks from stocking

The genetic harm¹² of stocking, with reference to both the released farmed types (or translocated wild stocks) and the recipient population, include:

- swamping native populations with genes from the farmed type (or translocated wild stock) thus depleting existing natural genetic diversity (including reduced heterozygosity), reducing N_e and promoting inbreeding;
- introducing maladapted genes from the farmed type into existing populations thus reducing their long-term fitness (a form of outbreeding depression);
- introgression through crossbreeding or hybridization between existing stocks and farmed types that create an inviable mixed group, e.g. sterile or inviable offspring that don’t reproduce or survive, or maladapted offspring that don’t thrive or survive well;
- undermining local adaptations through mixing of distinct genetic stocks; and

¹² Other risks such as competition, predation, behavioural displacement and disease transmission are not included here, but would be important to consider in an overall stocking programme.

- releasing inbred or maladapted farmed types that would not perform well when released.

Currently the main risks arise from hatchery produced stocks that have been genetically changed by domestication, selective breeding and crossbreeding or hybridization. However, in the future, risks may arise from the application of modern genetic tools including gene editing and the specific properties of harm arising from such genetic changes should be considered relative to those from more traditional breeding approaches.

Table 3 Example of a risk matrix for use in stocking (Source: FAO, 2015)

Likelihood of occurrence	Extent of harm				
	Insignificant	Minor	Moderate	Major	Significant
Rare	N	L	L	M	M
Unlikely	N	L	M	H	H
Possible	N	L	H	H	E
Likely	N	M	H	E	E
Almost certain	N	M	E	E	E

Note: N = negligible; L = low, M = moderate; H = high; E = extreme

The *likelihood* of an event occurring according to the ratings in Table 3 is further defined in Table 4.

Table 4 Explanation of likelihood ratings used in Table 3 (footnote 14)

Likelihood	Description	%
Rare	Event will only occur in exceptional circumstances	<5
Unlikely	Event could occur but not expected	25
Possible	Event could occur	50
Likely	Event will probably occur in most circumstances	75
Almost certain	Event is expected to occur in most circumstances	>95

The level of risk is defined as the product, or combination, of the likelihood of harm and the consequence of that harm. Once risk has been determined, risk management and risk communication are two other essential components of risk analysis. Stakeholders would review the risk analysis and decide if the benefits would be worth the risk, i.e. determine an acceptable level of risk.

5.3.3. Risk management

Should an oversight authority determine that distribution and production of a farmed type (or translocated wild stock) poses risk to a receiving ecosystem or native population, the question turns to managing the associated risk. Risk management is the design, selection, and implementation of a programme of actions to minimize risk to an acceptable level. A good approach to minimize risk is to minimize exposure to the hazard, i.e. to the released farmed type (or translocated wild stock). Sonesson et al (2023) identified, four non-mutually exclusive approaches that can reduce the chance of exposure to the genetic hazard from aquaculture stocks including: (1) geographic location, (2) physically confining the farmed type on aquaculture facilities, (3) reproductively confining farmed types and (4) operations management. This summary was focused on risks associated from aquaculture escapes and thus some of these measures are not applicable in the context of stocking programmes and the feasibility of others would depend on the rationale for the stocking. The most effective mechanism for managing risk, where genetic harm is likely, especially where reproduction in the stocked environment is not a goal of the stocking (i.e., including stock enhancement and ranching) is to stock reproductively limited or reproductively sterile farmed types. This could be achieved based on habitat or environment, for example stocking a species outside its natural range where the conditions do not exist to allow natural reproduction (e.g., a temperature range where organisms can

survive but not become sexually mature or stocking a species that can only spawn in a riverine environment, into lakes). Alternatively, it could be achieved by stocking reproductively sterile organisms, such as a triploid, or in the future a gene edited sterile farmed type. However, for rationale where reproduction of the stocked farmed type (or translocated wild stock) is desired (i.e., for stock establishment, restocking, conservation and ecosystem restoration) it is generally not feasible to manage risk using these approaches.

Effective management of genetic resources should be included in strategies to mitigate and reduce the risks from stocking programmes (see section 5.4).

5.3.4. Risk communication.

Risk communication transmits the ongoing process and results of risk analysis to key stakeholders, who would include competent authorities, international, regional and national agencies/government officials (including FAO), aquaculturists and fishers, non-governmental organizations, academic researchers, and the general public. It is important to establish effective communication channels to deliver key information which can include stakeholder workshops and development and circulation of specific guidance or policy document (see Box 9). Different groups of stakeholders will be reached most effectively by different means (Dew, Berkson and Hallerman, 2003). Risk communication is important so that stakeholders become aware of how their actions, e.g. fishing, impact the resource. Genetic issues are often complicated and difficult to understand for most stakeholders therefore communication should be as straight forward and clear as possible.

5.3.5. Relative risks and benefits

The four objectives for stocking present different levels of risk to the receiving/target populations and these differences largely depend on the size of the population to be rebuilt or enhanced relative to the size of the introduction. Small receiving populations are more susceptible to inbreeding, to loss of genetic diversity through drift and to swamping of their genes through introduction of large number of genes from external sources; small population size can cause similar impacts in hatchery populations. Furthermore, the larger the size of the stocked population relative to the size of the receiving population the greater the scale of harm that can occur. However, demographic considerations will also influence success of stocking programmes and success is likely to be inversely proportional to relative risk of harm (Box 6).

Box 6

The role of demographics in relation to benefits vs risk

Stocking programmes must take into account population size and demographics. Risk analysis indicates that a large target population to be enhanced has a lower level of genetic risk from the stocking programme inversely proportional to the relative size of the stocked population. However, evidence has shown that stocking into such large populations is often not successful (e.g., Bell, 2008), partially because the carrying capacity of the habitat is limited. Thus, while the risk of genetic harm is low, the risk of a failed stocking programme is high. Creating a new stock or a new species from hatchery releases also has a high risk of failure when the receiving ecosystem is already very productive. Again, the risk of a failed stocking project is high.

In species recovery programmes or restocking, the target population is usually extirpated or very small. Often a genetically identical stock is difficult or impossible to find and a substitute stock must be used. A compromise may be necessary to ensure sufficient numbers of farmed types are released of a genetically similar, but not identical, farmed type (or translocated wild stock) as the target population. In these cases, some degree of genetic change in the receiving population may need to be considered as an acceptable risk in order to gain some benefit given that the stocking programme must release a sufficient number of individuals to ensure that enough males and females meet and mate in the target ecosystem.

In the case of introducing a new stock, the genetic risk to the ecosystem depends on the size and genetic status of the populations of wild relative with which the hatchery produced farmed type (or

translocated wild stock) could interbreed; the larger the size of the wild relatives' population the less genetic risk. There is also the risk that the stocked farmed type will have inappropriate genetic and phenotypic characteristics and thus be inviable or have reduced fitness and viability.

In restocking programmes it is assumed that the receiving population is small and thus vulnerable to genetic change from the hatchery stock. When reestablishing a population the genetic basis of the stocked farmed type is thus fundamental to the future genetic status of the recovered population. It is assumed that the receiving population is small or non-existent and thus vulnerable to genetic change or replacement by the stocked genetic resource. Additionally, it may be challenging to find a source for the founding stock that represents the original genetic diversity of the stock to be rebuilt. Inappropriate genetic resource management of the stocked farmed type or translocated wild stock e.g. inbreeding, low genetic diversity, or inappropriate genetic stock structure, would present a high level of risk to the population to be rebuilt.

To increase the size of an existing stock (stock enhancement), the relative genetic contribution of the hatchery reared farmed type vs the size and genetic resource of the receiving population needs to be considered. When increasing the size of an existing stock, there is a lower level of risk as it is assumed that the receiving population is larger so less vulnerable to change.

Ranching presents the lowest relative level of risk to genetic resources of the four stocking objectives. This low level of risk assumes that genetic management in the hatchery has effectively produced organisms that will not reproduce or breed once released either because they have been rendered unproductive, they will be incapable of spawning in the receiving environment or they are expected to be harvested before they reach a reproductive size or age. Because, in principle, the released organisms will not breed and recruit in the receiving environment, if harm is detected the hatchery can stop the release programme. This applies to a fishery objective such as put and take fisheries rather than conservation given the intent to harvest the stocked resource. Ecosystem services can also be provided by non-breeding organisms, e.g. triploid grass carp to control aquatic vegetation or mosquito fish to control aquatic insects in temporary water bodies (**Error! Reference source not found.**). Often the biological control organism is non-native and not meant to reproduce in the wild, but rather to be stocked at the appropriate season.

