

STOCK IDENTIFICATION METHODS WORKING GROUP (SIMWG)

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i Executive summary

The Stock Identification Methods Working Group (SIMWG) reviews new methods for the definition and investigation of stock structure and provides recommendations to other ICES expert groups on how to interpret patterns of population structure.

In 2022, SIMWG continued providing annual updates on recent applications of stock identification methods to species assessed by ICES and on advances in stock identification methods. Based on the wide expertise of SIMWG members, we provide reviews of recent literature on genetics, growth marks in calcified structures, life history parameters, morphometrics/meristics, tagging, otolith shape, otolith chemistry, parasites and interdisciplinary approaches.

A key activity of SIMWG is to address requests by ICES working groups for technical recommendations on issues of stock identity. In the current term, we reviewed the outcome of the Workshop on Stock Identification of North Sea Cod (WKNSCodID) and the report of a project on herring stock structure upon request by the ICES Herring Assessment Working Group (HAWG). Moreover, SIMWG members contributed to the preparation of the Benchmark Workshop for selected elasmobranch stocks (WKELASMO) and reviewed the outcome of the Workshop on Stock Identification of West of Scotland Sea Cod (WK6aCodID). In 2022, the group reviewed and discussed stock structure of hake and anglerfish stocks by request of the Working Group for the Bay of Biscay and the Iberian Waters Ecoregion (WGBIE) and of anchovy by request of the Working Group on Southern Horse Mackerel, Anchovy, and Sardine (WGHANSA).

SIMWG contributes to the general understanding of the biological features of the north Atlantic ecosystem through its work to describe fish population structure. Additionally, SIMWG's annual reviews on advances in stock identification methods keep ICES members abreast of best practices in this field of study. SIMWG expert reviews on questions of stock structure for particular ICES species are directly relevant to the appropriate definition of stock and contribute to the accuracy of stock assessment and effectiveness of management actions. We see an important role for SIMWG in the future as ICES copes with the shifting distributions of fishery resources and questions regarding the appropriate definition of fish stocks. Understanding stock structure is a fundamental requirement before any assessment or modelling on a stock can be contemplated and SIMWG will continue to work with ICES expert groups to address pressing stock identification issues.

ii Expert group information

Expert group name	Stock Identification Methods Working Group (SIMWG)
Expert group cycle	Multiannual
Year cycle started	2020
Reporting year in cycle	3/3
Chair	Christoph Stransky, Germany
Meeting venue(s) and dates	By correspondence in 2020
	By correspondence and online in 2021
	14–16 June 2022, Reykjavik, Iceland

1 Review recent advances in stock identification methods

Over the past years, there has been a wide use of applications of stock identification methods to ICES stocks, as well as several notable advances in stock identification methods with many results relevant to ICES science and advice. SIMWG has committed to providing annual updates on recent applications of stock identification methods to ICES species and on advances in stock identification methods. The group has focused on summarizing research in the focal areas listed below:

- a) Genetics
- b) Growth marks in calcified structures
- c) Life history parameters
- d) Morphometrics/ meristics
- e) Tagging
- f) Otolith shape
- g) Otolith chemistry
- h) Parasites
- i) Simulation approaches
- j) Interdisciplinary approaches
- k) Emerging issues

SIMWG's annual reviews on advances in stock identification methods keep ICES members abreast of best practices in this field of study. This review activity has served as a valuable contribution to the field and has formed the foundational material for two editions of the book "Stock Identification Methods: Applications in Fishery Science". This book was published first in 2005 and again in 2014. SIMWG members S. Cadrin, L. Kerr and S. Mariani edited the 2nd edition and several SIMWG members contributed chapters to this book. A 3rd edition is planned for 2023/2024, again involving several SIMWG members.

The details of annual reviews of advances in stock identification methods are summarized in Annex 4.

Furthermore, in 2022 there was a focus on new genomic methods for stock ID and practical implications for sampling and analysis, accompanied by two presentations summarised in Annex 5.

2 Technical reviews and expert opinion on matters of stock identification

SIMWG provides ICES expert groups and working groups expert feedback on questions of stock structure for ICES stocks. In 2022, SIMWG has contributed to ICES advisory needs by providing expert feedback on the status of stock structure of several species.

SIMWG members contributed to the preparation of the Benchmark Workshop for selected elasmobranch stocks (WKELASMO) and reviewed the outcome of the Workshop on Stock Identification of West of Scotland Sea Cod (WK6aCodID). During the 2022 meeting, the group reviewed and discussed stock structure of hake and anglerfish by request of the Working Group for the Bay of Biscay and the Iberian Waters Ecoregion (WGBIE) and of anchovy by request of the Working Group on Southern Horse Mackerel, Anchovy, and Sardine (WGHANSA). The detailed reviews are provided in Annex 3.

SIMWG expert reviews on questions of stock structure for ICES stocks are directly relevant to the appropriate definition of stock and contribute to the accuracy of stock assessment and effectiveness of management actions. Understanding stock structure is a fundamental requirement before any assessment or modelling on a stock can be contemplated and SIMWG will continue to work with ICES expert groups to address pressing stock identification issues. We see an important role for SIMWG in the future as ICES copes with the shifting distributions of fishery resources and questions regarding the appropriate definition of fish stocks.

SIMWG's recommendations have been well received by the requesting groups and there are a growing number of requests from different groups which speaks to the service that SIMWG provides to the ICES community. SIMWG's expertise should be continued to be used to address on specific questions of stock structure and should be considered in the advisory process in the context of whether the stock units are appropriate for accurate assessment and sustainable management of ICES fishery resources.

3 Review and report on advances in mixed stock analysis, and assess their potential role in improving precision of stock assessment

Contributors: Lisa Kerr, David Murray and Steve Cadrin

In recent years, there have been advancements in the field of mixed stock analysis and new applications of the approach, which are relevant to ICES science and advice. Here, we focus on describing examples of ongoing applications and offer practical recommendations for implementing mixed stock composition analysis.

US Atlantic cod stock structure

A review of the best available science on Atlantic cod stock structure and the available data to support stock assessment by the [Atlantic Cod Research Track Stock Assessment Working group](#) has resulted in a change in the current assessment units (i.e. Gulf of Maine and Georges Bank stock units). The WG recommended adoption of four spatial units for assessment: 1) eastern Gulf of Maine; 2) western Gulf of Maine (winter and spring spawners combined); 3) Georges Bank; and 4) Southern New England (including the Mid Atlantic Bight; Figure 2). The WG discussed the treatment of the combined winter and spring spawners in the western Gulf of Maine stock unit and suggested a short- and long-term approach to addressing this issue. In the short term, the presence of sympatric stocks will be considered in the assembly of input data and interpretation of trends. In the long-term, mixed stock composition of survey and catch data may be possible to monitor and assess the abundance of winter and spring spawners in the Gulf of Maine. The WG plans to develop a work plan outlining the investments and protocols needed to implement this approach. The rationale for the 4-unit structure is an improvement in the alignment between the scale of cod stock assessment units and biological stock structure that can be supported with available information.

North-East Atlantic cod (NEAC) and Norwegian Coastal cod (NCC) stocks

The Institute of Marine Research (IMR) in Norway uses real-time genomic analyses to manage a mixed-fishery of abundant North-East Atlantic cod (NEAC) stock and a depleted, yet rebuilding Norwegian Coastal cod (NCC) stock. The NEAC stock is the single largest cod stock worldwide, with an annual total allowable catch of approximately 900 000 tonnes, worth millions of Euros to the Norwegian economy (Bertheussen *et al.*, 2019; Dahle *et al.*, 2018). IMR uses the *Pan IB* allele, which is present in NEAC but absent in NCC stocks, as population marker to assess their relative abundances within fishing grounds. Atlantic cod are sampled twice per week to test for the presence of these genomic markers (Dahle *et al.*, 2018). Only when the frequency of *Pan IB* allele is high enough to assume that the NEAC have arrived on fishing grounds does the Norwegian Directorate of Fisheries open the sector for fishing (Dahle *et al.*, 2018). Sampling continues throughout until the *Pan I* alleles frequency decreases, signalling the arrival of the vulnerable NCC stock which initiates the Norwegian Directorate of Fisheries to implement strict fishing quotas (Dahle *et al.*, 2018).

Salmon in the Baltic Sea

Mixed stock catches of the salmon in the Baltic Sea have been analyzed using a combination of microsatellite markers and smolt-age data since the early 2000s (ICES, 2021a; Koljonen *et al.*, 2021). The results of the mixed stock analysis have been used to guide area and temporal

management of salmon fishery in the Baltic Sea. For example, in 2021, the ICES advised that the commercial and recreational salmon catch in the Baltic Sea should be zero, according to MSY approach, but considering the results of the genetic mixed stock analyses, which showed that no salmon from endangered stocks end up in the catches in the Gulf of Bothnia, the advice for the Gulf of Bothnia was to allow the catch of 75 000 salmon (ICES, 2021b).

Atlantic salmon

Sheehan *et al.* (2021) described the international sampling program developed by ICES to determine continent of origin and biological characteristics of Atlantic salmon collected at West Greenland in 2018. Approximately 12% by weight of the reported landings were sampled. Sampled or processed data include length, weight, freshwater and marine age from scale samples, as well as continent and region of origin from genetic analysis of tissue samples. Fish were also examined for fin clips, external marks, external tags, and internal tags. Adipose-clipped fish were sampled for coded wire tags. Since the mid-1990s, a large proportion of the harvested stock was of North American origin (83% of the 2018 fishery) with a smaller contribution of European origin. A total of 5508 tag recoveries in the Greenland fishery have been reported since 1963, and the 142 tag recoveries since 2003 suggest that most fish (73%) are of North American origin.

See also: <https://repository.library.noaa.gov/view/noaa/32910> and ICES 2018. Report of the Working Group on North Atlantic Salmon (WGNAS), 4–13 April 2018, Woods Hole, MA, USA. ICES CM 2018/ACOM:21. 383 pp.

Western and Eastern Stocks of Atlantic bluefin tuna

Management of the commercially important Atlantic bluefin tuna (ABFT), *Thunnus thynnus*, considers two stocks separated at the 45°W meridian, relying on the assumption that individuals born at each of the two main spawning grounds (Gulf of Mexico and Mediterranean Sea) do not, or rarely, mix (Fromentin and Powers 2005). Yet, tagging (Arregui, *et al.* 2018, Galuardi, *et al.* 2010, Lutcavage, *et al.* 1999) and otolith chemistry analyses (Rooker, *et al.* 2014, Rooker, *et al.* 2008) have demonstrated regular and frequent trans-Atlantic migrations of ABFT adults while also suggesting that individuals return to their birthplace to spawn (Block, *et al.* 2005, Rooker, *et al.* 2014). This process, termed “natal homing” would imply that ABFT should be managed as a mixed stock fishery (i.e., that composed by spatio-temporally defined aggregations of individuals from different biological populations), which would require individuals caught in the mixing areas be assigned to their birth location. Currently ICCAT has developed a Management Strategy Evaluation (MSE) framework, for which estimates of mixing are required for the development of Operating Models (Butterworth *et al.* 2016). The mixing proportions are determined by the stock of origin data (genetics and otolith chemistry).

Further case studies of applied mixed stock analysis

- Greenland cod: Year-round genetic monitoring of mixed-stock fishery; implications for management (Christensen *et al.* 2022);
- Baltic cod: Mixed stock analysis is conducted using otolith or genetics; stock composition integrated into assessment and management;
- North Sea cod;
- Herring: genetics-based splitting of data (HAWG assessment); vertebral counts or otolith microstructure, genetics revealed additional complexity compared to other techniques (*cf.* Annex 5);
- Ongoing work for horse mackerel, Atlantic mackerel, blue whiting, anglerfish (*cf.* Annex 5).

Recommendation for implementing mixed stock composition analysis

SIMWG members discussed some of the obstacles to integration of mixed stock composition information into the stock assessment and fishery management process. It was suggested that outlining practical recommendations and developing plans for operationalizing application of mixed stock analysis could be helpful to outline a roadmap forward for communication with data and assessment scientists, as well as fisheries managers.

Identified needs for monitoring and assessment include:

- Development of plans for operational data collection that incorporates sampling for stock identification applications (e.g., tissue sampling for genomics, otolith sampling for shape or chemical analysis)
 - Fishery independent and dependent sampling (survey, landings, discards, sampling by age to inform age composition)
 - Alignment with data streams for stock assessment
 - Spatial and temporal coverage as to be representative
 - Database management (who holds the data, when is it submitted, who can access)
- Prescribed methods for data analysis (i.e. stock of origin assignment method)
 - Common R-scripts to be shared
 - Work flow and timing of data analysis to align with timing requirements of stock assessment
- Considerations: costs of tools and accessibility of methods.
- Examples: New methods for high volume, consistent sampling of genetics (see Annex 5), e.g. herring acoustic survey data, all biological subsamples (100 fish) analyzed for genetics.

Helpful tools for management include:

Simulation modelling can be a helpful tool to understand the impacts of adjusting management approaches to align with new information on biological structure. In particular, a management strategy evaluation framework which models stock mixing and fishery harvest of a mixed stock catch can be helpful to demonstrate performance of alternative fisheries management approaches and the potential cost of not acknowledging population structure. Model scenarios can be defined to test the impact of ignoring population structure (i.e. lumping, misspecified boundaries) and addressing population structure through redrawing of management boundaries or spatial management approaches (e.g. closed areas).

Next steps

This topic will be explored further in the coming year as a chapter in the next edition of the Stock Identification Methods book in 2023.

SIMWG Recommendation: SIMWG recommends enhanced communication between geneticist and data and stock assessment scientists collaborating on a roadmap for integration of mixed stock composition into data collection and application to stock assessment.

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Annex 2: Resolutions

2019/FT/HAPISG10 The **Stock Identification Methods Working Group (SIMWG)**, chaired by Christoph Stransky, Germany, will work on ToRs and generate deliverables as listed in the Table below.

	MEETING DATES	VENUE	REPORTING DETAILS	COMMENTS (CHANGE IN CHAIR, ETC.)
Year 2020	By correspondence			
Year 2021	17 June & by correspondence	Online meeting		
Year 2022	14–16 June	Reykjavik, Iceland	Final report by 1 August to SCICOM	

ToR descriptors

TO R	DESCRIPTION	BACKGROUND	SCIENCE PLAN CODES	DURATION	EXPECTED DELIVERABLES
a	Review recent advances in stock identification methods	a) Tracks best practices in stock ID b) Promotes new technologies Relevant to all ICES species	1.4, 1.8, 5.1, 5.2	3 years (and continued)	EG report
b	Provide technical reviews and expert opinions on matters of stock identification, as requested by specific Working Groups and SCICOM	a) Contributes to understanding of structure and connectivity of fish populations/stocks b) Highly relevant to assessment and management	1.4, 1.8, 5.1, 5.2	3 years (and continued)	EG report and updated table of species reviews
c	Review and report on advances in mixed stock analysis, and assess their potential role in improving precision of stock assessment	Relevant to resolving mixed stock composition issues in assessment and management.	1.4, 1.8, 5.1, 5.2	3 years	EG report and contribution to ICES ASC; methodological paper in international journal
d	Provide a review of recent studies (Aguirre-Saraiba <i>et al.</i> , 2021, and Abad <i>et al.</i> , 2021 in WGBIE 2021 report) with regards to the stocks' population structure of the northern and southern white anglerfish and hake	Request from the Working Group for the Bay of Biscay and the Iberian Waters Ecoregion (WGBIE)	1.4, 1.8, 5.1, 5.2	1 year	Chapter in EG report

species in the northern (areas 3a46), north-southern (areas 78abd) and south-southern (areas 8c9a) shelves to validate and improve the current ICES definitions for these stocks and propose revisions, if necessary, based on the new findings.

Summary of the Work Plan

Year 1	Address terms of reference through work by correspondence in 2020 .
Year 2	Organise a physical meeting for SIMWG for summer 2021.
Year 3	Address terms of reference through work by correspondence in 2022.

Supporting information

Priority	Understanding stock structure is a fundamental requirement before any assessment or modelling on a stock level can be contemplated. SIMWG liaises with ICES expert groups and working groups on stock identification issues and continues to review new methods as they develop
Resource requirements	SharePoint website and clear feedback from expert groups.
Participants	The Group is normally attended by some 10–15 members and guests.
Secretariat facilities	Standard EG support.
Financial	None
Linkages to ACOM and groups under ACOM	ACOM
Linkages to other committees or groups	SIMWG has recently worked closely with a range of ICES working groups including WGWIDE, WGBIE, WGHANSA, and NWWG; benchmark workshops including WKPLE and WKHAD, and advice drafting groups such as ADGDEEP, and in previous years SIWMG connected with many more ICES groups to fulfill requests.
Linkages to other organizations	There are no obvious direct linkages, beyond the SIMWG members' affiliation and commitment to their own employers.

Annex 3: Responses to requests from ICES Working Groups

WGBIE request

Background

SIMWG received the following request from the Working Group Bay of Biscay and the Iberian Waters Ecoregion (WGBIE): “Provide a review of recent studies (Aguirre-Saraiba *et al.*, 2021, and Abad *et al.*, 2021 in WGBIE 2021 report) with regards to the stocks’ population structure of the northern and southern white anglerfish and hake species in the northern (areas 3a46), north-southern (areas 78abd) and south-southern (areas 8c9a) shelves to validate and improve the current ICES definitions for these stocks and propose revisions, if necessary, based on the new findings” (SIMWG ToR d).

Hake

European hake, *Merluccius merluccius*, are currently managed as two stocks, the northern and southern stock, with the boundary located at the Canyon of Cape Breton. In the past, they were managed as three stocks, but in 1979, two areas in the North were joined to create the current Northern stock with the Southern stock remaining a separate unit.

SIMWG found that past studies, including genetic studies, do not support the current ICES stock areas. However, the occurrence of panmictic structure for this species is also not fully supported. Rather, it seems that there is support for “phenotypic stocks”. However, further research is needed to know whether these ‘phenotypic stocks’ have different demographic influence on the population dynamics and/or there is evidence of more complex structure.

Moving from current management and assessment units to an alternative structure would require further mixed stock analysis and evaluation of the consequences of integrating this information into the assessment and management process. There is ongoing research to address these concerns (e.g. <https://www.azti.es/gifaman>).

Testing the implications of a change in stock structure on the performance of assessment and management would be needed. Simulations considering different mixed-stock structures (population heterogeneity, metapopulation, and panmictic) might be simulated and tested before proposing a new stock structure that will persist for many years. For instance, whether the stock-recruitment relationships (SRR) are spatially differentiated or whether recruits settle in common pools. Whether connectivity occur as adults (migration rates estimation), early life stages, or both is not a trivial question and requires further research before a decision is taken. Dispersion experiments are needed for all the species to assess the degree of connectivity at early life stages.

Recent review of European hake stocks in the Mediterranean

FAO (2020) reviewed information on stock identity to determine the most appropriate spatial units for stock assessment of Mediterranean hake, which are adjacent to ICES hake stocks. The information available provided evidence of a distinct boundary between European hake populations in the Atlantic Ocean and Mediterranean Sea. Several studies using a range of genetic characters including allozymes, microsatellites and single nucleotide polymorphisms (SNPs) offer strong evidence of separate Atlantic and Mediterranean populations (Castillo *et al.*, 2004; Cimaruta *et al.*, 2005; Lundy *et al.*, 1999; Milano *et al.*, 2014; Roldán *et al.*, 1998). Population structure

within the Mediterranean was less certain. The STOCKMED project provides extensive information on spatial patterns of Mediterranean European hake, but did not have sufficient information for several areas, and work was in progress to fill some information gaps (e.g., TRANSBORAN, MED_UNITS). First results for hake published within the [MED_UNITS](#) project suggest a continuous longitudinal gradient, but consistent with the occurrence of both several phenotypic stocks and complex spatial structure.

