



Study to produce an International Manual of Procedures (IMP) to be used in the NAFO Regulatory Area to guide the collection of samples from fisheries products for genetic analysis

European Maritime and Fisheries Fund (EMFF)



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Specific Contract No 15
EASME/EMFF/2019/1.3.2.2

FRAMEWORK CONTRACT
EASME/EMFF/2016/008

**Provision of Scientific Advice for
Fisheries Beyond EU Waters**

**Study to produce an
International Manual of
Procedures (IMP) to be used
in the NAFO Regulatory Area
to guide the collection of
samples from fisheries
products for genetic analysis**

Final Report

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Contents

EXECUTIVE SUMMARY	1
RÉSUMÉ EXÉCUTIF	1
RESUMEN EJECUTIVO	2
1. INTRODUCTION	4
1.1 GENERAL INTRODUCTION TO THE SPECIFIC CONTRACT	4
1.2 OBJECTIVE AND STRUCTURE OF THE FINAL REPORT	6
2. OBJECTIVES, METHODS, PROGRESS AND MAIN RESULTS BY TASK	7
2.1 TASK 1 – LITERATURE REVIEW	7
2.1.1. OBJECTIVES	7
2.1.2. METHODOLOGY	7
2.1.3. RESULTS	8
2.1.3.1. BACKGROUND	9
2.1.3.2. ALTERNATIVE METHODOLOGIES FOR FISH PRODUCT AUTHENTICITY	10
2.1.3.3. DNA-BASED SPECIES ASSIGNMENT	18
2.1.3.4. ONGOING STUDIES AND RESEARCH PROJECTS	21
2.1.3.5. EMERGING TECHNOLOGIES	21
2.1.3.6. CHALLENGES AND MITIGATION	22
2.1.3.7. FUTURE PERSPECTIVES	23
2.2 TASK 2 – MANUAL OF PROCEDURES	24
2.2.1. OBJECTIVES	24
2.2.2. METHODOLOGY	24
2.2.3. RESULTS	25
2.2.3.1. SUB-TASK 2.1 - ANALYSIS OF THE PORTUGUESE MANUAL OF PROCEDURES	25
2.2.3.1.1. TRANSLATED AND REVISED PORTUGUESE MANUAL OF PROCEDURES	25
2.2.3.1.2. CRITICAL APPRAISAL OF THE PORTUGUESE MANUAL OF PROCEDURES	26
2.2.3.2. SUB-TASK 2.2 - PREPARATION OF DESCRIPTIVE ANALYSIS OF FISHING FLEETS	27
2.2.3.2.1. STATUS OF THE SUB-TASK 2.2	27
2.2.3.2.2. INTRODUCTION TO NAFO	28
2.2.3.2.3. NAFO CONSERVATION AND ENFORCEMENT MEASURES (NAFO, 2019A)	31
2.2.3.2.4. NAFO FLEETS/FISHERIES	38
2.2.4. SUB-TASK 2.3 – ELABORATION OF MANUAL OF PROCEDURES	79
3. TIMETABLE FOR THE IMPLEMENTATION	85
4. LIST OF DELIVERABLES	87
5. LIST OF MEETINGS	88
6. LIST OF MILESTONES	89
7. REFERENCES	90

1. EXECUTIVE SUMMARY

Within the scope of the Specific Contract No 15 EASME /EMFF/2019/1.3.2.2/02/15/SI2.807287 FRAMEWORK CONTRACT EASME/EMFF/2016/008, several actions were undertaken with the final purpose of generating an International Manual of Procedures (IMP) on “FISH PRODUCTS SAMPLING FOR DNA TESTING”. This involved three main previous steps: conduction of a thorough Literature Review on the state-of-the-art of the analytical methodologies and related sampling issues; translation of the Portuguese Manual of Procedures (PMP) to English with the necessary adjustments to the EU wide reality and incorporating Portuguese Inspectors’ criticisms and experience in the application of the PMP; and preparation of a Descriptive Analysis of Fishing Fleets operating in NAFO RA. On the basis of these three components, the IMP was prepared. However, further component incorporation, refinement, and improvements were necessary, namely, concerning response to the shortcomings of the PMP document and inspectors’ criticisms as well as detailing sample preparation and DNA analysis procedures for the production of a holistic and integrated IMP, which will be able to serve as an effective guide to all professionals involved in the identification of fish species in the fishing fleets operating in the NAFO Regulatory Area. Finally, it must be stressed that the generated IMP is open to additional changes as experience in the field is gathered and more information is collected. Hence, this IMP is above all other things a working tool for EU inspectors, analysts, other involved personnel, competent authorities, and other sector stakeholders.

2. RÉSUMÉ EXÉCUTIF

Dans le cadre du Contrat Spécifique n° 15 EASME /EMFF/2019/1.3.2.2/02/15/SI2.807287 CONTRAT-CADRE EASME/EMFF/2016/008, plusieurs actions ont été entreprises dans le but final de générer un Manuel International de Procédures (MIP) sur "L'échantillonnage des produits de la pêche pour les tests ADN". Cela a impliqué trois étapes préalables : la réalisation d'une analyse documentaire approfondie sur l'état de l'art des méthodes d'analyse et les questions d'échantillonnage connexes ; la traduction du Manuel de Procédures portugais (MPP) en anglais avec les ajustements nécessaires à la réalité de l'UE et l'intégration des critiques

et de l'expérience des inspecteurs portugais dans l'application du MPP ; et la préparation d'une analyse descriptive des flottes de pêche opérant dans la Zone de Régulation OPANO. C'est sur la base de ces trois éléments que le MIP a été préparé. Toutefois, d'autres éléments ont dû être incorporés, affinés et améliorés, en l'occurrence en ce qui concerne la réponse aux lacunes du document du MPP et aux critiques des inspecteurs, ainsi que le détail des procédures de préparation des échantillons et d'analyse de l'ADN pour la production d'un MIP holistique et intégré, qui pourra servir de guide efficace à tous les professionnels impliqués dans l'identification des espèces de poissons dans les flottes de pêche opérant dans la ZR OPANO. Enfin, il convient de souligner que le MIP généré est ouvert à des modifications supplémentaires à mesure que l'expérience sur le terrain est acquise et que davantage d'informations sont recueillies. Par conséquent, cette MPI est avant tout un outil de travail pour les inspecteurs UE, les analystes, les autres personnels concernés, les autorités compétentes et les autres acteurs du secteur.

3. RESUMEN EJECUTIVO

En el marco del Contrato Específico N° 15 EASME /EMFF/2019/1.3.2.2/02/15/SI2.807287 FRAMEWORK CONTRACT EASME/EMFF/2016/008, se emprendieron varias acciones con el propósito final de generar un Manual Internacional de Procedimientos (MIP) sobre "MUESTREO DE PRODUCTOS DE PESCADO PARA LA PRUEBA DE ADN". Ello supuso tres pasos previos principales: un examen exhaustivo de la bibliografía sobre el estado de las metodologías analíticas y las cuestiones de muestreo relacionadas; traducir el Manual de Procedimientos portugués (MPP) al inglés con los ajustes necesarios a la realidad de toda la UE y la incorporación de las críticas y la experiencia de los inspectores portugueses en la aplicación del MPP; y preparar un análisis descriptivo de las flotas pesqueras que operan en el Área Regulatoria NAFO. Sobre la base de estos tres componentes, se preparó el MIP. Sin embargo, fue necesario incorporar, perfeccionar y mejorar ciertos componentes, sobre todo en lo que respecta a la respuesta a las deficiencias del documento del MPP y las críticas de los inspectores, así como a la preparación detallada de las muestras y los procedimientos de análisis del ADN para la elaboración de un MIP holístico e integrado, que pueda servir de guía eficaz a todos los

profesionales que participan en la identificación de las especies de peces en las flotas pesqueras que operan en la zona de regulación de la NAFO. Por último, cabe destacar que el MIP generado está abierto a cambios adicionales a medida que se reúna más experiencia en la materia y más información. Por lo tanto, este MIP es, ante todo, un instrumento de trabajo para los inspectores de la Unión Europea, los analistas, otro tipo de personal implicado, las autoridades competentes y otros interesados del sector.

1. INTRODUCTION

1.1 GENERAL INTRODUCTION TO THE SPECIFIC CONTRACT

EASME has commissioned the IPMA led consortium (IPMA, AZTI, and IEO) to fulfil the request under the Framework Contract EASME/EMFF/2016/008 on "Scientific advice for fisheries beyond EU waters". The present draft final report refers to the Specific Contract (SC) N° 15 within this framework, aiming at the production of an International Manual of Procedures (IMP) to be used in the NAFO Regulatory Area to guide the collection of samples from fisheries products for genetic analysis.

Indeed, the Northwest Atlantic Fisheries Organisation (NAFO), in its 2019 edition of the Control and Enforcement Measures (CEM), has included the provisions for DNA analysis in an effort to develop a solid approach to fight issues related with species misidentification. NAFO has also emphasized the capacity of inspectors to take samples for DNA analysis as an additional tool in fighting illegal, unreported and unregulated fishing and fishing fraud by vessels operating in NAFO Regulatory Area (RA). Thus, a protocol to guide the collection and the chain of custody process of the samples to ensure the integrity and reliability of the results has been ensured through the preparation of the International Manual of Procedures (IMP) "Fish Products Sampling for DNA Testing".

On the other hand, the EU experience with DNA analysis is at the forefront of the experiences of other contracting parties. As such, as a NAFO contracting party, the EU wanted to build on previous experience and bring forth an international protocol for the collection of samples for DNA analysis in the NAFO RA. This international protocol will be presented for adoption during the 2019 NAFO Annual meeting, in its STATIC Committee.

Precisely, the Specific Contract No. 15 has drawn on previous experience and expertise and put forward an IMP to guide in the collection of samples from fisheries products for genetic analysis. In doing this, it has performed a literature review that comprised already established methodologies and emergent techniques, representing the state-of-the-art of the genomics.

The final report is a key piece in the process of accomplishing the activities programmed for the Specific Contract No. 15, making a balance of what has been done and highlighting the challenges that were overcome in the preparation of the IMP.

In order to fulfil the main purpose mentioned above, the following tasks were proposed and were developed:

Task 1 - Literature review:

Preparation of an updated review on molecular DNA-based analysis adequate to assign species to fish product and how these tools have been used worldwide to support fishing traceability and compliance. This review took into account the most recent advancements, including the identification of ongoing studies and research projects, taking special care in analysing the state-of-the-art in the field. This enabled the identification of the strengths and weaknesses of these techniques and emergent ones that are relevant for consideration in future developments. Additionally, this document contained a section dedicated to identify the practical problems that are faced when implementing the procedures and identify relevant mitigation measures as well as measures to prevent possible future problems.

Task 2 - Manual of procedures:

Development of an International Manual of Procedures (IMP) to support the collection of samples from fisheries products by inspectors during their operation in NAFO vessels. This manual of procedures is meant to be easily applied by inspectors from any NAFO Contracting Party in the context of their duties.

The manual set scientifically grounded principles for the collection of samples on-board fishing vessels. The manual took into consideration the specificities of each fishing fleet operating in the NAFO RA such as stowage plans, volumes of fishing products by species, type of processing presentation, type of fishing gear, and other characteristics deemed relevant. This was essential to ensure that the sampling protocol may be applied in different situations, not only conforming to the best scientific guidelines, but also producing the best results in practice.

This IMP took into account the experience and current practices of the EU MS in the NAFO RA for the collection of samples to perform DNA analysis and on possible extend build on the available experience. For such purpose, the Portuguese Manual of Procedures currently in place was reviewed.

The contractor has met the objectives of this task through undertaking the following specific sub-tasks:

Sub-task 2.1:

Analysis of the Portuguese manual of procedures to identify potential weaknesses to be addressed and the areas still requiring development and improvement in order to produce a Manual universally applicable to any fishing activity in NAFO RA.

Sub-task 2.2:

Preparation of a descriptive analysis of the fishing fleets operating in the NAFO RA, their characteristics and the identification of relevant factors to take into account in each of those for the designing of specific sampling schemas and other specific necessary adaptations to each type of fleet.

Sub-task 2.3:

Production of the Manual of Procedures, taking into full consideration the results from the previous two sub-tasks, therefore expanding in the areas the current manual of procedures does not deliver, and ensuring the implementation of appropriate sampling techniques (e.g. collection process, setting sample dimensions) for each type activity performed in the NAFO RA.

On the basis of these tasks it was possible to have a practical and reliable IMP for the NAFO RA.

1.2 OBJECTIVE AND STRUCTURE OF THE FINAL REPORT

The main goal of this Final Report is to provide an update on the progress made and the main results and outcomes achieved, specifying in detail the work undertaken under the specific Tasks 1 to 2, comprising all Sub-Tasks 2.1-2.3.

In this report we also inform about any problems that have occurred during the course of the SC15, how the Consortium addressed them and the final objectives were achieved.

2. OBJECTIVES, METHODS, PROGRESS AND MAIN RESULTS BY TASK

2.1 TASK 1 – LITERATURE REVIEW

2.1.1. OBJECTIVES

The objective of this Task was to prepare a literature review comprising available studies and methodologies describing the relevance of molecular analysis to reliably assign species names to fisheries products. This literature review provided examples of current application of these techniques in the world. The literature review comprised not only the genomic techniques, but also delved into the possibilities offered by proteomics, metabolomics, and lipidomics. Ongoing projects in this scientific area, practical applications, and case-studies were also included.

2.1.2. METHODOLOGY

This was basically a desk-based Task aiming at finding, revising, and organizing new knowledge available and data. The literature review was performed using scientific peer-reviewed studies, book chapters, and other relevant documents, such as reports and documents from International bodies, including Non-Governmental Organizations (NGOs). These documents were attained through a thorough search of the World Wide Web using Google as search engine and, in particular, taking advantage of the Google Scholar. Moreover, specific sources of scientific publications were screened, namely, ScienceDirect/Elsevier, Springer Verlag, Wiley Publishers, Taylor and Francis Publishers, the Royal Society of Chemistry, and OMICS International. Finally, the reference documents that were used to perform the literature review concerning the studies using molecular analyses to reliably assign species to fisheries products were collected from bibliographic databases of indexed journals such as PubMed (<https://www.ncbi.nlm.nih.gov/pubmed>) or Science Direct (<https://www.sciencedirect.com/>) and also from reports from FAO and the European Commission.

The list of references used for the preparation of this Task is presented in Table 1.

Table 1. List of references used in the preparation of the literature review.

Reference	Application
Cajka et al. (2016)	Book chapter dealing with all recent advances in mass spectrometry for food authenticity testing in an omics perspective
Ellis et al. (2016)	Review study addressing the subject of omics approaches for the detection of food fraud
Mazzeo and Siciliano (2016)	Review study covering the proteomics methods for the authentication of fish species
Mohanty et al. (2019)	Book chapter dealing with omics technology in fisheries and aquaculture
Ortea et al. (2010)	Study on identification of commercial prawn and shrimp species of food interest by native isoelectric focusing
Pinu et al. (2019)	Review study on systems biology and multi-omics integration
Salla and Murray (2013)	Study on matrix-assisted laser desorption ionization mass spectrometry for identification of shrimp
Sobolev et al. (2017)	Review study dealing with molecular fingerprinting of food authenticity

2.1.3. RESULTS

This subsection shows the results achieved on the review of the literature regarding the main issues that had been previously defined in the literature review index as worthy of attention and revision. The following items composed the literature review index:

1. Background;
2. Alternative methodologies for fish product authenticity;
3. DNA-based species assignment;
4. Ongoing studies and research projects;
5. Emerging technologies;
6. Challenges and mitigation;

7. Future perspectives.

These sections enabled a thorough revision of the state-of-the-art of molecular analysis for the purpose of food authenticity testing with a particular focus on fish products.