Box 7

An example of a low-risk stocking programme to provide ecosystem services, the case of Chinese carp for weed control and water quality management

Qiandao Lake is the largest artificial lake in China. The lake supports aquaculture, multiple industries, tourism and is a major source of drinking water. However, due to pollutant discharge and nutrient release from cage culture of species such as bass, mandarin fish, catfish and Chinese carps since 1995, water quality in the lake deteriorated and the lake became eutrophic with numerous blue-algae blooms (The Nature Conservancy, 2021). The deterioration in water quality decreased production from cage culture in the lake as well as adversely impacting domestic water quality. Efforts to improve the quality of the lake's water included elimination of intensive cage culture and reduction in upstream discharge of pollutants. In addition, silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*), were stocked. These two species are native to the region and consume phytoplankton and zooplankton, respectively. Since 2010 over 600 tonnes of Chinese carps have been released into the lake; monitoring of the lake revealed that in 2020 water quality had improved and had reached a high standard. These species of carp require long river systems in which breed and distribute eggs; therefore, they would not be able to reproduce in Qiandao Lake and thus the stocking programme should be repeated in order to retain the ecological services longer term. The stocking programme, along with other measures, provides economic benefits to the community and helps maintain an important aquatic ecosystem.

Source: The Nature Conservancy, 2021.

5.4. Founder stock selection

The genetic stock structure of the founder stock, i.e. farmed type (or translocated wild stock), should be tailored to the objectives and type of stocking. In re-establishing stocks and enhancing existing stocks the founder stock should match the genetic structure of the original stock as much as possible. This is best achieved by using wild-sourced farmed types, either wild caught seed or seed from wild caught broodstock drawn from populations that are genetically compatible with the receiving population (see Box 8). If wild caught broodstock are in short supply within the receiving population, as in the case of many endangered or locally extinct natural populations, genetic stock identification should be used to identify a very similar stock. Where genetic data are not available for stock identification, surrogate information can be used, e.g. choose stocks from same aquatic habitat (water body or specific watercourse, such as a tributary) and with similar life history, growth, colour, shape and behaviour characteristics. Transfers of stocks among different watersheds or ecoregions are to be avoided if possible.

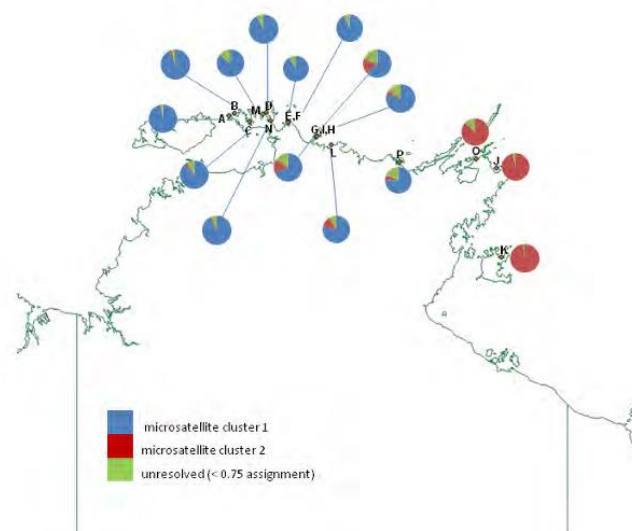
Box 8

Case study of the development of a genetic management plan ranching of the sea cucumber *Holothuria scabra* in Australia's Northern Territory

In the early 2000s a ranching programme introduced hatchery reared sea cucumber known as sandfish (*Holothuria scabra*) into shallow coastal areas in Grooyte Eyland, off the coast of Northern Territory of Australia was initiated with potential to expand the ranching to other parts of the nearby mainland. Native populations of the species existed throughout the areas identified for potential stocking and ranching activities. Prior to considering broader scale ranching activities a genetic management plan was requested in order to assess and mitigate the risk to the wild populations from the restocking of hatchery produced juveniles.

Microsatellite DNA markers were used to assess the population structure of wild stocks. This identified two distinct populations with a barrier to gene flow between them (Gardner, Fitch and Li, 2008).

Figure 3. Results from STRUCTURE cluster analysis of *H. scabra* populations in the Northern Territory identifying two discrete genetic clusters, the Eastern (Gulf of Carpentaria) and Western (Arafura Sea).



A policy recommendation was made to the NT government in the form of a genetic management plan to ensure that the ranching activities avoid unnatural alteration of the natural genetic diversity of wild stocks of the species in the Northern Territory. The plan, based on a broad risk analysis,

recommended key management steps to be taken. These included sourcing founder stock for the hatchery from the local populations (representing the respective genetic stocks) in the areas to be restocked, maintaining these separately in the hatchery, and restocking only with representatives of the same genetic stock that occurs in the wild at each location. The plan also recommended breeding strategies to manage effective population size for the ranched stocks with a minimum N_e of 10 per any one individual stocking event.

In establishing a new stock, the genetic structure of the founder stock should have sufficient genetic diversity to allow for adaptation to the new environment. In the case of translocating stock from a similar wild population this should involve collection of stock from different locations, and if for a species with an extended breeding season, collecting stock at different times. In the case of hatchery reared stock this usually means that an effective population (N_e) size of ~500 is required (see section 5.5.1) should be targeted. For ranching programmes the founder stock should ideally be sterile but should have enough genetic variation to survive release and reach harvest size. Where ranched stocks are not sterile, consideration must be given to the probability of them surviving and breeding either among themselves or with local wild relatives of the same or closely related species with which they could interbreed. This probability will be a function of their viability in the wild and the relative success of recapturing them.

5.5. Genetic management in the hatchery

Once an appropriate founder stock is selected, genetic management of broodstock in the hatchery is necessary to create and maintain the appropriate genetic stock structure of the organisms to be released.

The preference, if the objective is to minimize genetic impacts by using local populations, is to use broodstock harvested from the wild for each new cohort of seed for stocking, ensuring that adequate effective population sizes are maintained. This requires that a suitable stock or population exists in the wild that is feasible and cost effective to sample on a regular basis. Where this is not feasible it will be necessary to multiply generations of the captive propagated farmed types in captivity.

As outlined in Section 4, aquatic organisms will adapt (physiologically and behaviourally) in the short term to the captive environment and hatchery management practices and, in the longer-term over multiple generations, hatchery conditions will exert selective pressures (termed domestication selection) on them generating genetic change. Where practical, hatchery procedures should be designed so as to minimize these influences when the stocked material is intended to survive and/or to breed in the wild. Furthermore, it is preferred to use stock that has been domesticated in the hatchery for a limited number of generations although the number of generations that might be considered suitable for stocking would depend on how well or how badly they have been managed from a genetical perspective (e.g. what N_e has been achieved).

Typical modifications to hatcheries to minimize selection pressures, achieved primarily through minimizing the proportion of the stock that fails to thrive or dies,¹³ and avoiding selection pressures that differ substantially from those in the wild, that can thus decrease inadvertent and deliberate domestication selection, include (FAO, 2008):

- Avoidance of transfer of stock among hatcheries that are located in different areas, watersheds or tributaries in order to meet production goals.
- Spawn broodstock over the entire spawning season (i.e. do not simply collect lots of spawn when convenient in order to meet production goals);
- Provision of live food, from the wild if possible, rather than formulated feed;
- Provision of more natural habitat such as with gravel, plants, and shelters, rather than sterile tanks and raceways;

¹³ Any deliberate culling, for example to reduce numbers, should be applied randomly and broadly to minimize the possibility of elimination specific genotypes or genetic variation such as eliminating whole families.

- Provision of limited numbers of predators to teach predator avoidance;
- Use of natural diurnal and seasonal light/dark cycles; and
- Release of younger seed that have not adapted [been selected for] to hatchery conditions. However, this should be assessed as older seed may survive better once released.