Information from neutral genetic characters indicated basin-scale populations in the western Mediterranean, central Mediterranean and eastern Mediterranean that are reproductively isolated on an evolutionary timescale, with considerable advection of eggs and larvae among areas providing connectivity of early life stages among areas in those regions. Finer-scale differences in selected characters (e.g., allozymes, growth rates, size at maturity) and natural tags (e.g., parasites and otolith chemistry) suggested limited movement, local selection of post-larval life stages, and some demographic independence on an ecological timescale, which may be more relevant to stock assessment.

The apparent connectivity among areas at early life stages, intermittent larval transport, and limited movement of juveniles and adults among areas suggested that areas with different recruitment patterns could be appropriately assessed as unit stocks to estimate recruitment and survival of recruits for comparison with per-recruit reference points, but stock-recruit relationships may be confounded for small areas because of intermittent mixing of eggs and larvae. The large basin-scale population structure defined by oceanographic systems that involves evolutionary time scales, and smaller demographic units suitable for stock assessment on ecological time scales may also apply to European hake in ICES areas.

Stock status

In 2020, the Southern stock assessment was rejected due to a large retrospective pattern and an index-based method is now used with the current biological state in relation to reference points unknown (ICES 2021). The Northern stock assessment (ICES 2021) estimates that fishing pressure on the stock is at FMSY and spawning-stock size is above MSY, Btrigger, Bpa, and Blim.

A high increase in the biomass of the Northern stock in the 2010s was associated with a north east expansion of the stock (ICES, 2017). Choke stock issues are a concern due to recent increased abundance and low catch quota in a mixed fishery.

Stock development over time

The stock size and fishing pressure status relative to candidate reference points is unknown.

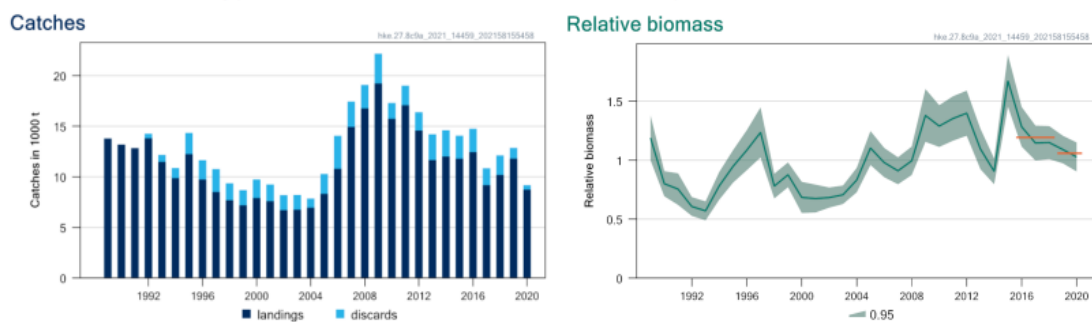


Figure A3.1. Southern hake catches and biomass trends.

Northern stock trends

Stock development over time

Fishing pressure on the stock is at F_{MSY} and spawning-stock size is above $MSY B_{trigger}$, B_{pa} , and B_{lim} .

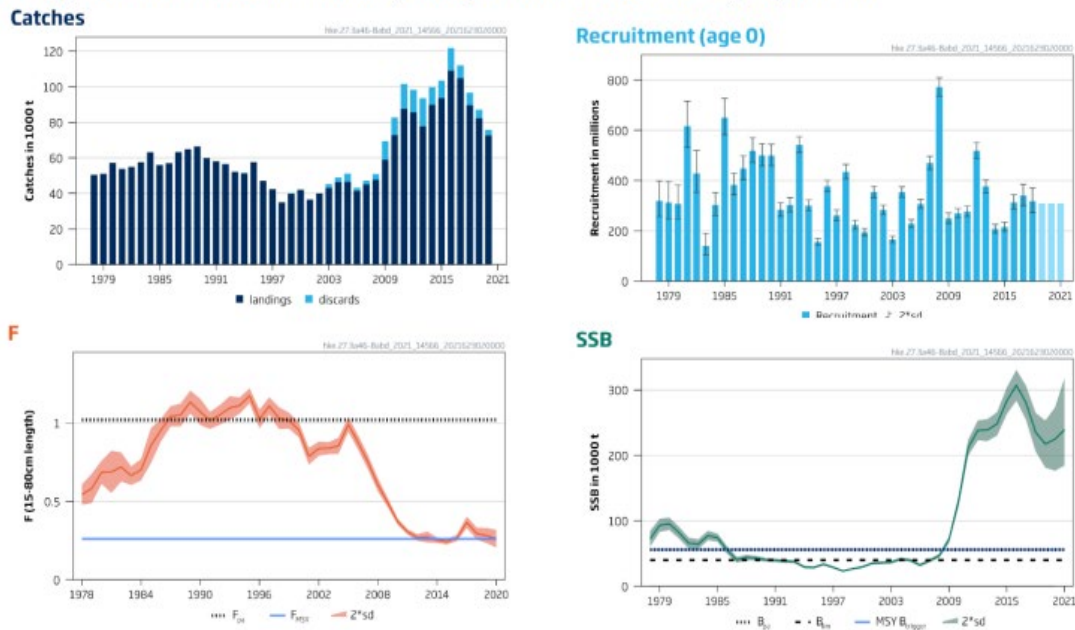


Figure A3.2. Northern hake stock trends.

Survey trends

The examination of correlation in survey trends revealed a general pattern of moderate to high positive correlations in total biomass across surveys, whereas recruitment time series were positively correlated across southern surveys, with some negative correlations seen across Northern surveys (Figure 12 in Abad *et al.* 2021).

Given the complete cover of the species by the International Bottom Trawl Surveys (IBTS), and availability of methods to filter the effects of contrasting seasons or different gears/vessels, a global analysis of the whole area for each species is needed to assess how spatiotemporal dynamics of the species are clustered (e.g., Lindegren *et al.*, 2022). Indeed, WKFISHDISH2 (June 2022) has modelled this species as one of the case studies comparing different species distribution models.

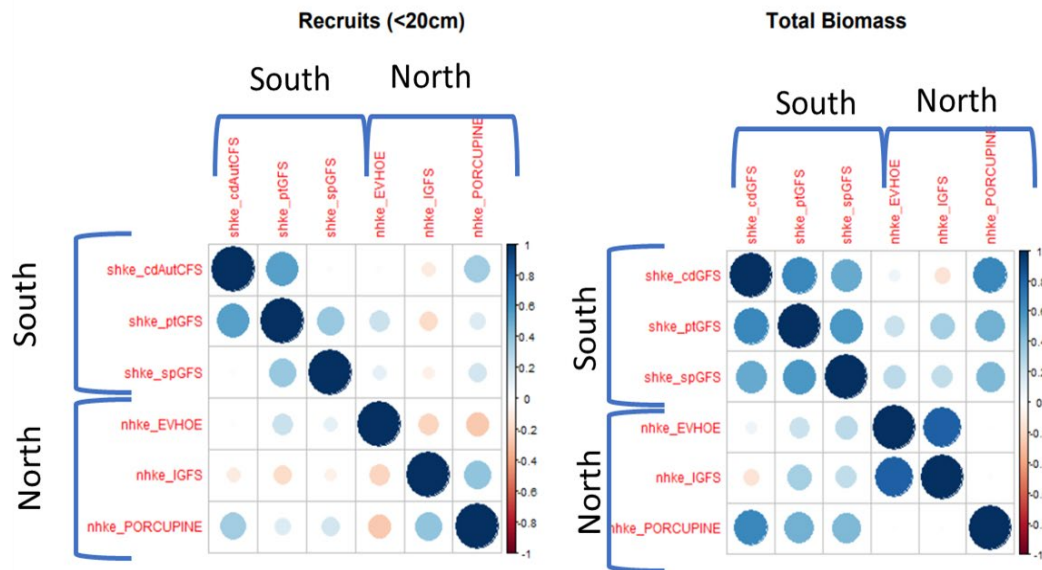


Figure A3.3. *Merluccius merluccius* in NE Atlantic. Correlation between abundance estimated from scientific surveys in the Bay of Biscay. The left-hand plot corresponds with the correlation of number of individuals smaller than 20 cm over time and the right-hand side with total biomass (= Figure 12 in Abad *et al.* 2021).

Life History Parameters

There are many life history studies on European hake in Atlantic waters, however, there is little evidence of significant differences, with the exception of reproductive differences identified between samples collected off the French and Galician coast. Domínguez *et al.* (2008) also found reproductive differences between areas (Galician and French desks) getting similar conclusions and suggesting that there is no reason to split both stocks although some kind of substock structure may be in play. In addition, the contrasting demographic and population dynamics influence different maturity schedules, mediated by an effect in the recruitment success (Hidalgo *et al.*, 2014). These two studies point to the existence of two phenotypic stocks with population dynamics implications, however, this must be tested. Thus, a panmictic scenario would not capture this stock heterogeneity (e.g. different SRR among other elements) and result in potential mismanagement. Simulation exercises and drift models are needed to resolve this question.

Tagging

Two recent tagging studies on European hake were reviewed. One reported: “Most fish were recovered near their release locations although a few of them travelled long distances (around 150 miles), suggesting that some exchange at a population level would be possible. However, none was recovered outside their original stock area.”. The second reported: “None of them was recovered far away from the release area. The maximum distance recorded was around 15 miles from release location after 347 days.” Thus, the results from both analyses supported homing behaviour and/or inshore residency with none of the hake tagged in Northern or Southern waters was recaptured out of their original stock area.

Genetics

A variety of studies, utilising a broad array of techniques (e.g. allozymes, mtDNA, microsatellites and SNPs), have examined the structure and connectivity of hake among northern (ICES subareas 4, 6, and 7, and Divisions 3.a, 8.a-b, and 8d) and southern (ICES Divisions 8.c and 9.a) stocks. In general, genetic studies failed to support the current ICES management units (Milano *et al.*,

2014; Westgaard *et al.*, 2017). Specifically, there is substantial evidence to reject the theory of reproductive isolation between current ICES hake stocks (Milano *et al.*, 2014; Westgaard *et al.*, 2017). However, this research also supports the presence of spatially complex sub-populations within both ICES hake advice units (Milano *et al.*, 2014; Westgaard *et al.*, 2017).

The most in-depth genetic analysis of hake population structures in the Northeast Atlantic included 299 neutral and 17 adaptive single nucleotide polymorphisms (SNPs) to delineate populations from the North Sea (Northern stock), west of Scotland (northern stock), south west Ireland (Northern stock), Galician coast (Southern stock) and North Portugal (Southern stock) (Fig. 3) (Milano *et al.*, 2014). Analysis of neutral genetic markers highlighted no significant genetic diversity among hake collected from west of Scotland, south west Ireland or the Galician coast (Milano *et al.*, 2014). However, this study did observe small, but significant genetic variation in neutral markers between North Sea hake and conspecifics collected from Ireland, Galicia and Portugal. Within the same study, analysis of the outlier loci increased the resolution enabling fine-scale population structure and revealed three genetically differentiated hake clusters: North Sea and North Portugal were identified as distinct genetic groups, separated from a cluster which included the west of Scotland, south west of Ireland and Galician coast hake (Milano *et al.*, 2014).

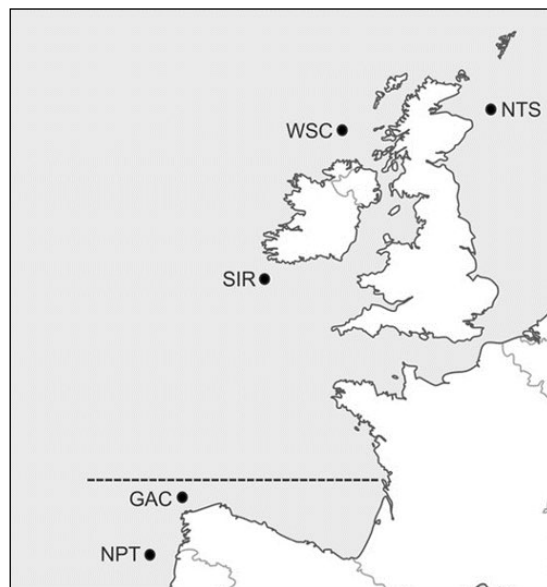


Figure A3.4. Map of hake sampling locations (NTS=North Sea, WSC=west of Scotland, SIR=south west of Ireland, GAC=Galician coast and NPT=north Portugal). Dotted lines indicate approximate boundaries between the Northern and Southern management stocks in the Atlantic (Milano *et al.*, 2014).

Despite the robust analyses with multiple discriminatory genetic markers used by Milano *et al.* (2014), the lack of temporal markers raises questions regarding the stability of these population structures. Additionally, the restricted geographical sampling may not encompass the full extent of genetic connectivity between northern and southern stocks. This level of detail would require additional sampling from areas such as the English Channel, Celtic Sea and Bay of Biscay.

Westgaard *et al.* (2017) undertook a similar study using a smaller SNP marker panel (47 neutral and 6 outlier loci). This study focused on the extreme northern and southern ranges of the northern hake stock (e.g. Bay of Biscay, North Sea and Fanafjorden). Population structure analysis using both neutral plus outlier loci, as well as only neutral loci found significant genetic variation between the Bay of Biscay hake and hake from the North Sea, Fanafjorden and Kattegat (Westgaard *et al.*, 2017). These results support the evidence provided by Milano *et al.* (2014) that sub-populations are present within the northern stock (ICES Subareas 4, 6, and 7, and Divisions 3.a, 8.a-b, and 8d).

Evidence of complex population structuring within and between stocks has been repeatedly observed during earlier studies which utilised allozymes, microsatellites and mitochondrial DNA (mtDNA) (Castillo *et al.*, 2004; Cimmaruta *et al.*, 2005; Lundy *et al.*, 1999; Pita *et al.*, 2014, 2016; Roldán *et al.*, 1998). For example, allozyme analysis using 21 loci, also observed significant genetic differentiation within the northern hake stock, specifically between fish collected from the south of Ireland and Arachon (Roldán *et al.*, 1998). Data generated by microsatellite markers revealed sub-population structuring within the southern hake stocks (Lundy *et al.*, 1999). Genetic samples collected from the southern region of the Bay of Biscay were significantly different from conspecifics collected off the coast of Faro in southern Portugal. However, the same study found no significant genetic difference between hake from the Celtic Sea (northern stock) and those from the southern end of the Bay of Biscay which is part of the southern stock (Lundy *et al.*, 1999).

In general, hake studies have highlighted substantial amounts of genetic connectivity between northern and southern ICES advice units, as well as evidence of complex sub-population structuring across the North East Atlantic (Castillo *et al.*, 2004; Cimmaruta *et al.*, 2005; Pita *et al.*, 2014, 2016). SIMWG have been made aware of an ongoing research project, led by AZTI, which will hopefully provide the temporal and spatial coverage to elucidate the full complexity of hake genetics within this region (Naiara Rodriguez-Ezpeleta 2022, correspondence). Results from such research should be considered before any decisions on hake population structures are agreed.

Detailed comments

- Page 20, last paragraph: “The migration hypothesis proposed by Pita *et al.* 2011 suggests that no barriers to migration seem to exist between the main Atlantic hake stocks, and there is a migrant flow of adult hakes from Porcupine Bank and Great Sole to the Bay of Biscay, the Cantabrian Sea and the Iberian Atlantic waters.” This supports population heterogeneity or metapopulation, but not a panmictic stock.
- Page 22, fourth conclusion: “Life history studies show differences between reproductive traits in both stocks. However, there are environmental and fishing pressure differences that can explain these phenotypic differences.” Evidence of phenotypic stocks with large contracting population dynamics implications in different population subunits.
- Page 22, fifth conclusion: “Current ICES stock structure with two stocks is not supported by any of the studies reviewed.” Pending additional analyses, a complex stock structure seems to be the most plausible scenario rather a panmictic one.

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Anglerfish

Genetics

Using single nucleotide polymorphisms (SNPs) as population markers, Aguirre-Saraipe *et al.* (2021) investigated the structure and hybridisation of white anglerfish (*Lophius piscatorius*) and black anglerfish (*Lophius budegassa*) in the north east Atlantic and Mediterranean. As specified in ToR d, this review will focus on population structures of white anglerfish in the North East Atlantic only.

During the study, 679 white anglerfish were collected from the ‘Northern platform stock’ (n=204), ‘Northern stock’ (n=453) and ‘Southern stock’ (n=22) (Fig. A3.5). For the purposes of ICES advice, white anglerfish from the Northern Platform Stock are part of ICES subareas 4 and 6, and division 3a, the Northern Stock are part of ICES Subarea 7 and Divisions 8.a-b and d, finally the Southern Stock are part of ICES Divisions 8.c and 9.a. Muscle tissues were sampled from each individual, DNA extracted and RAD-seq library prepared using a sub-sample of anglerfish DNA (n=306). After genotype by sequencing, including SNP quality control, between 16 712 and 23 126 SNPs were utilized to examine population structures within white anglerfish.

Results from Aguirre-Saraibe *et al.* (2021), ADMIXTURE analysis, a statistical analysis for inferring geographical origins, grouped the majority of anglerfish samples into ‘three clearly distinguishable groups’: Group 1 (anglerfish from all locations), Group 2 (anglerfish from all locations except the Southern Stock) and Group 3 (anglerfish from all locations except the Northern platform Stock and black anglerfish). Samples classified as Group 1 and 3 were not admixed, while samples statistically classified as group 2 were defined as admixed to both groups 1 and 3 (Fig. A3.6). However, PCA analyses, commonly used to visualize genetic clusters by examining variances matrices, could not group individuals into these three groups with the 1st axis (PC1) explaining 80% of the total variance (Fig A3.6). In general, group 2 showed a consistent pattern of admixture with approximately 75% or 25% of gene flow being contributed from Groups 1 and 3 respectively. Aguirre-Sarabia *et al.* (2021) concluded by stating that for white anglerfish there is ‘no genetic differentiation within the Atlantic’.

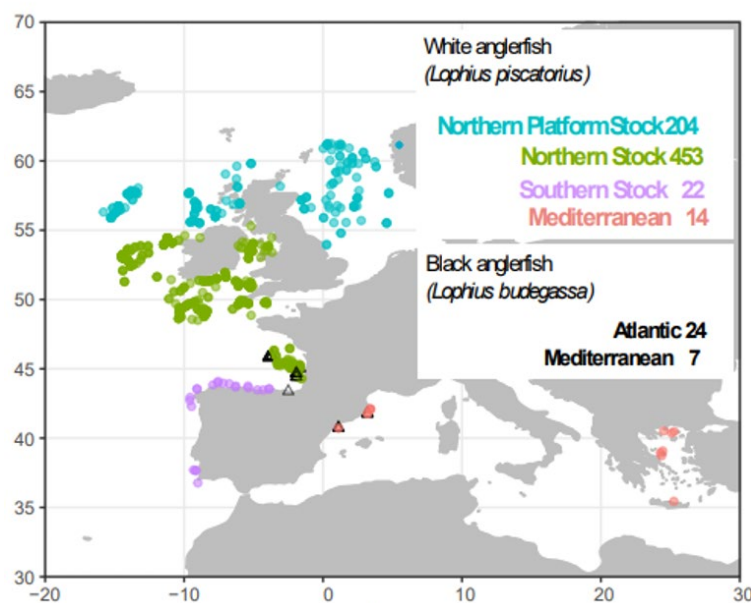


Figure A3.5. Location and number of samples collected for Aguirre-Saraibe *et al.* (2021) anglerfish study.