2.1.3.1. BACKGROUND

The challenges of the XXIst Century comprise an enhanced pressure on resources in order to feed the ever-increasing world population (FAO, 2018), which results in overfishing and expansion of fisheries towards previously unexploited species (Swartz et al., 2010). Illegal, unreported and unregulated (IUU) fishing exacerbate the problems derived from overfishing and mismanagement (Pauly and Zeller, 2016). Additionally, there has been an increase level of consumer awareness of the critical importance of food quality and safety for health and sustainability as species substitution not only decrease food quality, but can sometimes be harmful (Armani et al., 2015). Thus, authentication of fishery products is a central issue for both, sustainable management of resources and human health. Species substitution is a common form of fish fraud, which also include the use of undeclared and/or illegal additives, the addition of glaze water to frozen products, the mislabeling of ingredients, the use of falsified-brand products, and the mislabeling of geographic origin or of production method and/or fishing gear (FAO, 2018).

Species substitution is defined as the unintentional or deliberate misidentification of a fish product. Accidental substitutions may occur when two species have similar morphological features or when ambiguities with the species nomenclature exist and deliberate substitutions often involve the replacement of a high value species by a lower value one for financial gain (Spink and Moyer, 2011). Since 2000, the European Commission (Council Regulation (EC) No 104/2000, 1999) prepared directives and regulations regarding fishery and aquaculture products determining what must be written on the label: fish species, geographical origin, and production method (wild or farmed). Furthermore, the European Food Safety Authority (EFSA) established a detailed scheme of traceability for food (comprising fishery and aquaculture products) and feed companies in order to control food safety throughout the processing and distribution chain (Council Regulation (EC) No. 178/2002, 2002).

The identification of fish species using morphological characteristics is often not straight forward even for fresh fish because some species are very similar, especially at young age (e.g. the Yellowfin, *Thunnus albacares*, and the bigeye, *T. obesus*, tuna or the white, *Lophius piscatorius*, and the black *L. boudegassa*, anglerfishes), which translates into high misidentification rates (Nakamura and Séret, 2002; Laurenson et al., 2008). When it comes to processed products (filleted, canned, ...) the morphological identification becomes almost impossible as anatomical and morphological traits (e.g., head, fins, skin), of great importance for fish species identification, are absent. Thus, analytical methods not relying on morphological characteristics are required for ensuring unambiguous assignment of species to fish product. Moreover, in order to allow for the screening a large number of samples, these methods should be high-throughput and scalable. At present, the methods for species recognition that are based on the use of DNA and protein markers are considered the most suitable methods for fish authentication (Lavilla et al., 2013). Nonetheless, other methods based on proteomics, metabolomics, and lipidomics have made important progresses and deserve to be mentioned and reviewed.

In any case, DNA-based analyses fulfil these requirements and have been shown applicable and affordable for fish authentication. Moreover, as opposed to protein based biochemical analyses (e.g. isoelectric focusing, high performance chromatography or immunoassays), which are based on heat-sensitive proteins, DNA based assays can be applied to heated or highly processed products as, although DNA can also be degraded during the food processing process, this molecule is more thermostable than proteins, and short fragments, valid for authenticity tests, are generally recoverable (Chapela et al., 2007a). Consequently, the development of DNA-based methods to identify fish species is a growing field (Hellberg and Morrissey, 2009) that will allow improving at-sea inspection and surveillance of fisheries products.

2.1.3.2. ALTERNATIVE METHODOLOGIES FOR FISH PRODUCT AUTHENTICITY

The challenges of the XXIst Century comprise an enhanced pressure on resources in order to feed an ever increasing world population and higher levels of consumer awareness of the critical importance of food for health, being quality and safety of paramount importance. For fishery products, this poses specific problems, since there is

a clear pressure toward using undervalued species as other more valued species, especially after processing, and product authenticity has become a highly sensitive issue. Precisely, authentication of fishery products is a central issue for the evaluation of food quality and safety. In the fishery market, the most usual falsification is the replacement of more valuable fish species by inferior ones. Specifically, the identification of processed products by eye inspection becomes very challenging as anatomical and morphological traits (e.g., head, fins, skin), traditionally of great importance for fish species authentication, are impossible to recover after processing. These falsifications are constantly growing as a result of market globalization and, besides affecting product quality, pose also health risks to the consumers (Mazzeo and Siciliano, 2016). Since 2000, EU prepared directives and regulations regarding fishery and aquaculture products determining what must be written on the label: fish species, geographical origin, and production method (wild or farmed) (EC No 104/2000, 1999; EC No 2065/2001, 2001). Furthermore, the European Food Safety Authority (EFSA) established a detailed scheme of traceability for food (comprising fishery and aquaculture products) and feed companies in order to control food safety throughout the processing and distribution chain, as reported in EC regulation No 178/2002 (2002). Furthermore, there have been developments with the EU introducing a much more stringent regulation (EC No 1379/2013, 2013). This regulation dealt with different issues regarding the common organization of the markets in fishery and aquaculture products.

In general, genomics, proteomics, metabolomics and lipidomics are four alternative and in some cases suppletive systems corresponding to biological approaches that are often employed for food fraud detection studies (Ellis et al., 2016). An overview of these different approaches is given in Table 2. The analytical methods for fish authentication that were first put into use were based on the analysis of protein extracts by electrophoretic, chromatographic, and immunological methods (Mazzeo and Siciliano, 2016). More specifically, isoelectric focusing (IEF) using sarcoplasmic proteins has been able to distinguish different fish (Mazzeo and Siciliano, 2016) and shrimp (Ortea et al., 2010). This methodology was considered validated for species identification by the Association of Official Analytical Chemistry (Mazzeo and Siciliano, 2016). IEF profiles of sarcoplasmic proteins from various fish species have been assembled

together in a database by the U.S. Food and Drug Administration (FDA), being accessible in the internet library Regulatory Fish Encyclopedia. However, it should be stressed that the available databases for proteomics, metabolomics, and lipidomics are still not reliable to be used in identification efforts at the species level.

Table 2. Overview of the main approaches used for food fraud detection.

Technique	Description	Classification	Reference
Cytochrome b gene amplification	Amplification of a specific DNA fragment (cytochrome b gene) using Polymerase Chain Reaction (PCR)	Genomics	Chapela et al. (2007b)
mtDNA control region amplification	Amplification of a specific DNA fragment (mtDNA control region) using PCR	Genomics	Quinteiro et al. (2001)
12S rRNA region amplification	Amplification of a specific DNA fragment (12S rRNA region) using PCR	Genomics	Zhang et al. (2006)
Gas Chromatography (GC) mass spectrometry	Molecular profiling system applied to lipids using a MS technique	Lipidomics	Black et al. (2017)
Liquid chromatography (LC) mass spectrometry	Molecular profiling system applied to lipids using a MS	Lipidomics	Black et al. (2017)

		technique		
Nuclear Magnetic Resonance spectroscopy (NMR)		Molecular profiling system applied to lipids using a NMR technique	Lipidomics	Black et al. (2017)
Rapid Ionization Spectrometry (REIMS)	Evaporative Mass	Molecular profiling system applied to lipids using a MS technique	Lipidomics	Song et al. (2019)
Mass Spectrometry (MS)		Molecular profiling system applied to lipids and other metabolomes using a MS technique	Metabolomics/Lipidomics	Cajka et al. (2016)
Isoelectric focusing (IEF)		Method based on protein analysis and already applied to sarcoplasmic proteins	Proteomics	Mazzeo and Siciliano (2016)
Isoelectric focusing (IEF)		Method based on protein analysis and already applied to sarcoplasmic proteins	Proteomics	Ortea et al. (2010)
Ambient Spectrometry (AMS)	Mass	Molecular profiling system applied to proteins	Proteomics/other omics	Black et al. (2016)

	and peptides using a MS technique		
Desorption Electropray Ionization (DESI)	Molecular profiling system applied to proteins and peptides using a MS technique	Proteomics/other omics	Gerbig et al. (2017)
Electrospray Ionization (ESI)	Molecular profiling system applied to proteins and peptides using a MS technique	Proteomics/other omics	Gerbig et al. (2017)
Low Temperature Plasma ionization (LTP)	Molecular profiling system applied to proteins and peptides using a MS technique	Proteomics/other omics	Gerbig et al. (2017)
Matrix-Assisted Laser Desorption/Ionization-Time of Flight-Mass Spectrometry (MALDI-TOF-MS)	Molecular profiling system applied to proteins and peptides using a MS technique	Proteomics/other omics	Stahl and Schröder (2017)
Rapid Evaporative Ionization Mass Spectrometry (REIMS)	Molecular profiling system applied to proteins and peptides using a MS technique	Proteomics/other omics	Black et al. (2017)

Meanwhile, DNA-based methodologies that use the amplification of specific DNA-fragments —cytochrome b gene (Chapela et al., 2007b), mtDNA control region (Quinteiro et al., 2001), and 12S rRNA region (Zhang et al., 2006) using Polymerase Chain Reaction (PCR)— have gained much acceptance. In addition, DNA-barcoding has been also deemed as reliable for fish authentication and the FDA's Regulatory Fish

Encyclopedia has incorporated DNA barcode data (Khaksar et al., 2015). These methods have been shown to be sensitive and specific, even when species are closely related (Santacilara et al., 2015). These were tuna species (*Thunnus* species and *Katsuwonus pelamis*) in food products. However, processing of fishery products may involve industrial operations that affect DNA integrity (e.g. heat treatment, acidic conditions), thus causing non-specific identification. The difficulties in standardizing protocols for DNA analyses are also frequently cited as a potential cause of inconsistencies in results from different laboratories (Mazzeo and Siciliano, 2016). Of course, this could be problematic, leading to regulatory or legal repercussions. Moreover, though DNA analysis costs have been falling, relatively high costs of analysis should also be taken into account (Clark, 2015).

These drawbacks and the necessity of developing high-throughput methodologies have stimulated research into unambiguous biomarkers of authenticity and to set up techniques enabling the screening of many samples with minimal expenditure of time. Precisely, in the last years, within the so-called omics approach, proteomics has significantly contributed to investigate authenticity (Mazzeo and Siciliano, 2016). In the area of fish authentication, progress has been achieved through the classical proteomics that couples two-Dimensional gel Electrophoresis (2-DE) and mass spectrometry. This was followed by methods based on the analysis of peptides and intact proteins by high resolution and tandem mass spectrometry. These methods ushered in a new phase in biomarkers discovery and were applied in evaluating identity of fish-based food (Tedesco et al., 2014). What is more, molecular profiling schemes based on Matrix-Assisted Laser Desorption/Ionization-Time of Flight-Mass Spectrometry (MALDI-TOF-MS) have become an alternative tool to define biomarkers concerning fish authenticity (Salla et al., 2013; Stahl and Schröder, 2017).

More recently, Ambient Mass Spectrometry (AMS) is a new field of analytical chemistry and MS, which seems promising at detecting food fraud (Black et al., 2016). Rapid Evaporative Ionization Mass Spectrometry (REIMS) is one of the most recent forms of AMS and it was developed for medical research purposes. It operates using an electrosurgical knife, bipolar forceps or laser, thereby generating an aerosol (smoke) when cutting into a tissue sample (Black et al., 2017). Results may be obtained near-

instantaneously (2–3 s) and the technique appears to yield semi-quantitative results for solid samples without requiring any form of sample preparation using a liquid solution. Precisely, a very recent study applied REIMS analysis to 478 samples of five different white fish species using an electrosurgical knife (Black et al., 2017). The identification of 99 validation samples provided a 98.99 % correct classification, being species identification achieved near-instantaneously (≈ 2 s), thereby surpassing any other form of food fraud analysis. Additionally, Black et al. (2017) have shown that REIMS can also be applied in differentiating between capture methods of fishery products. Hence, REIMS seems to be an innovative technique to help in the detection of fish fraud (Böhme et al., 2019), being further studies essential. Furthermore, another recent trend involves the miniaturization of the mass spectrometer system in combination with different ambient ionization methods. Indeed, Gerbig et al. (2017) tested for food authentication —chemical fingerprinting of five fish species— three alternative ambient ionization methods: Electrospray Ionization (ESI), Desorption Electrospray Ionization (DESI), and Low Temperature Plasma ionization (LTP). These authors reported a classification accuracy of 100 % in the differentiation of fish species.

All these new methodologies show the large potential held by proteomics and are already delivering valuable and reliable results. Therefore, proteomics must be considered as a serious alternative to genomics whenever ascertaining the authenticity of fish products is of paramount importance.

Besides proteomics, metabolomics and lipidomics are two promising approaches that could enable to distinguish between different fish products. Sometimes there is some conceptual overlap between these two omics approaches. Hence, it should be remarked that metabolomics is the study of metabolomes, that is, small molecular end products of cellular regulatory pathways (Fiehn, 2002). It should be noted that metabolites are much smaller than proteins and smaller than most lipids. Though lipids may be classified as a subset of metabolites, their chemistry is quite specific and they must be treated separately, for instance, requiring different solvents. Therefore, lipidomics has developed as a particular and independent omics field, being defined as the systems-level analysis of lipids and their interactions (Smith et al., 2014). In comparison to genomics and proteomics, lipidomics is still in its infancy. Nonetheless, the extreme

variety of lipid structures and the correlation between this variety and taxonomical groupings mean that lipidomics holds a very promising potential. Indeed, lipids are grouped into eight categories that share common physical and chemical properties (Fahy et al., 2009), being reported more than 38,000 lipid molecules.

For food authenticity and fraud detection, metabolomics and lipidomics have been usually applied to matrices such as alcohol beverages (beer, wine, spirits), honey, olive oil, milk and cheese products, meat, spices, and juices (Cajka et al., 2016). Moreover, in comparison to proteomics, only a few metabolomics or lipidomics studies have also focused on the analysis of large numbers of samples (>100) in order to achieve robust statistical models. This means that there is still a huge knowledge gap concerning lipidomics (and metabolomics) and fish product authenticity. Though it is known that the particular lipid profile of a given fish species is very different depending on being wild or farmed, much more detailed knowledge is needed to make lipidomics a valid alternative to genomics and proteomics. There is some seminal research work that aims to close these knowledge gaps. Namely, the phospholipid profile is increasingly studied for fish authenticity (Black et al., 2017) using multiple analytical methodologies, which comprise nuclear magnetic resonance (NMR) spectroscopy, gas chromatography (GC) and liquid chromatography (LC) mass spectrometry.