For long-term restocking and enhancement programmes it is desirable to develop a rotational breeding plan where preferably wild-sourced, if available, broodstock, are used to produce material for stocking, i.e. farmed types, are released back into the wild after spawning, and then new wild-sourced broodstock are brought into the hatchery. The timing of this rotation will depend on the success of the programme and the availability of natural broodstock (see Box 9). For species that spawn only once (e.g. Pacific salmon) or where killing broodstock is necessary for achieving fertilization (e.g., some sturgeons) this rotation would not be possible. In species such as salmon that return to natal waters, genetic monitoring would be necessary to ensure that fish returning to the hatchery are the ones meant to be spawned. In some salmon enhancement programmes, hatchery produced fish returning to the hatchery are deliberately NOT spawned and wild sourced broodstock are used.

Box 9

Case study of genetic management of Goldstream coho salmon

The Goldstream River Hatchery (also known as the Howard English Hatchery named for its founder) is located on the upper reaches of the Goldstream River on the outskirts of Victoria, British Columbia, Canada. It is a small Community Involvement Project operated by volunteers with technical oversight provided by Fisheries and Oceans Canada. The focus of enhancement is directed primarily to Coho (*Oncorhynchus kisutch*) salmon. Broodstock is taken from the river with consequent progeny being released back into the natal river or transferred to adjacent watersheds to: (a) supplement existing stocks that are depressed; (b) replace stocks that have suffered extirpation; or (c) establish sustaining populations where none existed.

Every attempt is made in managing the hatchery environment to mimic natural conditions extant in the natal river. To achieve this, Goldstream River is the source of water for incubation and rearing ensuring that the thermograph and water chemistry in the hatchery match that of the river. All rearing containers are influenced by the natural photoperiod. Meeting these criteria is not difficult technically.

It becomes somewhat more challenging to optimize the overall fitness of the animal in the context of genetic integrity. Simply put, if the source of brood stock is hatchery fish, there is a very real possibility that undesirable genetic traits may be amplified over time. The reason for this is fairly straightforward. In natural spawning conditions in the river, the average number of eggs fertilized and successfully hatching averages approximately 20 to 30 percent of eggs deposited. If the population is to be self-sustaining, then a large number of spawning broodstock are required and this contributes positively to genetic diversity. When broodstock fish are artificially spawned in a hatchery setting, the process is so efficient that it is not unusual to achieve a 98 percent fertilization rate and the consequent number of parents contributing gametes is significantly less than in the wild. This can lead to a diminishing of genetic diversity.

To mitigate for the genetic challenge, the hatchery instituted protocols designed to achieve a broader genetic base for hatchery production. 100 percent of all Coho are externally marked so that the fish are readily recognized as having their origin in the hatchery even after recapture from the river. This is done by removing the adipose fin during the fry stage. While it is preferable to limit the number of brood animals of hatchery origin, it may not be possible in all cases. To cater to this, the hatchery utilizes what is termed a "PNI" (Percent of Natural Influence) factor for parental selection. For example: if a PNI factor is arbitrarily set at 50 percent and we require 100 adults, then at least 50 (male and female) must have an intact adipose fin. This indicates that they have been naturally produced in the river. Additionally, we harvest adults from an adjacent watershed that had a barrier to anadromy and consequently no native population of salmon. It now enjoys a self-sustaining and naturally produced stock of Coho because of early stocking efforts from the hatchery and this stock has been shown to share almost identical alleles with the Goldstream stock. However,

it has undergone a natural selection process in the wild. This reduces the possibility of an individual contributing undesirable genetic traits to the hatchery population.

Contributed by Peter McCully Goldstream Hatchery (salmonrule@shaw.ca)

For conservation focused reintroduction/re-establishment programmes consideration of genetic issues is critical but options may be limited by the source of material available to find the hatchery stock to be used for restocking. Attard et al (2016) present a useful holistic framework using carefully managed genetics-based breeding accompanied by genetic monitoring of survival and recruitment related to the re-introduction of species of pygmy perch into the lower Murray Darling Basin in Australia.

In ranching programmes there is no intention of creating self-sustaining populations. In these circumstances, genetic resource management should strive to optimize productivity and reduce negative impacts on the ecosystem. The production of sterile fish (see Box 10) is the best means to reduce the chance of stocked organisms breeding with local species. Creation of triploids, i.e. inducing the retention of an extra set of chromosomes, is the most common method of producing sterile fish. Triploidy can be induced by temperature, pressure or chemical treatments to gametes and developing embryos. This is easily accomplished in some species such as oysters, salmon and trout, but difficult on a commercial scale for others, such as tilapia. However, it should be noted that in some species triploids can have issues associated with health, welfare and product quality which could negate their use for stocking programs.

Box 10

Mechanisms to induce sterility and control reproduction in aquatic species already exist and next generation technologies are under development with considerable potential for application

Producing sterile or non-reproductive organisms for release has a number of potential benefits for stocking programmes, for example organisms may grow faster because energy is not diverted to reproduction, they may not exhibit secondary sexual characteristics or, assuming they are effectively sterilized, will be unable to breed with wild relatives. Several techniques are available to produce sterile organisms including ‘traditional’ approaches and more modern approaches utilizing genetic engineering, as reviewed in the context of aquaculture by Xu *et al.*, 2023.

Traditional approaches:

These include hybridization, triploidy and monosex induction. Crosses between some species yield viable but sterile hybrids, for example the cross between brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*), however such combinations among species in which stocking is desirable are relatively rare and thus this approach has its limitations. Triploidy is a more broadly applicable technology and has been induced in a large number of aquatic species using a range of approaches including chemical, pressure and temperature treatments applied to fertilized eggs. In some species such as Pacific oysters direct triploidy induction is performed in commercial hatcheries (Vignier, Adams and Lovatelli, 2024) but tetraploids have also been shown to be viable enabling mass production of triploids in diploid x tetraploid matings. Triploidy is most commonly applied to the stocking of salmonids.

For some species, most notably the salmonids, tilapias and some crustaceans, it is possible to produce exclusively monosex progeny, either all females (more common in salmon) or all males (more common in tilapias). This can be achieved through manipulation of established genetic sex determining mechanisms or via direct hormonal sex reversal treatment applied to sexually undifferentiated life stages. Hormonal treatments are mainly applied to tilapias whereas genetic manipulation of sex has been applied at scale mostly to salmonids. For a ranching programme where no con-specific population exists, this method may be successful in limiting reproduction but obviously, such an approach has its limitation where con-specific wild populations exist, particularly in the case of stocking of all female populations which would be capable of generating significant recruitment from interbreeding with wild con-specific males.

All these traditional approaches to control of reproduction have some limitations as they are not necessarily easy to apply in large scale and all change characteristics of the organism beyond simply ensuring that they do not breed. Also, not all mechanisms guarantee sterility creating the possibility that some stocked organisms could still have the potential to reproduce, although risks would be very significantly lower than for stocking of fully fertile mixed sex individuals. In some cases technologies can be combined to further reduce risk or deliver combined benefits of technologies such as in the stocking of triploid all female trout in British Columbia.¹⁴

Modern bio-engineering approaches:

Although, at the time of writing, not developed to the point of commercial application to aquaculture or used in stocking programmes, modern genetic manipulation techniques, particularly gene editing, offer the most significant potential. Transgenesis can be applied through disruption or reduction of genes that regulate production of reproductive hormones known as gene knockout or gene knockdown. Transgenic fish are almost universally referred to as GMOs and generally subject to regulations that would almost certainly prohibit their deliberate release into the wild. Gene editing approaches, typically using the CRISPR/Cas9 technique, can be used to insert, delete or substitute one or more DNA nucleotides to induce changes that can be transmitted by the germline to subsequent generations. Of course, in the case to induction of sterility, this would need to be reversible (for example through application of exogenous reproductive hormones) in order to be inherited, an approach which is deemed feasible. Gene editing research is targeting different parts of the reproductive pathway through the endocrine system or disruption of gametogenesis. Perhaps the most promising approach is disruption of the dead end (dnd) gene which encodes an RNA-binding protein that plays a role for migration of primordial germ cells (PGCs) to the gonadal region during embryogenesis and could be broadly applicable across vertebrates. This technology is perhaps some years from commercial application but may be subject to fewer regulatory constraints than transgenesis (gene edited products are not classed as GMOs in all jurisdictions and the classification and association regulation of this technology is still to be developed and harmonized globally).

