WORKING GROUP ON THE APPLICATION OF GENETICS FOR FISHERIES AND AQUACULTURE (WGAGFA)

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Contents

i Executive summary

In line with the Working Group on Application of Genetics in Fisheries and Aquaculture's (WGAGFA) research focus, which includes ecosystem-based fisheries and aquaculture management, conservation and biodiversity preservation and climate change forecasting, the scientific work presented in this report aims at i) exploring cutting-edge genomic advancements that show promise in realizing ICES vision, ii) facilitating the integration of genetic methods in fisheries and aquaculture management and iii) generating new genetic knowledge to better understand marine ecosystems. The specific questions addressed in the Terms of Reference tackled during this three-year term include the exploration of how genetic advances are revolutionizing stock identification, the review of the potential of adaptive variation for fisheries forecasting, the evaluation of the power of genetic tools to better understand the deep ocean and to explore the use of genetic methods for conservation program broodstocks. This work highlighted that despite the proven utility of genetics in fisheries stock delimitation and identification, DNA-based approaches are not routinely used, which could be due to a lack of understanding by stock assessment expert of genetic methods. Additionally, this work highlighted the power of using climate-associated adaptation information derived from genetic data to predict impact of climate change in marine species and develop consequent management measures, and the potential of a panoply of genetic methods to help understand the complex deep ocean at the individual, population, species, and ecosystem level. Concerning broodstock programs, our work reviews examples on how genetic gains are possible through genetically informed breeding and can be useful in long term conservation programs. Several review manuscripts derived from the Tors are in preparation and there will be a topic sheet and a scientific highlight produced. Future work should focus on continuing this discussion by including fisheries stock assessment and data collection experts as well as aquaculture researchers, which will be mandatory to ensure our results are applied in practice.

ii Expert group information

Expert group name	Working Group on the Application of Genetics for Fisheries and Aquaculture (WGAGFA)
Expert group cycle	Multiannual fixed term
Year cycle started	2021
Reporting year in cycle	3/3
Chair(s)	Naiara Rodriguez-Ezpeleta, Spain
Meeting venue(s) and dates	10-12 May 2021, Online (50 participants)
	17-19 May 2022, Online (40 participants)
	9-12 May 2023, Sukarrieta, Spain (40 participants – 25 online & 15 remote)

Scope and Remit

Under the remit of the ICES Aquaculture Steering Group, the Working Group on Application of Genetics in Fisheries and Aquaculture (WGAGFA) provides recommendations on methods to describe, conserve, and manage intraspecific biodiversity, focusing on the application of genetic and genomic analyses. WGAGFA works on management themes spanning from commercial fisheries to ecosystems, recreational exploitation, and aquaculture. This ICES Working Group also looks at several applications for genetic methods. Examples include identifying populations, tracing the origin of fish and fish products, tracing migratory behaviours and habitat use, determining the dynamics of non-indigenous species, and evaluating the effects of aquaculture escapees. Technological developments that have enabled environmental genomics are also considered, with recommendations given on application in species and ecosystem management. Recommendations focus on knowledge generated from applications of molecular genetics and genomic tools to identify, trace, restore, and manage local populations of fish and shellfish. The group also hindcasts and forecasts how drivers – for example physical, climatic, and fisheries ones – affect distributions. During this fixed-term cycle, from 2021 to 2023, in line with its objectives, the WGAGFA addressed through four Terms of Reference:

- How the rapid advances in genomics and analytical methods are revolutionising population identification in marine fish and invertebrate species;
- To review and evaluate the potential of adaptative variation for assessing fisheries;
- To evaluate available genetic-based solutions to better understand the mesopelagic ecosystem;
- Explorative cost-benefit analysis of genetic methods with emphasis on SME and conservation program broodstocks dedicated to aquaculture or natural population enhancement

1 Tor A: How the rapid advances in genomics and analytical methods are revolutionising population identification in marine fish and invertebrate species

1.1.1 Introduction

Fisheries are managed in units, or stocks. These are defined as self-sustained populations, where growth, recruitment, and natural and fishing mortality are not dependent on immigration or emigration rates (Cadrin, 2020). Yet, stock structure is often defined by geo-political boundaries rather than biological ones, hampering efforts for sustainable fisheries management. Signals of population structure have often been difficult to identify in marine species, because of a lack of physical barriers to adult or larval dispersal and large population sizes, and despite the many technologies applied (Mariani & Bekkevold, 2014). In more recent years, genetics has been used to successfully unravel patterns of population structure (Bernatchez *et al.*, 2017; Casey *et al.*, 2016). Nevertheless, the integration of genetic data into assessment and management has been lagging (Waples *et al.*, 2008). Given that stock structure is the starting point of a solid assessment, there has been growing interest in including genetics as a tool for stock delimitation and identification (Cadrin *et al.*, 2023).

The term 'stock' has many definitions depending on who is describing it (Carvalho & Hauser, 1994; Secor, 2014). When delineated using molecular approaches, they are referred to as *genetic stocks*, and are considered reproductively isolated units (Ovenden, 1990). It is important to highlight the difference between *genetic stock delineation* (finding stock structure) and *genetic stock identification* or GSI, which refers to the use of previously identified genetic markers for assigning samples to stocks of origin in mixed-stock analysis. Where genetics is successful in delineating stocks, it has often become part of the management toolbox. As illustrated below, after describing stock structure, genetics can be used to monitor catches through GSI in the case of mixed fisheries (like in cod, herring and tuna). In other cases, this might not be needed, and the genetic boundaries described are used to define the fisheries stocks, spatially and temporally.

The aim of this paper is to contribute to the discussion among fisheries and stock assessment scientists about how to standardise the use of genetic results so that their interpretation by non-geneticists becomes more straightforward. Here, we take the first step by presenting a framework that illustrates how to sample, generate, and analyse genetic data for stock structure and identification. We start with illustrating practical examples where genetics is now routinely employed for advising management and discuss best practices that should be applied to the interpretation of genetic data when deciding whether stocks should be managed separately or not.

1.1.2 Representative success stories

Nowadays, there is a growing number of cases where genetics is used for delineating stocks before they are assessed. Given the initial investment required by genetics at the onset, in terms of cost and skills, these are very often fisheries of very high commercial value which traditionally attract more research efforts.

In salmonids, the successful implementation of genetic measures is relatively straightforward due to their reproductive strategy, which limits gene flow and in many cases results in genetic

differentiation between regions, rivers, and tributaries within rivers higher than it is normally detected in marine species. The management of Pacific salmonids on the west coast of North America is the longest-running example of the incorporation of such genetic techniques into fishery management, with the Columbia River spring chinook salmon (*Oncorhynchus tshawytscha*) gillnet fishery applying genetic approaches since the 1990's. In the same period, genetics was used to delineate stocks for pink salmon (*Oncorhynchus gorbuscha*) in British Columbia, chum salmon (*O. keta*) in Alaska, and coho salmon (*O. kisutch*) off Vancouver Island (Shaklee *et al.*, 1999). For Atlantic salmon (*Salmo salar*), genetics has been incorporated into management strategies more recently, possibly because the genetic markers used in the '80s were not powerful enough to distinguish stocks (Griffiths *et al.*, 2010; Koljonen, 1995; Verspoor *et al.*, 2005). However, recent enhanced stock resolution between river groups in the Barents Sea has now allowed fishery management units to be re-defined (Anon, 2020).

In Atlantic cod (*Gadus morhua*), coastal and offshore populations have been identified genetically (Berg *et al.*, 2017; Bradbury *et al.*, 2013; Johansen *et al.*, 2020; Pampoulie *et al.*, 2011). In northern Norway (>62°N), these units are the Northeast Arctic cod (NEAC) stock in the Barents Sea, and the coastal cod (CC) along the coast and in the fjords. They differ in growth and maturity rates (Nordeide *et al.*, 2011) and genetically (Johansen *et al.*, 2018; Johansen *et al.*, 2020; Jorde *et al.*, 2021), and have been managed as separate stocks since 2000 (ICES, 2021;). In Spring, they are caught in a mixed fishery, so management in some areas is done in real-time, with the proportion of CC/NEAC estimated genetically (Dahle *et al.*, 2018; Johansen *et al.*, 2018), to keep the proportion of CC in the catch <30% over time.

In Atlantic herring (*Clupea harengus*), numerous attempts to identify population structure using genetics have been inconclusive up until the application of whole genome sequencing (Han *et al.*, 2020; Lamichhaney *et al.*, 2017). This approach revealed significant genetic differences between populations and has produced highly discriminatory genetic markers that are now routinely used to distinguish stocks in the North Sea-Baltic Sea (Bekkevold *et al.*, 2023), and around Ireland and the UK, where stocks have been redesigned (Farrell *et al.*, 2022).

Atlantic bluefin tuna *Thunnus thynnus* has traditionally been managed as two stocks separated at the 45°W meridian, the Gulf of Mexico and the Mediterranean Sea, despite tagging analysis demonstrating extensive trans-Atlantic migration (Arregui *et al.*, 2018). Both, otolith chemistry (Rooker *et al.*, 2014) and genetic data (Rodríguez-Ezpeleta *et al.*, 2019) sources of information are now used for mixed stock identification and are now integrated into management.

1.1.3   The way forward: a standardised approach

Here we propose a framework for using genetics for delineating population structure, with the aim of answering basic questions to those bodies interested in using genetics for fisheries, as well as clarifying the interpretation of published results.

1.1.3.1   Samples
Adequate sampling effort, tailored towards the species of interest, which considers the available biological knowledge on spawning and feeding seasons and migrations, population sizes, age structure and the geographic distribution, is important to ensure a successful outcome. Generally speaking, it is important to carefully design the sampling, in order to avoid bias: sampling of individuals should be random, and spatial and temporal replication are important factors in ensuring that any inference of population structure is stable and biologically meaningful (Waples and Naish, 2009).

Where? The number of locations sampled depends on the species' distribution range, migration patterns and reproductive behaviour (spawning areas and time), the budget and the time

available. Sampling grids like the ICES and NAFO statistical areas, represent a good starting point for sampling any species of interest, and the basic design should aim at collecting individuals from each area amongst those occupied by the species. If the species is already managed as separate stocks, they should be included in the sampling. If information on spawning areas and migration patterns between areas are known, these areas should be reflected in the sampling design as well. In general, it is always advisable to include samples 'outside' of the region of interest, to better understand the ecological dynamics causing the presence (or lack) of genetic structure.

What? When spawning grounds and seasons are known, spawners should be sampled because they likely contribute with genetic material to the next generation. If this is not feasible, due to a lack of knowledge or access, collect juveniles and adults (Gíslason *et al.*, 2023), making sure that most age groups are sampled.

How many? Historically, when only very few (<10) genetic markers were used to assess genetic diversity, between 30-50 individuals were collected per sampling location; now that it is feasible to look at thousands of loci (see section 2), estimates of population structure appear to be quite robust against the number of specimens collected per location, and sampling a *minimum* of 20 individuals per location is recommended (Foster *et al.*, 2021), depending on budget and time availability.

How? Tissue samples can be taken from fins, gill or muscle using clean scissors, scalpels or biopsy punch, and should be preserved in ethanol until processing. This assures DNA integrity which is important for all downstream applications. Collecting additional life history data such as length, weight, but also sex, maturity or age will greatly improve the ability to understand any pattern seen in the genetic results.

1.1.3.2   Sequencing and Markers

The more commonly used markers for stock delineation are microsatellites or SNPs (single nucleotide polymorphisms). Microsatellites have individually more resolution power, but they are less frequent and more difficult to discover and genotype. In contrast, SNPs are numerous, spread across the genome and easier to genotype. If markers are already available for the target species, and have been used to successfully identify structure, we recommend keeping using them. If no markers are available, SNPs development is recommended. The use of SNPs has improved the ability to resolve fine-scale population structure with reduced biases, when compared to microsatellite markers (Coscia *et al.*, 2013, 2020; McGill *et al.*, 2023). The spatio-temporal resolution needed might dictate the type of markers to develop through sequencing: if looking for structure at a fine spatial scale or between cohorts, SNPs are more likely to deliver than microsatellites, but *next-generation* sequencing (NGS) can, however, help discover hundreds of very useful microsatellites at once, which can inform on specific management issues – see (Bradbury *et al.*, 2018). There are several sequencing approaches to develop SNPs, often variations of the same protocols, aimed at optimising the trade-off between the number of markers obtained, cost and simplicity of the laboratory procedures. The whole genome of individual samples can be sequenced (*Whole Genome Sequencing* – WGS) or reduced-representation sequencing (*RADseq* and derived methods). These two approaches' main differences lie in the costs, complexity of the analysis, and amount of information they deliver (higher for WGS), all reviewed in da Fonseca *et al.* (2016). After markers have been developed, diagnostic SNPs (i.e., those best suited for addressing the question of interest from the many thousands identified) can be assembled as panels on *SNPchips* for routine, fast and cost-efficient monitoring.

How long will it take? The laboratory procedures needed for genomic methods (library preparation) can be completed within a month, even when several hundred specimens need to be analysed. The actual sequencing takes 2-3 days using common sequencing chemistry (e.g.,

Illumina platform) and delivery of raw sequencing data is usually accomplished within a month or two after sequencing. Bioinformatic processing of the raw sequencing reads using established bioinformatic pipelines can deliver a thorough population genomic analysis within 1-2 months. Consequently, a population genomic study can be completed within the timeframe needed for proper benchmarking (<2 years). Once SNPs have been discovered and assembled into panes, or SNPchips, the turnaround time is significantly faster (<1 year from DNA extraction to data).

1.1.3.3   Data analysis

Understanding the results of a genetic study, and transferring the insights gained into assessment frameworks is not a trivial task. This set of guidelines might help users interpret and make use of published results too. Genetic results are usually presented by sample groupings on simple x/y plots and cluster analysis. Principal component analysis (PCA) is the simplest method to visualise population structure, as it will group individuals based on genetic similarities on the multivariate space. In the last decade, Discriminant Analysis of Principal Components (DAPC) (Jombart, 2008; Jombart et al., 2010) has gained traction in population genetic studies. DAPC maximises the difference between pre-defined groups (i.e. sampling locations, cohorts, sampling years), while minimising the variability within them. These multivariate approaches are virtually assumption-free, quick to run, and the output is easy to interpret. If data points are well clustered in the multivariate space, and separated along at least one of the axes, it is an indication of stock structure that should be further investigated with individual-based assignment methods. This is done using different softwares (e.g., STRUCTURE, fastSTRUCTURE, Admixture, sNMF, NGSadmix) (Pritchard et al., 2000; Raj et al., 2014; Skotte et al., 2013; Tang et al., 2005), all with different underlying approaches and assumptions. The choice will depend to a degree on the type of data available. The results are usually depicted in a 'structure barplot', a stacked barplot where each vertical bar (an individual) represents the probability of assignment (admixture coefficient) to each identified, colour-coded genetic group (a 'cluster'). Below 80%, individuals cannot be assigned: these can be hybrids, or individuals with high amounts of missing genotypes, where the assignment power drops. A further step is F_{ST} analysis (Weir & Cockerham, 1984). F_{ST} estimates the level of genetic differentiation among pre-defined groups (usually sampling locations). In the marine realm, F_{ST} values are typically low and tend towards zero (i.e., no structure) (Hauser & Carvalho, 2008).

When sampling strategy has been conducted as recommended, and data analyses yield congruent results in that cluster analyses show distinct groupings, population assignment analyses can be done with great certainty (>80%) and F_{ST} values are significant after correction, then genetic stocks can be robustly defined. It is not unlikely to have scenarios that are in between these two cases (i.e., a low but significant F_{ST}). In this case, detailed knowledge of the biology of the species and oceanographic conditions might clarify the results (Knutsen et al., 2011).

1.1.4   Conclusions

Genetics has slowly but steadily been included in fisheries advice, but it is still not considered a tool that can help routine monitoring and assessment. We set out to devise a framework and general guidelines on the basic steps involved, hoping to simplify the process as much as possible for fisheries scientists that want to approach these methods, but lack the time to get themselves acquainted with the extensive literature on fisheries genetics. Of course, this framework is not the ultimate tool needed to use genetics, but it wants to represent a starting point to understand what is required in terms of sampling effort, bioinformatic capacity and analytical skills. Ultimately, consulting an expert in the field will give the best chances of success.

Genetics expertise will likely be required at the final stages, should results interpretation not be straightforward. This is possibly the one hurdle to the routine inclusion of genetics in stock

delineation studies. Results do need interpretation, as there is no 'metric' used to decide whether a set of samples screened with genetic markers represent one or more stocks. This is where future efforts should also be concentrated: developing an indicator, in the form of a threshold, a metric or a number, that can be used in stock delineation to guide the management decision of whether to split or group existing stocks.

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1.1.6 Glossary

A glossary (also included in the manuscript) has been compiled.