The multidimensional mass spectrometry-based short gun method is one of the main analytical platforms in current lipidomic studies. Nonetheless, the experimental operation of such methods is difficult, laborious, and time consuming. A more recent advance applies REIMS with an intrinsic coupled intelligent knife (iKnife). This technology is based on the production of gaseous ions, mostly from lipids and, in particular, from phospholipids. This has already been used in routine clinical trials and more recently has been extended to identify food authenticity because it offers real time analysis (Black et al., 2017). Recently, a method using the REIMS system with iKnife has been developed and optimized to discriminate between salmon and rainbow trout (Song et al., 2019). This efficient method promises to meet a pressing demand, since there are numerous claims that in China rainbow trout is often labeled as salmon. REIMS continues to be investigated and developed, given its potential to be used in other situations, for instance, in the distinction between line-caught and trawler-caught

seafood. Therefore, lipidomics application to fish product authenticity testing is an underdeveloped field that, as such, requires further study and intensive investment in the application of novel analytical methodologies to particular groups of lipid molecules.

The current state of affairs is characterized by a clear prominence of genomics and proteomics in the application to fish product authenticity, since it is buttressed in statistically robust studies comprising large number of species and products and involving challenging situations (taxonomically closed species or sampling from products with substantial degree of processing). Metabolomics and lipidomics hold an unmistakable potential, but remain quite behind the other omics.

2.1.3.3. DNA-BASED SPECIES ASSIGNMENT

DNA-based methods for species assignment consist on identifying variations in the DNA sequences that are unique to each species. These variations can involve base substitutions, insertions or deletions as well as structural variants comprising rearrangements, repetitions or insertion/deletions of larger DNA fragments (Liu and Cordes, 2004). These variations can be present in the mitochondrial or nuclear genomes and affect coding or non-coding regions. The mitochondrial genome presents advantages with respect to the nuclear genome, such as being haploid, presenting high number of copies per cell, or having a high substitution rate (Rehbein, 2013). Yet, for some species, mitochondrial markers are not suitable because of the presence of past introgression events from close relatives, such as in tunas (Diaz-Arce et al., 2016). Although insertion/deletions have been useful to distinguish among even closely related species such as for example *Mytilus edulis*, *M. galloprovincialis*, and *M. trossulus* (Inoue et al., 1995), most DNA-based species assignment rely on nucleotide substitutions.

Almost all DNA-based methods for species assignment rely on a previous amplification step by Polymerase Chain Reaction (PCR) to increase the concentration of the DNA fragment that contains the diagnostic marker(s). This PCR amplification is then combined with a method for screening the amplified diagnostic markers, so that different possible techniques exist, the most common being:

- i) Amplified Fragment Length Polymorphism (AFLP), whereby the amplified region differ between target species, which can be visualized in agarose or acrylamide gel electrophoresis (e.g. (Inoue et al., 1995));
- ii) Restriction Fragment Length Polymorphism (RFLP), whereby the amplified product is digested with restriction enzymes to create DNA profiles that can then be visualized in agarose or acrylamide gels (e.g. (Lin and Hwang, 2007));
- iii) Random Amplified Polymorphic DNA (RAPD), whereby the DNA is amplified using random primers to generate patterns of bands that can then visualized through electrophoresis (e.g. (Lakra et al., 2007));
- iv) DNA barcoding, whereby the amplified product is sequenced, and the obtained nucleotide string is compared against a database that contains the correspondence between species and DNA sequences (e.g. (Lockley and Bardsley, 2000));
- v) Real-time or quantitative PCR (qPCR), whereby the DNA sequences are amplified and quantified simultaneously by measuring the fluorescence resulting either from the binding of a binding dye to the double stranded DNA or that resulting from the binding of the reporter probe to the target DNA (e.g. (Lockley and Bardsley, 2000));
- vi) High resolution melting (HRM), whereby the qPCR amplified DNA product is heated until the two strands of DNA separate, which will be sooner or later depending on the sequence and which can be monitored by the loss of fluorescence (e.g. (Jin et al., 2014));
- vii) Microarrays, whereby many fluorophore-labeled oligonucleotide probes specific to the target sequences are disposed in glass microscope slides, whose signal is measured for species detection (e.g. (Teletchea et al., 2008)).

The selection of the method of choice will depend on several factors (Teletchea, 2009) such as:

- i) the prior knowledge of diagnostic markers for a given species: for some species, standard markers such as the cytochrome oxidase I gene are

available in reference databases (eg. most tuna species (Viñas and Tudela, 2009)), however, for others less studied markers need to be sequenced to determine their diagnostic capacity;

- ii) the amount of samples to be tested: if more than 100 samples will be analyzed, qPCR or electrophoresis assays are preferred to sequencing as, although they require less processing time and result more cost effective the more samples are analyzed;
- iii) the expected presence of closely related species: in these cases, the genetic marker that unequivocally discriminates among species needs to be selected (e.g Armani et al., 2012);
- iv) the knowledge of potential intraspecific variation, in which case the use of a combination of different markers might be necessary (Liu et al., 2017);
- v) the expected DNA quality or quantity to be obtained from the sample.

AFLP, RFLP and RAPD are methods considered obsolete for the purpose of species identification as they rely on visual inspection of electrophoretic gels and a prior knowledge of the expected patterns and are very sensitive to DNA degradation (Tabit, 2016). Among the above cited methods, recently, the popular one is DNA-barcoding, which relies on sequence variations within a short and standardized region of genome (barcode) that can be amplified using species or group specific primers (Hebert et al., 2003). The method has been used extensively for identification of seafood (Griffiths et al., 2014), including processed products (Pardo et al., 2018). In general, markers used for barcoding fish species are based on mitochondrial genes (Cytochrome oxidase I – COI, Cytochrome b – cytb or 12S ribosomal RNA – 12S rRNA) which are amplified using universal primers. DNA barcoding has the advantage of not requiring developing species-specific assays and allowing detection of intraspecific variability. This method is particularly interesting if no prior knowledge of the target species. However, if the main purpose is to detect if the product belongs to one or two species, the most efficient assay are the qPCR and the HRM. Although these methods require a prior investment of time in developing the assay and purchasing species specific probes, they become cost-effective if a large number of samples needs to be assessed. As opposed to sequencing, which requires a few days before the result can be available, qPCR and HRM results

can be obtained within hours. Additionally, both methods rely on a short DNA fragment and are thus less sensitive to DNA degradation. Concerning microarrays, these are suitable when several species (hundreds) need to be detected at the same time, which is unlikely in the case of food authentication. Compared to barcoding and qPCR/HRM methods, microarrays are more sensitive to contamination (Tabit 2016).

2.1.3.4. ONGOING STUDIES AND RESEARCH PROJECTS

DNA-based analyses are currently being used for food authenticity and, in particular, for fish traceability. Analyses based on DNA barcoding, qPCR and HRM are applied at different steps of the process, from vessels, ports, markets (Mariani et al., 2015) supermarkets (Harris et al., 2016) to restaurants (Pardo et al., 2018) worldwide in support of fishing traceability and compliance. Among the projects that are currently developing/using DNA technologies for fish traceability are:

FOODINTEGRITY: <https://secure.fera.defra.gov.uk/foodintegrity/>

LABELFISH: <http://www.labelfish.eu/>

SEATRACES: <https://www.seatraces.eu/publications/>

2.1.3.5. EMERGING TECHNOLOGIES

Although DNA barcoding and qPCR are the most commonly used methods for assigning fish product to species, there are new methodologies that are currently being developed and that could become a game-changer in the future:

- Portable high-throughput sequencing devices (e.g. Oxford Nanopore Minion), which allow the on-site sequencing of DNA for a quick response;
 - o Advantages: Small and portable; relatively inexpensive (two orders of magnitude cheaper than a bench sequencer)
 - o Disadvantages: Requires good quality DNA: high error rate
- Loop-mediated isothermal amplification (LAMP), a method that achieves amplification of the target sequences without the need of a thermocycler and in less than 90 min (Notomi et al., 2015);
 - o Advantages: Rapid, portable; almost no equipment required
 - o Disadvantages: Still incipient and developments needed; requires optimization for each species; can be unspecific
- Microfluidics based high-throughput DNA barcoding (Jiang et al., 2016).

- Rapid; can detect > 20 species simultaneously
- Requires costly assay optimization; species detectability is compromised by DNA quality

2.1.3.6. CHALLENGES AND MITIGATION

Using DNA techniques for species identification has inherent challenges that are impossible or very difficult to overcome even with advanced technological developments:

- i) It does not allow to determine age of the specimen, meaning that it does not allow controlling for catches of young specimens for example;
- ii) It does not allow detecting origin of the catch if no genetic differentiation among locations exist.

There are other challenges associated to DNA barcoding that can be mitigated:

- Database completeness: for DNA-barcoding to be useful, the DNA sequence of the target species should be available in the database; currently, the Genbank database (<https://www.ncbi.nlm.nih.gov/genbank/>) is where DNA sequences are stored; however, there are databases specific for some markers that have been curated by experts such as BOLD (<http://www.boldsystems.org/>) or (SILVA <https://www.arb-silva.de/>) which are preferred for barcoding studies;
- Universal primers used for fragment amplification are not equally efficient for all species;
- Intraspecific variability might induce errors in the species identification if these variants are not known;
- If DNA is too degraded, even short barcodes can be difficult to amplify;
- If contamination have occurred, false positives can be derived;
- If the product is mixed, only the most predominant one will be detected;
- Lastly, this document will identify potential practical problems directly related to the process of collecting and transporting the samples that could undermine the DNA analysis and concomitantly propose some relevant respective mitigation measures;

- If there are hybrids or species with introgressed DNA in their genomes, species identification might fail.

2.1.3.7. FUTURE PERSPECTIVES

Genomics, proteomics, metabolomics, and lipidomics are research fields which have developed tremendously in the last decades, bringing forth new knowledge insights as well as various practical applications. The increasing importance of the omics research and analysis is buttressed in the increasing sophistication of a large group of analytical techniques. In spite of the impressive developments, there are still large challenges, especially in the achievement of effective and reliable applications.

Namely, it should be stressed that most of the data generated over the past 25 years by the various omics platforms have been qualitative or semi-quantitative in nature. While qualitative data can be used to compare similar variables across samples within a single lab, they do not support comparisons across labs or across platforms. Likewise, qualitative measurements do not allow for consistent and accurate integration of multi-omics data where measurements typically have to be done on multiple platforms, across multiple laboratories, over extended time periods (Pinu et al., 2019). Achieving fully quantified omics data allows much more accurate comparison to reference values, more consistent inter-laboratory comparisons, greater reliability, and a more straightforward integration of data (Pinu et al., 2019). Moreover, the combination of the various omics and their data resources is a challenge that could bring large advances in the future. Instead of relying on a single field (for instance, proteomics), all omics could be applied and the generated data integrated in order to minimize uncertainty and enhance reliability, especially in the field of food authenticity. Of course, this requires the application of overarching bio-informatic algorithms based on applied mathematics and computer science (Smith et al., 2014).

It must also be pointed out that there can be issues and caveats that are specific to each of the omics fields, such as the measurement issues associated with molecular and mass spectrometry analysis of food matrices (Burns et al., 2016). In particular, food products present themselves as complex physical and biochemical matrices, the composition of which can be strongly dependant on a variety of environmental factors such as climate,

seasonality, and storage conditions. This reality represents a permanent challenge to the detection of food fraud. For the future perspectives of the omics application to food authenticity testing, the creation and development of MS/NMR/other techniques databases of authentic food samples can be useful for routine verification of authenticity. Another important point will be the development of widely shared standard protocols for MS/NMR/other techniques metabolite profiling of specific foodstuffs (Sobolev et al., 2017).

As conclusion, it must be mentioned that the particular omics approaches hold a great deal of promise for the detection of food authenticity and integrity, especially so when using an integrated omics approach (in tandem with future technological and computational advances). Large benefits could also be accrued from the knowledge and expertise from a wide range of sources, thereby leading to valuable new insights and applications, which, in turn, may induce further technological leaps. These advances may lead to beneficial outcomes for an equally wide-range of areas with complex intrinsic and extrinsic links to global food systems (Ellis et al., 2016).

2.2 TASK 2 – MANUAL OF PROCEDURES

2.2.1. OBJECTIVES

The core objective was to prepare an International Manual of Procedures (IMP) to be used in the NAFO RA to guide the collection of samples from fisheries products for genetic analysis. This main goal was more specifically threefold leading to three Sub-Tasks: (i) To analyse and retrieve valuable elements of the Portuguese Manual of Procedures; (ii) To prepare a descriptive analysis of the fishing fleets; and (iii) To elaborate the IMP on the basis of the results of the previous Sub-Tasks results.

2.2.2. METHODOLOGY

This was also mainly a desk-based Task, but differently from the previous one, drawing from practical and empirical knowledge of the reality of the fishing fleets operating in the NAFO RA and of the template provided by a Portuguese Manual of Procedures, which already structured and organized all the measures and steps for a successful sampling of fish products for genetic analysis. More specifically, the outcome was

based on the collection of knowledge and experience during meetings with experts. These were considered experts due to their practical experience on fishing fleets operating in the NAFO RA and on using the template provided by the Portuguese Manual of Procedures. Moreover, an additional effort of literature revision —yielding the figures in the NAFO fleet section and based on the NAFO SC reports as well as others papers, which have been added to the reference list— and of networking with experts within the organization of the Consortium Partners enabled to deepen and mature the future IMP, ensuring a high level of quality and realistic approach to every detail.

2.2.3. RESULTS

2.2.3.1. SUB-TASK 2.1 - ANALYSIS OF THE PORTUGUESE MANUAL OF PROCEDURES

This Sub-Task is divided in the translation and adaptation of the current Portuguese Manual of Procedures and in the critical revision of the document with the purpose to improve it and make it a truly valuable contribution for the elaboration of the IMP.

2.2.3.1.1. TRANSLATED AND REVISED PORTUGUESE MANUAL OF PROCEDURES

The Portuguese Manual of Procedures previously prepared by the Portuguese governmental department, DGRM (Direção-Geral de Recursos Naturais, Segurança e Serviços Marítimos – General Division for the Natural Resources, Security, and Maritime Services) was duly translated to English taking into account the specific nomenclature of the discussed subjects and the need to adapt to a slightly differing reality of an international nature.

This manual was structured along the following main themes:

- I - Introduction and Objectives;
- II - Definitions;
- III - Types of Sample;
- IV - Kit for the Collection of Samples;
- V - Procedures for Sampling, Collection, Registration, Transport, and Delivery;
- VI - Costs;

- VII - Forms;
- VIII - Certification of the Fishery Inspectors;
- IX – Versions;
- X – Legal References;
- XI – Annexes.

The Portuguese Manual of Procedures, after translation to English, was restructured and revised with the final purpose of constituting a valuable contribution to the IMP.

2.2.3.1.2. CRITICAL APPRAISAL OF THE PORTUGUESE MANUAL OF PROCEDURES

The translated Portuguese Manual of procedures was considered a starting point for the elaboration of the IMP. This had to do with its specific scope and novelty in the Portuguese framework. Indeed, such novelty and absence of previous work entailed that many practice-related issues were not incorporated in the build up of the Portuguese Manual of Procedures. These manuals are always improvable with the progressive trickle down of field experiences in direct contact with the fishers and fleets. The reality forces new adaptations of the manuals and also presents new problems —that were not initially foreseen— that lack solutions, thereby leading to novel approaches and modifications of parts or even of whole procedures.

Among those problems, it should be mentioned:

- Adequate random sampling of different lots;
- Appropriate sampling dimension for any given lot;
- Proper training in handling samples, especially avoiding any cross-contamination.