The release of individuals of a single sex, i.e., monosex, has also been used to reduce chance of reproduction. Combining induced triploidy with monosex production would further reduce chances of unwanted breeding. The advent of powerful molecular techniques, particularly gene editing, hold out some prospect of being able to produce sterile farmed types for stocking across a broader range of species provided that regulatory hurdles can be overcome (see Box 10).

It is also possible to control breeding through non-genetic means, for example by controlling fishing effort and through choice of the habitat that is stocked. Aquatic organisms are sometimes stocked in temporary waterbodies that dry up before they can reproduce, in enclosed water bodies that lack connection to critical spawning habitats, or in areas with intense fishing pressure where 100 percent of the stocked fish are taken. However, these conditions are not always 100 percent effective at preventing reproduction. The use of sterile fish in these circumstances would further reduce the chance of unwanted reproduction and recruitment.

5.5.1 Broodstock management

Broodstock in the hatchery must be managed over one or more generations to meet the genetic objectives of the stocking programme (Table 5), which could be to produce farmed types with:

- similar genetic structure to a wild population;
- a genetic structure different from a population of wild relatives;
- specific genetic markers;
- a specific effective population size (N_e); and
- the inability to breed (sterile or monosex).

¹⁴ www.gofishbc.com/Blog/Science-and-Research/Why-We-Stock-Triploid-and-All-Female-Trout.aspx

Table 5 Genetic objectives of broodstock management in hatcheries producing progeny for stocking (Adapted from Bartley, Kent and Drawbridge, 1995)

Objective	Applicable circumstances	Required action
Increase genetic diversity	Small or founder populations have lost genetic diversity	Single pair mating of large numbers of unrelated organisms
Reduce inbreeding	Small populations or hatchery stocks derived from close relatives	Single pair mating of large numbers of unrelated organisms
Reduce genetic drift	Small populations especially with rare alleles	Single pair mating of large numbers of unrelated organisms
Conserve rare alleles	In restocking or enhancement of natural stocks where rare alleles are present	Specific mating of adults with the rare alleles
Genetically mark fish	In all stocking programmes	Select broodstock with specific genetic markers, e.g. SNPs
Produce specific genetic stock (farmed type)	As needed to meet objectives	Broodstock mating with specific genetic stock structure
Produce sterile organisms	When producing organisms not meant to breed after release	Triploidy, gene edited sterility, hybridization sterility
Increase effective population size (N_e)	To improve genetic diversity and viability of farmed type and recipient population	Increase numbers of unrelated broodstock, mate equal numbers of unrelated males and females and standardize variance in offspring production

In general, the management and optimization of genetic variability and/or the monitoring of the genetic status of farmed types (or translocated wild stocks) for stocking programmes, required the use of genetic markers. One of the major hazards in hatchery breeding programmes is losing genetic variation through inbreeding or genetic drift. Both inbreeding and drift can be reduced by increasing or managing N_e in the group of organisms to be stocked through appropriate broodstock management.

Also of concern is outbreeding depression where broodstock are so different genetically that offspring (for example that could arise from interbreeding of genetically distinct stocked fish with the resident receiving population), are inviable or have reduced viability. This is less of a risk with accurate species identification in broodstock, but can be an issue in areas where broodstock are identified by morphological rather than genetic characters or when broodstock are mated from very different geographic areas or habitats.

The concept of effective population size (N_e) is important to understand with regard to genetic management of stocks in a hatchery as it is an important tool to monitor and optimize the retention of genetic diversity from one generation to the next in a captive population. N_e is a measure that refers to the number of individuals that actively contribute to the next generation within a population i.e. the number of male and female broodfish and produce viable offspring able to contribute to the next generate.

Effective population size can be managed in the broodstock by breeding and retaining progeny from the maximum number of broodstock and mating equal numbers of unrelated males or females as can be determined from the application of equation 1 (from FAO, 2009). This is best achieved by using single pair matings and standardized brood size from each mating. In this way a few productive organisms won't be over-represented in the farmed types to be stocked. There are a number of ways to estimate N_e but the most commonly used estimate is equation 1.

$$1 \quad N_e = \frac{4 \times (\text{number of females})(\text{number of males})}{(\text{number of females}) + (\text{number of males})}$$

Where: number of males and females are those that leave viable offspring that can contribute as broodstock to future generations.

An important point that can be demonstrated using this formula is that N_e is sensitive to the sex ratio at which organisms are bred. This is illustrated in Figure 3 (from Tave 1999):

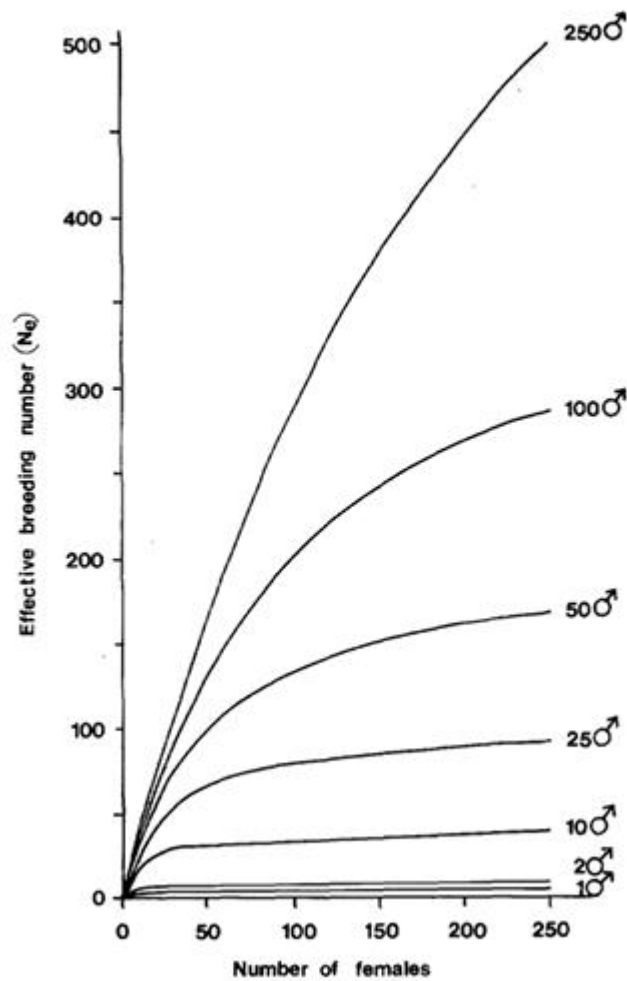


Figure 3 Illustration of the effective population size based on various proportions of males and females (from Tave 1999) illustrating that equal sex ratios produced the optimum N_e .

N_e is inversely related to the rate of inbreeding (ΔF) based on the following formula:

2

$$\Delta F = 1/2N_e$$

Based on this formula it can clearly be illustrated how N_e determines the accumulation of breeding per generation (Figure 4).

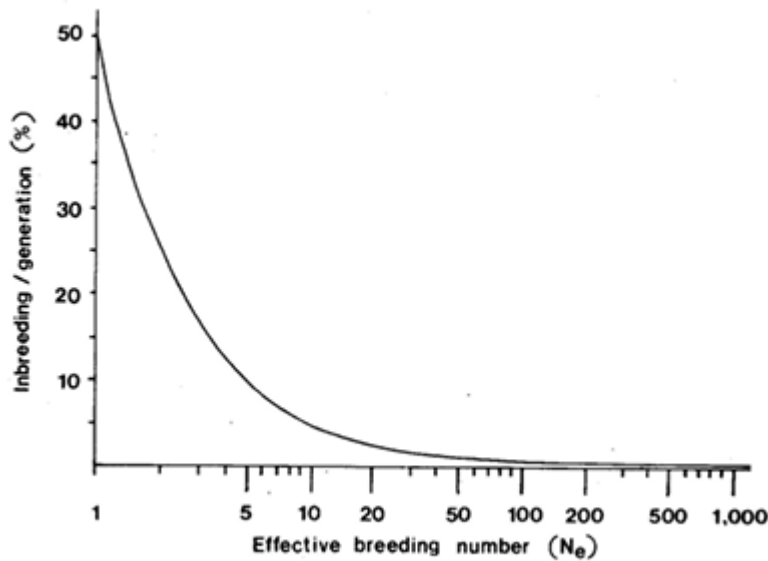


Figure 4. The relationship between N_e and average rate of inbreeding (from Tave 1999)

5.5.1.1. Inbreeding

Because inbreeding (F in equation 2) is accumulated through the mating of close relatives, PBT is used so that broodstock can be given unique tags or genetic markers. Thus, it is easier to prevent inbreeding or to minimize it by preventing parent-offspring, brother-sister, and half-sib matings. If the closest mating allowed is between second cousins, inbreeding will never become a problem. Inbreeding problems, i.e. inbreeding depression, may take a while to be reflected in changes in gene frequency the next generation.