DCF – Data Collection Framework	An EU agreement that provides the standards for sampling, managing and storing of samples to be used for fisheries.
Restriction site-Associated DNA Sequencing (RAD; e.g. ddRAD, 2bRAD)	A reduced-representation genomic method to discover SNPs using restriction-type enzymes.
FIS	A coefficient that measures the levels of inbreeding of individuals compared to the expected levels in the population. E.g. Fis<0, no inbreeding; Fis>0, inbreeding
FST	A coefficient that measures the levels of differentiation between two populations. E.g. Fst=0, no differentiation; Fst=1, completely isolated populations
genetic diversity	The genetic makeup of a population or species over which evolution acts on. Higher genetic diversity is essential for allowing a population to adapt to changes in their environment (e.g. climate change).
genetics	Field that studies the genetic components of individuals and how they are inherited in an evolution framework. "Population" genetics is a branch that focuses on changes in the genetic traits within individuals of a population.
genomics	The study of the whole DNA sequence of an organism, including its genes, genomic architecture and functionality.
genotyping-by-sequencing (GBS)	Reduced-representation genomic method to discover and genotype SNPs by using restriction enzymes.
GSI – Genetic Stock Identification	Identification of the population/stock of origin of individuals as well as the estimation of the stock proportions
Illumina Technology	A type of Next-Generation Sequencing (NGS) platform that allows to determine nucleotides from a DNA sequence using dye sequencing technique.
Genomic library preparation	A laboratory protocol to prepare DNA prior for sequencing, which apart from DNA extraction, it often includes shearing of the DNA, adaptor ligation, etc.
Microsatellites	A type of genetic marker that consists of a repetition of a specific motif of nucleotides that is variable within individuals of a population.
population structure – stock structure	The study of genetic differentiation and connectivity levels (dispersal, gene flow, etc.) ongoing between the individuals of putative populations, to identify genetically distinct units within a sampled area/time.
Reference genome	An assembled genome of an individual that is taken as a representative of the DNA of the species
SNPchip	A panel of SNPs that have been selected from a wider set of genome-wide markers because considered to be the most informative for a specific purpose (e.g. animal breeding, stock identification).
SNPs	A base pair (C, G, T, A) in the DNA where there is variation within individuals of a population and between populations
Locus/loci	Points or regions of the genome where there is variation within the individuals of a population and between populations

2 Tor B: To review and evaluate the potential of adaptative variation for assessing fisheries

2.1.1 Introduction

Globally, climate change continues to result in severe environmental change with recent years being the warmest on record since the industrialized period (Bush & Lemmen 2019). This global increase in average temperature has been accompanied by a suite of environmental changes including increases in regional temperatures, atmospheric water vapor, ocean heat content, and decreases in pH, dissolved oxygen and land and sea ice cover. For marine ecosystems, climate change poses several challenges with a range of downstream impacts that are expected to influence biodiversity and ecosystem function. Current and expected impacts include sea level rise, ocean acidification, perturbations to freshwater inputs due to increased glacial runoff, and increased sea temperatures (He & Sillman 2019). The consequences of these impacts are evident in changes in the distribution patterns across a range of taxa (Hammerschlag *et al*. 2022; Poloczanska *et al*. 2013; Phillips & Perez-Ramirez 2018) with a contraction of trailing range edges and the leading range edges tracking climate shifting poleward or deeper (Polyakov *et al*. 2020; Pinsky *et al*. 2020). In fact, marine species and the fisheries they support appear to be more vulnerable to climate change than terrestrial species (Pinsky 2021). Given the reliance on marine resources globally, these changes will undoubtedly have impacts on food security and economic stability (Payne *et al*. 2021). Consequently, there is a need to 'future proof' marine communities and conservation areas through an understanding of the ecological and genetic impacts of climate change (Tittensor *et al*. 2019).

As such, there is a pressing need to predict climate change impacts in marine species and ecosystems to inform conservation strategies and these predictions are particularly relevant to global fisheries and aquaculture. In their report on the impacts of climate change on fisheries and aquaculture, the Food and Agriculture Organisation (FAO) recognized that changes in the availability of sea products due to global warming are likely to have important economic, social and geopolitical consequences (FAO 2016). In Europe, fisheries in the United Kingdom and southern Europe are at highest risk of climate impacts (Payne *et al*. 2021). Ocean warming has also been associated with a significant decrease in production capacity and shifts in the distribution of fisheries targeted species, such as tuna (Monllor-Hurtado *et al*. 2017) and arctic charr (*Salvelinus alpinus*) (Layton *et al*. 2021), which will likely cause or exacerbate conflicts between users at a local, national, and international scale (Mendenhall *et al*. 2020).

Predicting range shifts in marine taxa in response to climate change remains a significant challenge and ultimately requires information on existing ranges, predictions of future environments, capacity for adaptive and plastic responses to environmental change, dispersal potential, and ecosystem structure and function. The latitudinal range of a species is hypothesized to directly result from its thermal tolerance as formulated in the climate variability hypothesis (Stevens 1989). While this hypothesis has been challenged for terrestrial species, marine species' range seems to better conform to their thermal limits (Sunday *et al*. 2012). This is the case for marine ectotherms, particularly tropical ones, for which the thermal safety margin (TSM; i.e., the relative proximity of an organism's range of experienced temperatures to its heat-tolerance limits) is narrower compared to terrestrial ectotherms (Pinsky *et al* 2019). Therefore, small changes in the thermal profile of a given marine region/ecosystem are expected to have measurable consequences on population survival and subsequent local extinction or migration.

A growing body of research has been focusing on developing tools to predict the impact of climate change on wild populations. Ecological niche modelling - akin to species distribution modelling (SDM) - has been used in the last 20 years to map the probability of occurrence of a species across a landscape (Elith *et al*. 2006; Kearney & Porter 2009). However, traditional SDMs do not consider the evolutionary response of populations to environmental change, for instance through multigenerational selection of advantageous genomic variants (e.g., standing genetic variation), or through the acquisition of epigenetic variants leading to acclimation via phenotypic plasticity. More recently, genomic offset - previously termed genomic vulnerability (Bay *et al*. 2018) has been applied to both terrestrial and marine taxa to predict climate change responses (Fitzpatrick & Keller 2015; Laruson *et al*. 2022; Layton and Bradbury 2021). This approach maps contemporary genomic variation onto future environmental conditions and calculates the offset between present and future climate-associated genomic variation as a measure of maladaptation to climate change. Despite some limitations and challenges (Laruson *et al*. 2022), the approach seems to accurately predict climate change vulnerability when compared to experimental predictions (e.g., Fitzpatrick *et al*. 2021). Given the potential of genomic offset estimates to inform climate change predictions, and the observation that marine species are already being impacted by climate change, an evaluation of potential applicability of the method in marine species is warranted.

There are several reasons to speculate that genomic offset calculations may be well suited to marine ecosystems and species. The poikilothermic nature of most marine fish, invertebrates, and plants means that temperature is likely a dominant selective pressure driving adaptive diversity across populations. The low genetic structure and generally large effective population sizes (Ne) often observed within marine species (Bradbury *et al*. 2008), means that the influence of genetic drift may be minimal compared with that of natural selection. Moreover, many marine species occupy large latitudinal ranges and genomic approaches have repeatedly demonstrated adaptation across these variable environments (e.g., Drinan *et al*. 2018; Hoey & Pinsky 2018). Taken together, our ability to resolve the genomic basis of climate-associated adaptation may be elevated in marine taxa compared with terrestrial taxa and it may translate into improved power to predict climate change responses in these systems. Here, our goal is to discuss the potential for genomic offset estimates in marine species to inform climate adaptation and mitigation strategies. Specifically, we (1) review the literature on the genomic basis of climate adaptation in marine species; (2) evaluate current examples of genomic offset estimates in marine species; and (3) discuss the potential for validation of these estimates and their utility in informing climate change and fisheries policy.

2.1.2 Genomic basis of adaptation in marine species

Accurate prediction of evolutionary potential and climate change response requires an understanding of how species are adapted to their environments. The number of potential drivers of adaptation in the marine environment is broad and includes numerous environmental variables such as temperature, salinity, oxygen, pH, and depth. Although theory would predict that extensive gene flow facilitated by long pelagic larval durations (PLD) in marine species would erode and prevent local adaptation, recent studies employing genome-wide data have demonstrated clear evidence for climate-associated adaptation in fish, invertebrates, and macrophytes (e.g. Sanford & Kelly 2011; Drinan *et al*. 2018; Vranken *et al*. 2021). The prevalence of climate-associated genomic variants in marine organisms provides support for a role in adaptation. For species with high connectivity and gene flow, like cod (*Gadus morhua*), herring (*Clupea harengus*), horse mackerel (*Trachurus trachurus*), and northern sand lance (*Ammodytes dubius*), a few "large effect" chromosomal rearrangements (e.g. inversions) seem to explain most of the climate-associated genomic variation (e.g., Barth *et al*. 2017; Kess *et al*. 2020; Fuentes-Pardo *et al*. 2023a).

However, different genomic architectures can underpin local adaptation even in species with similar life history traits, and some species may be more susceptible to this 'genomic island' scenario based on their ecology and biology (Yeaman 2022). In other cases, a signature of local adaptation is often restricted to many regions of "small effect" in the genome, fitting a polygenic model and requiring dense genomic data for detection, as seen in Arctic charr (*S. alpinus*), Atlantic silversides (*Menidia menidia*) and the Baltic copepod (*Eurytemora affinis*) (Wilder *et al*. 2020; Kess *et al*. 2021; Stern *et al*. 2022). The genomic underpinnings of local adaptation can also vary across environments and climate gradients, and this is especially complex in marine systems where species are distributed across both latitudinal and bathymetric clines.

Genomic signatures for thermal adaptation have repeatedly been reported and appear to be stronger in marine systems than in terrestrial or freshwater systems (Sasaki *et al* 2022). For example, a thermal break across the Scotian Shelf in the northwest Atlantic (Stanley *et al*. 2018) has been shown to drive strong population structure among northern and southern populations of northern sand lance (*Ammodytes dubius*) (Jones *et al*., 2023). This temperature-driven structure has been observed in other marine species, including sea scallop (*Placopecten magellanicus*) (Van Wyngaarden *et al*. 2017), northern shrimp (*Pandalus borealis*) (Stanley *et al*. 2018), invasive European green crab (*Carcinus maenas*) (Jeffery *et al*. 2017), capelin (*Mallotus villosus*) (Cayuela *et al* 2021), Tarakihi (*Nemadactylus macropterus*) (Papa *et al* 2022), and Atlantic herring (*Clupea harengus*) (Fuentes-Pardo *et al* 2023b). In the east Atlantic, a similar north-south genetic break has been observed in Atlantic horse mackerel (*Trachurus trachurus*) (Fuentes-Pardo *et al*. 2023a) and boarfish (*Capros aper*) (Farrel *et al*. 2016), which coincides with a biogeographical transition zone between temperate and subtropical waters off the coast of central Portugal (Gamito *et al*. 2016). Extreme thermal events can also result in a reshuffling of genetic clusters leading to local adaptation. For example, Coleman *et al*. (2020) demonstrated that an extreme marine heatwave caused a significant poleward shift in genetic clusters of kelp forests along the coast of western Australia, whereby 'cool water' alleles were replaced by 'warm water' alleles, resulting in a genetic tropicalisation of the populations. While within a given species the same adaptive alleles can be selected multiple times in distinct geographic areas, resulting in parallel evolution of thermal adaptation (Bradbury *et al*. 2010), universality of the molecular processes involved in thermal adaptation is largely unknown in marine systems, partly due to the lack of genomic resources and functional validation of the markers under selection in published studies.

Climate change projections not only indicate a rapid increase in ocean water temperature in the next century, but also lower salinity in relation to glacier melting and higher precipitation in some regions, like the northern hemisphere (Luo *et al*. 2016; Kniebush *et al*. 2019). Additionally, many marine species use a variety of habitats throughout their life cycle, some spanning freshwater and estuarine environments and thereby experiencing steep salinity clines, but little is known about local adaptation in these contexts. A significant body of work supports salinity adaptation in marine organisms (Johannesson *et al*. 2020), particularly in the Baltic Sea. For instance, whole genome sequencing revealed hundreds of genetic regions across the genome of Atlantic herring (*C. harengus*) that showed significant differentiation between Baltic and Atlantic populations (Han *et al*. 2020). Equivalent comparisons of Baltic and Atlantic populations have yielded similar results in other species such as Atlantic cod (*G. morhua*) (Berg *et al*. 2015), sand goby (*Pomatoschistus minutus*) (Leder *et al*. 2021) and European plaice (*Pleuronectes platessa*) (Le Moan *et al*. 2021), indicating adaptation to low salinity levels. Within the Baltic, different species of mussels have also shown salinity-related adaptation between western and eastern regions (Zbawicka *et al*. 2014; Knöbel *et al*. 2021). Recently, Stern *et al*. (2022) showed experimental and genomic evidence of Baltic copepod (*E. affinis)* rapidly adapting to decreasing salinity. Similar to thermal adaptation, salinity adaptation can be both the result of parallel evolution or convergent evolution (Le Moan *et al*. 2021), and it can have a genomic background involving few genetic variants of large effect or many loci of small effect (e.g., Berg *et al*. 2015; Le Moan, *et al*. 2021).

2.1.3 Prediction of climate change impacts using genomic tools in marine taxa

As discussed above, local adaptation to ocean climate appears to be widespread across marine species in a variety of taxonomic groups and ecological contexts. However, climate change is expected to quickly disrupt local adaptation in the coming years, resulting in maladaptation as the speed of change outpaces evolution in many species and populations (Davis & Shaw 2001; Razgour *et al*. 2019). The ability to resolve the genomic basis of adaptation, in conjunction with fine-scale climate projections, presents an opportunity to leverage these datasets to forecast future climate-associated genomic variation and maladaptation. Genomic offset, defined as the difference in current and future adaptive potential, was first introduced by Fitzpatrick and Keller (2015) and later by Bay *et al*. (2018) and has been used to identify vulnerable, or maladapted, populations. In the past few years, this method has been increasingly employed across various terrestrial systems (e.g., Capblancq *et al*. 2020), with more recent work focussing on *in situ* validation of these offset estimates (Fitzpatrick *et al*. 2021). To derive the offset estimate, allele frequencies (response variables) of putatively adaptive loci are used alongside environmental data (predictor variables) to model climate-associated genomic variation, using any number of modelling approaches (e.g., random or gradient forest, generalized dissimilarity modelling, redundancy analyses). Next, this model is used alongside environmental data from both current and future time periods and across the species' range to predict current and future adaptive indices, respectively. The difference between these values represents offset, with larger values indicating that a population is less likely to respond to future climate change and will thus be maladapted. This method has typically been used in species with strong population structure distributed across well-defined environmental gradients but its utility, and downstream interpretation, in marine species has been understudied. However, recent work has begun to address this gap by employing these methods in marine systems, demonstrating its potential utility in seven species of fish, invertebrates and marine plants (Table 2.1).

Table 2.1: List of current publications employing genomic offset estimates in marine species.

Organism	Region	Method	Included Climate Variables	Validated	Reference
Macrophytes					
Eelgrass (*Zostera marina*)	Coastal North America	RDA	Seasonal, annual bottom & surface temperature, salinity	No	Jeffery *et al.* (2023)
Seaweed (*Phyllospora comosa*)	Southeastern Australia	GDM	Sea surface temperature	No	Wood *et al.* (2021)
Kelp (*Ecklonia radiata)*	Western Australia	GF	Dissolved oxygen, sea surface salinity, sea surface nitrate, seawater velocity, attenuation	No	Vranken *et al.* (2021)
Vertebrates					
Arctic Char	Newfoundland/ Labrador	GF	Precipitation, air temperature	No	Layton *et al.* (2021)
Invertebrates					
Multi-species: Urchin (*Parechinus angulosus*), crab (*Cyclograpsus punctatus*) & limpet (*Scutellastra granularis*)	South Africa	GF	Sea surface temperature, sea surface salinity & air temperature	No	Nielsen *et al.* (2021)
Coral (*Acropora digitifera*)	Western Australia	GF & GDM	Sea surface temperature	No	Adam *et al.* (2022)
Tubeworms (*Galeolaria caespitosa* & *Galeolaria gemineoa*)	Southeast Australia	GF	Sea surface temperature	No	Gallegos *et al.* (2023)

Most of this work has focused on generating offset estimates in single species across their range, with variable patterns across taxonomic groups and geographic areas. For instance, in eastern Canada, offset estimates were highest in southern populations of Arctic charr (*S. alpinus*) (Layton *et al.* 2021), consistent with a scenario of contraction of the southern range limit of this Arctic species. Alternatively, genomic offset values were higher in mid-range (subarctic) populations of a widely distributed eelgrass species (*Z. marina*) across a similar area (Jeffery *et al.* 2023). Interestingly, offset estimates in *Acropora digitifera* in western Australia were more spatially complex, but populations living at the upper thermal limits (i.e. at lower latitudes) were predicted to require less of an adaptive shift than mid-latitude reefs (Adam *et al.* 2022). Similarly, Wood *et al.* (2021) show a spatially complex pattern of offset in a forest-forming seaweed (*Phyllospora comosa)* in Australia, demonstrating that offset estimates do not follow a simple latitudinal cline. Finally, Nielsen *et al.* (2021) in a comparative study of three marine invertebrates, explore genomic offset values, and demonstrate that temperature was strongly correlated with genomic variation in some invertebrate species but not others and that species-specific responses were a better predictor of evolutionary potential than environmental data alone. On the surface, these initial studies suggest broad generalizations of climate change vulnerability in marine species may be difficult even across similar species or geographic regions, but clearly many more examinations of genomic offset will be required.

It is important to note that the majority of studies exploiting genomic offset in marine species (and others) have relied solely on single nucleotide polymorphism (SNPs) allele frequency

datasets of various sizes. But it is increasingly clear that the basis of local adaptation in marine taxa extends beyond SNPs to larger types of chromosomal rearrangements, copy number variation, and even epigenetic variation. For instance, environmental adaptation in American lobster (*Homarus americanus*) was driven almost entirely by CNVs with little to no signal in the SNP data (Dorant *et al*. 2020). Similarly, Layton *et al*. (2021) report spatial similar trends in genomic offset calculated from CNV and SNP datasets in Arctic Charr populations from eastern Canada, In three-spined stickleback, differentially methylated regions (DMRs) were more significantly associated with environmental variation (salinity) than outlier SNPs (Ruiz-Arenas *et al*. 2017). Given these findings, Layton and Bradbury (2022) suggest a revised offset approach incorporating normalized read counts and methylation scores alongside allele frequencies may better reflect adaptive responses improving prediction accuracy.