Hence, all required changes resulted from the intersection of the research of the newest technical reports and scientific studies with the empirical experience made by the Portuguese inspectors in the application of the Portuguese Manual of Procedures (sampled through interviews with 2 Portuguese inspectors with a large experience, including in the application of the Portuguese Manual of Procedures), which, in turn, generated field notes that were collated in internal reports to the competent Portuguese Authority in the field (Direção-Geral de Recursos Naturais, Segurança e Serviços

Marítimos – General Division for the Natural Resources, Security, and Maritime Services). Whenever a procedure was revealed as empirically inviable, alternative (more viable) solutions were put forward on the basis of the most recent literature.

The issues previously mentioned led to the importance of the conduction of a critical appraisal of the Portuguese Manual of Procedures, taking into account some gathered information of the application of the Manual to the reality of Portuguese fisheries. There are also doubtful issues, such as the number of samples of the lot to collect and its relation to the quantity range of the fishery products' lot.

These issues were until the date of delivery of the Draft Final Report debated and some solutions were achieved. Namely, a formula for the estimation of the sample dimension was proposed and incorporated in the International Manual of Procedures, thereby replacing the Table that correlated sample dimension with the tonnage of the fish lots. There were also adaptations of the Manual of Procedures that were specifically done to meet the kind of fisheries of the particular NAFO RA, thus incorporating all the information conveyed by the Descriptive Analysis of Fishing Fleets, which is discussed below. Finally, a section comprising treatment of the samples and DNA analysis procedures was included in order to cover also aspects that are relevant for all professionals involved in the fish species identification activity.

2.2.3.2. SUB-TASK 2.2 - PREPARATION OF DESCRIPTIVE ANALYSIS OF FISHING FLEETS

The Sub-Task 2.2 encompasses a detailed and thorough analysis of the fishing fleets operating in the NAFO RA. This will be a major contribution to the preparation of the IMP, since this document will deliver the knowledge of the reality that exists in the sector and in the particular geographical area that are the addressees of the IMP. Indeed, this descriptive analysis will guide in the adaptation of general principles to the practical realities found in the fishing fleets.

2.2.3.2.1. STATUS OF THE SUB-TASK 2.2

This subsection shows the progress achieved on the review of the literature regarding between others, fishing fleets operating in the NAFO RA, type of fishing gear, catches -

volumes of fishing products by species, legislations about as stowage plans, type of processing presentation. Identification of relevant factors to take into account in each of those for the designing of specific sampling schemas and other specific necessary adaptations to each type of fleet.

- 1 – Introduction to NAFO;
- 2 – NAFO Conservation and Enforcement Measures;
- 3 – NAFO Fleets/Fisheries;
- 4 Literature.

This task has been completed, thereby reaching a highly detailed and refined level in the description and distribution of species. Furthermore, rules regarding the stowage plans have been researched and included below. On the other hand, it must be emphasized that directed fishing at the level of each national fleet/boat is difficult to track and fully monitor, being especially prone to changes over time (depending on national quotas).

2.2.3.2.2. INTRODUCTION TO NAFO

NAFO is an intergovernmental fisheries science and management body, to ensure long term conservation and sustainable use of the fishery resources in the Convention Area and, in so doing, to safeguard the marine ecosystems in which these resources are found.

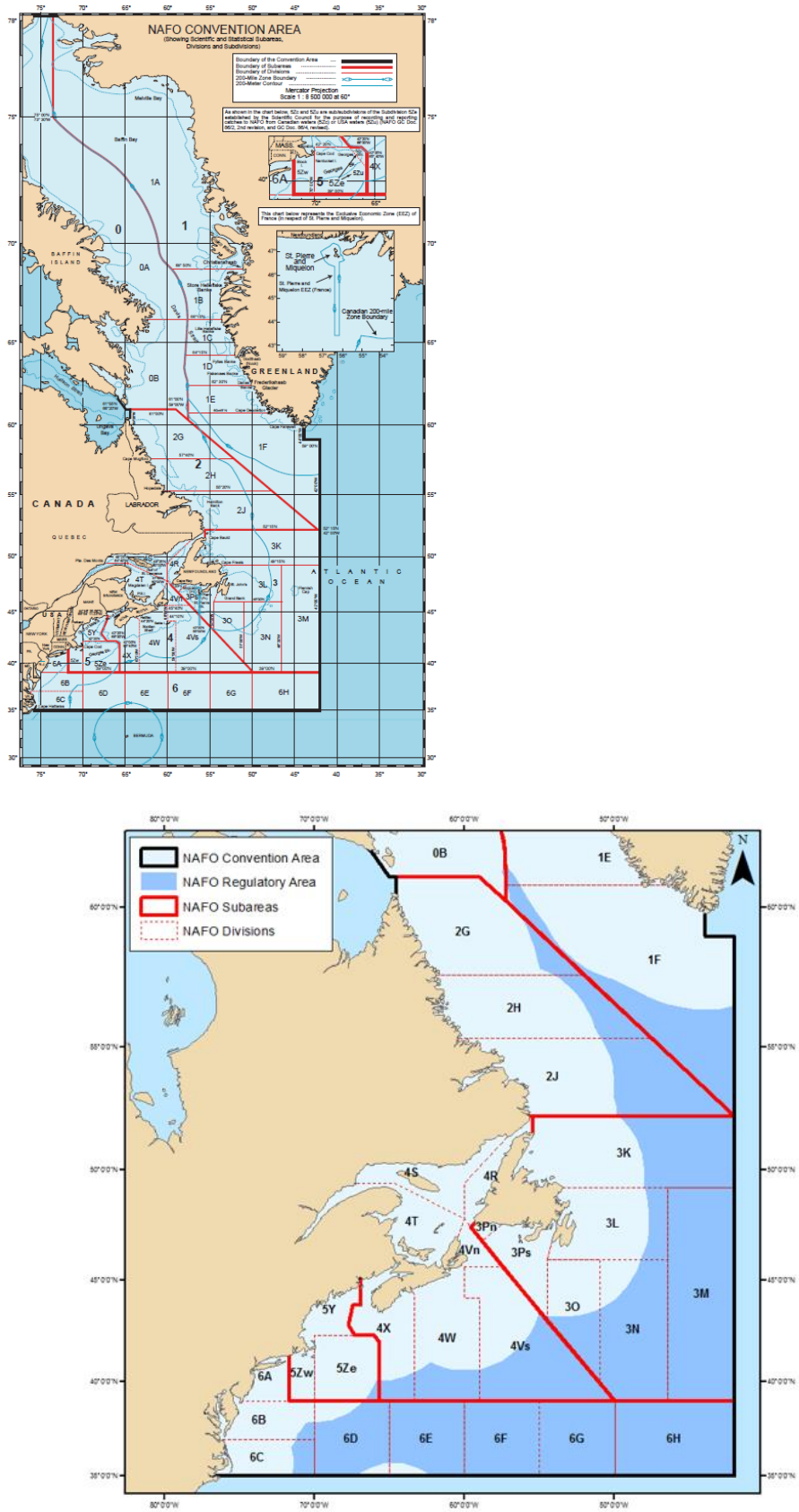


Figure 1. NAFO Convention Area and NAFO Regulatory Area maps. ©Northwest Atlantic Fisheries Organization (NAFO website: www.nafo.int).

Executive Agency for Small and Medium-sized Enterprises (EASME); European Maritime and Fisheries Fund (EMFF)
 EASME/EMFF/2016/008 Provision of Scientific Advice for fisheries beyond EU Waters
 "Study to produce an International Manual of Procedures (IMP) to be used in the NAFO Regulatory Area to guide the collection of samples from fisheries products for genetic analysis"

Currently NAFO Contracting Parties are: Canada; Cuba; Denmark (in respect of Faroe Islands and Greenland - DFG); European Union (EU); France (in respect of St. Pierre et Miquelon); Iceland; Japan; Norway; Republic of Korea; Russian Federation; Ukraine and United States of America.

Table 3. Stocks managed by NAFO.

Fisheries on:	Species	Scientific name	Stock	Management
Grand Bank	Cod	<i>Gadus morhua</i>	Divs. 2J3KL	TAC
			Divs. 3NO	TAC
	Redfish	<i>S. mentella</i> ; <i>S. fasciatus</i> ;	Divs. 3LN	TAC
			Div. 3O	TAC
	American plaice	<i>Hippoglossoides platessoides</i>	Divs. 3LNO	TAC
	Yellowtail flounder	<i>Limanda ferruginea</i>	Divs. 3LNO	TAC
	Witch flounder	<i>Glyptocephalus cynoglossus</i>	Divs. 2J3KL	TAC
			Divs. 3NO	TAC
	White hake	<i>Urophycis tenuis</i>	Divs. 3NOPs	TAC
	Thorny skate	<i>Amblyraja radiata</i>	Divs. 3NOPs	TAC
Capelin	<i>Mallotus villosus</i>	Divs. 3NO	TAC	
Shrimp	<i>Pandalus borealis</i>	Divs. 3LNO	TAC	
Flemish Cap	Cod	<i>Gadus morhua</i>	Div. 3M	TAC
	Redfish	<i>S. mentella</i> ; <i>S. fasciatus</i> ; <i>S. norvegicus</i>	Div. 3M	TAC
			Div. 3M	TAC
	American plaice	<i>Hippoglossoides platessoides</i>	Div. 3M	TAC
Shrimp	<i>Pandalus</i>	Div. 3M	Effort	

		<i>borealis</i>		
Widely distributed stocks	Greenland halibut	<i>Reinhardtius hippoglossoides</i>	SA 2 + Divs. 3KLMNO	TAC
	Pelagic Redfish	<i>S. mentella</i>	SA 2 + Divs. 1F+3K	TAC by NEAFC
	Splendid alfonsino	<i>Beryx splendens</i>	SA 6	TAC & Effort
	Short-finned Squid	<i>Illex illecebrosus</i>	SA 3+4	TAC

2.2.3.2.3. NAFO CONSERVATION AND ENFORCEMENT MEASURES (NAFO, 2019A)

The NAFO Conservation and Enforcement Measures (NAFO CEM) incorporate all NAFO measures presently in force as adopted by the Commission in accordance with provisions of Articles VI and XIV of the Convention on Cooperation in the Northwest Atlantic Fisheries. Every year the NAFO CEM is revised by the Commission. The document incorporates amendments which were adopted at the most recent NAFO Annual Meeting in September (NAFO website).

Some measures are relevant for this contract, such as:

Total Allowable Catches (TAC) for each stock in 2020 (NAFO, 2019e) as an example of the level of catches of the managed species/stocks, these TACs can change each year.

Table 4. 2020 annual quota values (NAFO, 2019e).

Report of the NAFO Commission, 23-27 September 2019

Annex 21. Quota Table and the Effort Allocation Scheme for the 3M Shrimp Fishery 3M for 2020

CATCH LIMITATIONS - Article 5. Total allowable catches (TACs) and quotas (metric tons in live weight) for 2020 of particular stocks in Subareas 1-4 of the NAFO Convention Area.

Species	Cod				Redfish					American plaice		Yellowtail
	Stock Specification	COD 3L	COD 3M	COD 3NO	RED 3LN	RED 3M	RED 3O	REB 1F_2_3K (i.e. Sub-Area 2 and Divs. 1F+3K)	PLA 3LNO	PLA 3M	YEL 3LNO	
% of TAC			% of 3M Cod TAC			% of 3LN Redfish TAC						
Contracting Party												
Canada		68	0.80	0	7 710	42.60	500	6 000	0 ¹	0	0	16 575
Cuba		316	3.70	-	1 774	9.80	1 750		0 ¹	-	-	-
Denmark (Faroe Islands and Greenland)		1 907	22.35	-	-		69 ¹⁰		0	-	-	-
European Union		4 865 ⁵	57.03	0 ⁴	3 300 ⁴	18.23	7 813 ⁴	7 000	0 ⁷	0	0 ⁴	-
France (St. Pierre et Miquelon)		-	-	-	-		69 ¹⁰		0 ¹	-	-	340
Iceland		-	-	-	-		-		0	-	-	-
Japan		-	-	-	-		400	150	0 ¹	-	-	-
Korea		-	-	-	-		69 ¹⁰	100	0 ¹	-	-	-
Norway		789	9.25	-	-		-		0	-	-	-
Russian Federation		552	6.47	0	5 207	28.77	9 137	6 500	0	-	0	-
Ukraine								150	0 ¹			
United States of America		-	-	-	-		69 ¹⁰		0 ¹	-	-	-
Others		34	0.40	0	109	0.60	124	100	-	0	0	85
TOTAL ALLOWABLE CATCH	*	8 531	100.0 ¹³	*	18 100	100.0 ¹⁴	8 590	20 000 ¹¹	0 ¹⁹	* ⁸	*	17 000 ⁹

Report of the NAFO Commission, 23-27 September 2019

Species	Witch			White hake	Capelin	Skates	Greenland halibut	Squid (<i>Illex</i>)	Shrimp		Alfonsoino
	Stock Specification	WIT 3L	WIT 3NO	HKW 3NO	CAP 3NO	SKA 3LNO	GHL 3LMNO	SQI 3.4 (i.e. Sub-areas 3+4)	PRA 3L	PRA 3NO	ALF 6 (i.e. Sub-area 6)
% of TAC			% of 3NO Witch TAC								
Contracting Party											
Canada			705	60.00	294	0	1 167	1 881	N.S. ²	0	
Cuba			-			0		-	510	0	
Denmark (Faroe Islands and Greenland)			-			-		216	-	0	
European Union			156 ⁴	13.27	588	0 ⁵	4 408	7 353 ⁶	N.S. ² 611 ⁵	0 ⁶	
France (St. Pierre et Miquelon)			-			-		206	453	0	
Iceland			-			-		-	-	0	
Japan			-			0		1 286	510	0	
Korea			-			-		-	453	0	
Norway			-			0		-	-	0	
Russian Federation			302	25.73	59	0	1 167	1 600	749	0	
Ukraine								-	-	0	
United States of America			-			-		-	453	0	
Others			12	1.00	59	-	258		794	0	
TOTAL ALLOWABLE CATCH		* ¹¹	1 175 ⁸	100.00 ¹⁵	1 000 ⁸	* ⁸	7 000 ¹²	12 542	34 000 ¹¹	0 ⁸	*

* Ban on fishing in force.

- ¹ Quota to be shared by vessels from Canada, Cuba, France (St. Pierre et Miquelon), Japan, Korea, Ukraine and USA.
- ² The allocations to these Contracting Parties are as yet undetermined, although their sum shall not exceed the difference between the total of allocations to other Contracting Parties and the TAC (= 29,467 tonnes).
- ³ Should NEAFC modify its level of TAC, these figures shall be adjusted accordingly by NAFO through a mail vote.
- ⁴ Including allocations to Estonia, Latvia and Lithuania in accordance with the sharing arrangement of the former USSR quota adopted by the Fisheries Commission in 2003 (FC WP 03/7), as applied by NAFO since 2005 following their accession to the European Union.
- ⁵ Including allocations to Estonia, Latvia and Lithuania in accordance with the sharing arrangement of the former USSR quota adopted by the Fisheries Commission in 2003 (FC WP 03/7), and to Poland, as applied by NAFO since 2005 following their accession to the European Union.
- ⁶ Including allocations to Estonia, Latvia, Lithuania and Poland, as applied by NAFO since 2005 following their accession to the EU.
- ⁷ Allocation of 17.85% to Lithuania and 2.15% to Latvia following their accession to the European Union.
- ⁸ Applicable to 2020 and 2021.
- ⁹ If an increase in the overall TAC as defined in footnote 3 leads to an increase in these shares, the first 500 tonnes of that increase shall be added to the quota share referred to in footnote 1.
- ¹⁰ Notwithstanding the provision of Article 5.3(b) and without prejudice to future agreements on allocations, these quotas may be fished in their entirety by these Contracting Parties.
- ¹¹ Applicable to 2020, 2021, and 2022.
- ¹² Should catches exceed 5 000 tonnes, additional measures would be adopted to further restrain catches in 2020.