5.5.1.2. Genetic drift

The relationship between N_e and genetic drift can be described by this formula:

$$3 \quad \sigma^2_{\Delta q} = pq/2N_e$$

where $\sigma^2_{\Delta q}$ is the variance of the change in gene frequency (the way genetic drift is measured) and p and q are the frequencies of alleles p and q for a given gene (FAO, 2009).

As was the case with inbreeding, genetic drift is inversely related to N_e ; the smaller N_e , the more likely that genetic drift will change gene frequencies. The effect that a reduction in N_e can have on gene frequencies via genetic drift is immediate.

Because it is difficult to prevent genetic drift in managed populations, genetic drift must be partitioned into acceptable and unacceptable changes for management purposes. A change in the frequency of an allele from 0.4 to 0.38 might not be critical and can thus be classified as acceptable, but the disappearance of an allele or change in the frequency of a common allele to that of a rare allele could be critical and should be classified as unacceptable and thus, where possible, prevented from occurring. Consequently, N_e must be managed to minimize the loss of alleles; since rare alleles are more likely to be lost than common ones, preventing the loss of rare alleles via genetic drift could be a specific management goal.

The probability of losing an allele of frequency q via genetic drift in a single generation is:

$$4 \quad P = (1 - q)^{2N_e}$$

When managing a population's N_e to minimize genetic drift, one must determine what genetic risk is acceptable; in this case, it is the desired guarantee of keeping an allele at a specific frequency or range of frequencies after a given number of generations. Geneticists and population biologists consider that an allele whose frequency is 0.01 contributes significantly to polymorphism, so a general goal of fisheries management and conservation programmes could be to save alleles whose frequency is 0.01 (if this is done, more common alleles will also be saved); other hatchery stocking programmes save alleles of frequency 0.10 based on baseline sampling of the population to be enhanced. For example, the constant N_e s needed for 20 generations in a hatchery range from 378 (for a moderate risk with $F < 5$ percent and a 99 percent guarantee of keeping an allele whose frequency is 0.01) to 1 000 (for little risk with $F < 1$ percent and a 99 percent guarantee of keeping an allele whose frequency is 0.005).

In recovering or enhanced populations stocking can help increase N_e through the addition of large numbers of genetically diverse unrelated males and females. However, stocking can also reduce N_e when large numbers of related individuals, individuals of only, or predominantly, of one sex, or individuals with reduced genetic diversity contribute to the spawning of small recovering or enhanced populations. This is especially crucial for marine species that are often highly fecund; hatchery production may inadvertently produce large numbers of organisms from only a few parents and thus reduce N_e .

The discussion about calculating N_e assumes that the population is fairly stable, which is often the case at a hatchery. When this is the case, the N_e for inbreeding and genetic drift is calculated as described above. However, when the population is small, fluctuates wildly over generations, or is declining, the N_e for inbreeding and genetic drift are different, because the effect of N_e on the population can be immediate (genetic drift) or delayed (inbreeding). When this is the case, N_e for genetic drift is called variance effective number (N_{ev}) and is described in equation 5 (FAO, 2009):

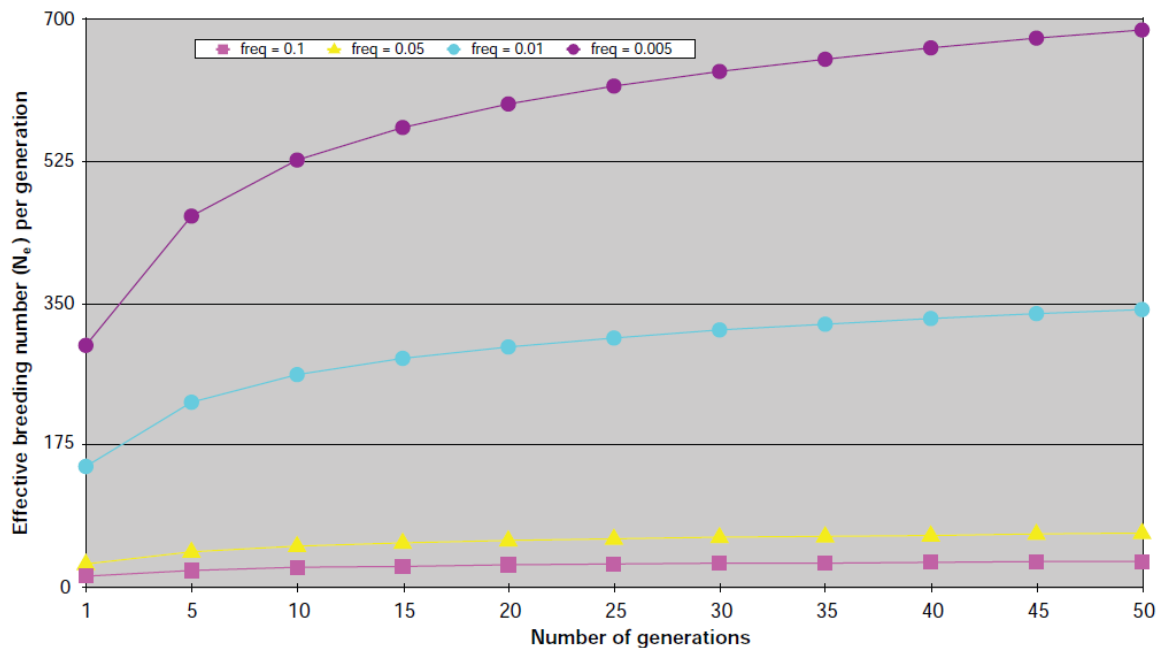
$$5 \quad N_{ev} = (4N-2)/(V_k + 2)$$

where N is the number of fish in the parental generation and V_k is the variance of offspring production.

N_{ev} is more important for fisheries and conservation management than it is for food fish culture, because genetic drift can have a more damaging effect on the ability of the species to survive in the wild. N_{ev} is estimated for each generation, independently from those of the previous generations. The common recommendation of N_e , i.e. $N_{ev} = 500$, will do a good job of minimizing genetic drift from parents to their offspring for >50 generations

Figure 4. However, if N_{ev} of the population was small in previous generations, an $N_{ev} = 500$ in a closed population will only keep genetic drift-induced problems from getting worse; it will not reverse genetic damage, e.g. loss of alleles and inbreeding, that has already occurred. This only applies to closed populations; addition of new alleles and unrelated broodstock from other sources would be able to increase N_{ev} . Means to increase N_e involve broodstock management and monitoring (Table 5).

Figure 4 Chart illustrating the N_e per generation, for 1 – 50 generations, to produce 95% guarantees of saving alleles whose $F = 0.1 - 0.005$. (Source: FAO, 2009)



5.6. Release strategies

Aquatic organisms often have specific migration times or behaviours that are in part controlled by their genes. Therefore, release strategies should take into account the movements of the released organisms and seasonality of movement or behaviour. Release strategies can also involve different life history stages, e.g. larvae, fingerlings, juveniles or adults. Larger organisms tend to survive better after release than younger ones. However, the longer a group stays in an aquaculture facility the greater the risk of the group being less able to survive and reproduce after release due to the behavioural and physiological adaptation to the artificial culture environment and the loss of individuals that may be well adapted in the wild but failed to thrive in captivity. This can be mediated to some extent by careful and prolonged acclimatization process upon introduction/reintroduction. In many ranching programmes for recreational fisheries, adult organisms are often released to satisfy anglers' immediate desires to catch fish as the main release strategy. Release of organisms into areas where they will have reduced chance of survival (see section 5.4) could be a strategy where fish are not intended to create self-sustaining populations. For some stocking programmes, release strategies involve acclimating organisms to the receiving environment or moving organisms away from geographic areas where they may not survive. For example, in some salmon enhancement programmes where there are genetically discrete stocks, release strategies correspond to the timing of the specific stock's juvenile or smolt migration time.