The ICES working document from Abad *et al.* (2021) summarized molecular studies carried out by Crozier (1987), Charrier *et al.* (2006), O’Sullivan *et al.* (2006) and Blanco *et al.* (2008). These studies utilized previous generations of genetic techniques, such as allozymes, mitochondrial DNA and microsatellites, to infer white anglerfish stock boundaries.

Using nine microsatellites to delineate white anglerfish populations, O’Sullivan *et al.* (2006) collected samples from Iceland, Rockall, Norwegian coast, west of Ireland and the North Sea. Results from this study revealed no differentiation between anglerfish collected within the northern component of the ICES advice units. O’Sullivan *et al.* (2006) did not collect anglerfish samples from the southern range of the species (e.g. Bay of Biscay, Portuguese coast), so no inference of connectivity between these ICES advice units can be made. Blanco *et al.* (2008) also used microsatellite markers (n=8) to examine gene flow between the northern and southern white anglerfish populations. In this particular study, samples were collected from the west of Ireland to southern Portugal. The study highlighted that white anglerfish from the Bay of Biscay were significantly differentiated (F_{st} values) from conspecifics, suggesting a sub-population of anglerfish within this region (Blanco *et al.*, 2008).

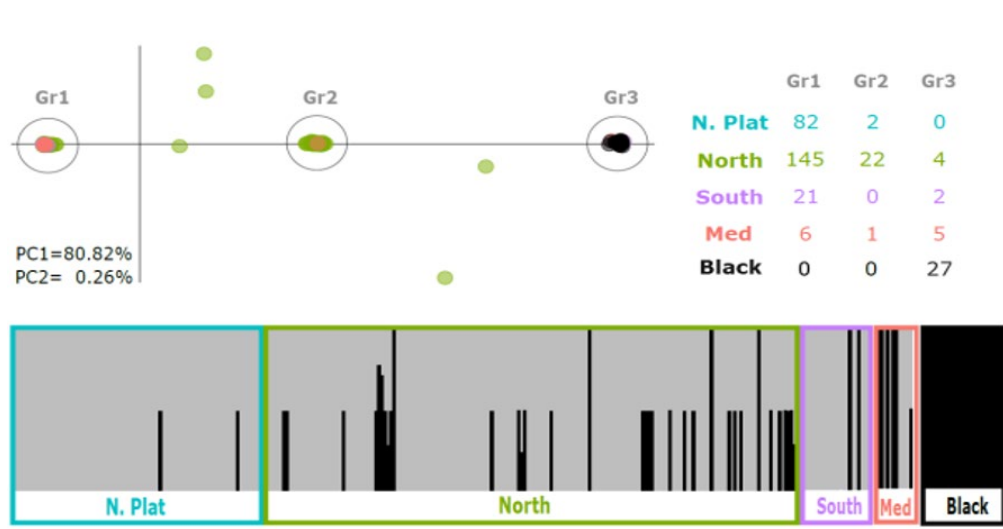


Figure A3.6. Principal components analysis (PCA) where differentiated groups are circled and number of individuals per group for each stock are indicated. Individual ancestry proportions estimated by ADMIXTURE when assuming two ancestral populations (Aguirre-Sarabia *et al.* 2021).

Results from Charrier *et al.* (2006) mtDNA analysis of white anglerfish was also limited to the northern stock unit, with the possible exception of one sample collected near the Cap Breton Canyon. In general, their results highlighted a lack of genetic differentiation among white anglerfish, except for samples collected from Northern Scotland which were significantly differentiated from conspecifics collected from other localities. Finally, Crozier (1987) sampled white anglerfish from 5 locations from the west coast of Scotland and Irish Sea. Using 24 loci to analyse genetic differentiation, the study revealed homogeneity within the sampling locations suggesting that white anglerfish were panmictic in this region.

To date, the working document submitted by Aguirre-Sarabia *et al.* (2021) provides the most robust evidence for delineating the spatial structure of white anglerfish within current ICES advice units. This is supported by the review document, containing results from previous molecular studies, submitted to SIMWG by Abad *et al.*, (2021). In general, these reports do not support the current ICES advice units which separate the northern and southern white anglerfish populations for WGBIE. The robust sampling by Aguirre-Sarabia *et al.* (2021) in combination with tens of thousands of population markers highlights significant gene flow between advice units. However, recent ICES workshops (e.g. WK6aCodID (ICES, 2021); and WKNSCodID (ICES, 2020)) have also based their recommendations on the results of outlier loci analysis. So far, this type of analysis is lacking for white anglerfish but could potentially reveal sub-populations within both advice units. There is some evidence of spatially complex sub-populations provided by the Abad *et al.* (2021) review, particularly within the northern ICES advice stock unit (Charrier *et al.*, 2006; Blanco *et al.*, 2008).

Detailed comments

- The fact that the actual ICES Divisions are not supported by past studies, basically genetics studies, seems well argued and well supported. However, the occurrence of panmictic structures is not yet supported.
- It seems that there is sufficient basis that 'phenotypic stocks' are in place. However, further research is needed to know whether these 'phenotypic sub-units' have different demographic influence on the population dynamics a potentially large delineated stock.
- Moving from current management/assessment scenarios to a panmictic (unique stock) without further investigating and testing the potential occurrence of complex stocks

structures cannot necessarily be a better scenario than the current one, particularly in scenarios of low stock production. Testing the implications in terms of the assessment outputs and implications of all possible and plausible scenarios would be needed. Simulations considering different mix-stock structures (populations heterogeneity, metapopulation, and panmictic) might be simulated and tested before proposing a new stock structure that will persist for many years. For instance, whether the SRR are spatially differentiated or whether recruits settle in common pools. Whether connectivity occurs as adults (migration rates estimation), early life stages, or both is not a trivial question that requires further research before deciding.

- Dispersion experiments are needed to assess three degrees of connectivity as early life stages.
- Given the complete cover of the species by the IBTS surveys, and availability of methods to filter the effects of contrasting seasons or different gears/vessels, a global analysis of the whole area for each species is needed to assess how spatiotemporal dynamics of the species are clustered, (e.g. Lindegren *et al.*, 2022).
- Page 10, last paragraph: ‘on the sea surface, could have a strong effect on dispersal distance and population connectivity (Fariña *et al.* 2008).’ In which sense? Reduced dispersal patterns? If a panmictic signal is suggested, it is needed to know the relative role of early life stage dispersal, or juvenile or adult movement.
- Page 11, end of second paragraph: ‘The impact of this divergence in an efficient management of the resource should be explored and, also, it should be tried to find a balanced definition of stock that includes biological, environmental, and political factors.’ More than ‘political’, they could consider management reasons.
- Page 11, end of fourth paragraph: ‘In contrast with previous results, the morphometric analysis showed a high segregation of the Portuguese coast (division 9a) and a north-south gradient, pointing to a more complex population structure than the current one.’ Which ones? More clarification on this point is needed. At which level, these phenotypic differences may be the consequence or have consequences at demographic level?
- Page 12, first line: ‘to confirm that black anglerfish in NE Atlantic is a panmictic population.’ Strong implications of assessing and managing as a panmictic population if the stock has a complex structure.

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WGHANSA request

Background

SIMWG received a request from the ICES Working Group on Horse mackerel, anchovy and sardine (WGHANSA) to provide a review of their recent report “Population structure of the European anchovy (*Engraulis encrasicolus*) in ICES Division 9a”.

The anchovy has two stock components: 9.a West (ICES 9aN, 9aCN and 9aCS) and 9.a South (27.9.a.S - Portugal and 27.9.a.S - Spain); (Figure A3.7).



Figure A3.7. ICES Statistical Divisions and Subdivisions in Southern Europe.

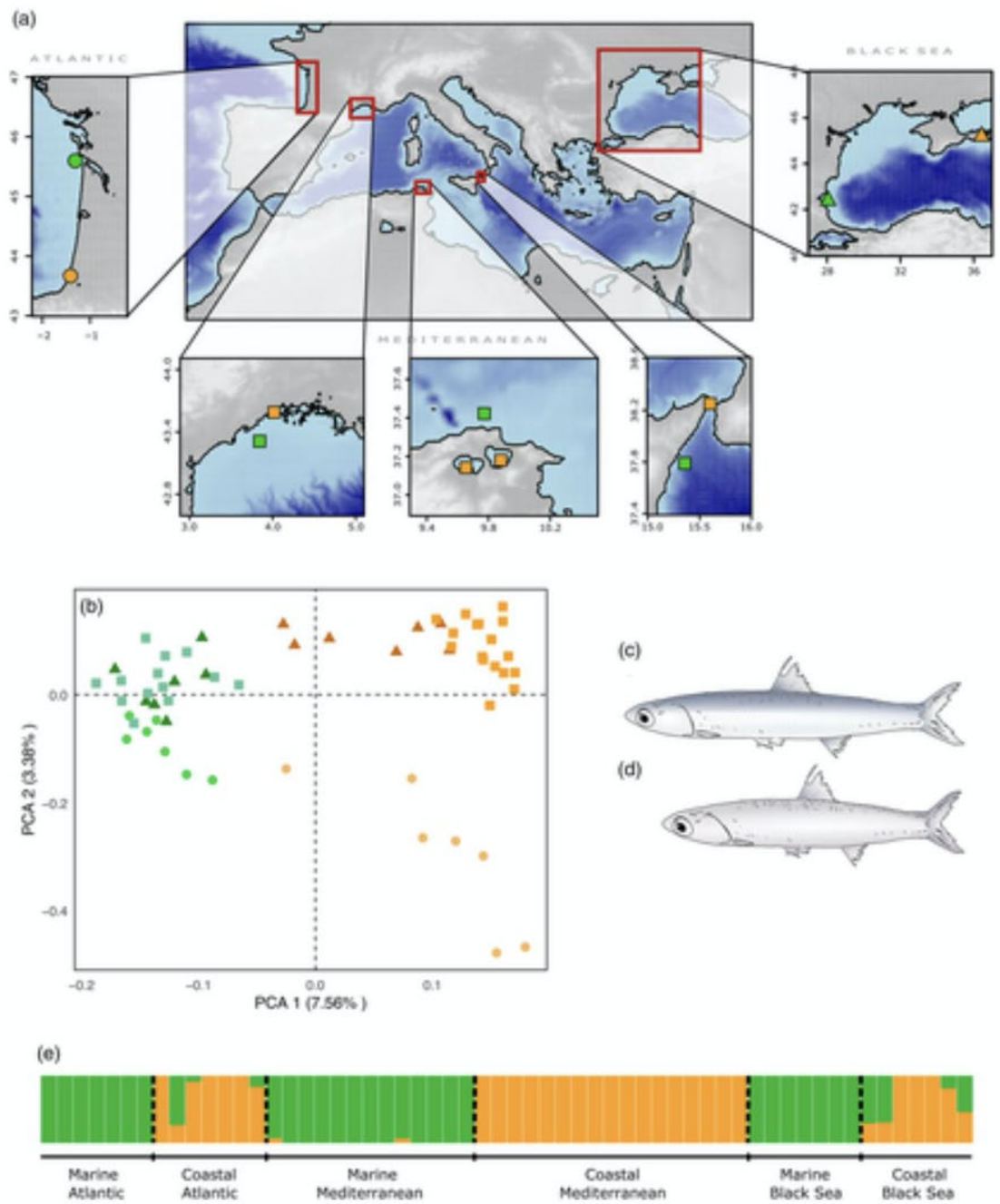


Figure A3.8. a) Sampling locations of *Engraulis cf. encrasicolus*. Symbols represent locations (●, Atlantic; ■, Mediterranean; ▲, Black Sea) while colours represent habitat type (green, marine; orange, coastal). (b) Principal component analysis based on 2952 SNPs in 58 individuals. Schematic morphological representations for marine anchovies (c) and coastal anchovies (d). (e) Individual ancestry proportions as determined by FastSTRUCTURE with K = 2 clusters. (from Bonhomme *et al.* 2022).

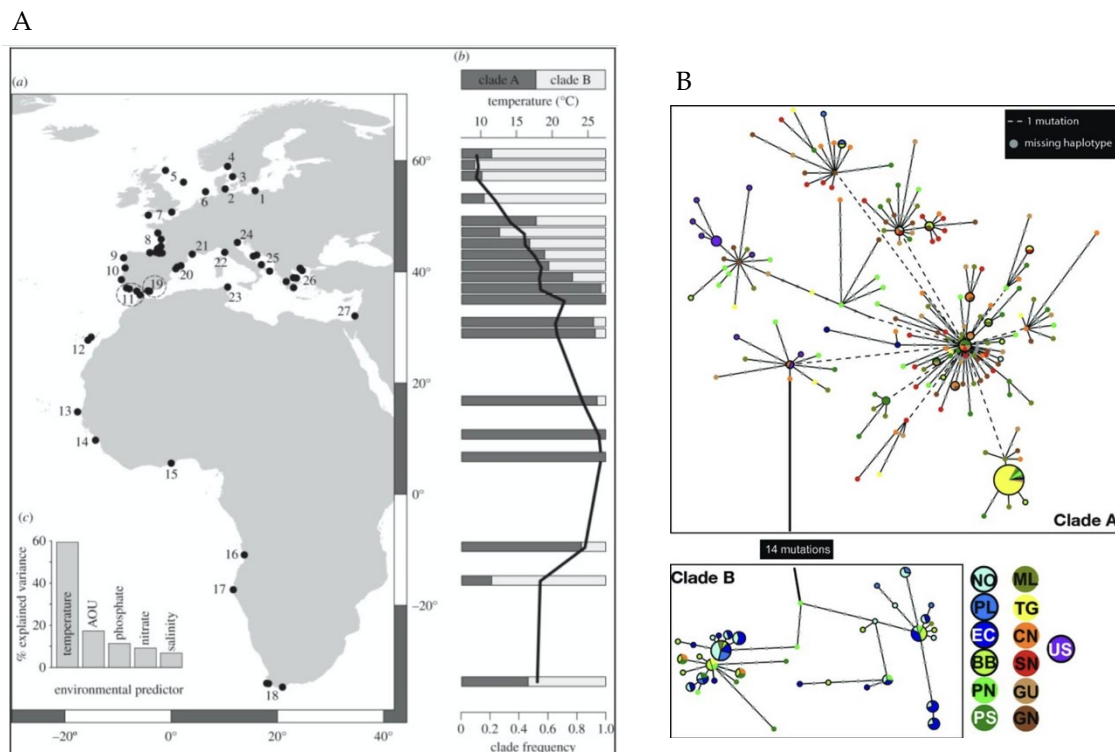


Figure A3.9. A (a) Locations used for environmental correlates of mitochondrial clades frequencies. **(b)** Sea surface temperature 2009 (black line) and clade frequency (clade A, dark grey; clade B, light grey). **(c)** Importance of environmental variables after hierarchical partitioning analysis; AOU, apparent oxygen utilization. (Silva *et al.*, 2014a and Silva *et al.*, 2014b). **B.** Mitochondrial DNA cytochrome b haplotype network of *Engraulis encrasicolus* and *E. eurystole* constructed with a median-joining algorithm. Colors indicate geographic origin of haplotypes. A black outline of haplotypes indicates their origin from recently colonized areas (USA and the north of Europe, English Channel and Bay of Biscay). Dashed lines indicate one mutation.

The species has been extensively studied in the past and the report under review recognizes the conflicting results regarding population structure. These conflicts arise from the confounding presence of two ecotypes that differ morphologically and genetically (Figure A3.8) (LeMoan *et al.* 2016, Montes *et al.* 2016, Bonhomme *et al.* 2022) and two mitochondrial lineages (Figure A3.9), one with low frequencies at higher latitudes, whereas the other has an anti-tropical presence (Magoulas *et al.* 2006; Borrell *et al.* 2012, Viñas *et al.* 2013, Silva *et al.* 2014a, Silva *et al.* 2014b) that do not correspond to the ecotypes.

From the previously published studies, there is evidence for population differentiation between the North Sea + English Channel and the Bay of Biscay (Petitgas *et al.* 2012; Montes *et al.* 2016, Huret *et al.* 2020), although this is not always the case (Petitgas *et al.* 2012; Montes *et al.* 2016, Huret *et al.* 2020). Differentiation further south between West Galicia + North of Portugal and the Gulf of Cadiz has been detected (Silva *et al.* 2014a, Zarraindia *et al.* 2012) but because no provisions were made to detect ecotypes, results may be biased by different proportions of each ecotype in the samples used.

There is a need for future monitoring programs to include sampling that considers the ecotypes presence and to further use genomic markers that display an appropriate level of resolution both geographic and genetic. Sampling should aim at building a genetic baseline of individuals from different spawning grounds and if there is no reference anchovy genome published meanwhile, assembling a genome will bring many advantages: (1) higher resolution power in subsequent analysis, (2) possibility of identifying genes involved in differentiation among and within ecotypes, (3) select markers that have the highest level of discrimination between those different populations and ecotypes, and use those into a panel for developing an genetic assignment model.

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Annex 4: ToR a) Review recent advances in stock identification methods

Advances in Stock Identification Methods in 2022

In 2022 (review period mid-2021 to mid-2022), there were several notable advances in stock identification methods and a proliferation of applications, with many results relevant to ICES science and advice. Here, we summarize advances and results accounting for research in genetics, life history parameters, growth marks in calcified structures, morphometrics, tagging, otoliths, parasites, simulation approaches, and interdisciplinary approaches.

Genetics (Contributors: Rita Castilho, Florian Berg, Stefano Mariani, Christophe Pampoulie and Lovisa Wennerström, David Murray, Naiara Rodríguez-Ezpeleta)

Our annual monitoring of the usage patterns of molecular markers in fisheries stock identification detects a small fluctuation in published studies around 250–270 papers, affecting both microsatellites and SNP papers (Figure A4.1). This year, we decided to introduce another search term to the markers, genom*, to capture mainly genome re-sequencing studies on stock identification. Numbers show a consistent increase in genomic studies, which in 2021 represented 44% of the three categories. There is the caveat that some of the identified as genom* papers may also be considered as SNP papers, and may be counted twice.