Historical statements

- ¹³ The allocation key of this stock is based on the 1998 Quota Table. In 1999, a moratorium on cod in Division 3M was declared.
- ¹⁴ The allocation key of this stock is based on the 1997 Quota Table. In 1998, a moratorium on redfish in Division 3LN was declared.
- ¹⁵ The allocation key of this stock is based on the 1994 Quota Table. In 1995, a moratorium on witch flounder in Division 3NO was declared.

**Effort Allocation Scheme for Shrimp Fishery in the
NAFO Regulatory Area Div. 3M, 2020**

CONTRACTING PARTY	NUMBER OF FISHING DAYS¹
Canada	114
Cuba	25 ³
Denmark	
– Faroe Islands	402
– Greenland	129
European Union²	823 ³
France (in respect of St. Pierre et Miquelon)	25 ³
Iceland	N/A
Japan	25
Korea	25
Norway	496 ³
Russia	525 ³
Ukraine	25 ³
USA	25
TOTAL	2 640

- ¹ When the scientific advice estimates that the stock shows signs of recovery, the fishery shall be re-opened in accordance with the effort allocation key in place for this fishery at the time of the closure.
- ² Including fishing entitlements transferred from Poland (25 fishing days), Estonia (416 fishing days), Latvia (123 fishing days) and Lithuania (145 fishing days) following their accession to the European Union.
- ³ In derogation of CEM Article 5.11 and CEM Article 9.4, the European Union will transfer 25 fishing days of its fishing days allocation for 2020 to France, in respect of St Pierre et Miquelon; Norway will transfer 25 fishing days of its fishing days allocation for 2020 to Ukraine; and the Russian Federation will transfer 25 fishing days of its fishing days allocation for 2020 to Cuba. The above transfers are without prejudice to the effort allocation key and are only for the year 2020 only. The 2020 catches under this interim regime will not create any catch history.

Mesh Sizes: No vessel shall fish with a net having a mesh size smaller than prescribed for each of the following species (NAFO, 2019a):

- (a) 40 mm for shrimps and prawns (PRA);
- (b) 60 mm for short finned squid (SQI);
- (c) 280 mm in the codend and 220 mm in all other parts of the trawl for skate (SKA);
- (d) 130 mm for all other groundfish, as defined in Annex I.C.;

(e) 100 mm for pelagic *Sebastes mentella* (REB) in Subarea 2 and Divisions 1F and 3K; and

(f) 90 mm for redfish (RED) in the fishery using mid-water trawls in Division 3O, 3M and 3LN. Within this fishery mid-water trawl means trawl gear that is designed to fish for pelagic species, no portion of which is designed to be or is operated in contact with the bottom at any time. The gear shall not include discs, bobbins or rollers on its footrope or any other attachments designed to make contact with the bottom. The trawl may have chafing gear attached.

Article 27 - Product Labelling Requirements (NAFO, 2019a)

When processed, all species harvested in the RA shall be labelled in such a way that each species and product category is identifiable. All species must be labelled using respectively the following data:

- (a) the name of the capture vessel;
- (b) the 3-Alpha Code for each species as listed in Annex I.C;
- (c) in the case of shrimps the date of capture;
- (d) the Regulatory Area and Division of fishing; and
- (e) the product form presentation code as listed in Annex II.K.

Labels shall be securely affixed, stamped or written on packaging at the time of stowage and be of a size that can be read by inspectors in the normal course of their duties.

Labels shall be marked in ink on a contrasting background.

Each package shall contain only:

- (a) one product form category;
- (b) one division of capture;
- (c) one date of capture (in the case of shrimps); and
- (d) one species.

Table 5. Product form presentation for each 3-Alpha Codes (Annex II.K).

3-Alpha Code	Presentation	Description
CBF	Cod butterfly (“escalado”)	HEA with skin on, spine on, tail on
CLA	Claws	Claws only

DWT	ICCAT code	Gilled, gutted, part of head off, fins off
FIL	Filleted	HEA + GUT + TLD + bones off Each fish originates two fillets not joined by any part
FIS	Filleted and skinned fillets	FIL+SKI Each fish originates two fillets not joined by any part
FSB	Filleted with skin and bones	Filleted with skin and bones on
FSP	Filleted skinned with pinbone on	Filleted with skin removed and pinbone on
GHT	Gutted headed and tailed	GUH+TLD
GUG	Gutted and gilled	Guts and gills removed
GUH	Gutted and headed	Guts and head removed
GUL	Gutted liver in	GUT without removing liver parts
GUS	Gutted headed and skinned	GUH+SKI
GUT	Gutted	All guts removed
HEA	Headed	Heads off
HET	Headed and tailed	Heads and tails off
JAP	Japanese cut	Transversal cut removing all parts from head to belly
JAT	Tailed Japanese cut	Japanese cut with tail removed
LAP	Lappen	Double fillet, HEA, skin + tails + fins ON
LVR	Liver	Liver only
OTH	Other	Any other presentation
ROE	Roe (s)	Roe(s) only
SAD	Salted dry	Headed with skin on, spine on, tail on and salted dry
SAL	Salted wet light	CBF + salted
SGH	Salted, gutted and headed	GUH + salted
SGT	Salted gutted	GUT + salted
SKI	Skinned	Skin off
SUR	Surimi	Surimi
TAL	Tail	Tails only

TLD	Tailed	Tail off
TNG	Tongue	Tongue only
TUB	Tube only	Tube only (Squid)
WHL	Whole	No processing
WNG	Wings	Wings only

Article 28 – Monitoring of Catch (NAFO, 2019a)

Recording of Catch and Stowage: For the purposes of monitoring catch, each fishing vessel shall utilize a fishing logbook, a production logbook and a stowage plan as defined below, to record fishing activities in the RA:

Fishing Logbook: Each fishing vessel shall maintain a fishing logbook consistent with Annex II.A that:

- (a) accurately records catch of each tow/set related to the smallest geographical area for which a quota has been allocated;
- (b) indicates the disposition of the catch of each tow/set, including the amount (in kg, live weight) of each stock that is retained on board, discarded, offloaded, or transhipped during the current fishing trip; and
- (c) is retained on board for at least 12 months.

Production Logbook: Each fishing vessel shall maintain a production logbook that:

- (a) accurately records the daily cumulative production for each species and product type in kg for the preceding day from 00:01 UTC until 24:00 UTC;
- (b) relates the production of each species and product type to the smallest geographical area for which a quota has been allocated;
- (c) lists the conversion factors used to convert production weight of each product type into live weight when recorded in the fishing logbook;
- (d) labels each entry in accordance with Article 27; and
- (e) is retained on board for at least 12 months.

Stowage of Catch: Each vessel shall, with due regard for safety and navigational responsibilities of the master, stow all catch taken in the NAFO RA separately from all catch taken outside the NAFO RA, and ensure that such separation is clearly demarcated using plastic, plywood or netting;

Each fishing vessel shall maintain a stowage plan that:

- (a) clearly shows:
- (i) the location and quantity, expressed as product weight in kg, of each species within each fish hold;
 - (ii) the location in each hold of shrimp taken in Division 3L and in Division 3M that includes the quantity of shrimp in kg, by Division;
 - (iii) the top view of product within each fish hold;
- (b) is updated daily for the preceding day from 00:01 to 24:00 UTC; and
- (c) is retained on board for each day fished until the vessel has been unloaded completely.

In this context, it is worth mentioning that there is a potential problem because the level of reporting catches is not at the level of the Product Labelling Requirements or the stowage plan.

2.2.3.2.4. NAFO FLEETS/FISHERIES

The term fleet can be interpreted in different ways: a set of vessels of the same tonnage; a set of vessels using the same gear; set of vessels owned by the same flag State; set of vessels directed to the same species and others combinations.

The term fishery can be associated with the gear used, depth, the target species, etc.

Fisheries in the NAFO RA/Fishing effort by gear type (NAFO, 2018a: STATIC Report)

NAFO traditionally identifies three main fisheries in its RA: the groundfish (GRO - primarily in Div. 3LMNO), shrimp (PRA - primarily in Div. 3LM) and pelagic redfish fisheries (REB - primarily in Div. 1F and 2J). The PRA and the REB fisheries have been under moratoria. In 2017, fisheries in the NAFO RA were limited to GRO. There were 112 trips by 45 fishing vessels spending a total of 3,872 days in the NAFO RA (Table 6). Additionally, a single vessel (class size 5) spent 14 days, as part of its fishing trip, in Division 6G catching alfonosinos. According to the observer report, the fishing gear used was a mid-water trawl.

Smaller vessels (<500 GT) tend to fish in Divisions 3NO using mainly longlines. The vast majority of the effort comes from larger vessels (> 500 GT) which account for 96 % of fishing effort in terms of days. The larger vessels use bottom trawl and fish in

Divisions 3LMNO. The major species caught by the bottom trawlers are cod, Greenland halibut, redfish, and thorny skate (Table 6).

Table 6. Fishing effort in the NAFO RA for trips that ended in 2017.

Vessel Class	# of fishing vessels	# of fishing trips	Main Gear	f = Days present in the NRA	Fishing Trip Range (days)	Main Species	Fishing Area
Class 3-4 vessels (less than 500 mt)	7	17	Longline	205	1-18 days	Cod, Yellowtail flounder	Flemish Cap (for cod); Tail of the Grand Banks (for yellowtail flounder)
Class 5 vessels (500-1000 MT)	10	31	Bottom Trawl	1051	9-71 days	Cod, Greenland halibut, redfish, skates	Flemish Cap; Tail and Nose of the Grand Banks
Class 6 vessels (1000-2000 MT)	26	60	Bottom Trawl	2435	2-100 days	Cod, Greenland halibut, redfish, skates	Flemish Cap; Tail and Nose of the Grand Banks
Class 7 vessels (> 2000 MT)	2	4	Bottom Trawl	181	28-57 days	Cod, Greenland halibut, redfish, skates	Flemish Cap; Tail and Nose of the Grand Banks
Total	45	112		3,872			

Fisheries in the NAFO RA/Effort Distribution by depth of GRO vessel (NAFO, 2018a: STATIC Report)

The requirement of providing the speed and course information in the position reports of Vessel Monitoring System (VMS) is satisfied. Hourly positions are required to be transmitted. Speeds between 0.5 and 5 knots were assumed to be fishing speeds in this analysis. In Figure 2, the distribution of fishing effort in hours of GRO vessels is presented. It shows that about half of all GRO effort is at depths 400 meters and below (skates, redfish and cod). Figure 2 also shows a concentration of fishing effort around 1000 meters and this can be attributed to the Greenland halibut fishery.

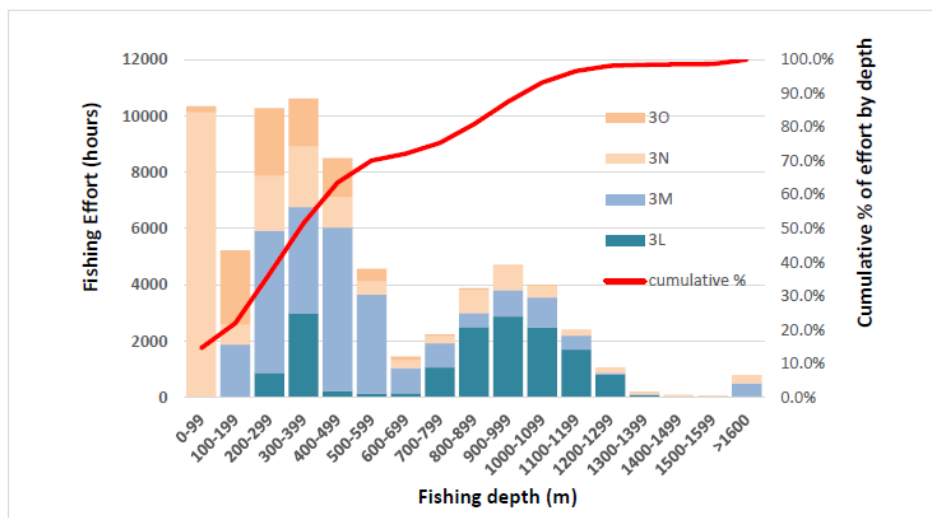


Figure 2. Distribution of fishing effort (in hours) by depth (m) in the NAFO RA in 2017. Vessels are assumed to be fishing at speed in the range of 0.5-5.0 kt.

Fisheries in the NAFO RA/Catch totals (NAFO, 2018a: STATIC Report)

In 2017, a grand total of 59,533 t of fish (58,141 t retained + 1,392 t rejected) were caught by NAFO-registered vessels (as reported in the daily CATs) authorized to fish in the RA (Tables 7 and 8). In terms of quantities caught, the stocks 3M Cod, 3LMNO Greenland halibut, 3M Redfish, 3LN Redfish, 3O Redfish, 3LNO Yellowtail flounder and 3NO Skates constitute the major GRO fishery in the NAFO RA.

Table 7. Total reported retained catches (in tonnes) of species (in FAO 3-alpha code) by Division for trips that ended in 2017 (Source: CA field of CAT Reports).

Division	3L	3M	3N	3O	6G	TOTAL
<i>Species subject to catch limitations (as listed in the Quota Table)</i>						
COD	98.6	14196.5	350.8	227.9		14873.9
GHL	6594.3	1562.0	1094.4	8.6		9259.3
HKW	0.0	1.9	56.2	113.8		171.9
PLA	82.9	158.7	622.4	254.0		1118.1
RED	3729.3	7079.3	4595.0	7484.9		22888.5
SKA	77.4	43.3	3695.8	425.5		4242.0
SQI	0.0	2.8	0.0	11.5		14.4
WIT	38.1	181.7	94.2	219.0		533.0
YEL			3821.3	44.7		3866.0
<i>Selected species not listed in the Quota Table</i>						
ALF					54.5	54.5
ANG			2.7	19.7		22.3
CAT	2.6	5.9	3.3			11.8
HAD		4.2	6.0	23.3		33.4
HAL	103.3	132.9	219.0	176.8		632.0
RHG	71.0	24.8	24.5			120.4
RNG	12.7	5.8	0.1			18.6
<i>Sharks</i>						
DGX			0.1			0.1
GSK		2.6	1.5			4.1
<i>Other Species</i>	3.4	11.5	8.9	250.9	1.7	276.4
TOTAL	10813.7	23413.8	14596.2	9260.7	56.2	58140.7

Table 8. Total reported rejected catches (in tonnes) of species (in FAO 3-alpha code) by Division for trips that ended in 2017 (Source: RJ field of CAT Reports).