5.7. Monitoring and evaluation

Monitoring and evaluation are key elements often neglected in stocking programmes. As with all fisheries, monitoring of all stocking programmes is essential and for this the stocked material must be identifiable. Hatchery marking programmes are being mandated in many parts of the world to assess hatcheries' contributions to fisheries or endangered populations (Box 11 and Box 13). Physical tags can identify initial stocked individuals, but if stocked fish reproduce in the wild, as is often an objective, only specifically selected genetic markers can indicate hatcheries' contributions to subsequent generations. McKenzie et al (2012) comprehensively review the properties of methods of fish tagging and marking which are broadly applicable to molluscs but options for crustaceans are

more limited due to their habit of moulting as they grow. Ramya and Behera (2023) review molecular markers and their applications in fisheries and aquaculture.

Increase in abundance of stocked organisms is sometimes, but not always, an indicator of hatchery contribution to a fishery. However, favourable changes in the environment or better fishery management may also promote natural increases and genetic monitoring can assist in identifying the relative contribution of stocking programmes to the health of a population.

Box 11

The GeMoLaR project – an example of incorporation of monitoring for genetic management of fish stocks

All native German populations of Atlantic salmon became extinct in the 1950s due to a combination of water pollution, water diversion, overfishing and hydroelectric power plant construction. In the 1980s efforts to improve water quality and reintroduce Atlantic salmon were undertaken, particularly in the Rhine catchment. Today, more than 1 million Atlantic salmon are stocked yearly to support the return of salmon to the Rhine River basin. The stocked salmon are produced by six hatcheries, and broodstock from different genetic origins are used. Stocking schemes differ between the hatcheries stocking the numerous Rhine tributaries, with salmon being released at different developmental stages and different breeding protocols and broodstock sourced are used for the different locales.

Although many salmon reintroduction projects have been operating for more than 30 years, all individual salmon project rivers still depend on on-going stocking. The quantitative success rates of these very cost- and labour-intensive stocking measures remain largely unknown. With the various hatcheries using different broodstock, and breeding and release strategies; systematic monitoring and evaluation of these practices has not been carried out. Therefore, there was an urgent need to evaluate current management to maximize the effectiveness of stocking efforts.

In order to close the existing knowledge gaps, the German Ministry for Agriculture and Food is supporting “Genetic monitoring for the reintroduction of Atlantic salmon in the Rhine basin” – known as the GeMoLaR project.¹⁵ The project aims to evaluate and optimize the stocking procedures for Atlantic salmon in the entire German Rhine River basin.

The project can compare the genetic profile of salmon from the Rhine to other genetic stocks of Atlantic salmon in a European-wide dataset (the SALSEA-Merge project)¹⁶ and thus even salmon coming from distant waters can be identified. Tissue samples of all parental fish from the 6 hatcheries that breed salmon for stocking in the German Rhine system were taken and genetically analyzed with microsatellites. Further, sample were taken from all available fish returning to the Rhine and its tributaries, from the sea. This enabled parental assignments of the returning fish to the hatchery of origin, and in many cases, the exact date and stocking location of the returning fish can be determined.

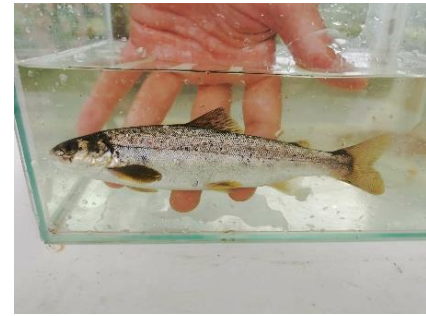
Genetic analysis revealed that almost all salmon from the upper Rhine can be assigned to parents originating from the “Allier” (river in France) genetic stock and most salmon from the middle and lower Rhine can be assigned to parents originating from the “Ätran” (Sweden) genetic stock or to the genetically similar population from the Danish Center of Vildlaks hatchery (a hatchery that also breeds the Ätran-origin salmon). Remarkably, 10-20 percent of salmon in the middle and lower Rhine were assigned to a stock of Irish origin, despite that Irish salmon have not been bred and stocked directly in the river Rhine system for more than 15 years.

The method also permits discrimination between stocked salmon and salmon originating from natural spawning. For example, in the river Speyerbach the parents of 55 out of 71 parr and smolts were identified to be of hatchery origin and thus the remaining 16 specimens would be naturally spawned. This information is very important as it shows where the amount of natural spawning is high and can thus help to improve management decisions.

¹⁵ <https://gemolar.fish/>

¹⁶ <http://salmonatsea.com/the-salsea-merge-project/>

Since humans were responsible for the disappearance of the salmon from the Rhine, the goal of several management programmes is to re-establish self-sustaining populations of the iconic Atlantic salmon in the Rhine. The genetic stock structure of Rhine River Basin Atlantic salmon populations and the stocking strategies of the hatcheries that are necessary to support them are now being assessed and evaluated. The GeMoLaR project is still running and information from detailed, small-scale studies is expected to be analysed and evaluated in the near future to inform management practises.



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Box 12

The role of genetic analysis and monitoring in support of oyster stocking programmes in Europe – The Native Oyster Restoration Alliance

The Native Oyster Restoration Alliance (NORA) is a non-profit Foundation and a network organization, uniting stakeholders interested in and working towards native oyster reef restoration in European waters. NORA's key objective is to support the protection and ecological restoration of the native European oyster (*Ostrea edulis*) and its habitat in areas of its current or historical distribution. NORA seeks to support responsible restoration practices, in compliance with global principles of ecological restoration including sustainable management of genetic resources. With its working groups dedicated to European native oyster genetics and production, scientists and practitioners are focusing on four key areas:

1. Phylogeography, population genetics, and identification of evolutionary forces shaping past and present population structure, with special attention given to populations at the fringe of the geographical range and genomic signatures of selection in populations under selective pressures.
2. Optimization of reproduction in captivity and monitoring of the progeny genetic diversity. Indeed, *O. edulis* reproductive strategies can easily lead to low genetic variability and subsequent inbreeding depression, therefore attention is currently given to pair-mating and marker-assisted parentage assignment to manage genetic diversity.
3. Selective breeding methods to improve traits of interest is showing promising results for disease resistance (to bonamiosis). Recent results from QTL mapping studies and Genome Wide Association Studies have also demonstrated the potential of marker-assisted and genomic selection albeit this remains to be implemented in breeding programs.
4. Connectivity between populations and positive/negative interactions between wild, restored, and farmed populations remain to be investigated. Direct quantification of larvae and hydrodynamic modeling can now be complemented by eDNA data to study larval dispersal in coastal areas and connectivity between populations.

These areas are notably being translated into practice by the development of a dedicated genetic program at the "Native Oyster Hatchery" at DTU Aqua (Technical University of Denmark). DTU Aqua is the sole Danish shellfish hatchery producing European native oyster for both restoration and aquaculture purposes.

Since 2019, the hatchery started two programs where incoming oysters used as broodstock were genetically screened for the presence of the disease and to establish genetic diversity. *Bonamia*-free oysters are stored in individual tanks and successively screened on a yearly basis to verify their disease-free status prior to being used for reproduction. A method for genetic diversity and parental

contribution in the production of spat has been successfully developed to compare broodstock status with the local wild population. This provided key indication on the effective number of breeders (N_b) to be used (Alves Monteiro *et al.*, 2022).

The number of oysters in a broodstock tank and broodstock batches are now selected on the basis of a compromise between: i) minimizing biosecurity risks, i.e. reducing the number of breeders in the same tank (10 to 20), and ii) ensuring acceptable genetic diversity of the spat, by increasing broodstock batches numbers (Alves Monteiro *et al.*, 2022). In order to safeguard an effective population (N_e) $\gg 50$ and limit broodstock adaptation to captivity, new individuals are also added to the oyster stock every year, and parental contribution from the broodstock and genetic diversity of the offspring are checked for each swarming event. All batches of spat are traceable, and genetic diversity is monitored once released into the wild. In the future, genetic tools will be applied also to guide management and selection of disease resistant broodstock.