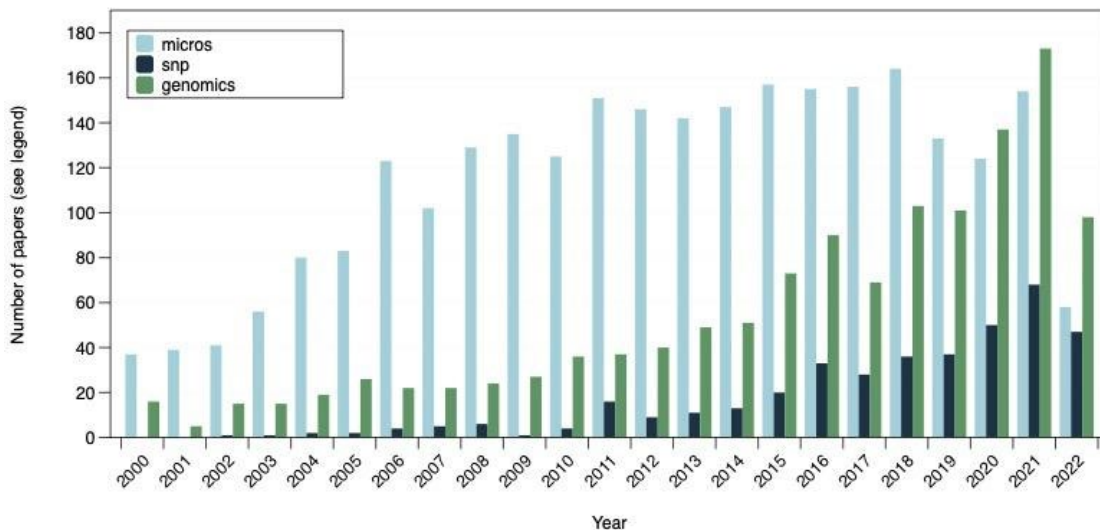


Figure A4.1. Scientific publishing trend since 2000, comparing outputs of studies using microsatellite, SNPs and genomics, as listed in the Clarivate Analytics Web-of-Science. The search criteria were: “fish* AND gene* AND (population OR stock) AND ‘molecular marker*’,” where ‘molecular marker*’ means “Microsatellite*”, “SNP*” or “Genom*”. Only papers in the following disciplinary areas were considered: ‘Fisheries’, ‘Environmental Sciences’, ‘Ecology’, ‘Biodiversity Conservation’, ‘Marine & Freshwater Biology’ and ‘Oceanography’. Data for 2022 only represent papers published through to the present date (July 2022).

Climate influence on genomic structure

The white seabream *Diplodus sargus* and the striped red mullet *Mullus surmuletus* displayed contrasting structure patterns based on a panel of SNPs, although some highly differentiated genomic regions (i.e. outliers) could be identified (Boulanger *et al.*, 2021). The authors tried to disentangle the relative contribution of space, dispersal and environmental variables (climate, marine primary productivity) on the outliers' genetic structure to test the prevalence of gene flow and local adaptation. Their contrasting observations for both species were presented as examples of how adaptation capacities and environmental niches can serve to implement evolutionary processes into the management of exploited marine species (Boulanger *et al.*, 2021).

Reference genomes input into stock structure

Reference genomes of non-model organisms such as the Atlantic cod and herring have contributed significantly to a better understanding and detection of population structure of these species across their distribution range. In the Atlantic cod, the availability of a reference genome has confirmed the presence of four chromosomal inversions also called supergenes (Matschiner *et al.*, 2022). These supergenes span several Mb and contain hundreds of genes likely to reflect local adaptation and selection processes at various geographical scales. They clearly differentiate migratory and non-migratory ecotypes of Atlantic cod across the North Atlantic (Matschiner *et al.*, 2022). Petterson *et al.* (2021) have also identified such a supergene in the Atlantic herring, a 7.8-Mb inversion located on chromosome 12, which is suspected to reflect local ecological adaptation processes of the Northern and Southern haplotypes.

Genome-wide analysis

Maroso *et al.* (2021) used a panel of 1152 SNPs among which 18 were outliers to investigate the structure of gilthead sea bream (*Sparus aurata*) across most of its range, genotyping a total of 958 samples. They detected weak subdivisions into three genetic clusters using neutral SNPs: Atlantic, West, and East Mediterranean. When the 18 outliers SNPs were used alone, they revealed further discrimination of the East Mediterranean Sea into an Ionian/Adriatic and an Aegean group. Seascape genomics and genomic functional analyses related this latter differentiation to salinity differences in the habitat.

A recent study on cod (*Gadus morhua*) in the eastern Atlantic using whole genome resequencing showed that stabilising selection on three supergenes maintains ecological stasis of ecotypes, coastal and offshore cod despite millennia of extensive exploitation (Sodeland *et al.*, 2022). Similarly, a study performed on cod on both sides of the Atlantic showed no substantial loss of genomic diversity and high effective population sizes despite years of exploitation (Pinsky *et al.*, 2021).

A study of European whitefish (*Coregonus lavaretus*) in the British Isles, identified a deep divergence between Scottish populations and English, Welsh and other populations using over 25 000 polymorphic SNPs. The split between Scottish and other populations reflects a pre-last glacial maximum separation, subsequent isolation and different colonisation routes to present distribution after the last ice age (Crotti *et al.*, 2021).

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Growth marks in calcified structures (Contributor: Richard McBride)

Calcified structures in fish, particularly the ear bones (otoliths), but also scales, spines, and fish rays, are used for identifying stock structure as well as for a variety of research applications (Brophy, 2014). Notably, for well over a century, they have been used to age fish when interpreting daily and annual rings (annuli). More recently, in combination with ability to preserve growth marks, these same calcified structures have been used to define stock structure based on their shape or their chemical composition.

Sanchez *et al.* (2022) is a recent example of an indirect use of otolith annuli as life history markers to delineate measurements of microchemistry in an otolith. They employed otolith microchemistry to investigate population connectivity of snowy grouper (Serranidae: *Hyporthodus niveatus*) across marine regions of the western Atlantic Ocean and Gulf of Mexico. They arranged the sampling (ablation) locations along a transect of the otolith to three life history periods: (1) the otolith core, representing the young-of-year (age-0) period, (2) the distal edge of the otolith, representing the most recent 2–3 years, and (3) across the otolith, representing the lifetime period. Because the life history of many reef fishes is bipartite – involving a highly dispersive early life stage and a more settled adult phase – this otolith sampling strategy allows interpretation of microchemistry results in the context of the bipartite as well as an integrated life periods.

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Life history parameters (Contributor: Richard McBride)

Life history parameters define vital rates, such as growth, mortality, and maturity (McBride, 2014). These traits define population structure as applied to a range of marine fishes, from the data-poor chondrichthyans (Green *et al.*, 2022) to one of the most data rich species: Atlantic cod (*Gadus morhua*).

In the former case, Nelson *et al.* (2022) advance the genetic evidence (SNPs) demonstrating population structure of the clearnose skate (*Raja eglanteria*). From a life history point of view, these skates lay large eggs, initially enclosed in a case (a “mermaid’s purse”), that has little capacity for dispersal. In addition, these skates show site fidelity and limited migrations throughout their wide geographic range – along the east coast and in the Gulf of Mexico of the United States. Not surprisingly, Nelson *et al.* (2022) observed a “crinkled connectivity” (Ovenden, 2013; Marandel *et al.*, 2018). This constituted a latitudinal gradient of genotypes with notable discontinuities around two known faunal breaks: Cape Hatteras, along the US east coast, and Cape Sable, which separates the Atlantic and Gulf of Mexico sampling sites.

In the latter case, Wright *et al.* (2021) examine genotypic and phenotypic traits of cod in the North Sea with a goal to integrate population diversity with subunit productivity. They report fine-scale isolation-by-distance genetic patterns. This genetic structuring was coherent with spatially-explicit life history patterns, such as cod on Viking Bank matured later and at a larger size. Wright *et al.* outline how these results are relevant to both re-interpretations of historical changes in maturation schedules, as well as current efforts to re-align mismatches between stock divisions and population units in this region.

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Morphometrics/meristics (Contributors: Emma White and Christoph Stransky)

Morphometrics and meristics remain fundamental methods of identifying stocks or populations of fish species. A review of the papers published on this topic between August 2021 and June 2022 are detailed below.

A review of the literature on distance-based morphometry used in ichthyology to identify species or identify subpopulations within species was conducted by Bánó and Takács (2022). The study aimed to collated information on the most widely used morphometric variables and answer questions on the processes used to select morphometric variables and what effect the chosen set of variables can have on the results. In addition to the literature review, three datasets were analysed to demonstrate findings of the study. Despite the availability of a large number of variables, most studies use “common” variables regardless of the species or aims of the study. Variable selection was rare in most studies and the datasets were often not standardised, which is a concern for accuracy and usability of the results. Through analyses of the author’s own datasets, it was demonstrated that the number of variables and the set of variables used can have a significant impact on the discrimination of stocks/populations and the percentage of correctly classified individuals. This study highlights the need for careful consideration when selecting morphometric variables, and the selection should be based on the species being studied and the goals or hypotheses of the study.

Jawad *et al.* (2021) examined meristic variation among stocks of greater lizardfish *Saurida tumbil* through the western coasts of the Arabian Gulf and Sea of Oman using meristic characters. Statistical analysis of meristic traits proposed that there is constrained migration of populations of greater lizardfish along the western coast of the Arabian Gulf and Sea of Oman. Overlapping of the two samples from the northern part of the Arabian Gulf (Iraq-Kuwait waters), three samples from the middle region of the Arabian Gulf (Bahrain-Qatar-Saudi Arabia) and two samples from the southern part of the Arabian Gulf/Sea of Oman (United Arab Emirates–Sultanate of Oman) suggested that there are three self-recruiting populations in the studied area. Inspection of the role of each meristic trait variable to Canonical Discriminant Analysis showed that changes among samples appeared to be linked with the pattern of distribution of water temperature and configuration of current in both the Arabian Gulf and Sea of Oman areas.

The horse mackerel (*Trachurus trachurus*) stock in the Moroccan-Saharan area is poorly investigated, especially in comparison to the Euro-Mediterranean stocks. El Mghazli *et al.* (2022) investigated the possibility of subunits within this stock using morphometric and meristic characters of this pelagic fish species. Variables including body weight, body dimensions and meristic characters were collected from fish from three areas. Discriminant Function Analysis (DFA) and Principle Component Analysis (PCA) were applied to the data to reveal slightly different results from the morphometric and meristic approaches, but both methods identified three subunits within the study area providing important, new information about the Moroccan-Saharan horse mackerel stocks. Further work using different stock identification methods, including genetics and otoliths, was suggested by the authors. A successful start has been made in understanding the population structure of horse mackerel in this area which will contribute to more accurate assessments and better management for these stocks.

Samples of the bogue, *Boops boops*, were collected from four sites off the coast of Tunisia to investigate morphometric and meristic variability of populations within the study area (Ben Labidi *et al.*, 2021). Significant variation between the four sites was detected from 13 morphometric characters and 4 meristic characters. A Canonical Variant Analysis (CVA) was applied to the morphometric variables and determined a 95% correct classification of individuals to their population of origin. The discrimination between the populations was less certain using meristic variables, with 57% correct classification reported. The morphological differences observed in this

study have been attributed to physico-chemical water properties at the different sites. This gives an insight into the influence of environmental conditions on characteristics of fish and the structure of a population.

The population structure of the Brazilian sardine, *Sardinella brasiliensis*, was investigated using body geometric morphometrics and otolith shape analysis (Schroeder *et al.*, 2022). Two distinct population units were observed in this study, which is covered in more detail in the otolith shape section.

The stock composition of round sardinella, *Sardinella aurita*, along the Algerian Coast in the Mediterranean Sea has been investigated using three methods, including body morphometrics, meristics and otolith shape (Ferhani *et al.*, 2021). Analysis of the count of vertebrates (meristics) showed no difference between the three areas for this species. A Linear Discriminant Analysis (LDA) was applied to the body morphometric variables and resulted in a 70% classification rate. The otolith shape analysis is covered in the otolith shape section. The results from this study can be used to better inform the management of round sardinella in this area.

Sabbir *et al.* (2021) provided new information on the demersal species, Hooghly croaker (*Panna heterolepis*), that were sampled from the Bay of Bengal. No information was available on the morphometric or meristic characteristics of this species so this study set about building a profile of this species by recording meristic counts of fin rays and producing length-weight relationships and total length-standard length relationships. This information can be used for species identification and may contribute to stock or population identification studies in the future.

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Tagging (Contributors: Steve Cadrin, Karin Hüsey and Douglas Zemeckis)

Introduction

Tagging studies published since the last SIMWG report in 2021 represented a range of tagging techniques and applications. The main objectives addressed by the studies were movement analyses, interdisciplinary stock identification (i.e., tagging compared to or integrated with other approaches), several comparisons of conventional to parentage-based tagging, and tag-integrated spatial stock assessments.

Movement patterns

The primary role of tagging for stock identification has been to describe movement patterns among areas and possibly quantify movement rates.

Mion *et al.* (2022) investigated seasonal movement of Eastern Baltic cod from historical tagging data (1955–1988) and recent tagging (2016–2019). Historical movement patterns included two movement patterns (resident or seasonally migratory), with larger distances moved by cod released in northern and central Baltic areas than cod released in southern Baltic areas. Recent tagging data indicated persistent resident cod in the southern Baltic.

Stamp *et al.* (2021) tagged 146 juvenile European bass with acoustic transmitters and tracked the for one year across three locations in the southwest UK. Tagged fish were resident within 2–20 km of the tagging location for 43–76% of the year, but some fish were resident throughout summer and winter. Other fish moved up to 317 km to other coastal locations, 81% of which returned to their original tagging location

Aalto *et al.* (2021) used decades of archival and satellite tagging to derive movement patterns for two discrete stocks of Atlantic bluefin tuna, and derived stock-specific catch and catch rates from monthly distributions. Catch rate of the eastern increased in the past two decades, and catch rates of the western stock decreased.

James *et al.* (2022) tagged young-of-year white sharks with satellite and acoustic tags in 2016 and 2017. Nine white sharks tracked over two consecutive winters over consecutive years occupied continental shelf waters (<100m bottom depth) off the coasts of North and South Carolina with mean sea-surface temperatures of 15–21°C. The observed winter habitat extended over 950 km south of the previously considered essential fish habitat.

Siwicke *et al.* (2022) inferred spawning behaviour of Greenland halibut in the eastern Bering Sea and Aleutian Islands from archival tag data. Spawning activity was indicated by abrupt vertical rises of females and predominantly occurred in January and February. Females rose to 200–350 m below the surface, indicating that eggs could be released shallower than previously assumed. Females had a single spawning rise annually, supporting single-batch spawning. Large females (>80 cm) had spawning rises in consecutive years, indicating annual spawning. Males exhibited spawning behaviour one or more times for an average of 20 days.

Klein *et al.* (2021) studied stock structure, site fidelity and movement patterns of common smooth-hound shark off southern Africa based on genetics and tag-recapture data. Eight microsatellite markers and two mitochondrial genes did not reveal significant genetic structure among locations, but tagging data suggest site fidelity (e.g., 76% recaptured within 50 km of tagging location).

Talwar *et al.* (2022) tagged Caribbean reef shark in the Bahamas from 2008 to 2020 to characterize movement and life history. Sharks were relatively sedentary (moved 0–8 km, 2 days to 7.1 years

at liberty), with no apparent seasonal migration. Growth increments suggest slower growth in the Bahamas than off Venezuela.

Grilly *et al.* (2021) tagged Antarctic toothfish in the Southern Ocean from 2001 to 2019 to study long-distance movements. Results indicate that adults are relatively sedentary, but some (~7%) move > 200km. There was no apparent relationship between long-distance movements, time at liberty or sex, but most long-distance movements were counter-clockwise and presumably influenced by oceanographic processes and other life-history traits.

Taylor and Johnson (2021) estimated connectivity of Eastern King Prawn in a spatial management network designed to protect small prawns from a multi-jurisdictional Australian trawl fishery. More than 7500 prawns were tagged intermittently from 1991 to 2019 in three spatial management areas of one jurisdiction, and 786 tagged prawns were recaptured. Results indicate high connectivity between all spatial management areas, fishing grounds and spawning areas. Most tagged prawns were recaptured in deeper waters adjacent to or north of the spatial management areas. Prawns tagged early in the growth season had lower recapture rates but moved further. Tagged prawns reached the adjacent jurisdiction within 54 days, but most were recaptured in the jurisdiction where they were released.

Conventional and Parentage-based Tagging

North Pacific salmon management routinely applies genetic stock composition and coded wire tagging. These approaches offer complementary information for stock identification, stock assessment and fishery management. Genetic sampling and analyses have been expanded to parentage-based tagging, which is essentially a natural tag.

Delomas and Hess (2021) developed a method to correct for bias from expansion of tagging rate for natural-origin fish in mixed-stock analysis using parentage-based tagging. Parentage-based tagging allows for large numbers of hatchery fish to be efficiently tagged and identified after recapture.

Jensen *et al.* (2022) used coded wire tag recoveries for stock assignments and stock-specific harvest to support management of mixed-stock Chinook salmon fisheries. They compared performance of alternative approaches to improve accuracy of harvest estimates. Bayesian models with time-lagged priors improved harvest estimates, but performances of mixed-stock harvest estimators varied. Results suggest that the optimal approach is stock- and fishery-specific.

Jensen *et al.* (2021b) also applied genetic stock identification and parentage-based tagging to the 2017–2018 recreational fishery for Chinook Salmon in the Columbia River and found that genetic stock identification and parentage-based tagging produced similar stock assignments.

Beacham *et al.* (2022a, 2022b) demonstrated that genetic sampling and analyses can improve on coded wire tagging programs for assessment and management of Chinook salmon fisheries and hatchery broodstocks off British Columbia. Genetic stock identifications and parentage-based tagging using SNPs were analyzed for 8208 Chinook salmon sampled from 2019 fisheries and hatchery broodstocks and identified 2237 parent-based tags (27% individual identification rate), but exploitation rates derived from coded wire tags did not provide an accurate representation of the catch (Beacham *et al.* 2022a). Genetic stock identifications and parentage-based tagging using SNPs were analyzed for 9234 Chinook salmon sampled from 2020 fisheries and hatchery broodstocks and identified 2250 parent-based tags (24% identification rate), and exploitation rates derived from coded wire tags and genetic analyses for 13 populations were strongly correlated ($r=0.98$; Beacham *et al.* 2022b).

Tag-Integrated Spatial Models

Spatially-structured models offer an alternative for assessing and managing spatially complex populations. Information from stock identification can be used to define spatial boundaries for conventional stock assessment as well as spatial strata for spatial assessments.

Goethel and Cadrin (2021) reviewed spatially-structured population models, including theoretical basis and recent advances in spatially explicit data and statistical parameter estimation. Simulation studies show that conforming to or revising spatial assumptions (e.g., revised stock boundaries based on stock identity) is essential for accurate estimation.

Bosley *et al.* (2021) evaluated performance of spatially stratified stock assessment models when population structure is mis-specified. They simulated a single uniform population, a single population with spatial heterogeneity, and a metapopulation and tested performance of several model assumptions, including a panmictic population, representing areas as spatially defined fleets, and a spatially explicit tag-integrated model. When population structure was incorrectly specified in the assessment model, parameter estimates were generally unbiased at the system level, but were often biased for sub-areas. Correctly specifying population structure led to better estimates, and mis-specified spatial assessment models performed better than spatially aggregated alternatives. Allowing for flexible parametrization of movement estimates, such as age-varying movement rates, performed better than correctly specifying population structure, and integrating tagging data helped to estimate movement.

Thorson *et al.* (2021) developed a movement model involving diffusion (random movement), taxis (movement towards preferred habitat) and advection (passive drift following ocean currents) and demonstrate an application to Pacific cod in the Bering Sea, with tagging, survey and fishery data from 1982 to 2019. Sensitivity analyses showed that tagging data were necessary to identify diffusion rates, and survey data were informative about movement among biogeographic strata.

Patterson *et al.* (2021) analyzed pop-up satellite and conventional tag data for broadbill swordfish in the western and central Pacific using a continuous time Hidden Markov model to describe movement and estimate transition rates among spatial strata defined by the stock assessment. They also evaluated an additional spatial boundary within the assessment area. Estimating variances in the 3-region model was limited by low data coverage in one region, demonstrating the need for data collection across all regions in a stock assessment.