2.1.4 Validating genomic offset estimates in marine systems

Projections of climate change response via genomic offset are correlative in nature and as such, these models can be prone to errors when the underlying genomic data, climate projections, or their associations are of low resolution or inaccurate. Moreover, these models largely ignore much of the underlying complexity of the evolutionary response including effective population size and rates of mutation and recombination (Rellstab *et al*. 2021). The models also assume that a population currently exists at its adaptive optimum and that the genotype-environment association tracks spatial patterns in local adaptation, an assumption that can be impacted by local demographic patterns and genetic drift (Rellstab *et al*. 2021; Laruson *et al*. 2022). For instance, spatial changes in allele frequencies may reflect neutral drift rather than adaptation, and this is especially likely when population structure is aligned along environmental gradients. Laruson *et al*. (2022) employ simulations to demonstrate that although genomic offset is broadly correlated with fitness, it could be confounded by demography, genomic architecture, and the nature of the offset-fitness relationship.

Beyond this simulation study, few have attempted to validate the assumptions inherent, or the projections of population response based on genomic offset calculations. In theory, validation may come from experimental studies, or comparison of predictions with existing demographic data. The only example of experimental validation to date is from a terrestrial species, the balsam poplar (*Populus balsamifera*), where genomic offset estimates were compared to population performance in common gardens (Fitzpatrick *et al*. 2021). Here the authors report a negative relationship between genomic offset and common garden performance consistent with significant power of offset estimates to predict population response to climate change, exceeding that of climate differences alone. In contrast, Bay *et al*. (2018) used genomic offset to explore climate change variability in the yellow warbler (*Setophaga petechia*) and compared offset estimates to historical trends in abundance. Although they report a correlation between offset projections and demographic decline, this approach assumes consistency among historical and future trends, and can be biased by non-selective / demographic influences on the offset prediction (Laruson *et al*. 2022). Interestingly, no direct attempts to validate genomic offset predictions in marine species have been published to date.

Although very informative and likely the gold standard, the potential for experimental validation of offset estimates through either reciprocal transplant or common garden experiments in marine species are limited by comparison with terrestrial plants or animals. This is largely due to the added complexity of these experiments in the marine environment, but some exceptions exist (see Jacobs *et al*. 2022). However, marine systems, particularly exploited species, often have extensive stock assessment and long-term monitoring programs with decades worth of time series data. Rather than comparing future estimates of climate change impact with past demographic data, this allows direct comparison of past demographic trends with predictions of past

climate-associated genomic change. This approach, although not perfect, allows for some degree of validation if offset calculations are hindcast to the period over which population monitoring has occurred. In fact, due to the extensive population abundance data available for many economically and ecologically relevant marine species, the potential for this sort of retrospective validation in marine systems is unprecedented. Furthermore, sampling programs spanning decades present the opportunity to retrospectively test for temporal changes in allele frequencies in response to climate change. Beyond validating offset estimates, identifying genomic regions linked to environmental variation provides additional support for climate-associated adaptation.

2.1.5 Recommendations for marine management

As genomic offset models are refined and applied to additional species of conservation interest, there will be a need to integrate predictions into management and decision making. While validating genomic offset models, through experiments or observations over time, will foster trust in the method, there are consequences to inaction as well (Salafsky and Redford, 2013). Different standards of proof of a model or prediction may be required depending on the relative consequences of action versus inaction, and whether management or mitigation strategies are reversible; a relatively low standard of proof may be required if inaction would lead to long-term ecological consequences when there is at least some certainty about a beneficial outcome for a population or species (Salafsky and Redford, 2013).

To integrate genomic offset into management decisions, we recommend:

1. Comprehensive sampling across the range of marine species, including locations and habitats of interest (e.g., latitude, conservation areas, spawning areas, temperature regimes, depth, etc.)
2. Suitable sampling for the genomic method to produce allele frequencies (i.e., minimum 30 individuals per sampling location, high-resolution analysis ideally whole genome resequencing at moderate to high depth >5-10X)
3. High resolution climate data for the study region, including biologically-relevant environmental variables and preferably validated with in situ measurements accounting for depth and seasonal variation.
4. Future climate change scenario models of the same resolution as contemporary climate data, usually with a trade-off of resolution and geographic scale
5. Genomic offset can then be used for 'assisted evolution' or restoration through transplantation, enhancing resilience to a changing climate (e.g., Wood *et al.*, 2021).

2.1.6 Conclusions

Climate change poses a variety of significant challenges across marine ecosystems likely to influence both the suite of biodiversity present and ultimately ecosystem function. The global reliance on marine resources means that these changes will undoubtedly have clear repercussions on food security and economic stability (Payne *et al*. 2021). Our ability to mitigate these changes and "future proof" management decisions and conservation actions will depend on a thorough understanding of the basis of climate adaptation and accurate predictions of the impacts of climate change in marine species. Our review of the existing literature reveals a wealth of examples demonstrating climate-associated adaptation in marine taxa resolvable with genomic analysis and marine climate data. The results suggest that our ability to resolve the genomic basis of climate-associated adaptation may be elevated in marine taxa compared with terrestrial taxa, providing improved power to predict climate change response in these systems. However, there remains a general lack of validation of genomic offset estimates, heightening the uncertainty of these predictions, both in terrestrial and marine systems. As such, there is a pressing need for

improved genomic and environmental sampling of marine species and targeted validation studies to quantify error and uncertainty in these estimates. Looking forward, significant methods development will also be required to integrate across multiple climate-associated genomic variants which are likely to improve prediction accuracy (i.e., Layton and Bradbury, 2022).

2.1.7   Bibliography

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3 Tor C: To evaluate available genetic-based solutions to better understand the mesopelagic ecosystem

3.1.1 Abstract

Marine ecosystems are among the most exploited and threatened habitats on Earth, and the deep sea is no exception. At its upper limit (from 200 to 1000 m depth) lies the mesopelagic or twilight zone, a poorly known but ecologically key oceanic region. This ecosystem is unique with tens of biogeographic zones and a wide taxonomic diversity of organisms that provide essential ecosystem services, sustain the entire marine ecosystem (including populations of commercial fish) and regulate climate. Yet, exploitation of resources of the twilight zone has recently gained increased interest, which calls for urgent research on the twilight zone so that sustainable management actions can be implemented before it is too late. Knowledge on the twilight zone remains incipient due to the challenges in exploring this deep and dark ecosystem. The continuous development of modern sequencing technologies, coupled with the new sources of data such as environmental DNA (eDNA), have opened a whole new horizon of big data omics information. It is expected to speed up and facilitate the compilation of missing basic knowledge about the mesopelagic ecosystem that will be essential to maintain its biodiversity and associated ecosystem services. Here, we showcase how molecular tools are essential to understand the mesopelagic ecosystem and provide genetic solutions for both basic and advanced biological questions at the individual, population, species, and ecosystem levels of biodiversity.

3.1.2 Background

Marine ecosystems are among the most exploited and threatened habitats on Earth. The deep sea, comprising the oceanic region between 200 m depth and the seafloor and representing 95% of the ocean's volume (Danovaro *et al.*, 2017), is no exception. At the deep ocean´s upper limit lays the mesopelagic zone, colloquially known as the twilight zone, which extends approximately to 1000 m depth, covers 60% of the planet's surface and represents about 20% of the ocean's volume (Bopp, 2021). This realm is characterised by decreasing gradients in light intensity, which are not enough for supporting photosynthesis (Arístegui *et al.*, 2009), and temperature with depth, and by an increasing gradient in salinity and pressure, as well as by the presence of low oxygenated areas (oxygen minimum zones, OMZs) (Ramirez-Llodra *et al.*, 2010; Robinson *et al.*, 2010).

The mesopelagic zone is home to a wide taxonomic diversity of organisms (Robinson *et al.*, 2010; Sutton *et al.*, 2017), which are involved in global biogeochemical cycles, essential for sustaining the marine ecosystem and for climate regulation. The mesopelagic zone displays microbial activity comparable to the epipelagic zone (Arístegui *et al.*, 2005; Gasol *et al.*, 2009)et due to absence of photosynthesis, the food web is heterotrophic, although primary production through chemoautotrophy has been reported (Arístegui *et al.*, 2009). Mesopelagic bacteria and archaea consume most of the organic matter that reaches the twilight zone in the form of degrading dissolved and/or particulate organic matter (DOM and POM, respectively) produced in the upper layers. DOM/POM reach deeper layers through particle or aggregate sinking and faecal pellets excreted at depth by vertically migrating mesopelagic organisms after feeding at upper layers (Buesseler and Boyd, 2009; Calleja *et al.*, 2018; Morán *et al.*, 2022), or through the release of living POM by viral infection (Laber *et al.*, 2018). This prokaryotic activity also promotes sinking organic matter to return to the trophic web through the microbial loop, underpinning a complex, well-

established food web that involves a wide array of planktonic and nektonic organisms including heterotrophic protists (e.g., pico- and nanoflagellates, ciliates), invertebrates (e.g., crustaceans, salps and cephalopods), and fish (Steinberg and Landry, 2017; Silva *et al.*, 2022). In the mesopelagic food web, zooplankton act as primary and secondary consumers and detritivores (Steinberg and Landry, 2017). Nektonic mesozooplankton (from 0.2 to 20 mm, mainly comprised of metazoans such as copepods or amphipods) are in turn preyed upon by mesopelagic fish and cephalopods, thus transferring energy from lower to higher trophic levels, and ultimately sustaining commercial fish and top marine predators (pinnipeds, cetaceans, seabirds, and tunas) (Naito *et al.*, 2013; Battaglia *et al.*, 2020; Braun *et al.*, 2022). Through their activity, zooplankton and nekton are key regulators of the biological carbon pump, i.e., the transport of the organic matter produced in euphotic layers to the ocean depths (Legendre *et al.*, 2015), by attenuating sinking of organic matter through feeding and fragmentation of particles, and by repackaging the organic matter into dense, quickly sinking faecal particles (Turner, 2002; Wilson *et al.*, 2008). This whole process regulates the ocean's carbon cycle through carbon and nutrient recycling and release of dissolved inorganic carbon (Arístegui *et al.*, 2009).

Mesopelagic organisms are also crucial in the vertical connectivity of marine food webs. Although the strong vertical community structuring reported for many microscopic groups suggests that mesopelagic species with limited movement capability across the water column such as protists and prokaryotes permanently inhabit mesopelagic depths (Countway *et al.*, 2007; Canals *et al.*, 2020; Giner *et al.*, 2020), the mesopelagic trophic web highly connected with the surrounding oceanic zones, especially with the epipelagic zone (0 to 200 m). In a phenomenon known as "The largest migration on Earth", hundreds of mesopelagic species perform a vertical journey every night to feed at epipelagic depths (Hays, 2003; Aumont *et al.*, 2018). This diel vertical migration (DVM) is observed worldwide, including in the high latitudes despite the limited darkness in summer months. During the DVM, mesopelagic organisms are preyed upon by epipelagic species, such as sea mammals, seabirds, and commercially important fish species (Giménez *et al.*, 2018; Komura *et al.*, 2018). Ontogenetic migration is also a common trait of many mesopelagic species, based on which early development stages inhabit epipelagic depths and move down to the mesopelagic zone at subsequent phases (Steinberg *et al.*, 2008; Yamaguchi *et al.*, 2019; Olivar and Beckley, 2022).

The mesopelagic zone is increasingly experiencing direct and indirect human interference. Indirect interference relates to increasing levels of pollutants (e.g., plastics and eutrophication) and to climate change-derived effects through seawater warming, acidification, deoxygenation, and changing circulation patterns (Levin and Le Bris, 2015; Rogers, 2015; Brito-Morales *et al.*, 2020; Yang *et al.*, 2020). In the epipelagic layer, a reorganization of marine ecosystems with poleward movements of species is already evident (Richardson, 2008; Benedetti *et al.*, 2021) and this will eventually affect the mesopelagic trophic web due to a close relationship between mesopelagic biomass and epipelagic productivity and carbon export. In turn biomass and productivity are dependent on local phytoplankton community composition (Guidi *et al.*, 2016; Hernández-León *et al.*, 2020). Seawater warming is also expected to significantly impact deep-sea ecosystems (Brito-Morales *et al.*, 2020) due to the adaptation to stable conditions and therefore limited tolerance to change (Levin and Le Bris, 2015).

Direct interference is related to interest in exploiting mesopelagic resources, which has increased mainly due to recent estimates pointing to total mesopelagic fish biomass of 9–19.5 gigatonnes (Irigoien *et al.*, 2014; Proud *et al.*, 2019; Martin *et al.*, 2020). The latter represents a new, huge potential source of marine lipids and protein in supply of the nutraceutical, food, and feed industries. Industrial-scale mining also represents a threat to mesopelagic ecosystems due to the release of sediments plumes to the water column, in addition to habitat (and associated biodiversity) destruction (Ramirez-Llodra *et al.*, 2010; Mengerink *et al.*, 2014). An uninformed management of mesopelagic resource extraction could lead to significant local and global

consequences on its biodiversity (Mengerink *et al.*, 2014; Danovaro *et al.*, 2017; Martin *et al.*, 2020), e.g., through ecological tipping points and cascading effects, and ultimately alterations and loss of biodiversity, impacting ecosystem services (Worm *et al.*, 2006; Crist *et al.*, 2017).

Despite the current threats of the mesopelagic zone and its critical role in regulating global bio-geochemical cycles and climate, it is barely understood, especially in comparison to the epipelagic layer. The gaps of knowledge about the mesopelagic zone are immense. Even the most basic biological and ecological questions such as which organisms live there, how many species are present, or how abundant they are, remain largely elusive (Webb *et al.*, 2010; Danovaro *et al.*, 2017; Martin *et al.*, 2020; Bopp, 2021). More complex matters, such as the role of mesopelagic organisms in regulating the biological carbon pump and climate or predicting the impacts in marine ecosystems derived from the potential extraction of mesopelagic resources or from climate change, are even more challenging and still subject to high uncertainties (Buesseler and Boyd, 2009; Burd *et al.*, 2010; Costello and Breyer, 2017; Bode *et al.*, 2018; Brito-Morales *et al.*, 2020). Hence, the mesopelagic zone does not feature prominently on international fora of management and governance, not the least because of poor documentation (Schadeberg *et al.*, 2023).

The mesopelagic realm is not a trivial environment to study, mainly due to logistic hindrances and economic demands related to sampling. Data on the mesopelagic zone remain sparse, with very few time series, often consisting of single site measures with few physical samples and always obtained at a large effort. However, research on the mesopelagic realm is currently experiencing a notable increase (Figure 2.1), partly thanks to government-funded projects such as the EU Horizon 2020 MEESO (https://www.meeso.org) and SUMMER (https://summerh2020.eu) projects or the Joint exploration of the twilight zone ocean network (JETZON) UN Decade program (https://jetzon.org/), coupled to the advent of new technological developments (Butler and Pagniello, 2021). In addition, research opportunities have never been so promising; molecular tools have opened a whole new horizon of big data omics information, which is expected to speed up and facilitate the obtention of missing, basic knowledge about the mesopelagic ecosystem, as well as to provide alternative basics for biodiversity and ecosystem services management (Heuertz *et al.*, 2023).

While the development of molecular tools for studying organismal evolution and functioning has become firmly established in the previous century, the advent of high-throughput sequencing tools developed since the end of last century has greatly benefited the study of non-model organisms (Bernatchez *et al.*, 2017; Casillas and Barbadilla, 2017). Questions addressing the organisation of a single genome, up to the evolution of populations and conservation management are feasible. A consequence is that the sparse samples from the mesopelagic zone offer uncharted opportunities for study at a high level of detail and understanding. For example, each animal harbours in addition to its genome a sample of the local prey and parasite assemblage, and its external and gut microbiome, thus providing information at the cellular, species, population, community, ecological and evolution level. It is therefore not surprising that there has been a marked increase in the number of studies in the mesopelagic zone using genetics in recent years (Figure 2.1).
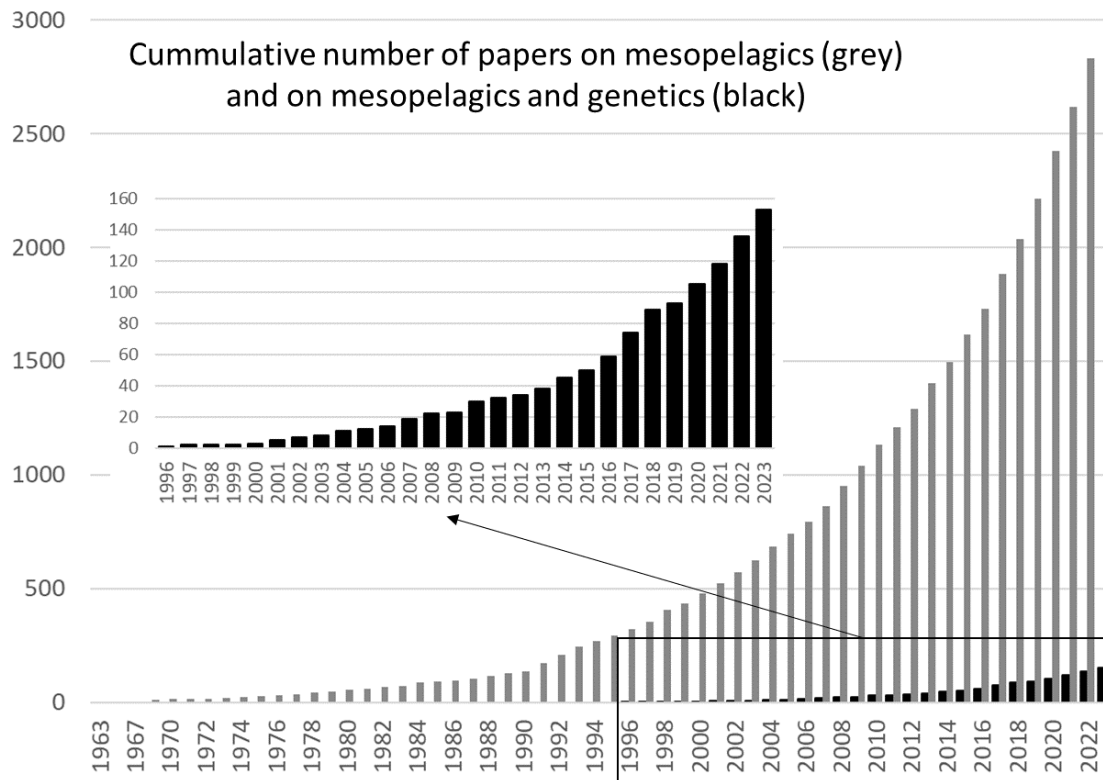
Figure 2.1. Cumulative number of peer-reviewed primary literature publications mined from Web of Science for *mesopelagics* (grey bars), with keywords [mesopelagic OR mesopelagics OR "ocean´s twilight zone" OR "ocean twilight zone"] and for *mesopelagics* and *genetics* (black bars), adding keywords [DNA OR genomics OR eDNA OR "high-throughput sequencing" OR "next generation sequencing"] as of June 12th 2023.