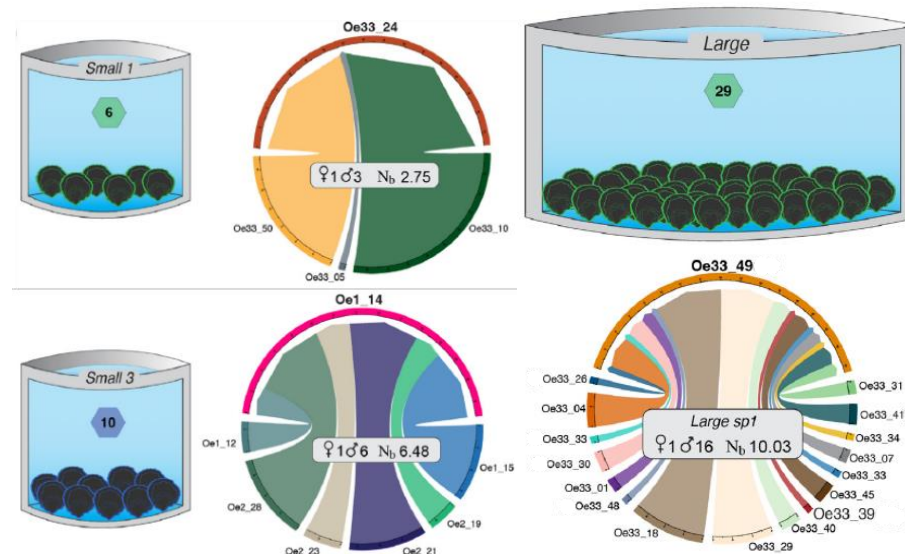


Figure 5 adapted from Alves Monteiro *et al.* 2022. Hatchery setting and breeding pairs. Experimental set-up in 3 parental broodstock tanks ('Small 1, 3' and 'Large') and offspring. Hexagons show broodstock sizes. For individual swarming events, each colored segment of the chord diagram represents a paternal full-sib family, and the thickness of the chord represents the number of offspring identified, as shown by peripheral tick-marks. Tags inside chord diagrams give the total number of observed breeders (dam and sires) as well as the number of effective breeders N_b .

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Hatcheries may simply report numbers of organisms released with no indication of the impact of those releases on the target populations. Member Countries of FAO have agreed to provide fishery and aquaculture data to FAO in order to better understand global status and trends; they fulfill this agreement by answering a FAO questionnaire. The FAO questionnaire on reporting fishery production has a question on the number of organisms of early life history stages that are released into natural environments (i.e. stocking). Although this is a very poor indicator of the impact of stocking programmes, most countries, even countries with large hatchery release programmes, generally do not complete this question.

Monitoring of the success and genetic impact of stocking programmes should make use of standardized questionnaires to collect reliable information and should be continued beyond the hatchery and release reports to include tracking genetic status, using genetic markers, over time in the rebuilt or enhanced populations (see Box 12, as well as in the populations of wild relatives with which the stocked organisms may interbreed. A range of genetic tools are available that can be used for such

monitoring. For several marker types such as SNPs and microsatellites, numerous markers are needed to distinguish stocks or for parentage assignment. Fortunately, researchers have developed SNP panels for a range of species with numerous markers that can be used although marker systems generally need to be developed specifically for individual species or species groups. Application of genetic marker analysis has become progressively less expensive as technology and capacity has advanced making it more affordable, particularly for the more routine analysis, once the marker panels are already developed.

As stated above, baseline genetic information on these stocks is essential to assess the impact of the hatchery releases and can provide additional information over and above what is possible from physical tags (see Box 13). Given the rapid development of useful genetic marking tools, additional and more powerful genetic markers will probably be developed in the near future.

Box 13

Use of genetic markers in assessing impact of stocking of white sea bass (*Atractoscion nobilis*)

White seabass, *Atractoscion nobilis*, is a marine finfish that supports commercial and recreational fisheries along the California/Mexico coast. The range of white seabass extends from Magdalena Bay, Baja California, Mexico to Juneau Alaska, USA with the center of abundance between Point Conception, California and Ballenas Bay, Baja California. In light of the fact that natural populations of the fish have declined, the California legislature initiated a stock enhancement project in 1983, carried out by the *Ocean Resources Enhancement Program (OREHP)*, with the aims of conserving natural levels of genetic diversity and increasing the population of white seabass in the wild. In 1986 the first farmed types were released into the coastal waters of California by Hubbs SeaWorld Research Institute in San Diego, California.

White sea bass are mass-spawners, i.e. they breed in large spawning aggregations. Hatchery breeding involves placing numerous wild-sourced adults in large tanks at spawning time and collecting the eggs. Early genetic studies demonstrated that the N_e of the broodstock was lower than the census (N) number of adults in the tanks and that adults did not contribute equally to the next generation. Thus, there was a great potential for hatchery releases to reduce N_e in the wild populations. Hatchery protocols were developed to ensure N_e large enough to conserve rare alleles and maintain genetic diversity similar to wild populations (Bartley *et al.*, 1995). The protocols involved sourcing broodstock from the wild, genotyping adult fish, rotating adults among different spawning tanks, and eventually releasing adults and replacing them with new wild-sourced broodstock. Modifications to early protocols have been made based on advances in genotyping fish and to overcome specific culture problems with raising adult white seabass in captivity (Gruenthal and Drawbridge, 2012). Since 1990 hatchery contribution to the wild population and recreational and commercial fisheries has been tracked using coded-wire tags (CWT); assessment has also involved genetic monitoring (Reiber, 2022).

Genetic monitoring based on a new 15 marker microsatellite panel indicated that CWT monitoring underestimated the programme's contribution to the sub-adult population of white seabass from California waters: 7.4 percent contribution estimated by CWT data vs 27.7 percent contribution based on the microsatellite markers. The hatchery contribution to the adult population taken from Mexican waters was 7.4 percent based on genetic markers and no CWT were found, although the sample size was very small ($N=3$) (Reiber, 2022). Genetic monitoring revealed no significant differences in population structure from Mexico to southern California (Reiber, 2022). With further refinement of genetic monitoring, it should be possible to more accurately determine the contribution of farmed types to the wild population and determine if farmed types are breeding in the wild. This example clearly illustrates that genetic monitoring can provide information not available with physical tags. The OREHP continues to date with *inter alia*, improvements in culture technology and husbandry, controlled breeding of white seabass, release of farmed types, checking of broodstock to ensure no farmed types are used for breeding and monitoring.

Monitoring may need to change over the course of a stocking programme. For restocking programmes monitoring may need to be fishery independent as the recovering population becomes established. For

enhanced populations or where populations can support harvesting, fishery dependent sampling would be possible. Fishery dependent monitoring of ranching programmes would also be appropriate.

Additionally, stocked fish have, in some cases, displaced local con-specific stocks. This is to be avoided and another reason why the ability to differentiate among hatchery and wild stocks is important in the overall assessment of stocking as a management strategy.

5.7.1. Precaution

In light of the risk associated with stocking, a precautionary approach (FAO, 1996) is advised and requires the development of reference points. Target reference points indicate desirable situations a hatchery or resource manager will strive to achieve and limit reference points indicate conditions to be avoided. Regular monitoring is required to determine to what extent the reference points are met or exceeded.

The reference points should relate to stated objectives, risk assessment and measures of success (Table 6). The reference points may be very restrictive, i.e. precautionary, for important wild populations to be enhanced or rebuilt, and/or where important fisheries may face harm, or they can be lenient for other situations. Reference points should be established in a participatory manner involving hatchery, fishery and conservation stakeholders as applicable. The reference points shown in Table 6 are only indicative examples; actual reference points should be decided by stakeholders. The last row of Table 6 on pathogens is not a genetic issue but demonstrates that reference points can be extremely restrictive depending on the type of risk.

The precautionary approach also calls for agreed actions or contingency plans to be put in place when the reference points are reached or breached. Actions may involve, *inter alia*, reducing or expanding the stocking programme, changing broodstock management, or increasing N_e of released organisms.