Kell *et al.* (2021) compared alternative stock assessment model structures for Indian Ocean yellowfin tuna, including a single-area biomass dynamic model, a spatial age structure production model with four areas, and a more complex integrated models with four areas. The age structured production model with four areas had the best prediction skill. The single-area production model and the more complex spatial model had lower prediction skill, presumably because of spatial heterogeneity for the more simple model and large sampling error of length compositions for the more complex model.

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Otolith shape (Contributors: Kélig Mahé, Christoph Stransky)

From August 2021 to June 2022, there were 17 papers dedicated to the otolith shape and two studies on morphometric analysis of the other calcified structures (Figure A4.2). For marine organisms, especially for fish species, the otolith is the main calcified structure to identify the stock structure. Eight studies focused on the difference among species (*Booops boops*, *Istigobius ornatus*, *Merluccius capensis*) with only otolith shape as descriptor of stock identification. In these studies, one paper (Quindazzi *et al.*, 2021) compared the 2D shape to 3D shape approaches applied on the same data. There are nine other studies using otolith shape with another descriptor (otolith chemistry, genetic and body shape) for stock identification of several species.

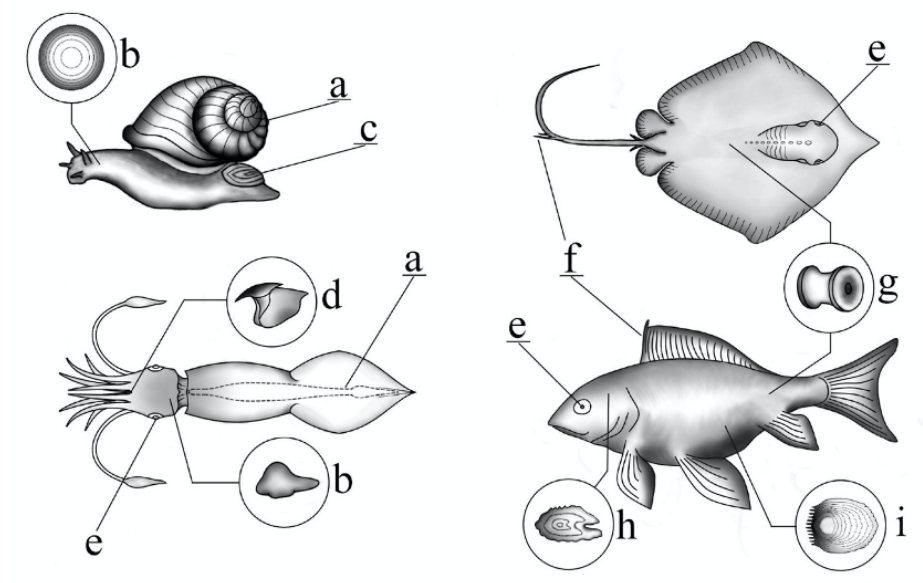


Figure A4.2. Schematic illustrating the location of various recording structures in marine species. (a) Shell (may be internal or external), (b) statolith, (c) operculum, (d) beak, (e) eye lens, (f) fin ray/sting ray, (g) vertebrae, (h) otolith, (i) scale. (In Lishchenko & Jones, 2021).

Otolith shape as tool for stock identification

Species/stocks in the ICES area

Saltamacchia *et al.* (2022) used the otolith shape of European sprat (*Sprattus sprattus*) to investigate large- and small-scale geographical variability across the Greater North Sea ecoregion. The otolith shape was extracted from digitised images and transformed into Wavelet coefficients to be analysed with multivariate statistics. Otolith shape was observed to follow the genetic population structure recently defined for the region, supporting the latest revision of the stock boundaries. Four main groups were identified based on phenotypic variability: (i) Norwegian fjords; (ii) North Sea and offshore Skagerrak–Kattegat; (iii) coastal Skagerrak–Kattegat; and (iv) Udddevalla fjord. However, 4-fold cross-validations based on Linear Discriminant Analysis resulted in low accuracy limiting at the moment the ability to use otolith shape analysis for population identification at an operational basis. Our results show the importance of coastal areas, which might be inhabited by distinct populations of sprat that are currently not acknowledged in the management and assessment.

Vasconcelos *et al.* (2021) analysed the otolith shape based on wavelet functions of *Trachurus picturatus* (blue jack mackerel) from mainland Portugal, Madeira, and the Canaries. They explored

the phenotypic variation throughout a latitudinal gradient, established a hypothesis to explain this variability based on the reaction norms, and determined how the use of average phenotype and/or morphotypes influence in the delimitation of stocks. Four morphotypes were identified in all regions, with an increase of phenotypes in warmer waters. The findings demonstrated that stocks were clearly separated with classification rates over 90%. The use of morphotypes revealed seasonal variations in their frequencies and per region. The presence of shared phenotypes in different proportions among fishing grounds may open new management approaches in migratory species. These results show the importance of the phenotypic diversity in fisheries management.

Species/stocks outside the ICES area

Ferhani *et al.* (2022) investigated the body morphometric characteristics and otolith shape described by elliptic Fourier descriptors on European anchovy (*Engraulis encrasicolus*) samples collected from six locations in Algerian waters during the acoustic survey ALPEL2018 (south-western Mediterranean Sea, GFCM-GSA04). The discriminant analysis was applied over twelve morphometric body distances and 31 normalised elliptic Fourier descriptors. It highlighted significant differences between areas but with high overlapping. No significant difference was detected between males and females, or right and left otoliths. Hierarchical clustering analysis conducted on the mean form of body and otolith shape showed a clear geographical cline. The results indicate that the geographical trend is present with a high level of overlapping between the near areas, suggesting that European anchovies in Algerian waters are not completely isolated.

Wujdi *et al.* (2022) investigated the variability of otolith shape as an efficient tool for stock discrimination of the Indian mackerel, *Rastrelliger kanagurta*. A total of 159 pairs of sagittal otoliths were sampled at four localities along the southern Java-Bali coastal waters. Otolith outline was modelled by using Wavelet coefficients and was compared between localities using ANOVA-like permutation test, Canonical Analysis of Principal Coordinates (CAP), and classification test performed using linear discriminant analysis. The results showed significant differences in the shape of otoliths between populations, reflected explicitly in the morphological feature of the excisura major. The differences in otolith shape were examined from all localities (ANOVA-like, $p < 0.001$, and CAP $p > 0.05$), except those otoliths between Palabuhanratu and Pacitan, thus, a mixed stock may occur in these two locations. These findings were also confirmed by the low classification success rate using LDA 44.26%. These results suggested that at least two distinct stocks potentially contributed to the fishery, impacting species management and conservation.

Chen *et al.* (2021) tested stock structure of *Gymnocypris selincuoensis* that may be distinguished using otolith shape from three rivers and eight lakes in northern Tibet. Shape indices (SDs) (form-factor, circularity, rectangularity, ellipticity, roundness, surface density and aspect ratio) and normalised Elliptic Fourier descriptors (NEFDs) were analysed by multivariate statistical procedures. To examine the differences of otolith shape from different sites, metric multidimensional scaling and a linear discriminant analysis were used for the SDs and NEFDs. The results of the linear discriminant analysis indicate that riverine otolith stocks had higher rates of successful classification compared with lacustrine otolith stocks, and stocks of otoliths from the Chargut Co and the Kyaring Co lakes had the highest rates of misclassification. Although a 70% classification success rate was produced by Bochu Tsangpo, the differences in otolith shape among 11 stocks were not very noteworthy. The information generated in this research can be used as a reference for stock discrimination of *G. selincuoensis* from the Tibetan Plateau. Further research is needed to explore the causes of shape variation.

Schroeder *et al.* (2021) evaluated the existence of distinct population-units of the Brazilian sardine (*Sardinella brasiliensis*) inferred from body geometric morphometrics and otolith shape analyses

using two and three-year-old individuals collected in Rio de Janeiro and Santa Catarina, the two main Brazilian fishery grounds. Univariate and multivariate statistics were performed for the entire dataset and separated by age groups. Re-classification success for combined ages achieved an overall re-classification rate of 84% and 89% for body and otolith signatures, respectively. Moreover, the combination of both techniques improved the overall re-classification success to 93%. However, when both techniques were conducted by age group, it allowed to unravel a more detailed population structure scenario, and a complete group separation (100% re-classification success) was obtained using otolith shape analyses for two-year-old individuals. Both techniques indicated the existence of two different population-units considering the Brazilian geographical extremes of the species' fishing area. Such regional differences are probably related to distinct oceanographic conditions affecting the feeding regime and fish growth. This study clearly shows that *S. brasiliensis* caught off southeast-south Brazil corresponds to two distinct population-units, and recommends a finer regional fishery management for the species.

Al Jufaili *et al.* (2022) investigated scale and otolith morphology and morphometry of Indian oil sardine *Sardinella longiceps* (Clupeidae) using light and scanning electron microscopy from eight different body regions for scales and the right and left otoliths. Scales of the Indian oil sardine show general characteristics of the other studied clupeids that are easily distinguishable from other fish groups, by having striae in the posterior field. The studied cycloid scales of *S. longiceps* were classified into three types based on the overall shape including circular (e.g. true circular and cordate), pentagonal and quadrilateral in the different body regions. The circular shape was the most common shape (87.5%), while the quadrilateral and pentagonal forms constituted 6.25% each. The results also showed that the relative scale size (J-index) plays a desirable contribution in separating the examined populations. The results showed that the mean (or relative) scale size for all the eight regions in the Oman Sea population is larger than the Arabian Sea population. Also, another scale variable, the scale shape index (Si index), demonstrated variation (a mean of 0.86 to 1.1) in different regions of both populations from the Oman and Arabian Seas. Interestingly, here, we found that scale characters of *S. longiceps* not only differ from its other congeneric species, but also differ in the populations from both sides of the Oman Sea (Iran and Oman) and the Arabian Sea. It shows a positive signal for the presence of different taxonomic and management unit in the Oman and Arabian Seas. The idea should be approved by using integrated molecular and morphological traits. The otolith morphology of *S. longiceps* from the Oman and Arabian Seas was more conservative than the scales, which can be due to its function acting primarily as a balance organ and also enhancing hearing. The overall shape of *S. longiceps* otolith was lanceolate, with an elongated morphology and a well-developed rostrum, an ostial sulcus acusticus that opens to the anterior/dorsal margin. These morphological characters are also found in the Iranian population of *S. longiceps*. However, otoliths displayed variation in biometric parameters among two populations and left and right otoliths and the RRL parameter were important characters to discriminate the Oman and Arabian Sea populations. Thus, the structural/biometrical variability of the otoliths may be used for population distinctness, especially in water bodies with various environmental factors, and the otolith has turned out to be a useful tool to track the life history of teleostean fishes in environments with physicochemical gradients.

Otolith 3D shape as tool to stock identification

Quindazzi *et al.* (2021) studied the otoliths of three populations of coho salmon (*Oncorhynchus kisutch*) with manual 2D, automated 2D, and automated 3D otolith measurement methods. The automated 3D method was able to detect an 8% difference in average otolith density, while 2D methods could not. Due to the loss of information in the z axis and the longer processing time, 2D methods can take up to 100 times longer to reach the same statistical power as automated 3D methods. Automated 3D methods are faster, can answer a wider range of questions, and allow fisheries scientists to automate rather monotonous tasks.

Otolith shape combined with other descriptors for stock identification

Singh *et al.* (2022) analysed the variations in the shape and elemental microchemistry of sagittal otoliths in *Channa punctatus* (Bloch, 1793) populations of three different rivers (Gomti, Ganga and Ken) of Gangetic basin sampled at three geographically distant locations (Lucknow, Kanpur and Banda) were analysed. In this study, otolith shape was analysed using shape indices, geometric morphometrics and Fourier analysis and tested through univariate and multivariate statistical procedures. Three shape indices, namely circularity, roundness and rectangularity out of six were significantly different in ANOVA ($p < 0.05$) between the three population groups. Principal component analysis of shape variables in geometric morphometrics and normalised elliptical Fourier descriptors (NEFDs) accounted for 82% and 80% of total variance, respectively. Visualisation of shape variation explained by principal components was depicted in the form of shape deformation grids (relative warps) and otolith contour reconstruction. The elemental concentrations in the otoliths of the three rivers also differed, and elements Sr and Ba, Mn and Pb, and Fe were significantly higher (ANOVA, $p < 0.05$) in the otoliths of river Ken, Gomti and Ganga respectively. Discriminant function analysis (DFA) of the shape variables, NEFDs and otolith elemental concentration clearly differentiated the otoliths of different rivers in scatter plots with isolated distant population groups and 100% classification of all the specimens into their respective river population. The study indicated regional variations in the shape and otolith microchemistry and also that fish from different rivers lived in different environmental conditions. These variations in the present study might be due to geographical isolation and river fragmentation due to the change in the habitat that limits fish movements. Thus, *C. punctatus* of river Gomti, Ganga and Ken sampled at Lucknow, Kanpur and Banda districts respectively existed as three different phenotypic stocks. This study is useful for the development of effective plans for sustainable fisheries management with the aim of conservation of this economically exploited fish in the Ganga basin.

Marval-Rodriguez *et al.* (2022) realised a population-based study concerning otolith shape and genetic analyses to evaluate if *Lutjanus campechanus* and *Lutjanus purpureus* are the same species. Samples were collected from populations in the southwestern Gulf of Mexico and the Venezuelan Caribbean. Otolith shape was evaluated by traditional and outline-based geometric morphometrics. Genetic characterization was performed by sequencing the mtDNA control region and intron 8 of the nuclear gene FASD2. The otolith shape analysis did not indicate differences between species. A nested PERMANOVA identified differences in otolith shape for the nested population factor (fishing area) in morphometrics and shape indexes ($p = 0.001$) and otolith contour (WLT4 anterior zone, $p = 0.005$ and WLT4 posterodorsal zone, $p = 0.002$). An AMOVA found the genetic variation between geographic regions to be 10%, while intrapopulation variation was 90%. Network analysis identified an important connection between haplotypes from different regions. A phylogenetic analysis identified a monophyletic group formed by *L. campechanus* and *L. purpureus*, suggesting insufficient evolutionary distances between them. Both otolith shape and molecular analyses identified differences, not between the *L. campechanus* and *L. purpureus* species, but among their populations, suggesting that western Atlantic red snappers are experiencing a speciation process.

Santos *et al.* (2022) investigated otolith shape and isotopes for stock identification of lane snapper (*Lutjanus synagris*) in the Southwest Atlantic. Fisheries on lane snapper have been growing in importance in the last decades, following declines in other snapper fisheries since the late 1970s, with evidence of overexploitation accumulating since the mid-2000s. Contrasting environmental conditions along the area are potential drivers of stock separation, yet no phenotypic approach has ever been applied to the stock identification of the species in the area. The study employs a multi-proxy approach based on otolith shape and isotopic composition to identify lane snapper stocks in the Southwest Atlantic and also investigates whether geographical limits of Large Marine Ecosystems and Marine Ecoregions are suitable surrogates for estimating stock

boundaries, which could be useful for application in stock assessments when stock boundaries are unknown. The spatial heterogeneity evidenced by all proxies used supported the existence of distinct lane snapper stocks, contrasting to previous genetic-based studies. The results reveal a strong influence of environmental and oceanographic conditions as drivers of stock separation. The Marine Ecoregions system was considered a more appropriate surrogate for delineating stock boundaries, representing a fast and feasible approach to facilitate stock assessments in other data-limited fisheries.

Maciel *et al.* (2021) evaluated spatial segregation of adult and juvenile stages of *Genidens barbus* from specimens collected from five coastal areas off Brazil (Paraíba do Sul River, Guanabara Bay, Itapanhaú River mouth, Paranaguá Bay), Argentina and Uruguay (La Plata Estuary) from otolith composition (edge *vs.* core: Mg/Ca, Mn/Ca, Zn/Ca, Sr/Ca, Ba/Ca) by LA-ICP-MS and Elliptic Fourier analysis. Fisheries of this diadromous catfish have largely collapsed in the southwest Atlantic coastal region due to overexploitation. An understanding of population structure is now critically needed for improved management strategies for this endangered species. PERMANOVA based on otolith edge chemistry showed significant differences ($p < 0.05$) between all sites, except Itapanhaú River and Paranaguá Bay. Shape, by comparison, found significant differences between all sampling sites, except Guanabara Bay and Paranaguá Bay, and Itapanhaú River and Paranaguá Bay. Discriminant analysis cross-classification success based on chemistry ranged from 33.3 (Paranaguá Bay) to 100% (La Plata Estuary), and 66.7 (Paranaguá Bay) to 100% (La Plata Estuary) for otolith edges (mean = 61.3%) and cores (mean = 78.9%), respectively. For otolith shape, the jackknifed rate (mean = 45.9%) was relatively low for all sites (32.1–44.7%) except La Plata Estuary (67.6%). Although otolith shape was not found to be particularly useful, otolith microchemistry supports the presence of different management units. The results revealed that on a small geographic scale (similar to 300 km) microchemistry might not be efficient to discriminate between some sampling sites.

Cabasan *et al.* (2021) utilised phenotypic- and model-based methods to discriminate stocks of *Plectropomus leopardus* in 3 LRFFT hubs in the Philippines (Taytay, Quezon, and Tawi-Tawi). Phenotypic variation among sites was assessed using shape descriptors and landmark data of otoliths, while patterns of connectivity were inferred from a dispersal model of coral grouper larvae. Inferences suggest (1) the presence of distinct phenotypic stocks and (2) limited larval connectivity among sites. There was an inconsistency with how otolith shape discriminated stocks. While shape descriptors identified Tawi-Tawi as a separate unit, landmark data differentiated Quezon from the other sites. This suggests that different processes may influence otolith shape, thereby presenting a caveat when using otolith morphometrics in stock discrimination. Meanwhile, the dispersal model showed that Quezon and Tawi-Tawi distribute larvae primarily to the West Philippine Sea and Celebes Sea, respectively. Although the model showed that Taytay supplies larvae to both Quezon and Tawi-Tawi, these connections were weak. Model inferences showing all sites as important larval sources to different reefs, coupled with the presence of distinct phenotypic stocks based on otolith shape, suggest that each LRFFT hub is an independent management unit. Thus, identifying key drivers of stock decline is crucial in developing site-specific fishery management approaches.

Jonsson *et al.* (2021) examined the effect of growth rate on the relationship between otolith shape and cod ecotypes (using the *Pan I* genotype as a proxy for ecotype), 826 archived sagittal otoliths collected over a 58-year sampling period were retrieved, the individual growth rate calculated, and otolith shape described using both Normalised Elliptic Fourier transform and Discrete Wavelet transform. Discriminant functions of otolith shape successfully classified ecotype, whether using Fourier or Wavelet descriptors, but only when excluding a heterozygous genotype from the analysis. The otolith shape variability of this genotype lowered the classification success, while otolith shape, in turn, was significantly affected by growth rate and cohort. Growth rate differences previously reported for the ecotypes were present, but were less marked

than expected and indeed, growth rate variance attributable to ecotype identity was dwarfed by cohort- and location-related variance in growth. Such a strong effect of growth rate suggests that cod ecotype discrimination based on otolith shape is sensitive to both temporal and spatial variations in growth, which can mask the effect of ecotype-related growth rate differences on otolith shape.