Here, we provide a synthesis review of how genetics could facilitate closing major knowledge gaps and increasing our understanding of the twilight zone diversity at the organismal, population, species, and ecosystem level. Then, we discuss how this knowledge is expected to inform management of ecosystem services and conservation. Finally, we provide recommendations to guide future research. By choice, we rely on studies carried out in the mesopelagic zone, yet when missing such studies, we take advantage of studies in other environments to showcase its potential application to the mesopelagic realm.

3.1.3 How genetic tools can help us understand the mesopelagic ecosystem

Revealing the inventory and functioning of the mesopelagic biodiversity is key for the sake of sheer knowledge and management. Genetics provides information at the individual, population, species, and ecosystem level. Since the turn of the century, access to the mesopelagic zone has benefited from the development of a diverse and handy molecular toolbox. The tendency to decreasing costs and well-managed databanks has given the molecular approach to genetics a phenomenal boost of which the following paragraphs pay testimony. What has been realised so far is only a part of the biodiversity the mesopelagic zone harbours. Here we provide an inventory by scientific discipline of what might be achieved in the near future.

3.1.3.1 Information at the individual level

Age and sex of multicellular organisms represents important information at the individual level to understand population dynamics, and ecological and evolutionary processes is the age and sex of multicellular organisms.

Genetic aging. Age determination is fundamental to population biology and thus highly relevant to fisheries management. Classically, seasonal and predictable growth marks in otoliths or other calcareous structures such as scales and fin spines have been used to estimate chronological age (Campana, 2001; Luque *et al.*, 2014). However, several harvested fish lack an accurate and practical method for estimating age. In addition, such techniques can be expensive, time consuming (Helser *et al.*, 2019), and of low accuracy (Campana, 2001). There are two alternatives under development. Vertebrate telomeres shorten as they age (Carneiro *et al.*, 2016). Hence telomere length functions as molecular clock, provided that a calibration curve is prepared (Nijland van Hal, pers. comm). So far, few marine organisms have attained the resolution required for field-based application. An alternative is epigenetics, which is defined as the ensemble of processes that alter gene activity without changing the DNA sequence and which can be heritable and reversible (Weinhold, 2006), the most common being histone modifications, RNA-based mechanisms, and DNA methylation. Among epigenetic mechanisms, DNA methylation, which consists of the addition of methyl groups to the DNA, typically cytosines within cytosine-phosphate-guanine (CpG) sites, has been considered as a potential predictor of age (Horvath, 2013). There is a relationship between methylation change and age (Field *et al.*, 2018), and increasing evidence support DNA methylation patterns as potential biomarkers for chronological age. This has led to the development of so-called epigenetic clocks (Field *et al.*, 2018; Beal *et al.*, 2022; Piferrer and Anastasiadi, 2023), which have been tested in several fishes (Venney *et al.*, 2016; Gavery *et al.*, 2019; Anastasiadi and Piferrer, 2020). By extension, the molecular aging developed in vertebrates, can be extended across the tree of life.

Sex identification. There is a remarkable diversity of primary sex-determining mechanisms in invertebrates and vertebrates (Devlin and Nagahama, 2002; Bachtrog *et al.*, 2014; Pennell *et al.*, 2018). Sex differentiation might be gonochoristic or hermaphroditic, maturing either first as males or as females. Hence, except for birds and mammals (with a heterogametic sex), sex identification requires species-specific approaches, which vary from poly- and monogenic sex determination to factors located on auto- or sex chromosomes. In fish, molecular markers have been used to understand sex determination and are effective to discriminate among sexes (Star *et al.*, 2016; Toli *et al.*, 2016). To our knowledge, no molecular information is available yet on any mesopelagic organism.

3.1.3.2 Information at the population level

Individuals grow, reproduce, and die; they form populations which expand and contract, move and adapt in response to environmental and interspecific interactions. Metapopulation theory, which considers patterns of dispersal and population dynamics, and population sources and sinks, provides an established and most useful concept to understand the dynamics of populations (Kritzer and Sale, 2004; Aiken and Navarrete, 2020). Species- and population-specific data on sex, age structure and life-history traits feed into population and production models, which provide the necessary information to assess ecosystem functioning. Taxa and functions revealed through (meta)barcoding and epigenetics from bulk DNA/RNA or environmental DNA/RNA reveal sex-and age-structured abundances (Yates *et al.*, 2021).

Genetic diversity and effective population size. A first measure to characterise populations is within-species genetic diversity, which is determined by the accumulation of novel genetic variation through mutation, its spread in space and time through gene flow, and processes leading to genetic drift and selection. Intraspecific genetic diversity represents the building blocks of evolution because genetic variation is a source for evolutionary responses to the changing

environment. Genetic variation is measured as indices of heterozygosity, inbreeding, allelic richness, and nucleotide polymorphism. The genetic variation of marine fish is higher than freshwater fish (Ward *et al.*, 1994), because the larger the population the larger the genetic diversity. There is no difference in genetic variation between coastal and deep-sea species (Taylor and Roterman, 2017). Mesopelagic organisms with their large population size fit the observations of high genetic diversity (Van de Putte *et al.*, 2012; Rodriguez-Ezpeleta *et al.*, 2017; Domingues *et al.*, 2019). However, behaviour, such as diel vertical migration, impact the genetic diversity of some mesopelagic organisms (Timm *et al.*, 2020). Hence, genetic variation determines the function and resilience of populations; the potential for adaptability of a population is proportional to its ecological strategy and genetic diversity (Romiguier *et al.*, 2014). Genetic diversity finds many applications in conservation biology (Hoban *et al.*, 2020). For example, consequences of small population sizes or overexploitation are first noticed through the loss of rare alleles (decrease of allelic richness) and at a later stage through the loss of heterozygosity and increased inbreeding (Pinsky and Palumbi, 2014; Rivera-León *et al.*, 2019).

Effective population size (N_e) quantifies the potential of a population for passing on genetic diversity to the next generation and hence quantifies population resilience. The value of N_e depends on factors such as census size and life history traits (Waples, 2022). Theory predicts N_e/N_c ratios of ≥ 0.1 but ratios of 10^{-4} or even 10^{-5} are not uncommon in highly fecund taxa (Portnoy *et al.*, 2008; Hare *et al.*, 2011). For example, (Domingues *et al.*, 2019) calculated an increase in N_e of the night shark over the past 50,000 years. Reliability of the N_e estimates is steadily improving with access to high quality genomic information (Davenport *et al.*, 2021).

Population structure and connectivity. Building further on the assumption that mesopelagic species have large population sizes and experience barriers in the ocean such as frontal systems, gyres and topographic features, genetic differentiation between populations is generally low compared to epipelagic and shelf populations (Kristoffersen and Gro Vea Salvanes, 2009; Van de Putte *et al.*, 2012; Rodriguez-Ezpeleta *et al.*, 2017; Domingues *et al.*, 2019). Spatially reduced gene flow between populations leads in many cases to isolation by distance (Taylor and Roterman, 2017; Gonçalves da Silva *et al.*, 2020). For example, Domingues *et al.* (2019) showed that populations of the night shark gradually diverge the further they live from each other (isolation by distance). In other cases, the underlying pattern may be attributed to selection (isolation by adaptation). Demographic instability or selective sweeps are common in benthos according to a meta-analysis by Taylor and Roterman (2017). Another explanation for constrained gene flow in an environmental context is isolation by resilience. Boussarie *et al.* (2022) argued that unlike geographical distance, dispersal distance and ocean depth are major factors explaining the distribution pattern of the grey reef shark on the coral reefs of the Indo-Pacific Ocean.

Functional connectivity is a cornerstone of viable populations. The exchange of genetic material probably represents the largest insurance against inviable populations. While demographic connectivity, also known as ecological connectivity, focuses on intragenerational phenomena, genetic or evolutionary connectivity considers multi-generational phenomena (Lowe and Allendorf, 2010). Each type of connectivity is studied with adapted methodologies and tools. Interestingly, a shallow water example on tropical clown fish shows that ecological and evolutionary dispersal, each measured with genetic methods, are comparable (Pinsky *et al.*, 2017). Dispersal of individual fish over long distances has been documented in the North Atlantic Ocean (McGill *et al.*, 2023). This opens excellent opportunities to understand the dispersal dynamics and interactions of mesopelagic organisms over a wide range of spatial-temporal scales. The information is crucial to identify management units and take conservation measures.

Tracing populations. An interesting application of the characterisation of population structure is the assignment of individual organisms to their source population. The genetic profile composed of large numbers of genetic markers, often SNPs, characterizes a population. When selecting a

highly discriminating set of markers characteristic for each population, the individual genotype reveals the spawning unit. The EU funded project FishPopTrace successfully assigned fish biopsies to their geographical source (Nielsen *et al.*, 2012). In another case, the fishery of two ecotypes of Atlantic cod is guided with a 5-day turnover test during spring spawning (Johansen *et al.*, 2018).

Phylogeography. During Pleistocene glaciations hydrodynamics and climate zones left a regional imprint on the genome of organisms. Postglacial periods led to range shifts and population expansions or contractions, with clades (and cryptic species) leaving geographical traces and revealing patterns of gene flow and suture zones of hybridization and introgression (Miyamoto *et al.*, 2010; Domingues *et al.*, 2019). Historical genetic patterns are scored with slow evolving organelle or nuclear loci, calibrated with fossil finds or parallel events for the era considered. For example, the mesopelagic night shark, which is often caught as bycatch in the pelagic longline fishery and listed 'vulnerable', differentiates in a southwestern and northwestern Atlantic lineage (Domingues *et al.*, 2019). Both lineages diverged during the Pleistocene with its fluctuating sea temperature, sea level and hydrodynamics. Phylogeographic and species patterns show considerable similarities; this is not unexpected because population-level genetic divergences are a starting point for macroevolutionary divergences between species (Bowen *et al.*, 2016). Oceanographic barriers affect concomitantly the distribution and connectivity of marine biota, leading to overlapping multispecies biogeographical and phylogeographical patterns (Antich *et al.*, 2023). All this information on historical patterns may be accounted for in conservation through management units (Avise *et al.*, 1987). Phylogeography of mesopelagic species can be studied "in bulk" through DNA metabarcoding (Turon *et al.*, 2020; Tsuji *et al.*, 2023) and species-by-species using SNP-based methods (Rees *et al.*, 2020).

Adaptation. Sampling of thousands to millions of genetic markers randomly spaced across the genome have transformed our understanding of genetic differentiation in marine systems. Consequently, cryptic but biologically significant patterns of genetic differentiation have been revealed in marine fishes. A toolkit consisting of genetic markers potentially under selection and structural variation (such as chromosomal inversions) that often capture multiple genes involved in local adaptation have been effective in delineating additional structure and hence connectivity. Deep-water conditions such as depth stratification and horizontally spaced structures (e.g., canyons, seamounts, and oxygen minimum zones) might induce species-specific adaptation. For example, signatures of adaptation in blue ling *Molva dypterygia* consisted in signs of selection in genes involved in growth, vision, and adaptation to lower temperatures (McGill *et al.*, 2023). Genetic differentiation at functional loci of the rock grenadier *Coryphaenoides rupestris* distinguishes individuals living at different depths, independent of horizontal spatial distance (Gaither *et al.*, 2018). A distinct pattern of cryptic genetic structure exists for putative functional loci, despite apparently high levels of gene flow in orange roughy *Hoplostethus atlanticus*. Juvenile orange roughy may have a distribution that is more mesopelagic than adults (Gonçalves da Silva *et al.*, 2020).

Seascape genomics. The ecological niche at the population level of genomically characterised marine organisms provides an integrated approach to generate analytical and predictive models of genetic differentiation. Seascape genomics helps to identify barriers to gene flow and connectivity corridors for incorporation into network design. It is achieved through statistical models that incorporate geographically referenced environmental features (the seascape) with genetic and phenotypic information (Selkoe *et al.*, 2016). It finds applications in the functional delineation of populations, management units and conservation areas. To our knowledge no such study in the mesopelagic zone has been published because the approach requires an extensive and detailed spatially anchored data matrix of environmental and genetic data. Examples from the coastal zone cover taxa from across the tree of life (Wood *et al.*, 2021; Jahnke *et al.*, 2022; Benestan *et al.*, 2023).

3.1.3.3   Information at the species level

Taxonomic diversity. Compiling inventories of species involves the most basic biological and ecological knowledge and foundation for a deeper understanding of the ecosystem. The large pool of taxa in the mesopelagic zone awaiting description makes that species descriptions represent a prime task (Bopp, 2021). Taxonomy and phylogeny, largely based on genetic and genomic tools, provide important support to delineate, compile and understand species diversity (Christiansen *et al.*, 2018; Lee *et al.*, 2022). Hence, for routine identification increasingly relies on DNA metabarcoding, implemented through high-throughput amplicon sequencing, or on DNA barcoding, if possible, in combination with well-established morphological tools (e.g., Govindarajan *et al.* (2021)). Due to the broader focus, multi-species methods are the most suitable to obtain insights on the community composition. Especially DNA metabarcoding is favoured due to its low cost, standardised pipelines, and the capability to target specific taxonomic clades. DNA metabarcoding has been successfully applied in the mesopelagic zone using different types of DNA source including filtered water, sediment, and bulk samples (Wangensteen *et al.*, 2018; Hirai *et al.*, 2020; Canals *et al.*, 2021). It provides a list of the sequences present in a sample, which are then taxonomically assigned using a reference database. DNA metabarcoding is a powerful tool to get insights on the rare and hidden diversity (diversity that remains to be described or sequenced) (Lindeque *et al.*, 2013; Logares *et al.*, 2014), thus offering a deep view of an ecosystem biodiversity. DNA metabarcoding can be also a basis for widely used diversity descriptors such as alpha-, beta-, and gamma-diversity measurements (de Vargas *et al.*, 2015). Just like DNA metabarcoding, long-read sequencing provides a list of species present in a sample with the advantage of being able to retrieve much longer DNA fragments, which may improve taxonomic assignment, haplotype identification, and improve the phylogenetic placement of taxa that remain unidentified (Tedersoo *et al.*, 2021). Metagenomes also provide information on the taxonomic diversity of a sample, with the advantage that the resulting data better reflects abundances (since this method does not include any PCR step) and avoids biases due to primer efficiency (retrieving biodiversity that cannot be captured through DNA metabarcoding) (Cowart *et al.*, 2018; Obiol *et al.*, 2020); yet it is not possible to focus on specific clades of interest. Finally, taxonomic diversity can also be assessed by analysing RNA instead of DNA, which provides a view of the organisms that are active in the moment of the sampling (Yates *et al.*, 2021).

Diversity in the mesopelagic zone is large and unknown, and presence of cryptic species (i.e., species morphologically very similar but constituting two different reproductive units) is likely to occur, especially at the microscopic level due to the lack of (or difficulty to identify) distinctive traits. This limitation does not apply to genetics, since it relies on the differences in nucleotides, which will always differ between species. Thus, the identification of cryptic species from an environmental sample (e.g., bulk, filtered water) increases with multispecies approaches such as DNA metabarcoding or long-read sequencing (De Luca *et al.*, 2021). Alternatively, DNA barcoding or whole-genome sequencing technologies can be used in specimens, tissue, single cells, or bacterial/archaeal colonies to confirm whether two similar organisms are cryptic species (Brasier *et al.*, 2016). In a similar way, genetics can help in matching early life stages with its corresponding adult phase (Webb *et al.*, 2006), which may be highly challenging in hard-to-reach, understudied marine environments such as the mesopelagic realm.

It should be noted that the accuracy of genetic methods for the detection and identification of species highly relies on the completeness of reference databases (Claver *et al.*, 2023). Just like many other marine ecosystems, they are far from completion in the mesopelagic zone (Bucklin *et al.*, 2021; Govindarajan *et al.*, 2021). Thus, there is the need to populate open access international reference databases (e.g., Ratnasingham and Hebert (2007); Guillou *et al.* (2012); Benson *et al.* (2018); Bucklin *et al.* (2021)) with sequences of mesopelagic species. One way to do so is by sequencing the DNA from tissues, single cells, or bacterial/archaeal colonies of, if possible, previously identified species. Reference sequences can be obtained from a relatively short genomic

region (DNA barcoding through Sanger sequencing; Hebert and Gregory (2005)), or from the whole genome or mitogenome (for instance, through whole-genome shotgun sequencing or the less expensive low-coverage whole-genome sequencing).

Distribution. Biodiversity is not found evenly but each species presents its own distribution range. This range sets the geographic limits where a species is found, which may be static or dynamic (e.g., ontogenetic, or seasonal migratory organisms). Genetic tools can help uncovering species distribution ranges through both single-species and multi-species methods (Yu *et al.*, 2022), since both methods provide presence/absence information. However, it seems that species-specific techniques such as quantitative PCR (qPCR) and digital PCR (dPCR) are most reliable for assessing the distribution of target species. The reason is that it does not depend on the proportion of target DNA in relation to the total DNA of the sample and is not affected by biases related to PCR, as occurs with DNA metabarcoding (Deagle *et al.*, 2019).