Division	3L	3M	3N	3O	Total
<i>Species subject to catch limitations (as listed in the Quota Table)</i>					
CAP	0.0		9.2	2.1	11.3
COD	4.9	7.1	30.0		41.9
GHL	0.0	0.0	1.1		1.2
HKW		0.0	14.9	0.6	15.5
PLA	5.5	1.3	58.6	3.7	69.1
RED	1.0	10.8	1.2	2.9	15.8
RJR	0.4	1.5	56.4		58.3
SKA	2.1	2.2	61.7	0.9	66.8
SQI		0.1	0.0	2.1	2.2
WIT	8.1	1.3	6.6	9.0	25.0
YEL	0.0		24.5	0.0	24.5
<i>Selected species not listed in the Quota Table</i>					
ANG			0.0		0.0
CAT	13.2	5.1	7.5	6.3	32.0
HAD		0.0	0.1	0.5	0.6
HAL	0.1	0.9	16.0	0.0	17.0
RHG	202.1	38.2	24.1	0.8	265.2
RNG	36.6	44.3	9.3	0.1	90.3
<i>Sharks</i>					
DGX	3.0	0.4	0.7		4.2
GSK	183.0	36.3	130.2	19.7	369.2
POR			1.4	1.6	2.9
SHX		0.1		1.2	1.3
SMA	0.2		1.5	0.7	2.4
<i>Other Species</i>	24.6	29.7	194.1	27.5	275.9
Total	484.9	179.3	648.9	79.7	1392.8

Main Commercial Species in NAFO Regulatory Area (NRA): Description; distribution and fishery

Cod (*Gadus morhua*)

Description

The Atlantic cod (*Gadus morhua*) is one of 59 species of the family gadidae. The cod family is the most numerous and best represented of fishes in the Canadian area. A marine fish which occurs mainly in cool waters in northern seas, the cod is soft-rayed, has three dorsal fins on its back and two anal fins behind its whitish-coloured belly, and generally has an elongated hair-like projection called a barbel on its chin (Figure 3). It is generally grey or green but may be brown or reddish, depending upon the habitat into which its colour will generally blend. The scales are small and smooth. The mouth is large with a projecting upper jaw and the gill openings are wide. The lateral line of the cod is pale, and the tail is slightly concave, almost square. Generally, cod average 2 to 3 kg in weight and about 60 to 70 cm in length. They usually do not exceed 30 kg, but there is one record of a cod that weighed about 96 kg and was more than 180 cm long (Lear, 1993).



Figure 3. Illustration representing cod (*Gadus morhua*).

Distribution



Figure 4. Geographical distribution of cod (*Gadus morhua*) (Lear, 1993).

Similar species in NAFO: Hakes (*Urophycis spp.*), cod, haddock (*Melanogrammus aeglefinus*), Pollock (*Pollachius virens*) and others round fishes.

Most common product presentation: CBF (cod butterfly - escalado), GUG (Guts and gills removed), GUT (All guts removed), GUH (Guts and head removed), SAL (CBF + salted), SGH (GUH + salted), SGT (GUT + salted) and all kind of fillets.

Fishery

Both stocks, Divs. 2J3KL; Div. 3NO, are under moratoria since the first half of the 90s. In the NAFO RA, the catches are bycatches of others directed fisheries (primarily caught in the redfish, yellowtail flounder and skate fisheries - NAFO Website). Canada by other hand has a sentinel fishery and a stewardship fishery, on the stock Divs. 2J3KL, inside of its exclusive economic zone (EEZ).

Divisions 3NO

A moratorium was implemented in 1994. Catches since that time are by-catch in other fisheries.

Last assessment June 2018 (NAFO, 2018b) - Stock status: The spawning biomass increased noticeably between 2010 and 2015 but has subsequently declined and the 2018 estimate of 18,537 t represents only 31% of Blim (60,000 t). The 2006 year class remains relatively strong and at age 12 in 2018 makes up more than half of the estimated SSB. Subsequent year classes are much weaker, suggesting that the medium-term prospects for the stock are not good. Fishing mortality values over the past decade have been low and well below F_{lim} (0.3).

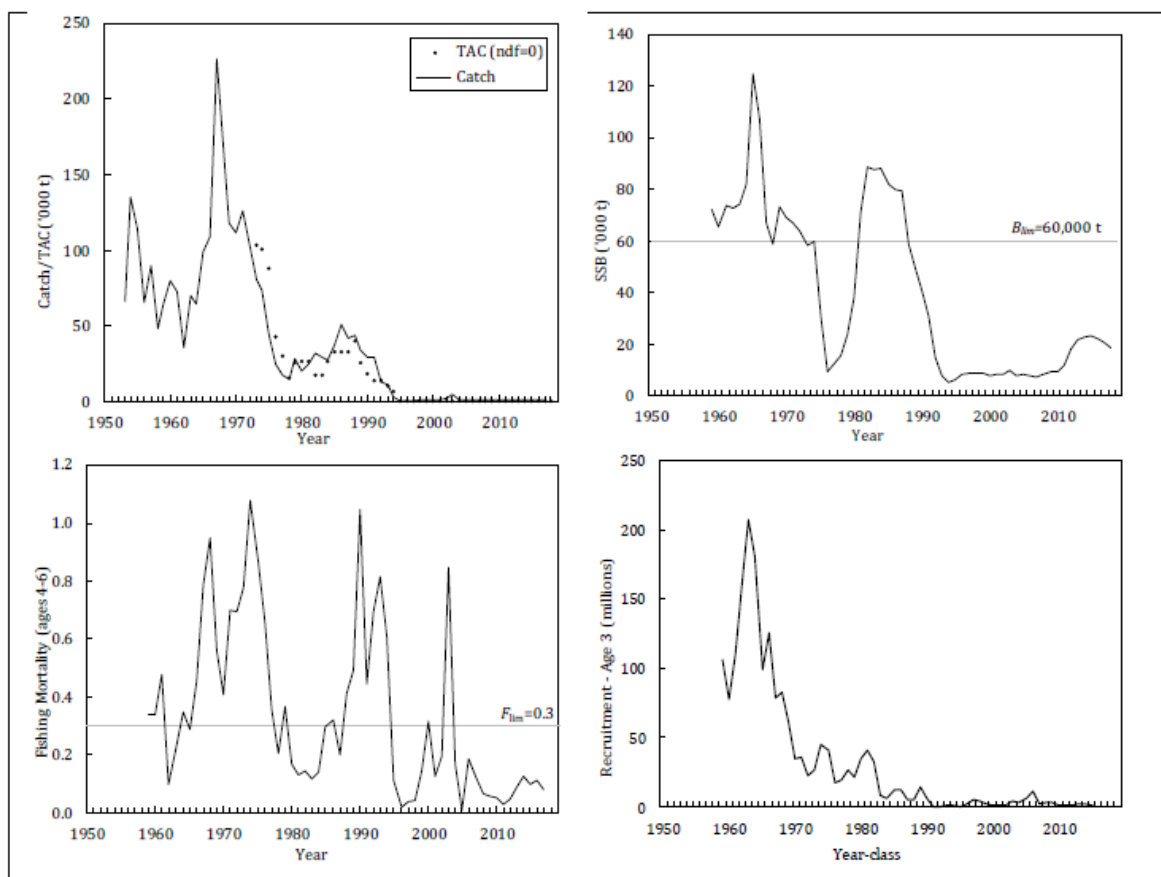


Figure 5. 3NO Cod: Catches and TACs; Biomass; Fishing mortality and Recruitment (NAFO, 2018b).

Division 3M

The cod fishery on the Flemish Cap has traditionally been a directed fishery by Portuguese trawlers and gillnetters, Spanish pair-trawlers and Faroese longliners. Cod has also been taken as bycatch in the directed redfish fishery by Portuguese trawlers. Estimated bycatch in shrimp fisheries is low. Large numbers of small fish were caught by the trawl fishery in the past, particularly during 1992-1994. Total annual catches from 1996 to 2010 were very small compared with previous years. From the reopening of the fishery in 2010, catches increased until 2013 to the TAC value, and remained at this level since (NAFO, 2019b). In recent years eight countries fished cod in Div. 3M, trawlers from EU-Estonia, EU-Portugal, EU-Spain, Japan, Norway and Russia and longliners from Norway, Faroe Islands and USA.

The Div. 3M stock is open and the TAC is set at 8,531 tonnes for 2020.

Last assessment June 2019 (NAFO, 2019b) - Stock status: Current SSB is estimated to be well above B_{lim} (15,177 t) although it is expected to decline rapidly in the near future due to poor recruitment since 2015. F increased in 2010 with the re-opening of the fishery but it has remained below F_{lim} (0.167).

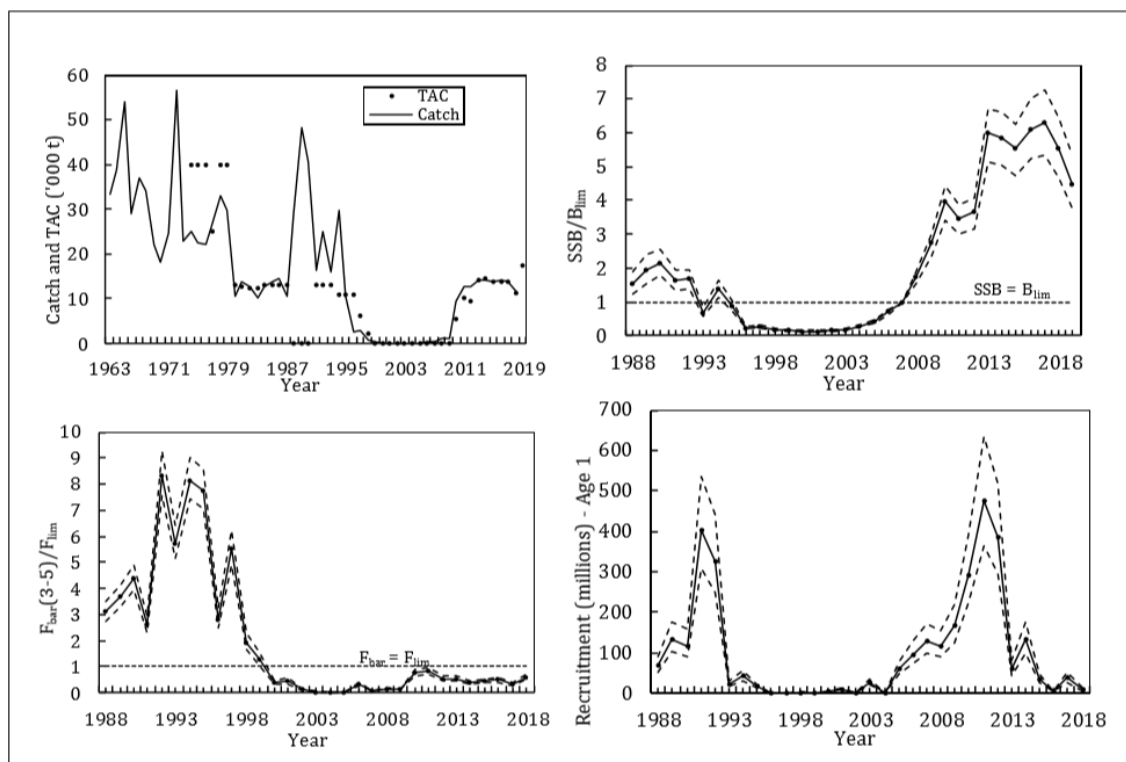


Figure 6. 3M Cod: Catches and TACs; Biomass; Fishing mortality and Recruitment. Dashed lines represents the 80% probability (NAFO, 2019b).

Redfish (*Sebastes* spp.)

Description

Redfish catches include three species of the genus *Sebastes*: *Sebastes mentella* and *Sebastes fasciatus*, known as "beaked redfish", and *Sebastes norvegicus* (= *S. marinus*).

Swimming in the deep waters at the edge of the great fishing banks and in deep channels carved in the ocean floor, redfish are mainly noted for the brilliant scarlet colour from which they take their name. Aside from its deep colour, which ranges from orange to flame-red, sometimes with a brownish cast, the beaked redfish is distinguished from other spiny-rayed fish by a bony protrusion on the lower jaw, by a fan of bony spines that radiate out from around the gill cover, and by its large eyes (Figure 5). Three types of redfish are known in the Northwest Atlantic, *Sebastes mentella* being the more common. It is found at depths of greater than 200 m, has a bright red colour, a relatively large eye and a long, well developed beak. The *S. marinus*, usually found at depths of less than 240 m, is orange in colour rather than red, has a smaller eye and a small, blunt beak that is relatively weak. It generally grows to a much larger size than its close cousins. As far as the commercial fishery or fisheries management is concerned, the two (or three) species are managed together as a single unit.



Figure 7. Illustration representing redfish (*Sebastes* spp.).

Distribution



Figure 8. Geographical distribution of redfish (*Sebastes* spp.) (McKone and LeGrow, 1991).

Similar species in NAFO: redfish, alfonsino (*B. splendens*).

Most common product presentation: JAP (Transversal cut removing all parts from head to belly), GUH (Guts and head removed) and GHT (Guts, head and tail removed).

Fishery

All three stocks, Divs. 3LN, 3M and Div. 3O, are open. Canada, Russia and Portugal are the main countries in this fishery.

In NAFO 2015 (WGESA report): The redfish fishery, in Divs. 3LNO, is conducted with 130 mm mesh size trawl bottom trawls with the primary areas being the slope area of Div. 3O, the east-central area of Div. 3N and the southeast area of Div. 3L near the border with Div. 3N in depths <600 m. Redfish comprise 90 % of the catch and the main by-catch species were American plaice (2 %), cod (2 %), silver hake (2 %) and Atlantic halibut (2 %) based on 2015 logbook information. Although mid-water trawling has comprised a significant percentage of redfish fisheries for principal

Russian fleet in the past, its use has diminished in recent years and only bottom trawls were deployed in 2013-14.

Divisions 3LN

There are two species of redfish in Divisions 3L and 3N, the deep-sea redfish (*Sebastes mentella*) and the Acadian redfish (*Sebastes fasciatus*) that have been commercially fished and reported collectively as redfish in fishery statistics.

A management strategy has been adopted for this stock based on a stepwise rule with biennial catch increases over the years 2015 to 2020. The TAC is set for 2020 at 18,100 t.

Last assessment June 2018 (NAFO, 2018b) - Stock status: The stock is currently in the safe zone of the NAFO precautionary approach framework and is estimated to be at 1.5 x B_{msy} . There is a very low risk of the stock being below B_{lim} . Fishing mortality is well below F_{msy} ($0.36 \times F_{msy}$), and the probability of being above F_{lim} ($= F_{msy}$) is very low. Recent recruitment appears to be low.