D'Iglio *et al.* (2021) analysed intra- and interspecific variation in sagitta morphology in three congeneric seabreams, to understand how such variability could be related to the lifestyles of each species. Therefore, the sagittae ($n = 161$) of 24 *Pagellus bogaraveo*, 24 *Pagellus acarne*, and 37 *Pagellus erythrinus* specimens, collected from the south Tyrrhenian Sea, were analysed using scanning electron microscopy and a stereomicroscope to assess morphometric features, variability between otolith pairs and the external crystalline structure of the sulcus acusticus. Statistical analysis demonstrated that, between the species, variability in sagittal otolith rostral length growth and sulcus acusticus features, correlated with increased fish total length and body weight. Moreover, slight differences between otolith pairs were detected in *P. acarne* and *P. erythrinus* ($P < 0.05$). The results confirm changes in otolith morphometry and morphology between different congeneric species and populations of the same species from different habitats.

Couillard *et al.* (2022) used otolith morphometry combined with microchemistry to generate new information on the diversity of spawning components was assessed in declining spring-spawning Atlantic herring *Clupea harengus* in the St. Lawrence Estuary (SLE), Restigouche Estuary and adjacent areas (RE), and southern and northern Gulf of St. Lawrence (SGSL and NGSL). Mature Atlantic herring (6–10 years old) were captured during the spring spawning season from 2013 to 2015 at nine sites with different salinity and temperature conditions along estuarine–marine and southern–northern gradients. Otoliths were measured by image analysis, and various shape indices were calculated. Elemental concentrations (Sr, Ba, and Mg) in the otolith cores were measured using laser ablation inductively coupled plasma mass spectrometry. Natal groups were inferred by hierarchical clustering analysis conducted on shape and microchemical variables separately or combined. Both types of analysis indicated the presence of two main natal groups: one predominant in the SLE and the other predominant in the RE. Proportions of fish having the characteristics of each natal source decreased with increasing distances from the head of the SLE and RE, respectively. Shape analysis provided higher discrimination in the SLE/NGSL, revealing temporal stability of a specific “pygmy” morphotype in the SLE that was previously described in the 1980s and 1990s. Microchemical analysis was more discriminating in the RE/SGSL, revealing a group of fish within the RE that had low Sr in the otolith core. Thus, otolith morphometry and microchemistry provided concordant and complementary information on natal groups, and both markers indicated the potential importance of estuaries as nursery grounds for the recovery of spring-spawning Atlantic Herring stocks in the SGSL and NGSL.

Aguilar-Medrano *et al.* (2021) studied the fish assemblage of two contrasting localities within the Gulf of Mexico, the Campeche Bank (CB) and the Perdido Fold Belt (PFB) to (1) analyse and compare the size, weight, trophic, and morphological (fish body and otolith) variation and (2) test if the otoliths morphological variation can be used to estimate the fish-body variation. Fish and otolith size, fish and stomach-intestine weight, and stomach content were determined and geometric morphometric methods were applied to the fish body and otoliths. The fish body shows a relationship with the weight measurement and the otolith size, no differences in these variables were found between the two localities. In both localities, Annelida, Mollusca, Osteichthyes, and Crustaceans are the most common preys; however, the CB assemblage was more diverse in both diet and morphology and six trophic groups and 18 ecomorphological groups were determined, while in PFB, four trophic groups and 13 ecomorphological groups were determined. Finally, according to the morphological comparison in both localities, the otolith variation can be used to estimate the fish-body morphological diversity. This study reports important information in the understanding of the links between morphological structures and highlights

the importance of the otoliths as a morphological variable that reflects a wide range of ecomorphological variation of the fish body.

Shape of other calcified structures as tool for stock identification

Ching *et al.* (2022) examined the genetic groups of *Sepioteuthis* spp. in southwestern Japan and northern Taiwan was examined using molecular markers, and the life-history traits and statolith morphology of the genetic groups were investigated. A total of 137 and 205 squid individuals from Japan and Taiwan, respectively, were examined. Three genetic groups of *Sepioteuthis* (S. sp. 1, sp. 2, and sp. 2A) were identified based on molecular evidence in both areas, among which *Sepioteuthis* sp. 2 was dominant in Japan (87%), whereas *Sepioteuthis* sp. 1 was dominant in Taiwan (70%). For the same genetic group, the mantle length, average growth rate, and size and age at maturity were greater for the squid in Japan than those in Taiwan. The statolith exhibited a wider lateral dome and longer rostrum for the squid in Taiwan than those in Japan. This study indicated the variations in the life-history traits and statolith shapes of the squid were greater between geographic populations (Japan and Taiwan) than among the genetic groups, which suggested that environmental effects on life-history traits and statolith shape of squid were greater than genetic effects. The results of this study provide insights into the population structure of *Sepioteuthis* spp. in the Northwest Pacific region, which is relevant for the sustainable management of the squid fisheries.

Lishchenko & Jones (2021) applied geometric morphometrics (GM) which is an alternative approach to the “traditional” method of collecting linear measurements and applying multivariate statistical methods to the Morphometric data of biogenic recording structures. Landmark- and outline-based GM methods are suggested to have several advantages over the “traditional” method. Due to the increasing popularity of GM methods in the modern literature, this chapter first compares different morphometric techniques, and then reviews the methods applied to recording structures, with a focus on GM outline-based analyses. It is clear that outline methods have become a popular method of analysis for structures such as otoliths, particularly for the purpose of distinguishing between population components. However, for other structures such as beaks this technique is only in its early stages of application and is more difficult to apply but shows great promise for future studies. The advantages of using a holistic approach, incorporating several techniques including outline analysis for stock identification purposes is discussed

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Otolith chemistry (Contributors: Lisa Kerr, Zach Whitener and Susanne Tanner)

Avigliano (2021) reviewed the methodology and assumptions of using otolith chemistry analysis for stock discrimination to optimize the methodological design. The author identifies that most studies do not validate the assumption that otoliths and water chemistries vary at appropriate spatio-temporal scale and goes into detail about how incorrect environmental assumptions and/or biological processes can lead to misidentification of stocks. The visual guides provided are excellent reference material for better understanding the potential relationships among environmental conditions, life history, and sample design. This article encourages further use and attention on age and growth data, dispersal ability and life history, but further integration of the issue of biology versus management, as well as how the integration of other approaches may assist, is recommended, as most likely there will be no single approach to resolve stock structure.

Fink-Jensen *et al.* (2022) investigated the migration and residency patterns of capelin (*Mallotus villosus*) in West Greenland with ICPMS-derived otolith concentrations of Li, Ba, and Pb taken from core-to-edge transects. Classification suggest that these capelin reside mostly within their home region throughout their lives and the Pb profiles in particular indicate local residency. The Pb levels in the environment are likely the result of local geology and anthropogenic sources and provide strong evidence of segregation of neighboring populations. The authors found that Pb levels were elevated at the otolith edge, indicating that the fish moved in shore for spawning, which is in contrast the other North Atlantic capelin stocks that are migratory on much larger scales. The authors suggest that the West Greenland capelin need a high spatial resolution framework of management that takes locally segregated populations into consideration.

Hüssy *et al.* (2021) used otolith microchemistry and genetic population assignment to investigate the stock mixing of Atlantic cod (*Gadus morhua*) stocks in the Kattegat. ICPMS was used to measure 25Mg, 43Ca, 55Mn, 65Cu, 66Zn, 88Sr, and 137Ba across transects of sectioned otoliths. Cod captured in Kattegat were genetically assigned to either the North Sea or the endemic Kattegat population and otolith chemical fingerprints differed significantly between populations during the larval and pelagic juvenile stage with higher strontium and lower barium and manganese concentrations in the North Sea population than the Kattegat population, indicating that North Sea cod are spawned in the North Sea or Skagerrak and drift into the Kattegat during the early life stages. The two populations were both most frequently distributed in the Kattegat (67%), with approximately 25% of both populations distributed in the western Baltic Sea and less than 10% in the Skagerrak–North Sea. This study demonstrates the usefulness of this approach to infer population-specific connectivity and migration trajectories for individual fish and its potential applications in basic and applied fields of fish ecology and fisheries sciences.

Macdonald *et al.* (2021) used simulations to derive quantitative rules for assessing the spatial and temporal scales of otolith chemical variation in a Bayesian modelling setting. These rules were then applied to empirical otolith chemistry data (elemental: Li, Mg, Mn, Zn, Sr, and Ba; stable isotopes: $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) from nursery-resident age-1 and age-2 Atlantic herring (*Clupea harengus*) from the Icelandic Summer Spawning stock. Bayesian multivariate linear models (BMLM) characterized source populations within the juvenile dataset. These sources were subsequently treated as baseline samples in mixture models that revealed 1) strong evidence for nursery-site fidelity between age-1 and age-2, and 2) multiple nurseries as contributors to a fished age-3 population, irrespective of presumed nursery quality. While additional sampling is required to confirm these results and their relevance for management of herring stocks in Iceland and further afield, they note that the rules and models presented here can be easily adapted for a broader suite of fisheries applications where reliable methods for evaluating differences in multiple attributes among individuals, populations or stocks are needed.

Metetski *et al.* (2021) used ICPMS derived otolith Ca: 43Ca, 55Mn, 85Rb, 86Sr, 138Ba, 208Pb and 66Zn ratios to see if hatchery-reared and wild brown trout (*Salmo trutta*) could be differentiated in the Baltic Sea. A mixed stock fishery occurs at sea and determining the origin of the fish is important considering the depleted state of the wild populations. Correct classification rates of trout parr back to their natal streams/hatcheries was 27–100%, with a mean of 73%, with the 87Sr:86Sr ratio being the most important. The authors also undertook an extensive study of the water chemistry in the same streams to determine underlying drivers of otolith chemistry, as they found significant variation among regions. Classification of parr back to natal stream/hatchery was best in Finnish streams, followed by Latvia and Estonia. This study will be the foundation for future research on the mixed stock fishery.

Milošević *et al.* (2021) investigated the otolith shape and chemistry of European eel (*Anguilla anguilla*) from three locations in the Adriatic Basin, including two rivers in Croatia and a lake in Montenegro, for a total of 62 samples. Otolith morphometric characteristics and shape indices were measured and elemental concentrations Ca: Cd, Cr, Cu, Fe, Mn, Ni, Zn, Ba, Sr, K, Mg, Pb, and Na were derived with ICP-OES and analyzed. Otolith shape was significantly different between riverine and lacustrine samples; canonical variate analysis showed no statistically significant differences in elemental concentrations between riverine and lacustrine samples. However, there was a relationship between sex and Mg concentrations, an element that is often related to physiology rather than habitat. The authors posit that the otolith elemental fingerprint of the European eel is homogenous and independent of ecological conditions.

Moreira *et al.* (2022) investigated population structure of blue jack mackerel (*Trachurus picturatus*) using chemical fingerprints (core and edge) of juveniles (age 0+ and 1+) and adults (age 3+) sampled off the Azores, Madeira, Canaries and Peniche (Portuguese coast) in 2013, 2016 and 2019. Variation among fishing grounds showed significant differences based on Li:Ca, Mg:Ca, Sr:Ca and Ba:Ca ratios. Multi-elemental fingerprints of the otolith core allocated 70% (2013), 43% (2016) and 43% (2019) to their capture location, while 63% (2013), 75% (2016) and 83% (2019) were correctly classified to their capture location based on the edge elemental signature. Based on natal signatures the authors suggest that there is some degree of self-recruitment in the fishing grounds, varying among cohorts, and that the subpopulations are connected essentially by dispersal of larvae/juveniles. Authors concluded that *T. picturatus* populations across the north-eastern Atlantic should be regarded as different population units and manage accordingly and suggest that future works should focus on the identification of natal sources and assess the impact of environmental change on larval dispersal.

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Parasites (Contributor: Ken Mackenzie)

Six publications dealing with the use of parasites as biological tags for population studies of marine fish were published in the year from June 2021 to July 2022, covering a wide variety of host and parasite taxa over a wide geographical range. The target hosts included an elasmobranch plus pelagic, benthopelagic, demersal and reef-associated teleosts. Study regions were Europe, the Southwest Atlantic, the Southwest Pacific, Japan, the Gulf of Mexico and Namibia.

Brickle *et al.* (2021) investigated the stock structure of the benthopelagic Patagonian rock cod, *Patagonotothen ramsayi*, around the Falkland Islands using elemental analyses of otoliths and parasites as biological tags. Analysis of trace elements in the edge of the otolith was extremely well resolved and indicated clear separation of samples from the northern and southern Falklands Shelf and the high seas outside the Shelf. Eighty fish were examined for parasites, all within the length range 20-30 cm and all caught in the same season to reduce the confounding effects of host length/age and seasonality on the results. Twenty-six parasite taxa were recorded, including 9 new host records. Parasites selected as most clearly fitting the biological tag selection criteria were the nematode *Anisakis* sp. and the myxozoan *Kudoa ramsayi*, with the acanthocephalan *Echinorhynchus* sp. having potential if its specific identity and life cycle can be elucidated. Analysing and comparing entire parasite communities from different sampling zones using multivariate techniques showed good separation between the southern Falklands Shelf and high seas samples. The results of the parasite study supported those from the elemental analyses in providing evidence of three separate stocks in the area – northern and southern Falklands Shelf and high seas north of the Shelf. The results also illustrate the value of the multidisciplinary approach to stock discrimination

Espínola-Novelo *et al.* (2021) investigated the spatial and temporal variability of parasite communities in three marine teleost species from samples collected over a period of 22 years from areas off the coasts of Chile and Peru. The fish sampled were two pelagic species – *Engraulis ringens* and *Trachurus murphyi* – and the demersal species *Merluccius gayi*. A high degree of spatial stability was found, but samples from different years were not well discriminated, underlining the need for awareness of the danger of combining data collected in different years or from different host age-classes when using parasites for fish stock discrimination.

Hidano *et al.* (2021) used *Anisakis* type 1 nematode larvae in a stock identification study of the pelagic teleost *Scomber japonicus* off southern Japan. An earlier study using these nematodes as biological tags had identified two separate stocks of *S. japonicus* – one to the east in the East China Sea and the Sea of Japan and the other to the west off the Pacific Ocean coast. The exact species of *Anisakis* found in these stocks had been identified using molecular methods: fish on the eastern side were infected predominantly by *A. pegreffii* and those on the western Pacific side predominantly by *A. simplex* sensu stricto. The present study examined samples of *S. japonicus* from an

intermediate area, the Bungo Channel in the Seto Inland Sea, which opens to the west into the Pacific Ocean. The authors found that 93% of the nematodes found there were predominantly *A. simplex* s. s., but that the prevalence of infection of only 6.8% was significantly lower than in fish caught in either the Pacific Ocean or East China Sea samples at 38% and 75% respectively. From these results the authors concluded that *S. japonicus* in the Bungo Channel may belong to a unique stock different from the two main stocks.

Villegas-Hernández *et al.* (2022) investigated the population structure of the reef-associated black grouper *Mycteroperca bonaci* in three natural protected areas off the northern Yucatan Peninsula in Mexico, using parasite assemblages and host genetics. Two of the areas studied are coastal and the other is around an offshore island. Intestinal helminth parasite communities of fish caught in the three areas were used in the parasite part of the study, and comparisons between localities were made using host fish of the same length. Three distinct parasite communities were observed, among which those most responsible for the differences were four species of adult trematodes and one adult acanthocephalan. This was supported by host genetics, which indicated three separate subunits. Despite the expectation that the island community would be the most distinctive, the eastern coastal locality of Dzilam was the most differentiated in terms of richness and abundance of parasites per host. The authors suggested that this was probably the result of oceanographic-driven low levels of host larval dispersal and adult migration and suggested that fishing regulations at Dzilam should be strengthened to prevent loss of diversity.

The study of Gérard *et al.* (2022) is one of the few examples of a parasite tag study on an elasmobranch host. The metazoan parasite fauna of juvenile starry smooth-hounds *Mustelus asterias* in the English Channel and off the south-west of England was investigated, one of the aims of the study being to examine the potential use of metazoan parasites as indicators of host diet and stock structure. Twenty immature specimens of *M. asterias* were examined and 12 metazoan parasites were recorded. From the stock identification point of view, the most significant result was the observed differences in prevalence and abundance of infection between male and female fish, one of the suggested explanations for this being that the two sexes may forage in different habitats as juveniles. Since there have been conflicting results with regard to the existence of a single biological stock unit of *M. asterias* in the study area, the authors suggested that parasites may help to identify different sub-populations.

A study of the parasites infecting the economically important benthopelagic teleost silver kob, *Argyrosomus inodorus*, caught off the coast of Namibia, was carried out by Amakali *et al.* (2022) in response to this fish being considered for mariculture in Namibia. The main aim of the study was to identify parasites that may cause problems under mariculture conditions, and five helminth parasites were identified as potential problem species. The authors also considered that the results of their study may be used as a baseline for the use of parasites as biological tags for stock assessment of *A. inodorus*.

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Simulation approaches (Contributors: Lisa Kerr and Steve Cadrin)

Spatial simulations continue to serve as an integrative tool (combining information from several stock identification approaches) to promote the application of stock identity in stock assessment (e.g., simulation-estimation testing to determine model accuracy) and fishery management (e.g., Management Strategy Evaluation).

Bosley *et al.* (2021) simulation tested performance of spatially stratified stock assessment models when population structure is mis-specified. They simulated a single uniform population, a single population with spatial heterogeneity, and a metapopulation and tested performance of several model assumptions, including a panmictic population, representing areas as spatially defined fleets, and a spatially explicit tag-integrated model. When population structure was incorrectly specified in the assessment model, parameter estimates were generally unbiased at the system level, but were often biased for sub-areas. Correctly specifying population structure led to better estimates, and mis-specified spatial assessment models performed better than spatially aggregated alternatives. Allowing for flexible parametrization of movement estimates, such as age-varying movement rates, performed better than correctly specifying population structure, and integrating tagging data helped to estimate movement.

Berger *et al.* (2021) explored the consequences of mis-matched management, stock, and population boundaries using a two-area simulation–estimation framework. They concluded that matching management areas with population structure is essential when fishing mortality is disproportionate to vulnerable biomass among areas, growth or maturity are not homogenous among areas, and their connectivity among areas. Bias was greater when management units include multiple populations than when they include a portion of a population, and estimates are even more biased when there is connectivity among areas.

Spatial management strategy evaluation for Atlantic bluefin tuna continues to progress toward adoption of a management procedure that is robust to population structure (distinct spawning populations in the Mediterranean and Gulf of Mexico) and mixed-population fisheries (ICCAT 2021, Carruthers 2022). A wide range of plausible operating models were structured based on stock identity (e.g., two genetic populations) and mixing rates conditioned on genetics, telemetry and otolith chemistry data. Performance indicators include conservation of both populations (e.g., biomass relative to unfished biomass) as well as fishery performance (e.g., regional catch, stability of catch). Although the process has been more complicated and taken longer than expected, it provides a valuable case study for evaluating the consequences of complex population structure and the development of management procedures that are robust to the complexity and uncertainty in population mixing.

In response to a recent synthesis of stock identity of cod off New England, and the finding that current management units are not aligned with population structure (McBride and Smedbol, in preparation), a series of workshops with stakeholders on how to represent the new perceptions of population structure (UNH 2021) and a provisional decision to revise stock assessment units (NOAA 2022), a management strategy evaluation is being developed to test performance of spatial management procedures, including the current management units, and several alternatives (Brothers *et al.* 2022).

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Interdisciplinary approaches (Contributors: Manuel Hidalgo, David Secor, Steve Cadrin and Lisa Kerr)

Using interdisciplinary methods to investigate stock identity is a continuing trend in the field. Below we have summarised applications which involved multiple techniques applied to address questions of stock identity.