Abundance and biomass. Estimates on the abundance and/or biomass of marine organisms is essential to evaluate ecosystem services and to manage harvesting. Genetics can provide information on both parameters by using both single-species and multispecies approaches. Single-species approaches (qPCR and dPCR) provide absolute estimates of the DNA abundance of a target species, which can serve as a proxy of organismal biomass and/or abundance (Techtman *et al.*, 2017; Shelton *et al.*, 2022). DNA metabarcoding is informative of how abundant a species is related to the others (Jing *et al.*, 2018; Zhang *et al.*, 2019; Ershova *et al.*, 2021), but data should be used with caution due to biases associated to PCR amplification (Deagle *et al.*, 2019), different gene copy number (Gong *et al.*, 2013; Milivojević *et al.*, 2021), and, in case of eDNA samples, different DNA shedding rates among species (Allan *et al.*, 2021). These biases can be however minimised and corrected (thus making abundance data more reliable) by adding internal DNA standards to the samples (Ushio *et al.*, 2018; Zemb *et al.*, 2020) and by using mock samples to correct the bias due to primer efficiency (Shelton *et al.*, 2023). Finally, close-kin mark-recapture (CKMR) is a method to estimate abundance of a species that provides a census count (N_c) based on genetic markers. It is being applied for highly commercial fish species (Bravington *et al.*, 2016; Trenkel *et al.*, 2022).

3.1.3.4 Information at the ecosystem level

Trophic web. Predator-prey interactions constitute the main pathway of energy transfer from lower to higher trophic levels and the key process on which trophic webs are built upon. Acquiring knowledge on the structure, energy flows, and dynamics of the trophic web is an important step for implementing ecosystem-based management approaches. DNA-based methods provide a reliable tool to obtain information on predator-prey interactions in the mesopelagic zone (Chua *et al.*, 2019). For instance, a comprehensive list of prey species in the stomach or faeces can be obtained by DNA metabarcoding (Clarke *et al.*, 2020; Carlisle *et al.*, 2021), and DNA barcoding can be applied to classify prey items that cannot be identified by visually inspecting the stomach contents (Dahl *et al.*, 2017). Finally, single-species methods such as digital PCR (dPCR) and quantitative PCR (qPCR) provide the added advantage to detect and quantify specific prey target in the gut or faeces (Cuende *et al.*, 2017). The modelling of mesopelagic food webs (Anderson *et al.*, 2019) might benefit from the molecular identification and quantification of hard to sample soft-bodied taxa such as cnidarians and salps (Riaz *et al.*, 2020). Even more important, the ocean metabolism has become accessible outside the wet lab through sequencing of bulk DNA (metagenomics) and bulk RNA (metatranscriptomics). The Tara Oceans project, focussing on the euphotic zone, provides an exemplary case on how global ecosystems biology has become a reality (Sunagawa *et al.*, 2020).

Metazoan parasite community. Metazoan parasites are highly diverse key actors in the regulation of the population dynamics and hence trophodynamics of the ocean (Poulin and Morand, 2000; Lafferty *et al.*, 2008). For example, parasites and microbial pathogens change food-web topology, shunt energy of infected hosts to the free-living microbiome, and might stabilize ecosystems. Parasite diversity is huge (Poulin and Morand, 2000) and hence metabarcoding contributes to the growing inventory. Molecular identification has been already used to identify fish parasites below the epipelagic layer (Klimpel *et al.*, 2008; Gaglio *et al.*, 2018). Some taxa are highly host-specific, such as monogenean parasitic flatworms, and hence are useful tags (Scheifler *et al.*, 2022). Overall, morphological studies on the parasite community of the mesopelagic zone could benefit a lot from metabarcoding (Woodstock *et al.*, 2020), especially with the latest developments of benchtop and field proof long-read amplicon sequencing (Farhat *et al.*, 2021). Meta-transcriptomics might contribute to document the thermal performance curves of host and parasite, which might shift with global change and affect ecosystem functioning (Byers, 2021).

Free-living microbiome and virome. The microbial and viral community of the world oceans is diverse, represents the biggest biomass of any taxon in the ocean, and is functionally of great importance, either as part of the food web, nutrient dynamics, or climate (Tara Ocean Foundation *et al.*, 2022). The state and functioning of the microbiome and virome of the mesopelagic zone is increasingly exposed thanks to an accelerated pace of discovery supported by molecular tools and bioinformatic analyses in analogy to research in the epipelagic (Salazar *et al.*, 2016; Sunagawa *et al.*, 2020). Several crucial features have been documented. The viral community regulates the population dynamics of bacteria and eukaryotes, and mediates the release of nutrients through the viral shunt (Brum *et al.*, 2015). The first RNA virus-based study of the tropical and temperate mesopelagic zone points to a rich diversity and temperature variation as key factor for shaping the communities (Dominguez-Huerta *et al.*, 2022). Deep-sea microbial communities are heavily influenced by epipelagic plankton communities (Ruiz-González *et al.*, 2020). Metatranscriptome studies learn that long-term degradation of hydrocarbons is enhanced in the mesopelagic zone compared to epipelagic (Kampouris *et al.*, 2023). It is expected that the full significance of the viral and microbial communities will be understood in the current decade.

Community dynamics. The interactions between taxa, from species sorting, metacommunity dynamics to eco-evolutionary feedback mechanisms are the focus of community dynamics (Gianuca *et al.*, 2018; Urban *et al.*, 2020; Govaert *et al.*, 2021). Environmental DNA (eDNA), once discovered that it is everywhere present in the ocean and provides a detailed view of the locally sampled mesopelagic community, has been embraced as a tool to document marine communities. Amplicon sequences of eDNA collected from bottle casts reflects day-night community patterns and species-specific vertical distributions consistent with the known diel migratory behaviour of many mesopelagic fishes (Canals *et al.*, 2021). In combination with acoustic analysis, the diel vertical migration of mixed consortia of organisms was observed in the Gulf of Mexico (Easson *et al.*, 2020). Consequently, community genetics has developed into a full discipline (Agrawal, 2003; Govaert *et al.*, 2021).

3.1.4   Bibliography

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4 Tor D: Explorative analysis of genetic methods with emphasis on SME (small and medium enterprises) and conservation program broodstocks dedicated to aquaculture or natural population enhancement

4.1.1 Introduction

Plant and animal agriculture have been shaped by domestication and directional selection for traits deemed beneficial to humans, dating back 13 000 years (Diamond *et al*. 2002). While more recent for most species, the same is true of aquaculture. Selection methods have evolved from the initial unintentional selection associated with domestication, to phenotypic mass selection, family selection, marker-assisted selection, and now genomic selection (see for review Boudry *et al*. 2021). The adoption of genetics and genomics in animal and plant breeding has been shown to provide objective, quantifiable benefits, both in general (Rexroad *et al*. 2019), and to aquaculture in particular (Houston *et al*. 2020). It allows the development of an understanding of the composition, diversity, and adaptive or selective potential of a population or strain, as well as linking genetic regions, pathways and ultimately single genes or variants to traits of interest. As such, adoption of genetics and genomics are important at all stages of aquaculture development, from descriptions of candidate species and populations, to the initial collection of broodstock or source material where individuals are collected annually from the wild, through to fully domesticated broodstocks which may be subjected to selective breeding, using the most sophisticated genomic selection methods.

Genetics and genomics are utilized in conservation programs where the aim is to maintain biodiversity by ensuring sustainable reproduction of endangered species or populations. They may be used similarly in aquaculture to define populations of interest, document their genetic/genomic characteristics, determine and keep track of family structure and pedigrees, and to monitor changes in genetic characteristics over generations. Inherent in this is the fact that the available and usable genetic tools, the complexity of laboratory, bioinformatic and genetic/genomic analyses, and the physical and staff infrastructure required will differ depending on the aim of the program (selective breeding or conservation) and on the species in question.

Genetics and genomics have been successfully used in aquaculture in a number of different settings, such as selection for disease resistance (Fuji *et al*. 2007, Houston *et al*. 2010, Moen *et al*. 2009, Yáñez *et al*. 2022, AquaGen's innOva BCWD selected stock), delayed maturation (Kause *et al*. 2003, Moghadam *et al*. 2007), growth rate (Tsai *et al*. 2015, Wang *et al*. 2017, Garcia *et al*. 2018, Gutierrez *et al*. 2018, Palaiokostas *et al*. 2018, Yoshida *et al*. 2018, Wang *et al*. 2018, Yoshida *et al*. 2019, Joshi *et al*. 2020, Gong *et al*. 2021, Vu *et al*. 2021, Wang *et al*. 2021, Jerry *et al*. 2022, Ke *et al*. 2022, Verbyla *et al*. 2021), feed efficiency (Besson *et al*. 2019, Barría *et al*. 2021, Besson *et al*. 2022), appearance (Kause *et al*. 2004, Colihueque 2010, Colihueque and Araneda 2014), production traits and flesh quality (Kause *et al*, 2011, Quinton *et al*. 2005, Nguyen 2016), survival (Vehviläinen *et al*. 2008, 2010, 2012) and parentage assignment (Vandeputte and Haffray 2014, Liu *et al*. 2016, Holman *et al*. 2017, Weng *et al*. 2021). In addition to traditional selection-based methods, genetic modification has been employed to introduce desired traits into fish for commercial, research and ornamental purposes; the discovery and operationalization of CRISPR-Cas9 (Doudna and Charpentier (2014) is likely to facilitate the development of novel lines, whose acceptability depends on consumers and local regulation (Qin and Brown 2006). Furthermore, the same tools

can be used in any breeding effort targeting restocking and conservation (Casey *et al.*, 2016, Waples *et al.*, 2020; Wenne, 2023).

There are many possible obstacles in the adoption of these approaches, particularly for small and medium enterprises (SMEs) focusing on aquaculture and conservation programs. The obstacles include the cost of development and implementation of genetic technologies, the availability of specific genetic tools for the species being raised, and the specialized knowledge or skill sets required to undertake laboratory, bioinformatic, and analytical work. Here we consider SMEs as programs where producers operate as smaller independent operations where there is no pooled investment or research direction, or genomic resource development. This scenario is common in programs developing newer species in commercial aquaculture and in conservation programs. It also may apply to more established species where, despite the availability of resources, groups must develop their own genomic resources due to proprietary factors. Across all scenarios, there are likely more significant limitations in terms of financial or infrastructure resources to develop and implement genomic resources to improve the aquaculture program.

Nevertheless, the use of genetic tools has benefits above and beyond the costs, and in some instances, may even be required by legislation. In light of this, we explore the uses of genetic and genomic tools across the spectrum of aquaculture developments, from species for which no genetic data exist and must be developed through to cases where genomic selection is routinely applied to select for advantageous traits, and finally we touch on the possibility of using gene editing in both commercial aquaculture and conservation settings.

4.1.2   States of genetic method application

Here we define and describe five states of development and application of genetic and genomic tools in aquaculture and conservation breeding programs. For each state, we characterize the genetic and genomic tools applied as well as the types of applications that may be common to that state for SME aquaculture and conservation-focused programs (Table 2.2). For each state, examples are highlighted for both SME aquaculture and conservation programs, where possible. The term "state" is purposefully chosen to remove connotations or implications that a program should or must advance from one state to the next; a program may find that the tools and data available at a given level may be adequate for their needs and thus remain at that particular state. In addition, current technologies make it much easier for programs to skip to higher states without sequential progression.

4.1.2.1   State 0: No previously developed genetic markers or genomic resources

This state refers to a situation where there are no specific genetic resources for a species, either for evaluating wild populations or characterizing cultured programs. Programs at this state may be collecting and rearing wild caught individuals in cages or tanks until they reach market size, or may be in the initial stages of determining whether a species, either for commercial or conservation purposes, is amenable to culture. While many species of interest have now been subjected to genetic studies, Tripletail (*Lobotes surinamensis*), is an example of a candidate aquaculture species in this state. This fish is of interest for culture in the U.S.A., and wild broodstock have been collected to identify and optimize spawning techniques for the development of commercial culture (Saillant *et al.* 2021). To date, there are no genetic resources available for Tripletail, and no genetic studies have been conducted on this species other than DNA barcoding using a universal mitochondrial marker approach (Sirisha *et al.* 2018). If a culture program wants to apply genetic methods for this species, then markers will need to be developed *de novo*.

For species in State 0, the long history of genetic and genomic tool development on which to rely has led to *de novo* genetic resource development no longer being an arduous process; it has become nearly trivial. The purpose of this document is not to describe how to develop the tools,

but to give examples of their use. The development of microsatellite markers, and their application to aquaculture was reviewed by Chistiakov *et al*. (2006), however since that time methods to identify microsatellites and SNPs have advanced considerably. Reduced representation sequencing (e.g., RADseq, and others) have been used to discover SNPs (single-nucleotide-polymorphism), microsatellites and more recently haplotypes. Where available, high-quality whole genome data allow for identification of SNPs and microsatellites using in-silico means. Development of an annotated reference genome is generally recommended because of the advantages it affords such as being able to link traits associated with phenotypes of interest to their underlying genes. This will be discussed in more detail in the section on "State Skipping".

For programs or species in this state, decisions as to what type of genetic or genomic marker development to pursue should consider both future goals and opportunity cost of limiting the amount and versatility of selected genomic tools. Even for programs that do not anticipate moving beyond State 2, individual genetic tagging (see below), pursuing the development of an annotated whole genome at this stage may be recommended. This is because it would allow the relatively simple development of goal-specific panels of molecular markers, as well as serving as the backbone for future marker assisted breeding and linking traits and markers to putatively causative genes.

4.1.2.2   State 1: Stock choice - Information available about populations genetics

Once a candidate aquaculture or conservation species has been identified, local regulations generally require permits or licenses for both collection of aquatic organisms from the wild as well as the aquaculture operations themselves. In many cases, the issuance of collection permits will be contingent upon the applicant demonstrating that the collection will not result in harm to the species or populations. Part of this process may involve determination of effective population sizes and structure. The delineation of population structure can be accomplished using genetics or genomics and this is often the first opportunity to develop and employ genetic tools and collect genetic information for the species that can be put to use in the other stages of aquaculture development.

There are myriad examples of population genetic and genomic studies in aquatic organisms. First based on allozymes, most studies now rely on microsatellite or single nucleotide polymorphisms (SNPs) as markers (see notably Wenne (2023) for a review about the applications of SNPs in conservation and exploitation of aquatic populations). Some studies have started to use structural variants such as copy number variants (e.g., Dorant *et al*. 2020), while others have begun to explore synteny (Schultz *et al*. 2023) and three-dimensional genomic conformation (Marletaz *et al*. 2023). Whatever the choice of marker, the goal is typically to define population units, determine degree of differentiation between them, and when collection and incorporation into aquaculture is the goal, determine the genetic diversity within the collection.

The choice of marker type, the degree of population divergence, the number of markers used in a study can influence the degree of genetic differentiation that is detectable. While studies historically relied on a handful of markers, advancements in genotyping technology have made panels of tens to hundreds of microsatellites (e.g., Bradbury *et al*. 2018) or hundreds to thousands of SNP markers the norm (e.g., Jeffery *et al*. 2018). Lehnert *et al*. (2023), used a weight of evidence approach and a combination of genetic and genomic data to identify previously unresolved landscape scale genetic distinction in Atlantic salmon. Similarly, using a panel of 101 microsatellites, Bradbury *et al*. (2018) were able to resolve 26 predominantly river-level Atlantic salmon genetic reporting groups across approximately 3 degrees of latitude in Labrador, Canada, where previous analyses using smaller numbers of SNPs were only able to detect three groups in the same area (Jeffery *et al*. 2018). Another example is seen in population genetic studies of lumpfish (*Cyclopterus lumpus)*, a species where both translocated and domesticated individuals are used in the aquaculture industry for biological control of sea lice. A study based upon 14 microsatellites

suggested that interbreeding between wild and aquaculture escapes would have little impact on the genetic composition of the wild stocks in Norway (Jónsdóttir *et al*, 2018), while a more recent study using thousands of genome wide SNPs demonstrated fine-scale population structure with the potential for breakdown of local adaptation if introgressed by aquaculture escapees (Jansson *et al*, 2023).

Available, or purposely produced, population genetic information can be used, often in conjunction with life history and morphological data, to select populations from which to collect individuals for culture. Ensuring that a wide range of genetic diversity, and thus adaptive and selective potential, is included may be more important for broodstock collection when the goal is to transition from State 1 to 2. A prime example of this is the Norwegian Atlantic Salmon breeding program history. In this program, life history and morphological data were used to select salmon from a diverse range of rivers, including populations that exhibited late maturity and large size at maturity (Gjedrem *et al*. 1991). Initial collections were spread across 41 rivers to maximize genetic variation and four separate breeding populations were formed (Gjedrem *et al*. 1991). The same approach was recently used during the establishment of the Norwegian Lumpfish breeding program, where milt from males collected along the entire Norwegian coastline were utilized in order to obtain genetic diversity for traits such as disease resistance and appetite for consuming sea lice (AquaGen NamGuard GEN2Boost strain).

Within conservation programs, a typical set of goals involve the maintenance of existing genetic characteristics of the species or population(s) of concern. This generally entails undertaking population genetic or genomic study to determine metrics such as genetic or allelic diversity, heterozygosity, and effective population size. Theoretical linkages can then be made to predict the species'/population's risk of genetic impact from inbreeding and drift over time, among other concerns. A special case in conservation, where populations have been extirpated and reintroduction is desired, the identification of genetically, and presumably adaptively, similar source populations is generally recommended, and these can be identified by screening nearby extant populations (e.g., Anderson *et al*. 2014). An adjunct to this for populations where reductions in genetic diversity is affecting their fitness and population resilience is genetic rescue. In this scenario, individuals are intentionally translocated into the imperiled population to restore genetic variation and adaptive potential (Kovach *et al*. 2022). While difficult to predict, the outcome of genetic rescue may rely in part on effective population size of the recipient and donor populations and their genetic divergence, metrics which can be measured using population genetics (Wells *et al*. 2019).