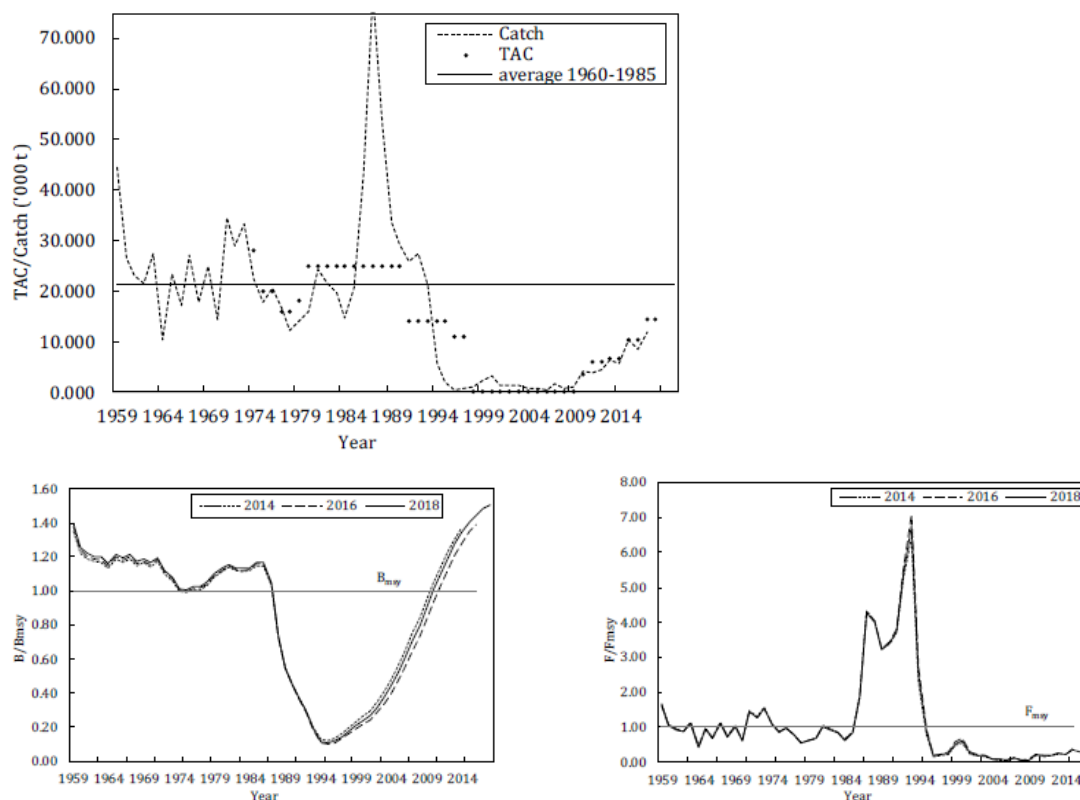


Figure 9. 3LN Redfish: Catches and TACs; Biomass; Fishing mortality (NAFO, 2018b).

Division 3O

Redfish are caught primarily in bottom trawl fisheries, but in the past, some landings were reported from mid-water trawl fisheries. In directed redfish fisheries, Atlantic cod, American plaice, witch flounder and other species are landed as bycatch. In turn, redfish are also caught as bycatch in fisheries directing for other species.

The fishery in NAFO division 3O is regulated by minimal mesh size and quota.

The TAC is set for 2020 at 20,000 t.

Last assessment June 2019 (NAFO, 2019b) - Stock status: Survey index values for the past three years were generally at or below their time-series average compared to relatively high values observed in 2010 to 2012. Current fishing mortality is low, and recent recruitment is unknown.

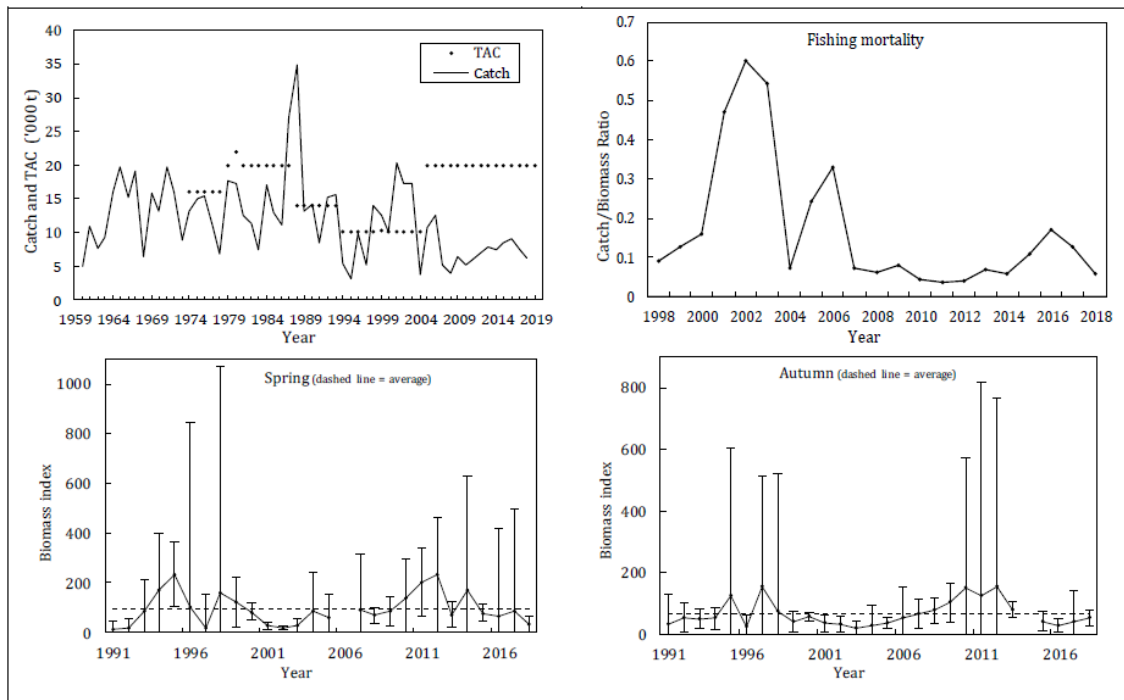


Figure 10. 30 redfish: Catches and TACs; Fishing mortality and Survey Biomass indices (NAFO, 2019b).

Division 3M

Redfish is caught in directed bottom trawl fisheries at intermediate depths (300-700m), but also as bycatch in fisheries directed for cod and Greenland halibut. The fishery in NAFO Div. 3M is regulated by minimum mesh size and quota.

The TAC is set at 8,591 tonnes for 2020.

Last assessment June 2019 (NAFO, 2019b) - Stock status: The stock is declining after a marked recovery that started in 2002-2003. High levels of biomass were maintained until 2014, supported by low fishing mortalities and individual growth of survivors, but could not be sustained. The decline in abundance is more pronounced, with no perspective to stop in the short term since year classes at recruitment continue to be extremely weak.

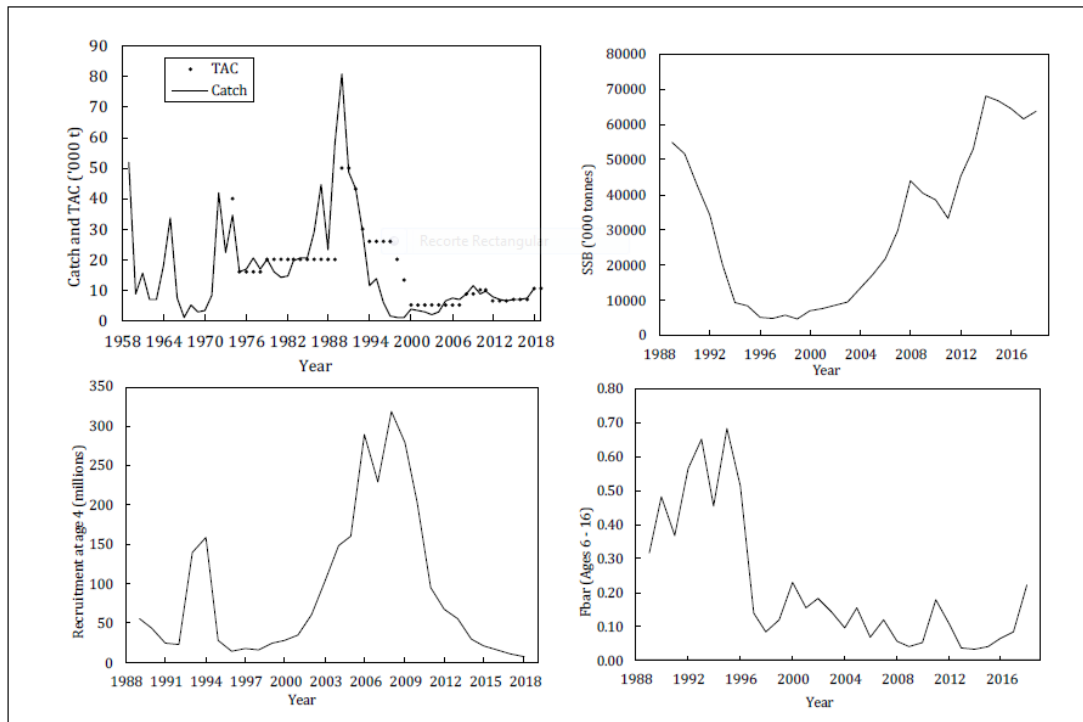


Figure 11. 3M redfish: Catches and TACs; Biomass; Recruitment and Fishing mortality (NAFO, 2019b).

American plaice (*Hippoglossoides platessoides*)

Description

American plaice (*Hippoglossoides platessoides*) belongs to the flatfish group of fishes, whose members are strongly compressed laterally and lie and swim on one side (Figure 7). When the young fish hatch from the egg, at or near the surface, they have the normal fish form. During development, as they settle to the bottom of the ocean, a change occurs in the body structure. The head becomes twisted so that the fish now swims and lies on its side. The upper side (which now has both eyes) is normally pigmented as compared to the lower side which lacks pigmentation. Plaice almost invariably have their eyes on the right side of the body. The fish has a large mouth that extends at least below the middle of the eye. The body is covered with relatively small scales, the tail fin is rounded, and the line that runs along the side of the body (the lateral line) is slightly curved just behind the gill openings. The colour is normally reddish to greyish brown on the upper pigmented side and white on the lower side.



Figure 12. Illustration representing American plaice (*Hippoglossoides platessoides*).

Distribution

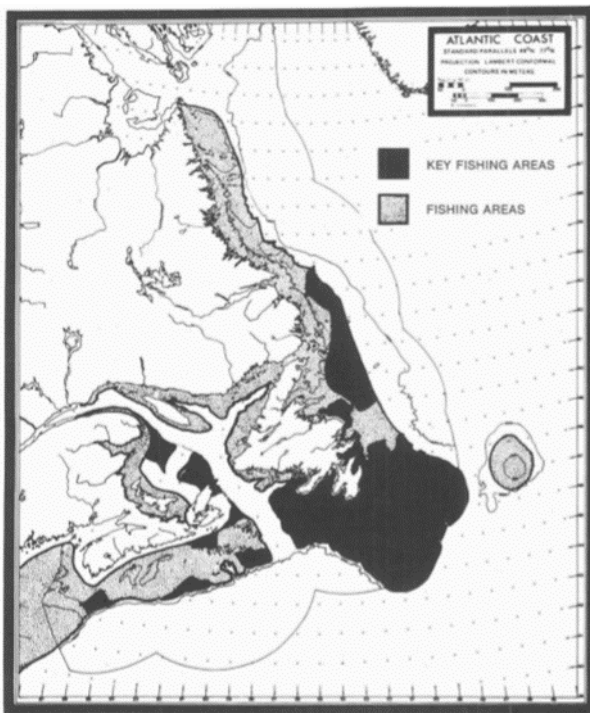


Figure 13. Geographical distribution of American plaice (*Hippoglossoides platessoides*) (Pitt, 1989).

Similar species in NAFO: Other flatfishes: yellowtail flounder, witch flounder, Greenland halibut etc.

Most common product presentation: GUH (Guts and head removed), GHT (Guts, head and tail removed) and all kind of fillets.

Fishery

Divisions 3LNO

The stock has been under moratorium (no directed fishery) since 1995. American plaice in recent years is caught as bycatch mainly in otter trawl fisheries of Yellowtail Flounder, skate, Greenland Halibut and redfish. A Conservation Plan and Rebuilding Strategy for 3LNO American plaice was created in 2011.

Last assessment was in June 2018 (NAFO, 2018b) - Stock status: The stock remains low compared to historic levels and is presently at 34% of the B_{lim} level. Recruitment has been low since the late 1980s, but Canadian surveys indicate a large number of pre-recruits in Div. 3L in recent years. Current estimates of fishing mortality are very low.

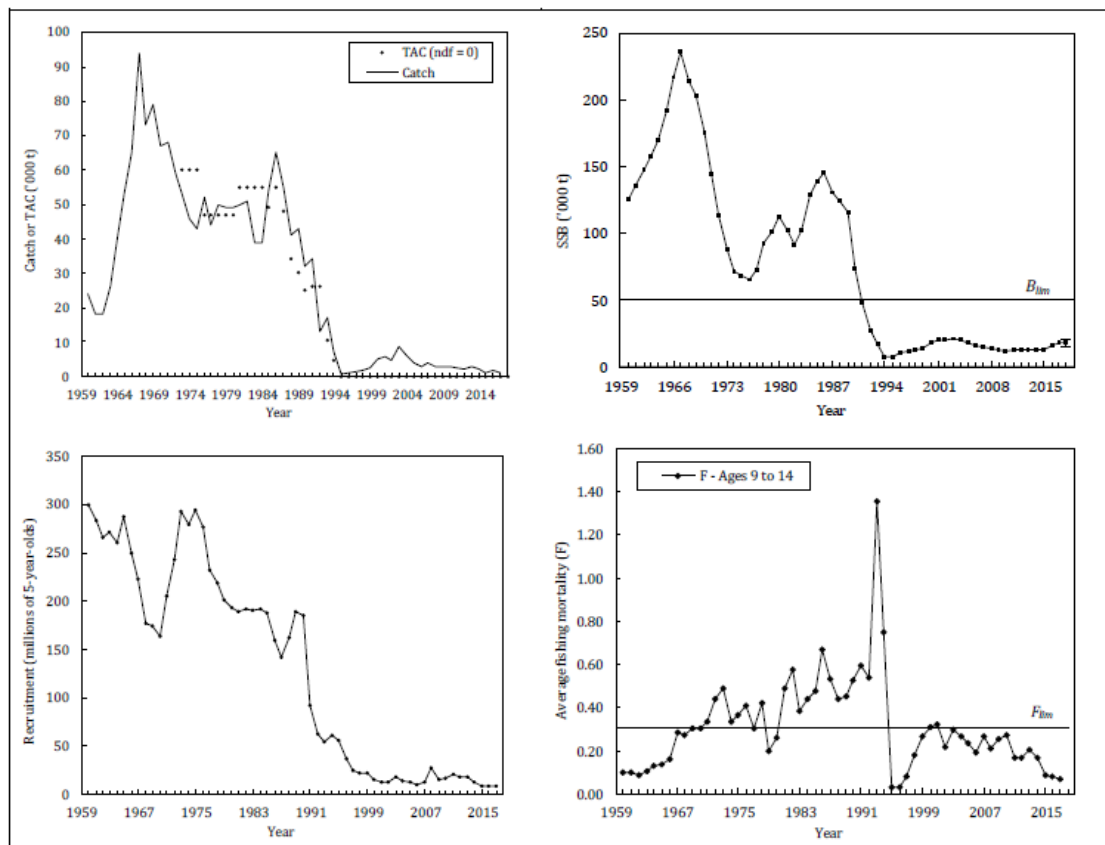


Figure 14. 3NO American plaice: Catches and TACs; Spawning Biomass; Recruitment and Fishing mortality (NAFO, 2018b).