Table 6 Possible reference points for the application of a precautionary approach to genetic resource management in stocking programmes. T and L are Target and Limit reference points, respectively.

Purpose of establishing a reference point	What to measure (hypothetical/potential reference point)
To establish acceptable levels of inbreeding (L)	Inbreeding coefficient ($F = 0.15$)
To establish acceptable level of gene flow/introgression between released organisms and wild stocks (L)	1. Number of wild and farmed organisms exchanging genes (<1/generation) 2. Change in gene frequency in wild stocks ($F = , 0.10$)
To establish acceptable number of organisms to be used as broodstock (T)	Effective population size (N_e) of broodstock (500 for long-term stocking programmes)
To ensure sterile aquaculture product (T)	1. Percent triploid fish in hatchery product (>90%) 2. Percent organisms producing gametes (<3%) 3. Number of released organisms breeding in the wild (<2)
To conserve rare genes in the wild (T)	1. Effective population size ($N_e = 500$) 2. Gene frequency in hatchery stocks (targeting identical frequency of rare gene in the wild)
To assess impact of stocking	1. Number of organisms stocked (poor indicator and usually set by administrators) 2. Percent change in recipient stocks (depends on management objectives and may take time to observe e.g. 15 percent) 3. Increase in hatchery-specific genetic marker in wild stocks (e.g. 15 percent)
To establish level of harm from stocking programme (L)	Reduction in N_e over a given period of time, e.g., 10 years or 3 generations (<10%; could be lower for rare and endangered species)
To establish acceptable fishery impacts (T and L)	1. Fishing mortality ($F = 0.20$ or could be set lower for recovering populations) 2. Yield (Maximum Sustainable Yield)
To establish risk of extinction (T)	1. Effective population size ($N_e = 500$) 2. Probability of extinction within a given time, e.g., 5 yrs) ($p < 5\%$)

	3. Decrease in population size (> order of magnitude decrease over a period of time)
To prevent spread of pathogens and disease (L)	Levels of specific pathogens in hatchery and wild populations (0 is often set as a target and limit reference point)

6. A decision support tree

This section presents a flow chart representing the key decisions processes in planning and implementing a stocking programme. Figure 6 is currently under development and is yet to be finalized.

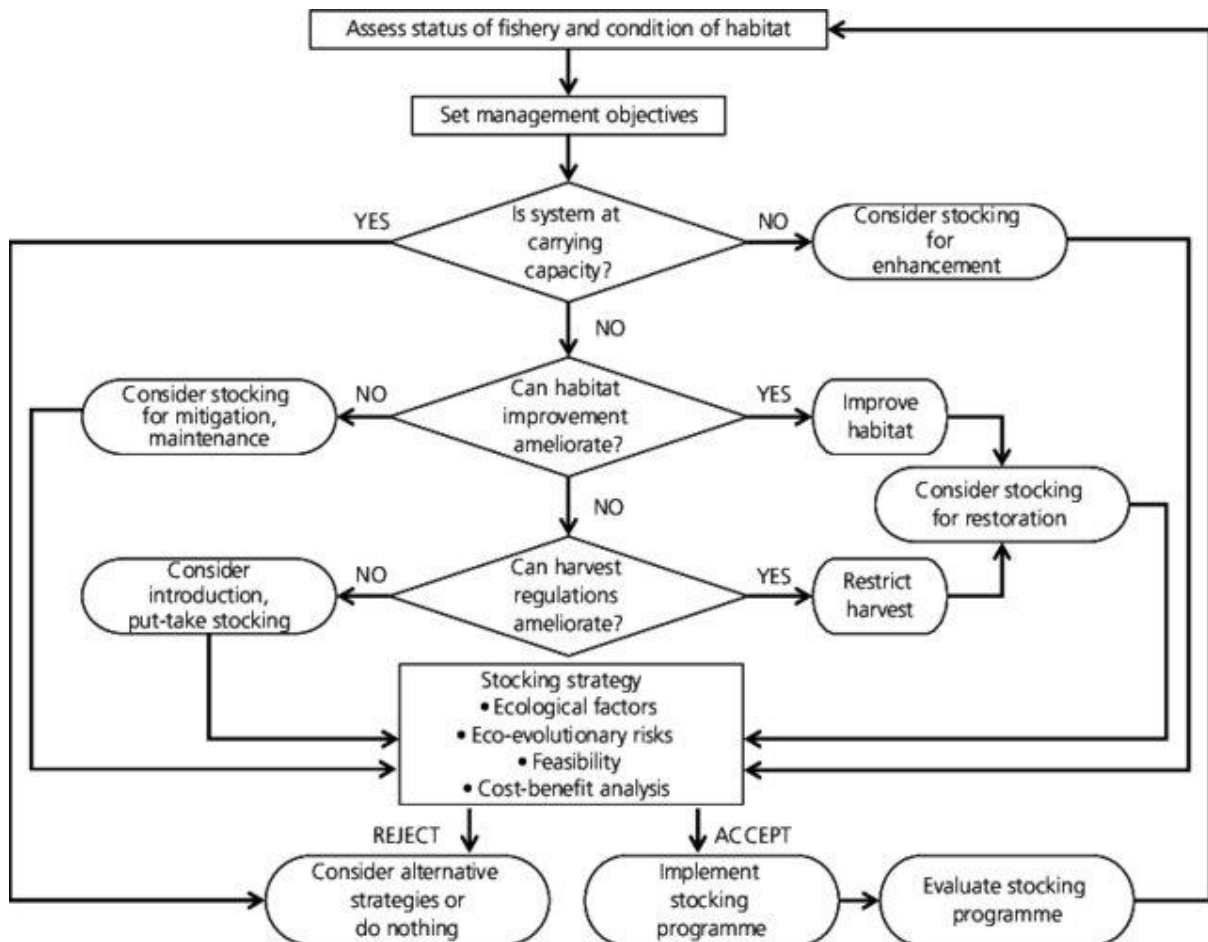


Figure 6 Flow chart representing the key review and decision-making processes in planning and implementing of a stocking programme (initial draft only and final version is being prepared with a graphic designer)

7. Summary and conclusion

These guidelines were prepared at the request of the Commission on Genetic Resources for Food and Agriculture. They represent practical guidelines on genetic management in stock enhancement which include culture-based fisheries and other types of stocking of hatchery reared farmed types, and translocated wild stocks, into the natural environment. There are several rationales for such stocking activities including stock establishment, restocking, stock enhancement and ranching. These carry varying levels of risk to the genetic resources in the receiving environment and this risk needs to be understood and mediated. The application of these guidelines in the planning and implementation of stocking programmes, for any of the reasons outlined above, will focus attention on minimizing the risks of harm arising from stocking events. The overall objective of the guidelines is to provide practical genetics related advice to hatchery, fishery and resource managers on how to responsibly and

sustainably use aquatic genetic resources for food in stocking programmes for enhancing fisheries, both commercial and recreational, habitat and ecosystem rehabilitation and conservation.

These guidelines are intended to be used by all stakeholders when considering initiating or continuing on-going stocking programmes. They can be incorporated into national strategies for fisheries and ecosystem management and used to inform stocking decisions and policies on a case-by-case basis and should be consulted prior to initiating any stocking.

These guidelines relate to the implementation by members of the *Global Plan of Action for the Conservation, Sustainable Use and Development of Aquatic Genetic Resources for Food and Agriculture* (FAO, 2022) and can be informed by data on related genetics resource entered in AquaGRIS, FAO's information system for aquatic genetic resources.

The guidelines cover stocking activities for a range of purposes including in support of fisheries, conservation and ecosystem services but exclude stocking for the purposes of aquaculture. Stocking events or programmes can be for stock establishment, restocking, stock enhancement and ranching which represent varying levels of genetic risk to the receiving population or genetic stock. This document provides specific guidance on the key elements of genetic management that need to be considered in the development, implementation and monitoring of stocking events for all of the purposes outlined above. These key elements include: relating genetic issues to the benefits of stocking programmes; the importance of baseline genetic information prior to initiation of stocking; the components of risk analysis to be applied during the planning process; the management of identified risk; the selection of founder stock; the application of genetic management in the hatchery prior to introduction for different stocking purposes; release strategies; monitoring and evaluation of stocking programmes once underway and the application of the precautionary approach.

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