In some situations, genetic approaches can be used across species (e.g., universal primers to PCR-amplify specific mitochondrial DNA fragments) or across related species (e.g., cross amplification of microsatellite markers). For example, because of the (relatively) conserved nature of many genes and stretches of the genome between closely related species, genetic resources that have been developed for one species can often be utilized between species. This can be seen in an examination of linkage maps created for salmonids which generally reveal the extensive use of markers developed for one species in many species (e.g., Nichols *et al*. 2003; Moghadam *et al*. 2007; Reid *et al*. 2005); within salmonids extensive cross amplification of microsatellites among species appears to be the norm (Cairney *et al*. 2000). Cross amplification of microsatellites between related species is widely observed: Hamilton and Tyler (2008) found that a set of microsatellites designed for roach (*Rutilus rutilus*) amplified in other cyprinid species; Leclerc *et al*. (2000) cloned microsatellite loci in Yellow Perch (*Perca flavescens*) which showed successful amplification in four other fish in the family Percidae; and King *et al*. (2001) developed microsatellites in Atlantic Sturgeon (*Acipenser oxyrinchus*) that showed amplification in other species in the Acipenseridae.

4.1.2.3 State 2: Individual genetic tagging

Founding aquaculture programs, by necessity, requires sourcing or collecting aquatic organisms from the wild; in many cases the culture of wild-caught organisms is the entirety of the program. This process is the norm for many shellfish species, as well as some finfish. Collection of individuals from the wild is also the norm in many conservation and supplementation programs which may operate as fully closed, fully open, or partially-open breeding programs. These types of programs differ in whether, or what proportion of, captive-bred individuals are incorporated into breeding programs. Where the program is not fully open, a method that can rapidly, accurately and non-lethally distinguish wild from hatchery-produced individuals is required (e.g., Horn *et al*. 2022). For commercial aquaculture, if organisms are collected from the wild each generation, this generally precludes the ability to undertake directed selection, although unintentional domestication selection may occur. However, it need not mean that the development of genetic tools and undertaking genetic work is not without its benefits.

One of the most immediately informative genetic approaches to utilize for a breeding program is individual genetic tagging. Individual genetic tagging refers to the use of genetic markers to unequivocally identify an individual, link an individual to genetic information pertaining to family relationships (e.g., parentage and pedigree analyses), and/or to trace the individual back to their breeding program, or origin (e.g., farmed versus wild, and/or location) (Norris *et al*. 2000, Chistiakov *et al*. 2006). Both parental assignment and pedigree reconstruction can be used to manage the level of inbreeding and genetic diversity in the culture program across generations (Meuwissen and Sonesson 2004, Wellmann *et al*. 2014, Vandeputte and Haffray, 2014, Gebregiwergis *et al*. 2020, Gautason *et al*. 2022), which are important parameters for both commercial and conservation breeding.

Genetic kinship data available across generations can be used for parental assignment (Lacy 2012) and pedigree reconstruction (Mendes *et al*. 2022). These data can be helpful in exploring spawning dynamics and assessing individual spawning success for naturally spawning species in culture settings (Herlin *et al*. 2007, 2008, Horreo *et al*. 2008). These analyses may also assist in tracing physical traits (due to genetic or non-genetic factors) back to individual broodstock. This type of application can be immensely helpful to quickly inform breeding designs and culturing approaches. In the California Yellowtail (*Seriola dorsalis*), for example, parentage analyses using a small number of microsatellites (developed for other *Seriola* species) revealed that only one or occasionally two female broodfish contributed to individual spawning events and dominated spawning seasons regardless of the number of females in a tank (Schmidt *et al*. 2021). This approach was also used to identify females contributing eggs of poor quality. In the White Abalone (*Haliotis sorenseni*) Recovery Program (a collaborative effort with the University of California, Davis, NOAA Fisheries Southwest Fisheries Science Center, and other partners) parentage and pedigree analyses, using microsatellite markers, informed crosses of gametes for the captive breeding program to maximize genetic diversity in this endangered species.

Supplementation or conservation program efficacy may also be evaluated using genetic marking and pedigree reconstruction (Araki *et al*. 2007). In order for a conservation program to be successful, the individuals removed from the wild population to be bred in captivity must produce at least as many adult offspring as they would have had they been left to reproduce in the wild. Whether the program meets this objective can be evaluated by genotyping the parents in captivity and subsequently sampling and genotyping the next generation and conducting genetic parentage assignments. This methodology may also reveal if the parents in captivity contribute to the next generation to such an extent that it ultimately reduces the effective population size and thus the genetic variation in wild populations. For supplementation programs, providing harvest opportunities may also be among its objectives. Such is the case for Canada's West Coast Salmonid Enhancement Program, which includes rebuilding vulnerable populations and providing harvest opportunities among its goals. The Salmonid Enhancement Program uses

parentage-based tagging to track fish captured in the fishery back to their hatchery (and thus population) of origin. While technically not a breeding goal, genetic tagging can be used for product traceability in production systems, and traceability to a producer or cage facility is a legal requirement for aquaculture in some jurisdictions (Håstein *et al*. 2001, Espiñeira and Vieites 2015, Holman *et al*. 2017).

From a conservation standpoint, the goals of captive breeding are avoiding loss of genetic variability and inbreeding, and avoiding genetic drift and accidental selection, or other program-specific targets such as retention of rare alleles or maintenance of a target effective population size (Waters *et al*. 2015, Rollinson *et al*. 2104, Attard *et al*. 2016, Wright *et al*. 2020, Marshall *et al*. 2022). Additionally, it is important to monitor both the genetic trends of reintroduced populations, and the effect of reintroduced individuals on the genetics of the wild remnants of the species/population (Russello and Amato 2004, Christie *et al*. 2012, Christie *et al*. 2014, Water *et al*. 2015, Hagen *et al*. 2020, Auld *et al*. 2021, Marshall *et al*. 2021), especially in order to avoid the Ryman-Laikre effect (Ryman and Laikre 1991), where hatchery fish increase the number of spawning individuals fish while simultaneously decreasing the effective population size (Morvezen *et al*. 2016). As an example, genetic approaches are used to identify the origin of Norwegian Atlantic Salmon. Due to declines in natural populations, Atlantic salmon is being stocked for conservation purposes in multiple areas of their natural range. Genetic screening of wild caught broodstock can be an effective tool for improving the accuracy of such programmes. From a 7kSNP-chip, a set of 59 SNPs have been identified with the purpose of separating Norwegian Atlantic salmon individuals of farmed and wild origin (Karlsson *et al*. 2011, 2014). Introgression of farmed salmon has been documented throughout Norway (Karlsson *et al*. 2016), leading not only to genetic but also phenotypic and phenological alterations in introgressed individuals (Bolstad *et al*. 2017, 2021, Besnier *et al*. 2022). It has furthermore been documented that farmed ancestry have been inadvertently selected for in supplementary stocking of Atlantic salmon (Hagen *et al*. 2019) and genetic screening for introgressed individuals in broodstocks used for conservation breeding is now routinely done in Norway.

As demonstrated in the examples above, it is still possible to use genetic technologies such as microsatellites, or sets of SNP markers for the applications described under this State. Some of these goals may benefit from the adoption of genomic technologies due to the greater precision potential they have for estimating population parameters (Supple and Shapiro 2018). The analytical and bioinformatic support required for these applications is not extensive, but will depend on how often they are required for the desired analyses.

4.1.2.4 State 3: Develop and apply linkages between phenotypes/traits and genetic markers

In State 3 genetic markers are used to develop linkages to traits or phenotypes of interest. Programs in this state may focus on targeting simple traits with Mendelian inheritance or phenotypes associated with genes (and markers) of major effect, and this may include family-based selection. Development of these linkages between phenotypes and genetic markers at this level allows programs to use marker assisted selection (MAS) to identify and screen individuals at genomic loci for those traits of interest. While many traits do not fall under this category, and are instead controlled by many genes or loci of small effect (discussed further in State 4), marker assisted selection and/or application of genetic screening for these traits offers a rapid approach to make improvements in the breeding program or inform breeding decisions.

If phenotypic markers have already been developed in other research projects or breeding programs, then the application of this approach may only require genotyping (SNPs, microsatellites, or PCR and gel electrophoresis) to assess the phenotype. Alternatively, if linkages between phenotypes and genetic markers do not yet exist, the identification of these linkages will require genome-wide association studies (GWAS) using linkage maps or genomic data to identify

regions in the genome associated with the trait(s) of interest. While linkage maps may be produced through various forms of reduced representation sequencing to obtain markers across the genome for this purpose, it is often nearly as cost effective and easy to generate a reference genome. Combinations of sequencing technologies (e.g., short-read, long-read, super-scaffolding approaches) can both provide good coverage depth and high contiguity (e.g., chromosomal level scaffolds) in the *de novo* genome assembly and result in a high-quality reference genome. However, the cost (in terms of sequencing, computational, and bioinformatic support) of assembling a reference genome will depend on the size and complexity of the species' genome. Some species may require modest increases in sequencing and assembly resources to generate due to larger or more repetitive genomes, for example, in the blue mussel (*Mytilus edulis*), and penaeid shrimp species (Corrochano-Fraile *et al*. 2022, Yuan *et al*. 2021, Zhang *et al*. 2019), while a few species may require considerably more resources and intensive assembly approaches to sort out duplicate or polyploid genomes (e.g., catfish, salmon and sturgeon) (Lebeda *et al*. 2020, Lien *et al*. 2016, Drauch *et al*. 2011). Regardless, a reference genome is an important investment for a cultured species (Yanez *et al*. 2023); this resource is valuable to guiding development of genetic tools that may be used across any of the genetic and genomic applications described in this report.

There are widespread examples of the use of genomic resources described in State 3, but frequent applications include identification of genetic sex, improvements in disease resistance, and indicating degree of introgression by aquaculture escapees in fish destined for breeding programs. For example, the reference genome developed for California Yellowtail (*Seriola dorsalis*) was used in conjunction with a GWAS on resequenced (i.e., lighter coverage whole genome sequencing) sexed individuals to identify a genomic region associated with sex. This approach identified both a genomic region associated significantly with sex, but also an insertion/deletion in the region. PCR primers spanning this region were amplified and visualized on an agarose gel to identify sexes of brood and offspring fish by the banding pattern (e.g., presence/absence with a positive control), which has become a useful tool for a species with no external sex-distinguishing characteristics (Purcell *et al*. 2018). At the NOAA Southwest Fisheries Science Center, in collaboration with Iowa State University, a similar approach is currently being attempted for the endangered white abalone (*Haliotis sorenseni*) to improve the breeding program. A recently assembled chromosomal-scale reference genome for this species is being used to inform a GWAS using genotyping-by-sequencing (GBS) data from sexed white abalone specimens. One highly significant genomic region associated with phenotypic sex has been identified, and genetic variants are currently being assessed and screened (as of the writing of this report) to determine if a sex-specific marker may be developed. Development of a sex-specific marker for this species would be tremendously important for the breeding program to identify appropriate individuals to bring into the broodstock population without the need to extensively handle wild abalone to determine sex (e.g., a swab and subsequent genotyping would work for these more-often-than-not stationary animals) and to inform outplanting groups of juvenile white abalone to ensure mixed sex groups or match-make with wild individuals in the region.

In Atlantic salmon, outbreaks of infectious pancreatic necrosis virus (IPNV) were a major concern to the aquaculture industry. Research groups identified a single major quantitative trait locus (QTL) that explained 80 to 100% of genetic variation in resistance IPNV. Marker assisted selection, utilizing a SNP-based genetic screening for the favorable, resistant, and dominant allele, was rapidly adopted by salmon breeding programs to help prevent further outbreaks of this virus (Houston *et al*. 2012). Similarly, disease outbreak of Ostreid herpesvirus 1 (OsHV-1) has greatly impacted aquaculture of Pacific oysters (*Crassostrea gigas*). A QTL on chromosome 8 of the Pacific oyster genome was determined to be associated with improved survival (13% phenotypic variance) to mortality events in Tomales Bay, California, where OsHV-1 is endemic. Marker-assisted selection for this QTL resulted in 47% greater survival in breeding values for families undergoing MAS than based solely on pedigree selection (Divilov *et al*. 2023).

Developing and applying genotype-phenotype linkages is also important from a conservation perspective. A good example is the European flat oyster, *Ostrea edulis*, which is currently a species of relatively low interest in aquaculture, particularly when compared with the Pacific oyster *Crassostrea gigas*. However, there is an increasing interest in restoration of oyster beds, particularly in northern Europe where populations have been severely depleted, or gone totally extinct due to overfishing, parasites and habitat degradation (see https://noraeurope.eu/). Genomic resources have been developed for this species, and these currently include several low- to medium-density SNP arrays (Lapègue *et al.* 2014, Gutierrez *et al.* 2017), RAD-Seq data, three independently developed chromosome-level assemblies and low-coverage whole genome sequencing (Bean *et al.* 2022, Boutet *et al.* 2022, Gundappa *et al.* 2022, Li *et al.* 2023). While most studies have been dedicated to population genomics aimed at studying population structure and identifying signatures of local adaptation (e.g., Vera *et al.* 2019, Lapègue *et al.* 2023), GWAS has also been applied in hatchery-produced progenies to target traits of interest for aquaculture (Peñaloza *et al.* 2022). In particular, molecular breeding to improve resistance against bonamiosis (an infection caused by a protozoan parasite that impacts wild and cultured populations) is a major objective in this species (Pouvreau *et al.* 2023). However, the development of selective breeding programs remains limited for *O. edulis* due to the small-scale nature of the aquaculture industry and to the constraints related to seed supply for restoration projects (Colsoul *et al.* 2021).

Determination of what constitutes a 'species' is foundational to conservation as well as endangered species legislation, especially given that most programs and legislation consider subunits below the level accepted as biological species. For instance, in determining designatable units, the Committee on the Status of Endangered Species in Canada considers the proposed group's discreteness and evolutionary significance (Lehnert *et al.* 2023). This is similar to the US evolutionarily significant unit (ESU) being based on "reproductive isolation" and "evolutionary legacy" (Waples *et al.* 2022). This determination is generally accomplished through investigation of population structure using panels of (typically neutral) markers (e.g., Lehnert *et al.* 2023). However, adaptive diversity at single markers exists within identified conservation units, and the appropriateness of considering designations based on variants at these markers has been debated (Waples *et al.* 2022).

For instance, within salmonids, variants for single genes have been detected that have major influence on important life history traits. In Atlantic Salmon, variants of the genes *six6* and *vgll3* have been found to have pronounced effects on age at maturity in some genetic and habitat backgrounds (Barson *et al.* 2015, Sinclair-Waters *et al.* 2020). While in Pacific salmon, *six6* has also been associated with age at maturity in Steelhead and sockeye salmon, but no link was found in Chinook and Coho (Waters *et al.* 2021). Of particular note for conservation programs are the links that have been detected between an approximately 200 Kb region of the Steelhead and Chinook salmon genomes between the genes *GREB1L* and *ROCK1* (Narum *et al.* 2018, Hess *et al.* 2016). Variants at this region are associated with the propensity to exhibit 'early' or 'late' migration, where the migration type allows individuals to access spawning habitats that are inaccessible under different flow/temperature conditions (e.g., sandbars can block river mouths during low flows). Despite being loci of large effect, the presence of both variants in a number of populations suggests that this diversity is being maintained by ongoing selection. The importance of selection in maintaining diversity is also demonstrated by allele frequency changes resulting from interbreeding when habitat that was previously inaccessible to one or the other type is made available through anthropogenic alteration. Waples *et al.* (2022) discuss loci of large effect in Pacific Salmon in relation to conservation and the definition of conservation units.

4.1.2.5 State 4: Genomic selection and/or genomic imputation and prediction using family- or pedigree-based selection.

In state 4, genomic selection and/or genomic imputation and prediction using population, or pedigree-based selection is executed. In this state many species will have been bred in captivity and undergone selection for generations, but genomic prediction methods are now increasingly being applied to wild populations of conservation interest as well. The theme unifying these approaches is to leverage existing genomic information to predict unobserved data, whether that is a trait of interest, signatures of environmental association, or genotypic state at a given locus.

Family- or pedigree-based selection relies on known family relationships to evaluate the breeding value of an individual and has been commonly used for many years. Although not required, the availability of genetic information has improved the effectiveness of pedigree-based selection. With advances in high throughput sequencing, genomic selection has emerged as a powerful tool in aquaculture to enhance selective breeding programs and improve trait outcomes. It relies on the analysis of a large number of markers distributed genome-wide to estimate the genetic potential, or genomic estimated breeding value (GEBV), of individuals for various traits, even those with complex genetic architectures. During the development of the prediction equation, phenotypic information for the traits of interest and genome-wide genetic information is necessary for a training population. However, once the prediction equation has been established, only genotypic information is required, which makes the subsequent application of genomic selection straightforward and cost efficient, despite the elevated initial labor- and economic- costs.

Several approaches and technologies now allow generation of genome-wide genotypes required for genomic selection, even for species with limited genomic resources available. Reduced-Representation Sequencing - such as Restriction site-Associated DNA (RAD) sequencing or GBS - or RNA sequencing have been as successful as candidate approaches with no prior genomic information for a species of interest. However, independently of how a new SNP panel is created, it is important to achieve a number of markers that are informative while not being redundant. Cross-validations tests carried out in multiple aquaculture species and traits indicated that the target number of these panels should be about 2000-7000 SNPs to achieve a satisfactory accuracy in genomic evaluation (Kriaridou *et al*. 2020). These efforts might be beneficial in conservation settings as well, depending on the genetic variability of the species being bred. As genomic resources become more available, it is advantageous to build upon pre-existing resources to create cheaper and more stream-lined tools and workflows, both in data generation and processing, such as RAD-cap (i.e., sequence capture of dual-digest RADseq libraries technologies; Hoffberg *et al*. 2016) or SNP chips. SNP chips normally require a much simpler processing pipeline, which is faster and cheaper. Additionally, SNP chips normally provide data that can be much more easily compared over time and/over datasets. On the other hand, the predictability and uniformity of SNP chips and many genotyping–by-sequencing approaches, mean that they only provide information for a predetermined set of genetic markers, this limitation may lead to missing important genetic information that might be present in the stock in analysis, due to ascertainment bias. The discovery of molecular markers in a limited set of samples can bias all downstream genetic diversity inferences in non-reference populations. The availability of off-the-shelf genetic tools is likely more challenging for conservation efforts aimed at species of little or no commercial value, y*et al*l the above considerations are nonetheless valid even in those specific circumstances.