Among these we can first highlight several reviews that have compared current available information about a given species from a diversity of techniques. Arretxe-Arrate *et al.* (2021) for instance have combined information from analysis of fisheries data, genetic markers, otolith microchemistry and tagging, among others, to revise the current available knowledge on the stock structure of Skipjack (*Katsuwonus pelamis*), yellowfin (*Thunnus albacares*) and bigeye (*Thunnus obesus*) tuna in the Indian Ocean. Shackell *et al.* (2021) do the similar exercise with the trans-boundary population of Atlantic halibut (*Hippoglossus hippoglossus* L.) across Atlantic US and Canada boundary, combining genetics, tagging and life history variation (including somatic growth rate). The review concluded that the current perspective of spatial structure of this

species is that there are two distinct populations, and within each, there are subpopulations composed of multiple migratory contingents. New interdisciplinary approaches combining environmental and habitat modelling with population genomics to estimate, and sometimes forecast, changes in population structures are referred to as seascape genomics (Jahnke *et al.*, 2022). Certain SNP markers within the genome act as signatures of selection often driven by environmental interactions (e.g. climate change, fisheries induced, pollution etc) (Bernatchez *et al.*, 2017). These are known as outlier loci and the association of these loci to environmental variables can provide information on how populations structures are formed and how they may change based on alterations to their environment (Russello *et al.*, 2011).

As a relatively new technique, few examples of its utilisation within ICES areas exist. Jahnke *et al.* (2022) used genome wide differentiation analyses with a Lagrangian biophysical model to examine how selection, driven by tidal regimes, influence population structures of shore crab larvae (*Carcinus maenas*). The model showed a strong effect of larval behaviour on long-term connectivity, and dispersal barriers that coincided, in part, with different tidal barriers (Jahnke *et al.*, 2022). Genomic analysis showed three clusters representing micro-, meso- and macro-tidal areas. The genetic differentiation was driven by outlier loci which showed strong allelic clines located at the limits between the three tidal areas. Combined, these analyses highlight that tidal regimes act as strong selection forces on shore crab population structures, consistent with larval behaviour affecting dispersal and recruitment success (Jahnke *et al.*, 2022). Mykscoff *et al.* (2021) used a similar approach combining laboratory experiments of saithe egg buoyancy and temperature-modulated development time, genetic field data, and high-resolution oceanographic models to disentangle the mechanisms causing isolation and mixing between the management units and the biological populations of saithe *Pollachius virens* over all known spawning grounds in the Northeast Atlantic. Saithe egg buoyancy and development data were included in an individual-based model to simulate transport. Strong retention of early life stages around Iceland could indicate an isolated population. However, active migration of adults westward from the Norwegian coast is likely counteracting this isolation - no genetic differentiation from other populations was found. Overall, the dispersal modelling supports the genetic analysis, showing a large and well-connected Central Northeast Atlantic population distributed across several management units. Authors stress that this mismatch between population structure and management units can potentially increase the risk for overexploitation of saithe.

Seascape genomics studies outside of ICES areas are relatively more common. In the Mediterranean Sea, Boulanger *et al.* (2021) examined how space, migratory behaviour and environment shape the population patterns of two sympatric fish species with contrasting dispersal abilities; the white seabream (*Diplodus sargus*) and striped red mullet (*Mullus surmuletus*). The study identified outlier loci and disentangled the relative contribution of space, dispersal and environmental variables (climate, marine primary production) on the outliers' genetic structure to test how local adaptations have driven stock structures. Seabream showed a distinct Alboran sea population and panmixia across the rest of the Mediterranean Sea (Boulanger *et al.*, 2021). The mullet revealed additional differentiation within the Mediterranean Sea that was significantly correlated to summer and winter sea surface temperatures, as well as primary production within population specific areas (Boulanger *et al.*, 2021).

Identification of outlier SNPs, associated with a range of environmental variables, can be fed into climate models to forecast population structure specific responses to environmental change. For example, Stanley *et al.* (2018) succinctly combined population genetics to habitat suitability models and climate forecasts to show that increases in sea surfaces temperatures may shift the distribution of several socio-economically important marine species, such as North Atlantic cod and European green crab, towards more northerly areas. Predicting how environmental variables can alter population structures offer an important tool for fisheries managers since it can

highlight which populations are particularly vulnerable to climate change and how stock boundaries may change under various climate scenarios (Benestan 2019).

Berg *et al.* (2021) combined and applied genetics and otolith information (microchemistry and microstructure) to Atlantic herring (*Clupea harengus*) caught at the same locations during spring and autumn to determine the coherence of methods in identifying the most common spawning modes (spring and autumn). Most herring (similar to 77%) had an otolith microstructure and genetic assignment coinciding with the phenotypically assigned spawning season, and only for 8% of spawning herring, the genetic and otolith assignment contradicted the phenotypically assigned spawning season (a similar percentage was found for otolith-typing contradicted the genetic and phenotypical). Although the viability of offspring of herring remains undocumented, this study suggests that the switching of spawning season may contribute to gene flow between herring populations.

Over the last three years, the Atlantic Cod Stock Structure Working Group summarised the available information on stock structure of Atlantic cod in US and adjacent waters (McBride and Smedbol, in preparation). The group followed an interdisciplinary approach, with six topical subgroups: early life history, genetic markers, life history, natural markers, applied markers and fishermen's ecological knowledge. Topical reviews were synthesised to evaluate the scientific support for alternative biological stock structure scenarios. Several mismatches between the current management US units (Gulf of Maine and the Georges Bank) were identified, including phenotypic and genetic heterogeneity within management units and extensive dispersal and movement between management units, and a mixed-population fishery in the southwestern Gulf of Maine. The group identified a biological stock structure that includes five biological populations in US waters: 1) Georges Bank; 2) southern New England stock; 3) western Gulf of Maine and Cape Cod winter spawners; 4) western Gulf of Maine spring spawners; and 5) eastern Gulf of Maine. In response to these findings, stock assessment units are being revised as 1) Georges Bank; 2) southern New England stock; 3) western Gulf of Maine and Cape Cod winter and spring spawners; 4) eastern Gulf of Maine (NOAA 2023).

In 2021, the Workshop on stock identification of West of Scotland cod (WK6aCodID) convened to recommend the most plausible scenario of population structure for stock assessment and fishery management advice (Albertsen *et al.* 2022). The review considered geographic variation and movements of cod life-stages inferred from genetic analyses, scientific surveys, fishery data, tagging, and otolith microchemistry and shape. Based on the review, several population structure scenarios were hypothesised (including the scenario assumed in the current advisory unit), and the plausibility of each scenario was evaluated. Practical implications of the most plausible scenario, including the derivation of a catch time-series, were considered to form recommendations for benchmark stock assessment workshops. Given linkages of the inshore and offshore subpopulations to cod in Division 4.a, it was recommended to combine the North Sea and West of Scotland cod assessments in a future benchmark.

Medeiros-Leal *et al.* (2021) tested the hypothesis that the European conger population off the Azores region represents a fishery management unit. They analysed movement patterns, length composition, and bathymetric distribution fishery and survey data. Abundance peaked at 300–600 m depths, with larger individuals around seamounts. Tagging data showed a strong sedentary behaviour. These results supported by previous genetic and otolith studies indicated that the stock in the Azores may represent a self-contained population.

Sheehan *et al.* (2021) described the international sampling program developed by ICES to determine continent of origin and biological characteristics of Atlantic salmon collected at West Greenland in 2018. Approximately 12% by weight of the reported landings were sampled. Sampled or processed data include length, weight, freshwater and marine age from scale samples, as well as continent and region of origin from genetic analysis of tissue samples. Fish were also

examined for fin clips, external marks, external tags, and internal tags. Adipose-clipped fish were sampled for coded wire tags. Since the mid-1990s, a large proportion of the harvested stock was of North American origin (83% of the 2018 fishery) with a smaller contribution of European origin. A total of 5508 tag recoveries in the Greenland fishery have been reported since 1963, and the 142 tag recoveries since 2003 suggest that most fish (73%) are of North American origin.

Bradbury *et al.* (2021) determined the region of origin and long-distance oceanic migration from 3891 archived samples of Atlantic salmon from 1968 to 2018 using range-wide genetic assignment, supported with long-term tagging data. Historical and recent tagging studies suggest that trans-Atlantic migrations of salmon are infrequent, except for feeding migrations to Greenland by some North American and European populations. Genetic assignments support the importance of the Labrador Sea as an aggregation area, where 73% of all genetic groups were detected. Salmon from six European groups were identified in the Northwest Atlantic, and six North American groups were detected in samples from the Faroe Islands. Estimates of transatlantic migration distance averaged 3861 km for North American salmon and 2889 km for European salmon.

Combining different information emerging from the otoliths (stable isotopes, otolith microchemistry, otolith shape or microstructure) was one of the first multidisciplinary approaches performed, and it is still one of the most observed in the literature. Nazir and Khan (2021) develop a comprehensive review of all the diversity of factors influencing the otolith chemistry and shape. Among the recent studies, Morales-Nin *et al.* (2022) discriminated European hake using otolith shape and microchemistry at two geographical scales within the Mediterranean: geographical sub-areas defined for fishery monitoring and stock assessment, and (2) three larger units defined by genetic variation (Western Mediterranean, Adriatic-Central Mediterranean, Eastern Mediterranean). Shape analysis was based on a large sample of juvenile hake ($N=1,656$) from forty sub-areas, and a subsample ($N=154$) from ten sub-areas was analyzed for otolith shape and microchemistry. Discrimination was relatively poor but better at the sub-area scale, and best when both shape and microchemistry were combined. Results are consistent with the hypothesis of a continuous gradient of population structure with no distinct boundaries. The same combination of techniques was applied by Coillard *et al.* (2022) to assess the spawning components in declining spring-spawning Atlantic herring (*Clupea harengus*) in the St. Lawrence Estuary, Restigouche Estuary and adjacent areas, and southern and northern Gulf of St. Lawrence. The two analyses indicated the presence of two main natal groups: one predominant in the St. Lawrence Estuary and the other predominant in the Restigouche Estuary. Proportions of fish having the characteristics of each natal source decreased with increasing distances from the head of each area. Each technique provides higher discrimination in different areas, otolith shape driven by the temporal stability of a some morphotypes, while microchemical analysis revealing a group of fish that had low Sr in the otolith core. The two techniques provided consistent and complementary information on natal groups, and both markers indicated the potential importance of estuaries as nursery grounds for the two stocks of Atlantic Herring in this area. Jenkins *et al.* (2022) combines information of otolith microchemistry and microstructure from spawning sources of King George whiting *Sillaginodes punctatus* populations in the states of South Australia and Victoria (south-eastern Australia). Differences in microchemistry were most evident for elements influenced by physiological processes and attributed to the influence of the different larval growth rates identified for the different areas, supporting that, according to the authors, the areas should be managed separately. Correia *et al.* (2021) analyzed both otolith microchemistry and stable isotopes from Atlantic chub mackerel (*Scomber colias*) collected off the Portugal mainland and in the Atlantic Oceanic Islands selecting only individuals of 2-year old at each location. Overall reclassification accuracy rate was 80 % suggesting that fish passed through their life enough time in heterogeneous water masses to have a distinct chemical signature. Moreover, fish from the islands were fully discriminated (100 % reclassification rate). Authors stress that

four population-units of *S. colias* in the NE Atlantic, namely Azores, Madeira, Canaries and Portugal mainland as their results provide support for treatment of these fisheries as different regional management units. Sanchez *et al.* (2022) used otolith chemistry analyses and stable isotopes from long-lived, slow-growing demersal fish snowy Grouper *Hyporthodus niveatus* one along the eastern Atlantic coast and three regions in the Gulf of Mexico to understand population connectivity throughout the region by analysing three life stages in the otolith: core (first-year deposition), edge (most recent deposition), and life (mean of lifetime deposition). Dissimilar element signatures were evident across regions for all three life history stages, with regional patterns in individual markers following expected differences in environmental chemistry based on the geographic location of dominant mesoscale features and associated physicochemical conditions. Authors stress that the close adherence in regional patterns of otolith chemistry with expected environmental chemistry indicates that movement of individuals is limited. Maciel *et al.* (2021) integrated information from otolith chemistry composition in the edge vs core and Elliptic Fourier analysis to evaluate spatial segregation of adult and juvenile stages of the diadromous catfish *Genidens barbatus* from five coastal areas off Brazil (Paraíba do Sul River, Guanabara Bay, Itapanhau River mouth, Paranaguá Bay), Argentina and Uruguay (La Plata Estuary). Although otolith shape was not very conclusive, otolith microchemistry supports the presence of different management units. However, their results also revealed that on a small geographic scale (ca. 300 km) microchemistry might not be efficient to discriminate between some sampling sites. Conventional tagging has been found to be combined with different techniques along the last year. Russell *et al.* (2021) compared estimates of connectivity for Mulloway between nursery and fishing grounds from conventional tagging and otolith chemistry. Multinomial Logistic Regression of otolith chemistry data suggested that two of eight estuarine nurseries sampled were important sources of recruits, and estimates of stock mixing were greater than those derived from historic tag-recapture studies. However, estimates were uncertain, possibly from inter-annual variability and flooding during the study period. The comparison demonstrates the need to consider multiple approaches and to interpret results in the context of their limitations. Klein *et al.* (2021) analysed information of the widely distributed demersal shark, *Mustelus mustelus*. While eight species-specific microsatellite markers analyzed and two mitochondrial genes did not reveal any significant genetic structure among neighbouring sites, tagging data demonstrate a remarkable degree of site fidelity with 76% of sharks recaptured within 50 km of the original tagging location. Authors argued that results show that dispersal and stock structure of *M. mustelus* are governed by a combination of behavioural traits and oceanographic features such as steep temperature gradients, currents and upwelling systems across across the Benguela-Agulhas transition zone separating the South-East Atlantic Ocean (SEAO) and South-West Indian Ocean (SWIO) populations.

Taylor *et al.* (2021) sampled bull trout for genetic analysis of 10 microsatellites and tagged 262 with a combination of conventional, radio, and PIT (passive integrated transponder) tags in the Nechako River and several tributaries of the upper Fraser River above the confluence of the two rivers. Genetic stock composition analysis suggested that five populations contribute 80% of the mixture. Radio telemetry confirmed that tributary populations contributing to the mixture estimated by genetic analysis also contained individuals that moved between spawning tributaries and overwintering sites.

Jensen *et al.* (2021a) compared coded wire tagging to phenotypics and genetics (SNPs) for stock identification of Chinook Salmon. They promoted phenotypic stock identification as a less time intensive alternative to coded wire tags or genetics. Their 'rapid phenotypic stock identification' included a visual identification guide for pigmentation (spotting), fin morphology, sexual maturity indicators, and muscle lipid content, and was developed from local ecological knowledge and understanding of life history differences to distinguish two dominant genetic lineages (lower river tules and upriver brights) over three years (2017–2019). Classification accuracy was

up to 90% and consistent over time within fishery seasons and between years but had bias between sexes. Compared with coded wire tags, phenotypic stock identification methods were more rapid but had greater assignment error.

Studies combining genetics and other natural markers displaying information at a shorter spatial scale are increasingly emerging. Zhang *et al.* (2021) use information of three natural tags (i.e., microsatellites, parasites, and otolith nucleus chemistry) in order to describe the population structure of the spinyhead croaker, *Collichthys lucidus*, and provide scientific support for the northern and southern Chinese stocks separation. In many cases, genetics and other markers provide contrasting but complementary information. Cortinhas *et al.* (2022) combines genetics information from mitochondrial DNA with phenotypic (morphometric) analyses for the endemic red crab *Chaceon notialis* in the Southwestern Atlantic (Brazil, Uruguay and Argentina). While genetic analyses revealed only a single panmictic population, with no genetic structuring among the analyzed stocks, morphometric results showed two distinct phenotypic groups (one from Brazil/Uruguay and the other from Argentina), with significant differences in female abdomen width and chela size. The authors strengthen that the management measures applied in each country will influence the population dynamics of the others, and that international cooperation is necessary. Also, in Argentina, Brickle *et al.* (2021) also analysed parasites community with otolith microchemistry to assess the potential segregation of The notothenid *Patagonotothen ramsayi* from the Falkland Islands Shelf and in an area north of the Falkland Islands on the high seas. The separation between the south of the islands and the high seas was the most clear and consistent among techniques while some intermixing during larval dispersal between the Falkland Islands subregions.

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Statistical tools

The increasing availability of large data sets from different countries (e.g. DATRAS) and beyond (Meauroud *et al.* 2021), along with the diversification of statistical models for different fisheries science needs, have improved the use of fisheries-dependent and -independent data. Lindegren *et al.* (2022) compiled and standardized multiple trawl-survey data sets on a widely distributed marine fish species, European sprat (*Sprattus sprattus*), and used a range of statistical tools to assess whether the current management boundaries adequately account for potential population structuring. Their findings are consistent with recent genetic studies of sprat, indicating reproductive isolation between the Baltic Sea/Kattegat and a larger cluster containing the North-, Irish, Celtic Sea, and Bay of Biscay of likely phenotypic subgroups.

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Emerging issues (Contributors: Lisa Kerr, David Murray)

Climate change and marine population structures

Climate change is shifting the productivity of fisheries, resulting in many species and populations moving out of traditional management zones into new areas, and the expectation is to considerably increase in the coming decades (Pinsky *et al.* 2018). Changes to variables, such as temperature, salinity, current patterns, influence evolutionary processes such as gene flow, drift and selection, each of which contributes to shaping species population boundaries (Boulanger *et al.*, 2021). Fundamentally, climate change can be viewed as a driver pushing populations to avoid extinction by either migrating away from areas experiencing climatic change or evolving life-history traits to adapt to climate change (Boulanger *et al.*, 2021). Environmentally induced evolution of life-history traits can be plastic (i.e. quick and reversible) and/or fixed, with research highlighting either type of evolution can be transferred to subsequent generations (Naish and Hard, 2008). Subsequently, either migratory behaviour or adaptation of life-history traits may alter current marine population structures and necessitate temporal re-assessments after specific climate events (i.e. El Nino, frequency and strength of Heatwaves, or extreme weather events, among others).

The field of stock identification will need to confront the impact of climate change on the performance of certain stock identification methods, potential changes in mixed stock composition, and shifting distributions relative to management boundaries. Otolith-based methods may be impacted by climate impacts such as warming. The uptake of certain stable isotopes (e.g. O-18) and trace elements (e.g. Sr, Ba) in the otolith are influenced by temperature resulting in possible changes in the stability of baselines for use in mixed stock composition analysis. Likewise, the application of otolith growth increments for stock identification will be sensitive to warming as fish growth is impacted. Furthermore, parasites have been connected to specific environmental conditions (Palm, 2011) and their application for stock identification will need to be considered in the context of environmental change. Beyond current patterns expected changes, pelagic larval duration and location of spawning aggregations will change due to warming altering the dispersal to settlement process. These changes highlight the need for continued baseline sampling to monitor and adjust for these changes in baseline signatures over time. It is important to understand the sensitivity of each stock id marker to climate change to understand if the tool is accurately reflecting stock structure or reflecting increased environmental heterogeneity.