The combination of long- and short-read technologies allows high-fidelity genomes to be produced that include repeated elements, inversions and other structural variants that have been shown to have fitness and phenotypic implications, but were not easily detected using older methods that relied more heavily on short reads alone. Having a high-quality genome for a species of interest opens up opportunities to produce genome-level data for individuals at a much-reduced cost through low coverage whole genome sequencing (Lou *et al*. 2021) or by sequencing

at a reduced number of loci and then imputing to the whole genome (Kijas *et al.* 2017). In recent years, whole genome sequencing approaches that leverage low-coverage data and imputation or error correction approaches have begun to be used in both aquaculture (Gundappa *et al.* 2023) and wild population studies (Kess *et al.* 2022) when a reference genome is available to maximize the number of informative markers for genetic analyses. Imputation methods leverage missing genotype information at the pedigree or population level to assign unobserved or low confidence genotypes therefore reducing rates of missing data (Marchini & Howie 2010). Alternatively, genotype likelihood approaches utilize the combined information across sequenced individuals to infer population genetic parameters (Kim *et al.* 2011). These methods have been generally found to out-perform reduced representation and SNP-array based approaches in both informativeness for detected trait associated loci (Jørsboe & Albrechtsen 2023, Homburger *et al.* 2019), and in accurately inferring population divergence (Szarmach *et al.* 2021). Imputation-based whole genome sequencing (WGS) approaches have been applied in Atlantic Salmon in aquaculture and wild population settings for both marker discovery and GWAS (Kess *et al.* 2022). Similarly, low coverage WGS and genotype likelihood methods have been applied across multiple wild species of management interest, including Atlantic Silversides (Therkildsen *et al.* 2019), Atlantic Eel (Enbody *et al.* 2021), Sandlance (Jones *et al.* 2023) and Cunner (Nugent *et al.* 2023), to uncover population differentiation relevant to species management. These methods represent the forefront of WGS approaches that can be utilized to optimize the tradeoff between marker and individual sample number, and provide relatively unbiased estimates of genomic diversity, population structure, and trait association. However, reference population divergence and local population structure and linkage can bias imputation accuracy (Lou *et al.* 2021). Genotype likelihood approaches should also be considered as a validation step in cases of low divergence and linkage, or high divergence from a reference population.

Genomic predictions of phenotype are now increasingly used in both wild and aquaculture populations, to infer unobserved phenotypes from observed genomic data. Similarly, polygenic risk score approaches, first utilized in human disease studies, have also been developed to summarize individual genomics-based phenotype probabilities (Dudbridge 2013). These methods have been utilized for prediction and trait improvement across many aquaculture species (reviewed in Song & Hu 2022), and the automation of this process is now also underway, with the development of reference genotype panels across many aquaculture species, as well as web-based infrastructure to support analyses (Zeng *et al.* 2023). Machine learning approaches are now also being deployed to better account for non-linearity of interactions among trait-associated loci (Brieuc *et al.* 2018), and have shown improvements beyond traditionally used additive genetic models under certain genetic architectures (Abdollahi-Arpanahi *et al.* 2020, Azodi *et al.* 2019). In wild populations, genomic vulnerability approaches provide a methodologically similar approach to using genomic information for predicting phenotypes (Bay *et al.* 2018, Capblancq *et al.* 2020), but phenotypic information is instead inferred indirectly through environmental or climate data, assumed to reflect local adaptation (Hoban *et al.* 2016). Polygenic score approaches have been applied in this context, predicting individual environmental associations in Eastern Oyster (Bernatchez *et al.* 2019) and Sea Cucumber (Xuereb *et al* 2018). Additionally, genomic vulnerability estimations of future climate impacts have been conducted in Arctic Charr (Layton *et al.* 2021), marine invertebrates (Nielsen *et al.* 2021), seaweed (Wood *et al.* 2021) and eelgrasses (Jeffery *et al.* 2022). Similarly, all-cause decline rate risk has also been estimated at the river level in Atlantic Salmon (Lehnert *et al.* 2019), indicating genomic prediction methods may show utility in conservation planning and prioritization.

4.1.2.6 State 5: Gene editing

Gene editing and genetic engineering are rapidly emerging technologies. However, their acceptance by the public and the legislation regarding their use in commercial, and especially conservation programs, varies considerably across the world. Therefore, consideration of local laws

and public perspective and acceptance should be carefully investigated before they are considered for use in a program.

There are multiple methods for performing gene editing, with Zinc fingers (ZFN) (Miller *et al.*, 1985, Klug 2010, Urnov 2010) and TALENs (transcription activator-like effector nucleases) (Joung and Sander 2013) being the first technologies developed. Today these techniques are considered to be both time consuming and expensive. The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas technology, first presented in 2012, is a simpler, inexpensive, and effective method for targeted gene editing, and can be used in all organisms and cells (Doudna and Charpentier 2014). Thus, compared to ZFN and TALENs, the CRISPR/Cas technology was identified early as a promising tool (Gaj *et al.* 2013). However, all three techniques are based upon the same principle; a protein that cuts DNA in a targeted place.

Compared to the traditional methods of gene modification, where whole genes (often from other organisms) are randomly placed into the genetic material of the organism, gene editing can be used to precisely modify genes already present in the organism, to inactivate (knockout) genes/genetic sequences or to add (knock-in) genetic material at specific locations of the genome. Thus, a prerequisite for editing genes with CRISPR/Cas is to know the sequence of the specific piece of DNA that is to be edited, as well as the sequence of the entirety of the genome to reduce the potential for off-target editing and effects. In this way, gene editing by knock-in can also be used to transfer genes from other organisms to specific sections of the genome, so called transgene knock-ins. The CRISPR technology also has the potential to switch off genes without altering the genetic code, thus to alter the epigenome (Nunez *et al.* 2021), although the full potential for CRISPR-epigenetic editing is still unknown.

As a potential solution for hindering gene flow from aquaculture escapees to wild conspecifics, the CRISPR/Cas technology can be used to block the ability for the aquaculture fish to reproduce, thus rendering them sterile. This can be accomplished by inhibiting the function of proteins that are important for germ cell development and/or survival (Wong and Zohar 2015), and has been demonstrated in Atlantic salmon (Wargelius at al. 2016, Güralp 2020, Kleppe *et al.* 2022). There are also several examples where CRISPR/Cas-induced knockout of genes have been shown to improve commercial important traits, such as growth, pigmentation, disease resistance and omega 3-production (see Roy *et al.* 2022). In 2019, the first CRISPR/Cas-edited aquaculture species, a strain of Tilapia (*Oreochromis niloticus*), became available for commercial sale in Argentina (Okoli *et al.* 2022). The producers state that an improvement in feed conversion ratio, growth rate and filet yield has been achieved, without it containing any foreign DNA (https://www.fishfarmingexpert.com/aquabounty-argentina-gene-editing/aquabounty-gets-argentina-go-ahead-for-edited-tilapia/1151140; Accessed July 19, 2023). More recently, CRISPR-edited for faster growth tiger puffer *(Takifugu rubripe)* and red sea bream *(Pagrus major)* (Kishimoto *et al.* 2018) were approved for the Japanese market.

The discovery of the CRISPR/Cas system has also begun to supplant the use of random transgenesis techniques. There is a relatively long history of development and production of transgenic fish in culture through multiple random transgenesis methods; the first transgenic aquaculture fish were produced over 35 years ago (Devlin *et al.*, 2006), and in that intervening period over 35 species have undergone transgenesis (Devlin *et al.*, 2015). Through traditional methods of gene modification transgenes have been introduced to aquaculture species to achieve a number of desired phenotypic outcomes such as: improved disease resistance, altered metabolism, and increased growth (Devlin *et al.*, 2006). CRISPR-edited transgenes have been produced in an effort to achieve increased disease resistance (Simora *et al.*, 2020), and today, effort is directed towards identifying genes or causative mutations in Pacific salmon related to sea lice resistance (Robinson *et al.*, 2023). If such genes are successfully identified, they could be transferred to Atlantic salmon, a species highly susceptible to sea lice, using the CRISPR technology.

The transition from the use of random transgenesis techniques to achieving transgenesis through CRISPR/Cas can also be seen within the ornamental aquarium trade, and supporting commercial culture systems. Incorporation of transgenes that express different fluorescent colour proteins, and thus result in novel colour phenotypes, has been undertaken through random transgenesis techniques for a number of species including the Black Tetra (*Gymnocorymbus ternetzi*) and Zebrafish (*Danio rerio*) (DFO 2018, 2019, 2020). More recently, CRISPR/Cas has been used to introduce the same genes and achieve similar colour phenotypes in Siamese fighting fish (*Betta splendens*; DFO 2021). The single gene to single trait nature of these modifications make the development of these types of ornamental fish relatively simple. As these technologies advance, the development of fish that incorporate more sites of modification and thus more complexity in the modification of phenotypes is likely.

Although few commercial CRISPER-edited species are available for human consumption today, the number might increase as regulations of their use are being evaluated in multiple regions. In conjunction with this, more commercial companies are increasing their research efforts in regard to this powerful technique.

The commercial applications of genome editing are clearer than its use in conservation programs. Moreover, public and legislative acceptance is more likely for genome editing in commercial settings than for conservation purposes, especially when the program will result in the release of gene-edited individuals into the wild. That said, uses of gene editing in conservation programs have been proposed, albeit mainly theoretically. For instance, a case wherein changes to a single region that results in increased immunity to a disease agent that is endangering a species is easily imaginable. Phelps *et al*. (2020), propose a number of other potential uses of gene editing in conservation ranging from relatively simple (i.e., inducing neutral changes to a single base pair for marking) to vastly more complex and likely controversial (e.g., utilizing synthetic biology to recreate genomes and species that have been lost through extinction).

4.1.2.7 State Skipping

In many ways the states presented here are a product of the history of the development of genetic, and subsequently genomic, tools in non-model species. While it is understood that aquaculture has been practiced for thousands of years, the broad adoption of selective breeding and expansion of aquaculture programs began in the latter half of the 20th century. This time period also corresponded to initial development and application of genetic tools in non-model species. Perhaps the prime example would be Atlantic Salmon: collections occurred from the wild in the 1970s to form large-scale family-based breeding programs in Norway (State 0; Gjedrem (1985)), around this time descriptions of genetic variation and population genetic studies began being conducted, including hatchery-reared populations (State 1; e.g. McKenzie and Paim (1969) Moller (1970); Payne (1974); Davidson *et al*. (1989)), followed by the development of markers to identify individuals and populations (State 2; e.g. Davidson *et al*.(1989); Taggart and Ferguson (1990)), the linkage of traits of interest with gene regions (State 3; e.g. Reid *et al*. (2005)), and more recently the use of genome-scale data in breeding programs (State 4; D'Agaro *et al*. (2021)) and the creation of both transgenic lines and gene editing (State 5; e.g. Du *et al*. (1992)), Wargelius *et al*. (2016), Güralp *et al*. (2020). However, this transition through stages is more a product of the progress of science and technology than it is a deliberate process. Because of the relative ease with which a *de novo* chromosome-level, fully annotated genome can itself be produced - from which most commonly used genetic and genomic tools can be themselves be developed - new programs can today rapidly begin operating at any state they desire, effectively skipping those states below the chosen state. State skipping, as we have termed this, has advantages in terms of cost (developing only tools needed), efficiency (tools optimally designed to the problem at hand), and time (application of tools nearly immediately; optimized tools allow for faster results). As such, we would recommend that new programs seek to develop genomic resources with broad

applicability such as whole genomes that will allow for the development of tailored tools. At the same time, developing program goals (e.g., testing for the maintenance of genetic diversity in a conservation program, undertaking genomic selection in an aquaculture strain, etc.) will facilitate conversations with experts to help determine the best set of tools to develop given the goal.

Taken another way, the advances in genotyping technology and the reduction in genotyping costs on a per-base basis mean that a candidate species can be moved from State 0 to practically any State of a researcher or SME's choosing fairly easily. For example, Cunner (*Tautogolabrus adspersus*) is a temperate reef fish native to the northwest Atlantic, that is currently being considered as a candidate species to be used as cleaner fish in Atlantic Salmon aquaculture in Atlantic Canada, and for which, until recently few genetic tools were available (Chen 2020; Costa *et al*. 2016, Monk *et al*. 2016). Nugent *et al*. (2023), describe the production and annotation of a chromosome-level genome assembly for this species, as well as the characterization of population structure in Atlantic Canada. This genome will allow the development of many different types of genetic tools (e.g., microsatellites, SNPs, copy number variants, etc) and because it has been annotated, functional relationships can be determined from linkages between traits of interest and genomic markers. Annotated reference genomes can improve the quality of genomic tools by allowing users to avoid targeting repeated elements and to optimally space their markers across the genome. Moreover, an annotated reference genome allows for the transfer of data and information between projects or groups because they can reference standardized positions and names for genetic loci. Finally, comparisons can be made between annotated reference genomes of different species to target loci or ecologically significant genes that have been identified as being important in a given species, habitat or context. An excellent example of the use of a *de novo* generated annotated genome alongside data from another project is described in Gao *et al*. (2023). These researchers outline the development of a 50K SNP array designed for North American Atlantic Salmon which was based on SNPs detected in the North American salmon genome they developed with the European salmon genome's annotation used as a proxy for the location of genic regions.

4.1.3 Considerations and constraints of genetic methods

The widespread adoption of genetic methods is constrained by economic and practical considerations. The economic considerations are centred on the overall costs associated with the use of these approaches, and whether the expected outcome or improvement will result in a net economic gain. The practical considerations range from what technology stage the species of interest currently is in, to availability of all ancillary resources (such as bioinformatic and analysis pipelines, data storage and management) required to actually translate raw genetic data into actionable breeding or conservation information.

Expense considerations will need to take into account the total cost of genetic/genomic approaches, including both genotypic and phenotypic data collection, data analysis, and data storage, on top of the actual cost of genotyping. Part of this consideration should be the development of a breeding objective which should note the traits that influence income, expenses, or both; where the goal is to improve multiple traits, they should be prioritized based on economic importance (Lhorente *et al*. 2019). It is worth noting that any cost/benefit analysis in the adoption of genetic/genomic tools will be dependent on the species and the technological stage the work can be carried out at – for instance, moving from a high-throughput sequencing approach to the use of an off-the-shelf genotyping solution, or moving from a dense genome-wide marker set to a low density marker set with genome-wide imputation, could substantially decrease costs and thus substantially change medium and long term cost-benefit predictions.

Technical constraints are likely to be less amenable to state skipping, especially in terms of data collection. Depending on the goals that are pursued, different types, and different quantities of

data will need to be collected and handled, starting from tissue sample collection for genotyping, up to full phenotypic and genotypic characterization of hundreds or thousands of samples, potentially over multiple generations. For applications in State 1 and 2, the infrastructure requirements are generally lower as the focus is on sampling wild individuals or broodstock and offspring. Genetic sampling tools and methods of preservation are usually inexpensive; methods are available to preserve genetic samples at room temperature, although in some cases, storage of DNA samples could require freezer space, or flash-freezing (e.g., liquid nitrogen). The best approach will depend on the species and on the genetic application. In most instances, staff can be easily trained to collect samples for genetic processing. However, careful record keeping will be necessary to match genetic samples back to individual organisms (e.g., utilizing physical tags) or families.

Beginning in State 3, and more extensively in State 4, trained staff will be necessary to accurately phenotype the animals. The importance of precise and standardized phenotyping approaches cannot be overstated. To a large extent, the success in associating traits to genotypes will depend on the ability to collect good phenotypic information. In State 3, phenotypes are often easier to collect (e.g., sex based on gonads, length of fish, presence of deformities), but become more complex in State 4 (e.g., fillet yield, color, oil/fat content, feed conversion rates, disease/parasite resistance), and require not only more highly trained staff but may also require specialized equipment for some traits. As mentioned above, applications in State 3 and State 4 also require a large number of individuals to be phenotyped and genotyped, and as trait complexity or the number of targeted traits increases, a greater number of samples are needed for analyses. The time, labor, and (if needed) equipment necessary to phenotype large numbers of animals (and repeatedly phenotype in successive generations - as is the case in State 4) must be considered. Often in State 3, and certainly in State 4, family and/or selected lines also need to be maintained, which increases the infrastructure required to maintain these populations within the aquaculture program. State 5 has similar infrastructure requirements as State 4; however, the intensiveness of phenotyping will depend on the specific target(s) of gene editing. In addition, State 5 may require close coordination with genomic scientists to obtain gametes, fertilized eggs, or larval stages needed for genome editing approaches. Therefore, the availability of both a skilled workforce, and access to a sufficiently large sampling population (and the ability to house it) needs to be addressed before any further action is taken. In these considerations, it is assumed that processing of genetic samples occurs offsite (*see Resources section*) since it is unusual for SME or conservation aquaculture programs to have their own genetic laboratory capacity.

Once costs and practical considerations have been addressed, it is also important to understand the limitations of each and every genetic approach. Individual identification (barcoding) requires a set of informative markers, but the information content of markers is dependent on polymorphism rates, a parameter itself dependent on population inbreeding and actual sample size. The information content of markers used for individual identification is therefore not stable across neither time nor space, requiring regular testing against a known standard to assess the usefulness of the markers used. Limitations of markers used in pedigree reconstruction largely follow the considerations mentioned in individual identification. In both cases it is also important to estimate genotyping error rates, and take them into consideration in the actual barcoding or pedigree reconstruction. For high throughput sequencing, it is extremely important to recognize that error rates are not constant across fragments, but normally increase as fragments are read. While large, genome-wide marker panels are unlikely to be error limited, smaller panels might be, depending on sample handling, and genotyping quality.

GWAS can provide an understanding of the genetic architecture of a trait, but with a number of caveats. The first is that sample size and marker density must match the effect size of any locus associated with the phenotype of interest to identify loci associated with a phenotype – if the marker set is too sparse, or if the sample size is too small, power might not be sufficient to identify

loci that are associated with the phenotype. Conversely, it is paramount to remember that at normal marker coverage GWAS identify genomic locations associated with a phenotype, generally covering many loci, and that association is not causation, and thus any locus identified in a GWAS cannot be considered causal for a phenotype. Independent GWAS, carried out on the same trait(s) but on different populations help validate the effect of a locus on a phenotype, obviously with increasing costs to support these experiments. A further complication has to be considered in breeding settings, where alleles associated with a desirable trait are selected into fixation, causing them to disappear in further GWAS, because all samples are selected to be the same at that locus.

The limitations of genetic approaches for genomic selection programs are closely associated with the ability to reconstruct pedigrees, and, more broadly, kinship across samples. Genomic selection calculations include the inverse of the kinship matrix between individuals to calculate variance components and breeding values. Informative markers genotyped at low, or very low error rates will enable calculation of those parameters with the greatest precision, whereas markers that do not enable correct calculations and precise kinship estimates will negatively affect the outcome of genomic selection approaches.

4.1.4 Resources for implementing genetic/genomic work

SME and conservation programs interested in the adoption of genomic technologies can find support through contact and/or partnership with aquaculture societies and organizations, research consortia, governmental institutes and academic institutions, and fee-for-service providers (Table 2.3). The support these partners or partnerships can provide range from advice, to access, to development and implementation of genomic resources, to funding dedicated to breeding or conservation, making it worthwhile for SME and conservation programs to investigate these resources, both at the national and supranational level.

Multiple aquaculture societies (such as, the European Aquaculture Society (https://aquaeas.eu); the World Aquaculture Society (https://www.was.org), the Food and Agriculture Organization of the United Nations (https://www.fao.org/fishery/en/aquaculture); the US Aquaculture Society (https://www.usaquaculture.org/); the American Fisheries Society (https://fishculture.fisheries.org); the Sociedad Chilena de Ciencias del Mar (https://www.schcm.cl); the Instituto Tecnológico del Salmón (https://www.intesal.cl); the Aquaculture Stewardship Council (https://asc-aqua.org/)), both national or international, with general or specific focus, can be contacted or joined to obtain specific know-how, to receive recommendations on service providers, or access to available funding. While the focus of aquaculture societies might be primarily business related, the importance of sustainability and conservation in the aquaculture sector means that aquaculture societies could be interested in collaborating or supporting conservation programs, especially those focused on the wild counterparts of farmed species.

A second source of support for SME and conservation programs are research consortia. Research consortia might be permanent bodies akin to aquaculture societies and organizations (such as Aqua Vitae (https://aquavitaeproject.eu/); Aquaexcel (https://aquaexcel.eu/); the Global Salmon Initiative (https://globalsalmoninitiative.org/en/); the Aquaculture Collaborative Research and Development Program (https://www.dfo-mpo.gc.ca/aquaculture/acrdp-pcrda/index-eng.htm); SYSAAF (https://www.sysaaf.fr/)), or might be limited in time and scope, and specifically created to apply for national or international funding opportunities. Drivers of the latter type of consortia is the availability of national or international funding, such as the EU Horizon funding instruments, which require the involvement of industrial/non-academic partners in consortia applying for funding. Active involvement in trade or research organizations and consortia could allow SME and conservation programs to increase their visibility and their professional network, facilitating their inclusion in consortia responding to funding calls.

Academic or institutional research organizations and institutes (e.g., NOFIMA, IMR, INRAE, Ifremer, DFO, NOAA, USDA, LUKE, DTU, The Roslin Institute, University of Santiago de Compostela) often have a sustained interest in aquaculture and/or conservation breeding, and thus a partnership with these research institutions can facilitate access to the pertinent know-how concerning the acquisition, use, and analysis of genomic data. Depending on funding, research institutes can provide access to specific infrastructure, and grant agreements can cover specific costs (such as genotyping, personnel, data analysis), decreasing the investment required for genomic work. It is important to remember that, unless working as service providers, research institutes and funding agencies expect dissemination of results, the details of which should need to be agreed upon before work commences.

In addition to these resources, fee-for-service providers are also available to help guide, develop, and implement the use of genomic approaches within the breeding programs. There are several that have extensive experience in, or are specifically focused on, the aquaculture sector, and many of these companies have worked on a broad array of species. Because providers may vary by region or change rapidly with this growing field, specific providers are not listed in this report. However, many of the above resources would be able to provide information on service providers working in a region or on a specific species. If the financial resources make this option a possibility, progress will likely be quickest using this route. This investment may be particularly worthwhile for emerging aquaculture species, where the species may not be on the radar of larger funding agencies, which could lead to challenges in developing institutional or academic collaborations, and where critical mass may not yet exist to form research consortia focused on that particular species. Although, it should be noted that there may be funding opportunities specifically geared towards development of newer species for both commercial and conservation programs.

4.1.5   Summary

Ultimately, the argument for the use of genetics and genomics by SME was stated succinctly by Gjedrem *et al*. (2012): "investments in well planned and managed breeding programs are unique, because genetic gains obtained in such programs are eternal and cumulative". Large genetic gains are possible and will be made more rapidly through the use of genetically-informed breeding. Long term conservation of threatened aquatic species cannot be achieved without considering genetic diversity and proper broodstock management.

4.1.6   Bibliography

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Table 2.2. Five states in the development and application of genetic and genomic tools in aquaculture and conservation breeding programs.

State	Description	Applications / categories of research questions	Tools
State 0	No previously developed genetic markers or genomic resources	No genetic resources exist for this species for wild or cultured programs; markers may be available in related species, or through the use of universal primers.	No species-specific tools are available to utilize for genetic / genomic projects. Newer technologies make it easier to develop these tools d*e novo.*
State 1	Stock choice - Information available about populations genetics	Characterize the population(s) or the species of interest (e.g., determination of effective population sizes, population genetic structure, degree of differentiation, genetic diversity, etc.)	Allozymes, Sanger sequencing of small sections of nuclear or mitochondrial regions, microsatellites, SNPs
State 2	Individual genetic tagging	Individual identification, parentage and pedigree analyses, trace the individual back to their breeding program, or origin, diversity estimates compared to wild populations	Microsatellites, SNPs, Sanger sequencing of small sections of nuclear or mitochondrial regions, RAD-Seq, low-coverage genome sequencing
State 3	Development and application of linkages between phenotypes/traits and genetic markers	Link genotypes to phenotypes for simple traits (Mendelian inheritance) or for genes (and markers) of major effect; implement marker assisted selection to guide broodstock development or to improve traits.	Genotyping-by-sequencing approaches (e.g., RADSeq, ddRADSeq, WGS); screening using SNPs, PCR, microsatellites.
State 4	Genomic selection and/or genomic imputation and prediction using family- or pedigree-based selection.	Implement genomic selection, utilizing family- or pedigree-based selection for complex polygenic traits, typically requiring breeding programs and/or genomic selection/imputation to improve trait outcomes.	SNP arrays, genomic resequencing (e.g., GenCove - use resequencing for genome imputation and prediction instead of developing SNP panel)
State 5	Gene editing	Utilize gene editing to precisely modify genes already present in the organism (or from another organism - transgenic), to inactivate genes/genetic sequences or to add genetic material at specific locations of the genome.	Zinc fingers, TALENs, CRISPR-CAS

Annex 1: List of participants

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Annex 2:   Resolutions

2020/FT/ASG02   The **Working Group on the Application of Genetics in Fisheries and Aquaculture (WGAGFA)**, chaired by Naiara Rodriguez-Ezpeleta, Spain, will work on ToRs and generate deliverables as listed in the Table below.

	MEETING DATES	VENUE	REPORTING DETAILS	COMMENTS (CHANGE IN CHAIR, ETC.)
Year 2021	10–14 May	Olhao, PT	E-evaluation to SCICOM	Chair: Naiara Rodríguez-Ezpeleta
Year 2022	17–19 May	Online	E-evaluation to SCICOM	Chair: Naiara Rodríguez-Ezpeleta
Year 2023	9–12 May	Sukarrieta, Spain	Final report by 30 June to ASG, SCICOM and ACOM	Chair: Naiara Rodríguez-Ezpeleta

ToR descriptors

TOR	DESCRIPTION	BACKGROUND	SCIENCE PLAN CODES	DURATION	EXPECTED DELIVERABLES
a	Documentation: How the rapid advances in genomics and analytical methods are revolutionising population identification in marine fish and invertebrate species	Stock identification has always been a major aspect of fisheries genetics. In the genetic context, the term "stock" means population or discrete breeding stock, and has biological reality. For populations to be accepted as the fundamental units on which assessment is based, it is essential to accurately classify these units, and ideally describe how they originated and are maintained. Until recently, population identification has been limited by the availability of sufficiently powerful molecular markers and analytical methods. Now however complete genome sequences are available for several commercial species, it is quick and economical to compile WGS for other species, and exponentially-increasing computer power has led to a plethora of new analytical methods. The aim of this proposed TOR would be to list and describe these methods, and their actual or potential application in population identification. It would be presumed that details would be constantly updated during the next three year period, thus ultimately producing an up-to-date document for publication. Power analyses would be invoked to calculate suitable sample sizes and locus number, and relative implications of different approaches would be compared. How these population entities were formed during post glacial range expansion and are maintained, for example, by heterogeneous spawning habitat, oceanic barriers and other factors would also be investigated. Many marine species, while homing to discrete natal areas to spawn, mix at other life history stages. These stages, usually involving harvest, would be investigated using mixed stock analysis (MSA) methods, presuming that sufficiently large differences can be demonstrated between component populations. Adaptive loci, under directional selection, might be particularly useful in the	2.7, 5.6, 6.1	3 years	Review paper and metrics for measures of indirect genetic impacts

ToR	Description	Background	Science Plan Codes	Duration	Expected Deliverables
		latter context, but also in investigating population response to climate change.			
b	To review and evaluate the potential of adaptative variation for assessing fisheries.	A growing body of evidence suggests marine species display local adaptation over moderate to fine spatial scales, and the genes and genomic regions contributing to adaptive diversity (e.g., temperature, pathogens, etc.) have been identified in a variety of marine species. Yet despite this knowledge and widespread biodiversity losses across the North Atlantic, we still lack an understanding of species responses to disturbance, such as future climate change, in many commercially, culturally, and ecologically important marine species. The overarching goal of this ToR is to evaluate the current capacity to quantify relevant adaptive diversity in marine species; and explore how this information may be utilized in predictions of future biodiversity response to change. Specifically, we will review the literature regarding the genomic basis of adaptation in marine species, and examine how genomic architecture (e.g., single loci, CNVs, and chromosomal rearrangements) influences phenotype associations and our ability to resolve relevant variation. Secondly, we will evaluate new methods that utilize genomic data to establish an evolutionary framework for understanding adaptive diversity and to predict future responses. These will include "genomic vulnerability", a metric that quantifies the shift in genomic variation required to adapt to future change and uses machine learning to incorporate genomic descriptions of adaptive diversity, climate projections, and ecological modelling. Such approaches have the potential to identify highly vulnerable marine populations and transform science advice regarding fisheries management and marine conservation. Thirdly, we will provide recommendations for how this information could be practically integrated with existing advisory and management frameworks in the Northern Atlantic. Ultimately, this ToR will directly inform the use of genomic approaches to both quantify adaptive diversity and to predict future responses to disturbance in marine species.	1.3, 1.5, 1.7, 2.2, 2.5, 5.2, 6.1, 6.3	3 years	Review paper and recomendations on the use genomic data to predict future population responses to environemtnal change and disturbance.
c	To evaluate available genetic-based solutions to better understand the mesopelagic ecosystem.	Recent estimates suggest that mesopelagic fish represent 90% of the fish biomass of the planet, which has raised interest in exploitation of this unknown ecosystem. Yet, the high estimated biomass also suggests that mesopelagic fish might play a key role in sustaining other commercially relevant species and carbon sequestration. Thus, there is an urgent need to understand this still pristine ecosystem before it becomes too late to take protecting actions. This ToR could be dedicated to explore and evaluate the different alternative genetic methods available that could be used for that aim such as environmental DNA	1.4, 1.6	3 years	Review Paper and non-technical review topic sheet.

ToR	Description	Background	Science Plan Codes	Duration	Expected Deliverables
		samples for estimating biomass and species identification, stomach content DNA analysis for understanding trophic networks, population genomics for species connectivity and diversity as proxies for resilience, etc.			
d	**WGAGFA & WGSEDA**: Explorative cost-benefit analysis of genetic methods with emphasis on SME and conservation program broodstocks dedicated to aquaculture or natural population enhancement.	Managing genetic relation-ships and diversity within broodstock enables a long-term basis for both selection of improved food fish material for aquaculture production and supportive augmentation of natural populations. The loss of genetic variability due to inbreeding is detrimental for the cost-effectivity of re-stocking and it may even be impossible to retrieve variability again from the wild. While the use of genetic tools is part of day-to-day routines in large breeding companies, the lack of logistically feasible and cost-effective tools has so far prevented proper broodstock genetic management in SME's and conservation programs. This ToR is planned as a shared ToR between WGAGFA and WGSEDA and has linking points to WGs with fish stock conservation focus (e.g. WGNAS) and contributes to the Science Plan topics "Emerging techniques and technologies", "Seafood production" and "Conservation and management science"	4.4, 5.5, 7.6	1 (initially. - Reserving the possibility to extend further)	Explorative study on market availability for genetic breeding consultation and genotyping services, evaluating the occuring costs and contrasting these to their benefits in report form.
e	Provide a review of the recent genetic studies on white anglerfish (Aguirre-Saraiba *et al*., 2021). Molecular genetic data have found widespread application in the identification of aquatic species' population and conservation units. For white anglerfish, the recent study shows that i) the species forms a panmictic population throughout the Northeast Atlantic (the two stocks belong to the same population), ii) there is hybridization between	Request from the Working Group for the Bay of Biscay and the Iberian Waters Ecoregion (WGBIE)	1.8	1 year	Provide input to SIMWG for further inclusion in contribution/ response to WGBIE.

ToR	Description	Background	Science Plan Codes	Duration	Expected Deliverables
	white anglerfish; iii) there is misidentification between the white and black anglerfishes even if the color of the peritoneum is used for taxonomic identification.				

Summary of the Work Plan

Year 1	ToR a) Review the literature, with special focus on the application of genomic data analysis to the study of population structure and connectivity in exploited (directly or indirectly) marine species (vertebrates and invertebrates). ToR b) Review the literature regarding the genomic basis of adaptation in marine species, and examine how genomic architecture influences phenotype associations and our ability to resolve relevant variation. We will identify approaches that build on this genomic understanding of adaptive diversity, to predict future responses of populations to disturbance. ToR c) Produce an overview of the mesopelagic ecosystem, identify key species and review the literature on different genetic methods available to study this ecosystem. In addition to this overview, focus will be on identifing where especially eDNA and stomach contant DNA analysis are being used or could be used in the mesopelagic ecosystem. Identify the key species in the mesopelagic ecosystm with respect to the trophic network – create a simple flowchart. ToR d) Report on explorative study on market available genetic advices and genotyping services, evaluating the occuring costs and contrasting these to their benefits in report form. Evaluation of outcome and value of further deepening of anlysis. Decision as to whether ToR will be carried on.
Year 2	**ToR a)** Identify analytical approaches used and evaluate their power and accuracy. Start drafting an "analytical framework" that will attempt at standardising the sampling/processing/ statistical approaches to be used when producing results that will feed into management measures. **ToR b)** Evaluate new methods which build on a genomic understanding of adaptive diversity, to predict future responses of marine populations to distburbance These will include but not be limited to an examine of genomic vulnerability. **ToR c)** Continue the evaluation and identification of genetic methods as well as key species for studies of the mesopelagic ecosystem, including any relevant studies describing the ecosystem. Evaluate any new genetic methods for utilisation in studies of the mesopelagic ecosystem. Start to formulate review paper manuscript. **ToR d)** To be determined. Pending decision of year 1. **ToR e)** Provide a review of the recent genetic studies on white anglerfish (Aguirre-Saraiba *et al.*, 2021).
Year 3	**ToR a)** Complete review paper for publication and develop recommendations. **ToR b)** Complete a review paper for publication and develop recommendations. **ToR c)** Finalise and update the evaluation: identify problematic areas requiring future research as well as identify areas where novel techniques show particular promise. Finish review paper and non-technical review topic sheet. **ToR d)** To be determined. Pending decision of year 1 and 2.

Supporting information

Priority	The WGAGFA Terms of Reference for the reporting period 2021 to 2023 will produce information, knowledge and advice in line with the ICES Science priorities. Particularly ecosystem science, impacts of human activities, observation and exploration, emerging techniques and technologies and seafood production, as well as conservation and management will be tackled and reported upon.
Resource requirements	The research programmes which provide the main input to this group are already underway, and resources have been committed.
Participants	The Group is normally attended by some 15-25 members and guests.
Secretariat facilities	None.
Financial	No financial implications.
Linkages to ACOM and groups under ACOM	Joint SCICOM/ACOM group.
Linkages to other committees or groups	There is a very close working relationship with EPDSG, EOSG and EPISG. Additionally, several EGs, particularly WGSEDA but also including WGITMO, WGBIODIV, WGBOSV.
Linkages to other organizations	European Commission; Scientific, Technical and Economic Commitee for Fisheries (STECF); European Fisheries Control Agency (EFCA); GFCM; FAO; IFREMER, NOAA, DFO Canada.