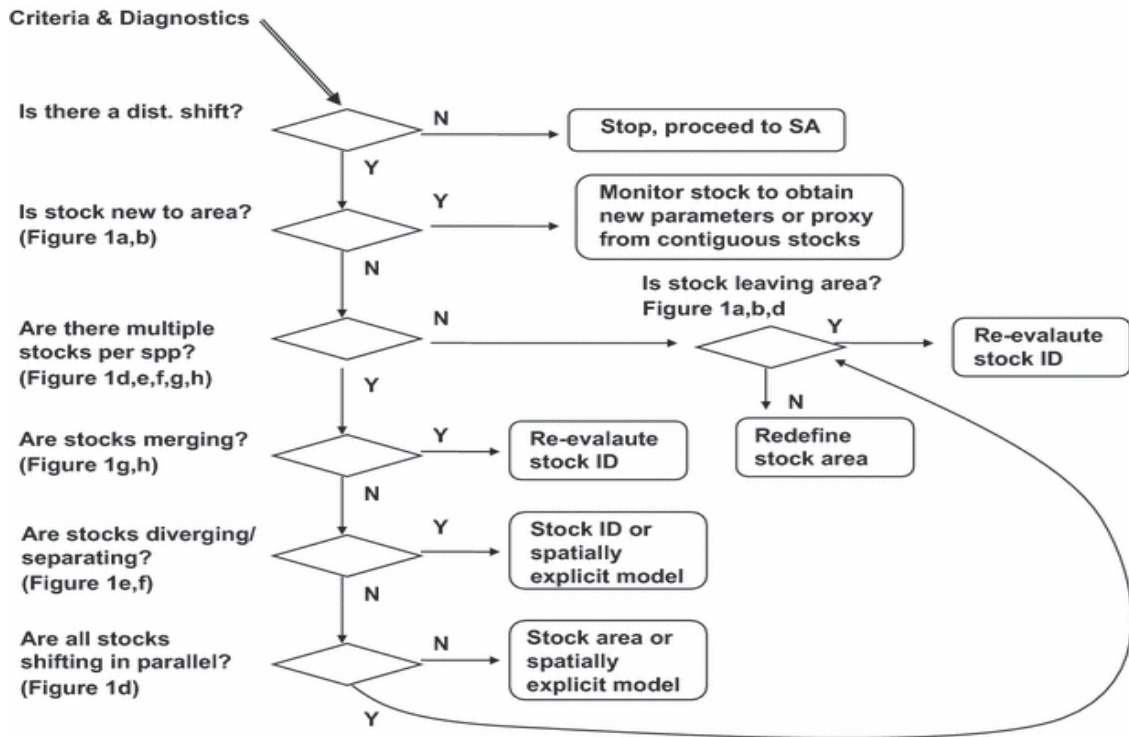


Figure A4.3. Decision tree for how to address whether a shift in stock distribution needs to be considered in an assessment context. Y = yes, N = no. SA = stock assessment, ID = identification, spp = species. From Link *et al.* 2011.

Research on the impacts of ocean warming on fish populations has focused on documentation of distribution shifts. Interpretation of these apparent shifts in distribution has typically been attributed to shifts (both geographic and bathymetric) in the species range due to thermal limits with little consideration of the potential for shifts in the relative abundance of populations along a spatial gradient. We can expect that populations that are genetically equipped (i.e. heat tolerance) to cope with warming and other impacts of climate change may be increasing in their relative abundance, changing stock composition in areas of mixing. Link *et al.* (2011) acknowledged this possibility of different drivers underlying apparent distribution shifts and how it could be dealt with in the context of climate change (Figure A4.3). Understanding what have been the impacts using temporal variation information in the past can also be used to foresee potential future impacts (e.g. artificial tagging, Shelton *et al.* 2021; genetics, Boulanger *et al.*, 2021). In addition to natural and artificial tags generally in stock ID, considering climate-induced scenarios in spatial population dynamics models and simulations will help to advance on potentially unexpected patterns (Goethel *et al.* 2020), or other scenarios tested under the management strategy evaluation framework (e.g. Jacobsen *et al.* 2022).

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Impact of Offshore Marine Renewable Energy (OMRE) technologies of fisheries population structures

Due to high offshore wind levels and adjacent shallow waters with low tidal stresses, much of the North Atlantic is particularly well suited for Offshore Marine Renewable Energy (OMRE) installations (Maxwell *et al.*, 2022). As such, Norwegian, EU and UK governments have rapidly begun to increase their capacity for harnessing offshore energy in order to reduce its dependency on energy imports and to meet policy targets on renewable energy generation (Maxwell *et al.*, 2022). Implementation of these offshore sites are often subject to extensive environmental impact assessments from various regulatory bodies, yet knowledge regarding impacts of offshore installations on basic ecological processes, such population structures remain limited.

During construction, pylons, concrete foundations and/or energy transfer substations are installed, resulting in soft sand substrate being replaced with artificial hard substrates (Vandendriessche *et al.*, 2015). Previous studies have observed that Installation of structures associated with offshore energy production causes epibenthic cascades, firstly attracting invertebrates quickly followed by species associated with higher trophic levels (i.e. crustaceans, cephalopods, teleost and marine mammals) eventually creating a network of interconnected small artificial reef systems (Causon and Gill 2018). Research regarding the impact of windfarms on biological diversity noted increasing abundances of species such as sea bass (*Dicentrarchus labrax*), pouting (*Trisopterus luscus*) and cuttlefish (*Sepia officinalis*) around established offshore wind farms (Vandendriessche *et al.*, 2014; Degraer *et al.* 2020). These studies highlight that altering areas of the seabed, previously devoid of diversity, creates artificial ecosystems and provides species with a tremendous choice of spawning habitats and feeding grounds beyond their traditional locations.

The inherent choice species make to migrate towards, spawn and feed within certain areas can lead to fine scale genetic differentiation through evolutionary processes such as DNA mutation, genetic drift and natural selection within relatively short time scales (Willi *et al.* 2022). For example, species which attach their eggs to hard substrates (i.e. cephalopods), both eggs and hatched juveniles will experience distinct ecological differences depending on whether adults choose to spawn on artificial offshore structures or natural rocky coastlines (Bloor *et al.*, 2013). This can lead to differences in survival, prey availability and physicochemical conditions (Bloor *et al.*, 2013). Additionally, offshore facilities impede fishing vessels, constraining crossing or circumnavigation, as well as sometimes excluding any fishing operations during operational phases (Schupp *et al.* 2021). All of the above-mentioned variables can result in incremental changes to gene flow within regions, which over time may lead to divergent genetic population structures (Willi *et al.* 2022).

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Annex 5: Summary of presentations on genomic methods for stock identification

Recent Advances and Implementation of Genetic Stock Identification

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The Issue: Lack of accurate fish stock identification

Fish stock identification is an essential prerequisite for fisheries stock assessment (1). The central fundamental weakness that remains in many existing stock assessments is the inaccurate recognition, definition and delineation of 'stocks' for data collection and assessment. Traditionally, exploited stocks have been defined, assessed and managed according to geographical and political features or regions. Such is the case in the northeast Atlantic (FAO Major Fishing Area 27) where the European Union (EU) defines the term 'stock' as 'a marine biological resource that occurs in a given management area' and delineates and names stocks using ICES Statistical Areas (2). It is increasingly evident that the temporal and spatial distributions of most fisheries resources are not aligned to these artificial divisions (3) and that biological populations are more dynamic and complex (4, 5). Whilst delineation by predefined area may be convenient for management and regulation purposes, accurately assessing the status, biomass and sustainable exploitation rates of stocks without knowing their biological (population) composition is impossible. Fisheries data may be confounded, which may mask changes in the abundance of individual populations and lead to biased estimates of population abundance and unsustainable exploitation of smaller populations (6). It is critical to identify the underlying population structure of fisheries resources in order to identify the appropriate level at which to aggregate or segregate data for defining assessment and management units. It is also important to be able to assign individuals in mixed survey and commercial catches to the population or assessment unit to which they belong (6, 7) in order to ensure the validity of data for inclusion in stock specific assessments.

The Solution: Whole Genome Sequencing based stock identification

Genetic assignment methods compare genetic data from individuals to genetic profiles of reference samples from potential source populations to determine population of origin for a given individual (8). Genetic stock identification methods have promised to address the deficiencies in other methods (e.g. morphometric, otoliths, parasites etc), yet until recently few studies have yielded results that have been integrated into effective management (4, 9, 10). Many existing genetic studies have been hampered by high cost, few analysed individuals, inadequate sampling coverage, low numbers of suitable molecular markers, laborious genotyping and low power to detect genetic structure. The advent of High-Throughput Sequencing (HTS) technologies has fundamentally changed the way in which genetic sequence data are generated and it is now possible to generate large Whole Genome Sequencing (WGS) data sets for non-model species, which facilitate the identification of genetic loci with high discriminatory power for specific population differentiation questions (11, 12). This is a more comprehensive approach than other commonly used approaches which rely on sequencing a subsection of the genome in the hope of finding informative genetic markers, e.g. Restriction site-associated DNA sequencing (Rad-Seq). Such non-exhaustive approaches may fail to identify important differentiation indicative of population structure and incorrectly conclude panmixia between what are in reality different

populations. This is a potentially dangerous outcome and may lead to the unknown promotion of unsustainable exploitation of smaller populations.

The WGS approach for commercial fish species has been pioneered by Professor Leif Andersson's research group in Uppsala University, Sweden on Atlantic herring (*Clupea harengus*) through the ERC funded *BATESON (Dissecting genotype-phenotype relationships using high-throughput genomics and carefully selected study populations)* project (ERC Advanced Grant, LS2, ERC-2011-ADG_20110310), and the subsequent Norwegian funded *GENSINC (GENetic adaptations underlying population Structure IN herring)* project (Research Council of Norway project 254774). These projects have shown that the WGS approach is the only approach that is capable of identifying the true extent of the genetic differentiation between different populations of marine fish such as herring. The majority of the herring genome shows no differentiation between multiple populations across the entire distribution of the species. However, through WGS hundreds of genes contributing to adaptation show that herring populations are highly structured and display a significant level of local adaptation (11). Recognition of this is key to the accurate identification of populations and consequently delineation of stocks for the purposes of stock assessment, which can lead to development of sustainable management.

The Proof of Concept: Resolving current stock identification issues & incorporation into assessments

The data gleaned in these projects has been used in an applied manner through the EU funded project 'Herring in Divisions 6.a, 7.b and 7.c: Scientific Assessment of the Identity of the Southern and Northern Stocks through Genetic and Morphometric Analysis' (EASME Service Contract EASME/EMFF/2017/1.3.2.1/SI2.767459). The primary objective of this study was to assess the identity of herring stocks to the northwest of Ireland and west of Scotland (ICES Divisions 6.a, 7.b-c) in order to develop genetic profiles of the northern (6.a.N autumn spawning) and southern (6.a.S, 7.b-c) stocks, which could be used to discriminate the two stocks during times of mixing (13, 14). To this end the most informative genetic markers were selected from the aforementioned WGS projects and used to analyse a large set of baseline spawning samples ($n = c. 4,800$) which represented the herring populations from around Ireland and Britain. An assignment model was developed for the populations found in ICES Division 6.a, 7.b-c and was used to split 8 years of Malin Shelf Herring Acoustic Survey (MSHAS) samples (2014–2021, $n = 5,725$). The analyses confirmed that the existing delineation of the stocks, based on geographic and statistical divisions, was inappropriate and consequently input data for the assessment were confounded. This resulted in the development of baseless catch advice and potentially unsustainable management over multiple years. Accurate splitting of the survey data into its constituent populations has enabled separate assessments to be developed for the stocks and this has recently been accepted by the Benchmark Workshop on North Sea and Celtic Sea stocks (15) and incorporated into the assessment of these stocks at HAWG (16).

Another example where a genetic stock identification method was applied are herring stocks in the North Sea, Skagerrak, Kattegat, and western Baltic Sea where stock splitting was historically conducted on a combination of otolith microstructure, otolith shape analysis, and vertebral counts. For the assessment of herring stocks catch data and survey estimates have been split into North Sea Autumn Spawners (NSAS), including the Downs winter spawners, and the Western Baltic Spring Spawners (WBSS) only. The abundance of other stocks in the assessment areas have been considered negligible. A detailed description of the applied genetic stock identification method is presented in Bekkevold *et al.* (Submitted). In contrast to the previous splitting methods, the genetics allow for a much more detailed small-scale population identification resulting in eight genetic distinct populations (Table A5.1). The genetic method was applied the first time for the 2022 assessment of these stocks. However, the detailed population identification raised several issues for the transition from previous methods to genetic assignments in the 2022 update

assessments. The new genetic information revealed that more herring stocks are present in the assessment areas than previously accounted for. Further, all stocks are utilizing larger parts of the assessment areas than previously modelled. Two options were considered for transitioning from the discontinued methods to genetic samples as the basis for estimating stock compositions.

The first option, which is preferable in the long term, was to split catches and surveys directly by genetic information into genetic NSAS-Downs, genetic WBSS, and other genetic stocks. In the short term, however, that would make the 2021 data incompatible with previous years which are based on spawning season. For example, genetic WBSS is only a subset of spring spawners present in the area. Moreover, this option would either leave parts of the catches unallocated to assessments or would require changes to herring assessments in several working groups.

Ideally, future work can move the assessments towards corresponding to the genetic populations, reflecting the relevant reproductive units. However, such changes would require corrections of data back in time, and close coordination between the assessments of all herring stocks. This was determined to be outside the scope of an update assessment and should be subject to the thorough peer-review of a benchmark. Instead, it was decided to keep the update assessment as consistent with the procedure decided at the last benchmark as possible. To be consistent with previous assessments, genetic stock identification was converted to the assignments that would be expected from the previous methods (Table A5.1). For microstructure, predominantly spring spawning genetic stocks were converted WBSS while predominantly autumn and winter spawning stocks were converted to NSAS. For vertebral counts, genetic stocks with VS lower than 56.1 were converted to WBSS while stocks with higher VS were converted to NSAS. We note that this conversion does not fully correspond to what would be obtained with the previous methods since inter-stock variability and the risk of misclassification is not accounted for. To account for this would require individual observations with both genetic information and vertebrae counts or microstructure, which was not available for the update assessment.

Table A5.1. Overview of genetically assigned distinct populations. Mean vertebral counts (VS) for each genetic populations were estimated based in Norwegian catches in 2021, total number of assigned individuals are presented. For consistency in the assessment, genetic populations were assigned to either North Sea autumn spawners (NSAS) or western Baltic spring spawners (WBSS) based on expected outcome from previously used splitting methods. Norwegian data was split by mean vertebral counts, whereas Danish data was split by otolith microstructure into different hatching season. Mismatch between assigned stocks based on Norwegian and Danish data is presented in *italic*.

Genetic population	VS	Hatching season	Stock assigned	
			Norwegian data	Danish data
North Sea autumn spawners	56.5 (n = 530)	Autumn	NSAS	NSAS
Downs	56.5 (n = 782)	Winter	NSAS	NSAS
Western Baltic spring spawners	55.7 (n = 206)	Spring	WBSS	WBSS
WBSS-Skagerrak	56.8 (n = 172)	Spring	<i>NSAS</i>	<i>WBSS</i>
Norwegian spring spawners	57.1 (n = 194)	Spring	<i>NSAS</i>	<i>WBSS</i>
North East Atlantic (Faroes, Iceland)	56.3 (n = 6)	Autumn	NSAS	NSAS
Central Baltic herring	55.6 (n = 54)	Spring	WBSS	WBSS
Baltic autumn spawning herring	55.6 (n = 23)	Autumn	<i>WBSS</i>	<i>NSAS</i>

Further work utilising the WGS approaches developed for herring has also been undertaken on Horse Mackerel (*Trachurus trachurus*), through an industry funded project, and a panel of genetic markers capable of identifying the different populations has been developed (18). Preliminary deployment of this tool has confirmed the distinctiveness of the North Sea stock and has shown that the current delineation of the Southern stock may not be appropriate (18). Further coordinated wide-scale sampling and analyses are required to progress this work to the stage where it can be considered by ICES in the context of improving the existing stock assessments. More recently work has begun to initiate baseline sampling of Atlantic Mackerel (*Scomber scombrus*) and Blue Whiting (*Micromesistius poutassou*), which will undergo WGS as part of the European Reference Genome Atlas (ERGA) initiative, which is funded under Horizon Europe.

In conjunction with the identification of population structure and the development of informative marker panels for the species above, significant effort has been directed at standardising the sample collection and genotyping processes. To this end the industry has been actively involved in the development of a new Genetic Sampling Tool (GST) with LVL technologies GmbH & Co. KG, Germany (www.lvl-technologies.com), which increases the efficiency and quality of genetic sample collection and ensures standardised samples are collected without disrupting existing sampling protocols. This is essential if genetic sampling is to be adopted on a large scale for analysing fisheries survey and commercial catches. Such an increase in the adoption of this technology also necessitates the development of a standardised approach to processing and genotyping samples, which may be implemented by multiple institutes to analyse samples of the same species collected in different areas, without inter-lab collaboration issues. For example if a single 'universal' panel of genetic markers for herring were available, which contained all informative markers for all herring populations identified through WGS, then it would be possible to develop an assignment model capable of assigning any herring of unknown origin back to its population of origin with a high degree of confidence and without the need to subjectively pre-select a subset of location specific markers to screen the samples with. This would represent a significant advance in the ability to ensure that the most appropriate data is included in stock specific assessments. To this end a new pilot multi-species SNP array (DNA TraceBack® Fisheries platform) has been developed by the Uppsala research team in collaboration with a commercial genotyping service provider. Version 1 of the array contains all the known informative markers, derived from WGS, for herring, horse mackerel and sprat (*Sprattus sprattus*) and the first trials are underway. It is possible to add genetic markers for more species to the array and to ultimately have a single array with all the genetic markers required for performing stock identification on any commercial species in the northeast Atlantic region. A significant benefit is the reduction in the individual cost per species and per sample and the simplification of the coordination of analyses between institutes.

The Future: Widescale implementation for all commercially important northeast Atlantic fish species

The approaches outlined above are universal in their application and may be implemented in any fish species of interest. There are now no technological limitations in the ability to identify what populations constitute stocks, as they are currently defined. At the very least the alignment of populations with these stocks should be investigated to confirm that the bases of current assessments are valid. If not, then the projects above have also demonstrated that large scale genetic stock identification is a tool that can be incorporated into regular data collection programmes and lead to major improvement in the input data for species-specific stock assessments.

There are two major limitations to fully implementing this across a wider range of species; lack of a long term sustained funding source and a lack of standard sampling coordinated by national fisheries institutes. There is a need to start to incorporate standard genetic sampling into the regular biological sampling programmes undertaken on survey and commercial catches. This

sampling should include both baseline samples and potentially mixed samples. Institutes should endeavour to build up archives of samples which can contribute to answering relevant stock identification issues once informative genetic markers become available. This proactive approach would rapidly increase the availability of suitable samples and increase the efficiency of future dedicated stock identification research programmes. In order to undertake the sample analyses there is a need for a sustained funding stream to be developed in close collaboration with the fisheries institutes. The WGS approaches should be used for identifying informative genetic markers and then suitable genotyping approaches be used to enable collaborative projects to be undertaken without any inter laboratory calibration issues.

Summary

- Stock identification is essential for stock assessment.
- Most assessments have inaccurate recognition, definition and delineation of stocks.
- As proven, this may be resolved through whole genome sequencing based genetic approaches.
- There are no technological limitations in the ability to identify what populations constitute stocks.
- The limitations are lack of sustained funding and lack of standard sampling.

Further information

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