Division 3M

American plaice is caught as bycatch in otter trawl fisheries, mainly the cod and redfish fisheries. From 1979 to 1993 a TAC of 2,000 t was in effect for this stock. A reduction to 1,000 t was agreed for 1994 and 1995 and a moratorium was agreed to thereafter.

Last assessment was in June 2017 (NAFO, 2017) - Stock status: The stock has increased slightly in recent years due to improved recruitment. Although the catches since 1996 have been low, this stock remains at a relatively low level.

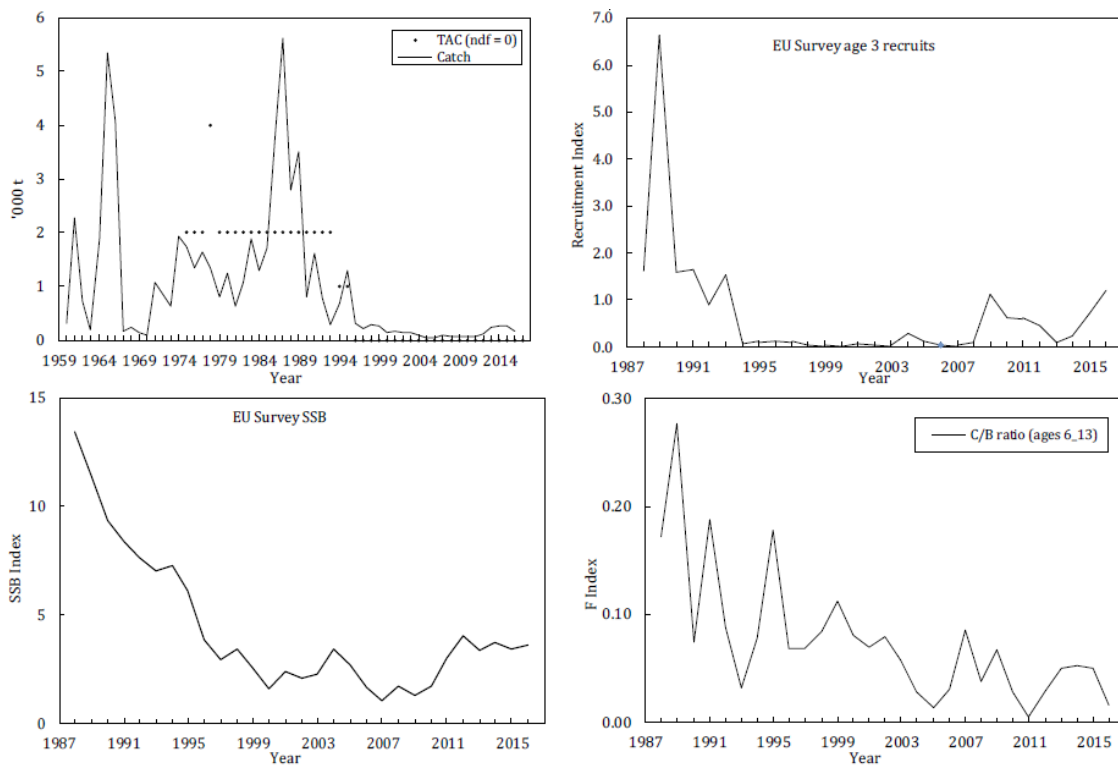


Figure 15. 3M American plaice: Catches and TACs; Recruitment; Spawning Biomass and Fishing mortality indices (NAFO, 2017).

Yellowtail flounder (*Limanda ferruginea*)

Description

The yellowtail flounder (*Limanda ferruginea*) has a remarkable adaptability to the colour of the bottom of the ocean on which it lies, and easily blends into the environment to become practically invisible. This is a trait shared with some other members of the flatfish family. In the early larval form, it is indistinguishable from other bony fish with respect to its body shape, but as development proceeds in the upper water layers, a change occurs in the body structure. The head becomes twisted and the

fish now lies and swims on its side (Figure 9). The upper side, which now has both eyes, is pigmented, while the lower side lacks pigmentation. Adult yellowtail fish almost always have eyes on the right side of their bodies. Yellowtail fish have small mouths, and the mouth region is turned up to give the appearance of a "snout". The line running along the sides of the body (the lateral line) is distinctly arched just behind the gill openings, and the tail is rounded. The upper side is usually brownish green, with many irregularly shaped rusty reddish spots, and white on the underside with yellowish colouration just in front of the tail fin.



Figure 16. Illustration representing yellowtail flounder (*Limanda ferruginea*).

Distribution

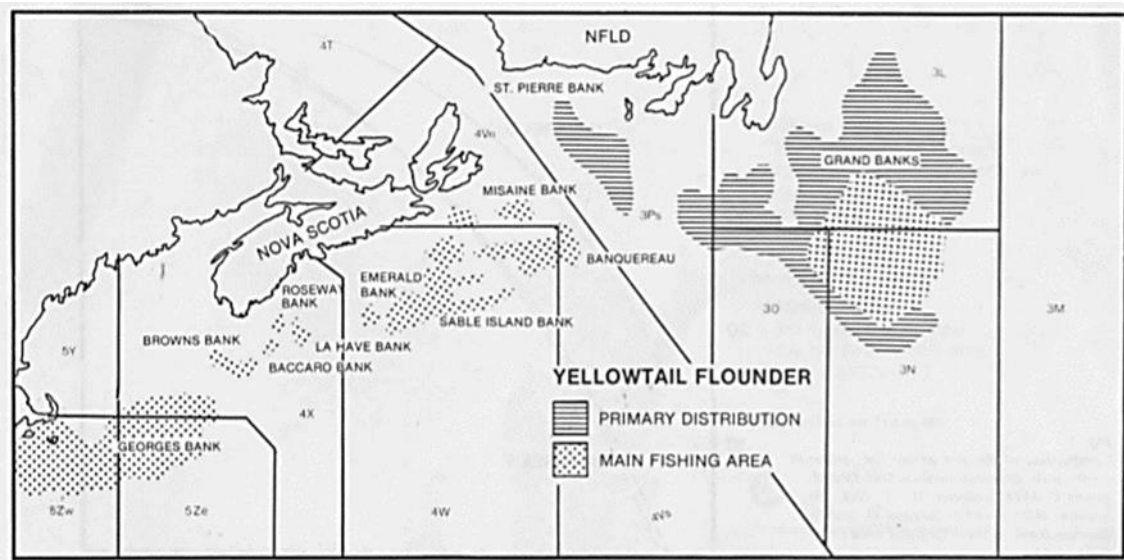


Figure 17. Geographical distribution of yellowtail flounder (*Limanda ferruginea*) (Pitt, 1993).

Similar species in NAFO: Other flatfishes: American plaice, witch flounder, Greenland halibut etc.

Most common product presentation: GUH (Guts and head removed), GHT (Guts, head and tail removed) and all kind of fillets.

Fishery

Yellowtail flounder are distributed within Divisions 3LNO and managed as a single stock.

The Div. 3LNO stock, is open with TACs for 2020 of 17,000 t. Canada, France St. Pierre et Miquelon, United States of America and more recently Japan are the main countries in this fishery.

Yellowtail flounder is caught in a directed trawl fishery and as by-catch in other trawl fisheries. The fishery is regulated by quota and minimum size restrictions. Catches in recent years have been low due to industry-related factors. American plaice and cod are taken as by-catch in the yellowtail fishery. There is a 15% by-catch restriction on American plaice and a 4% limit on cod (NAFO, 2018b).

Last assessment was in June 2018 (NAFO, 2018b) - Stock status: The stock size has steadily increased since 1994 and is presently 1.5 times B_{msy} ($B_{msy}=87.63$ Kt). There is very low (<1%) risk of the stock being below B_{msy} or F being above F_{msy} . Recent recruitment appears higher than average.

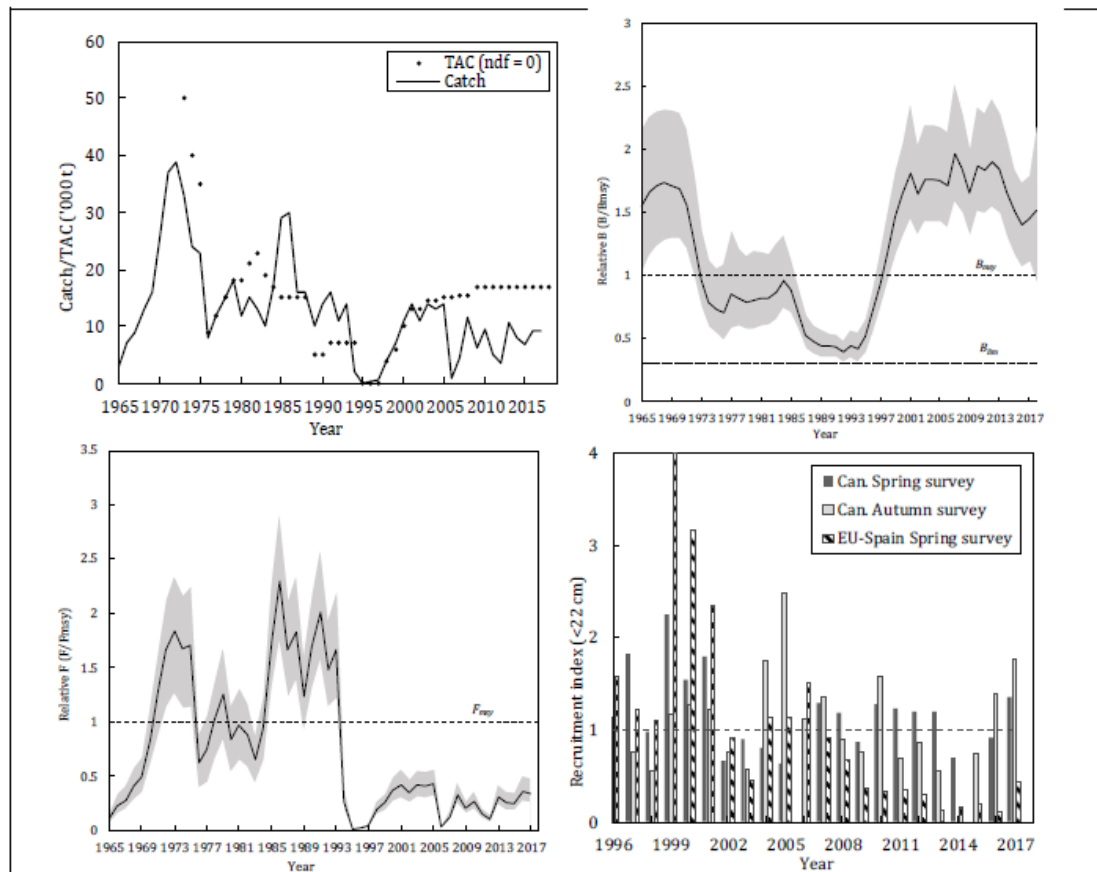


Figure 18. 3LNO Yellowtail flounder: Catches and TACs; Biomass; Fishing mortality and Recruitment. Biomass and fishing mortality with 90% confidence intervals (NAFO, 2018b).

Witch flounder (*Glyptocephalus cynoglossus*)

Description

Witch flounder (*Glyptocephalus cynoglossus*) or "greysole", as it is also commonly known, is a flatfish like American plaice, yellowtail flounder and Greenland halibut. It lies on its left side with the stomach and other visceral contents on the right. This species has several characteristics which make it easily distinguishable from other flatfishes (Figure 11). The body is relatively narrow compared to other flatfishes, with a very small head. On the blind side of the head are about a dozen large open mucous pits which are very noticeable. Its body is oval and very thin with the head occupying only about one fifth of the total body length. It has a very small mouth not unlike that of the yellowtail flounder. The body is covered by smooth scales which make it very slippery and extremely difficult to hold. The body colour of witch flounder is less variable than

most other flatfishes, with the eyed side a dark greyish brown and the under side white with minute dark points all over it, giving it a light grey appearance. It can grow as large as 78 cm in length, with a weight of 3.5-4.0 kg, although witch flounder beyond 60 cm in length and 2.5 kg in weight are uncommon.



Figure 19. Illustration representing witch flounder (*Glyptocephalus cynoglossus*).

Distribution

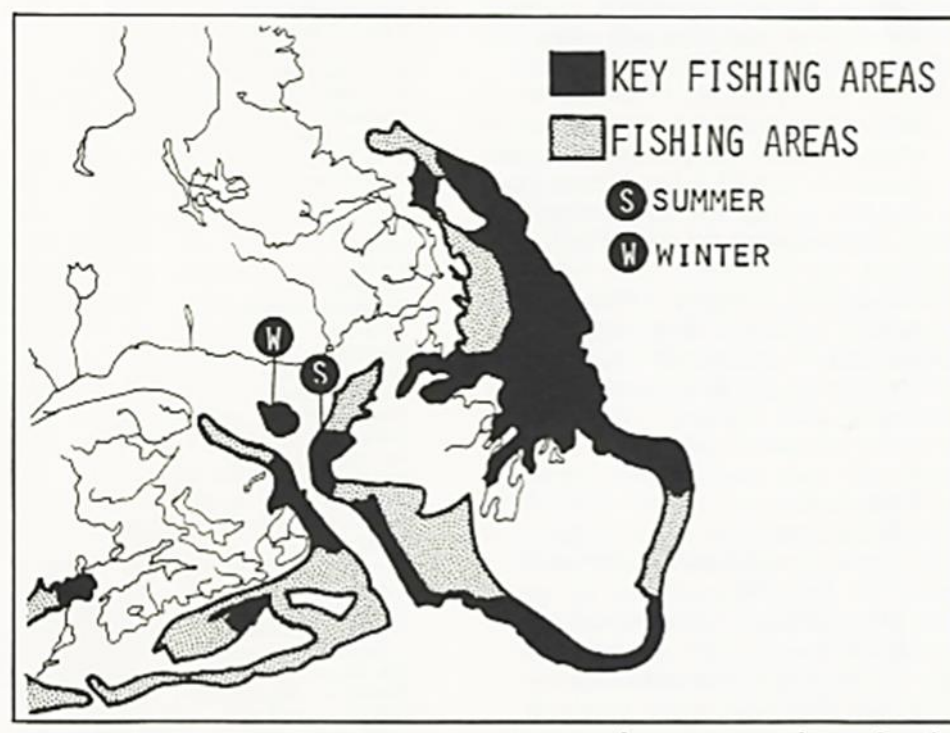


Figure 20. Geographical distribution of witch flounder (*Glyptocephalus cynoglossus*) (Bowering, 1990).

Similar species in NAFO: Other flatfishes: American plaice, yellowtail flounder, Greenland halibut etc.

Most common product presentation: GUH (Guts and head removed), GHT (Guts, head and tail removed) and all kind of fillets.

Fishery

Witch flounder are distributed within and managed as two separate stocks in Divisions 3NO and 2J3KL, though a TAC is only assigned for 3L.

Division 3L

A moratorium was implemented in 1995 following drastic declines in catch from the mid-70s, and catches and catches since then have been low levels of bycatch in other fisheries (e.g. Greenland halibut and redfish fisheries).

A moratorium (no directed fishery) has remained in place since it was declared in 2004. Last assessment June 2016 (NAFO, 2016) - Stock status: The stock remains below B_{lim} . (The probability of biomass being below $B_{lim} = 0.66$). Recruitment during 2013 to 2015 was above average and fishing mortality is current low.

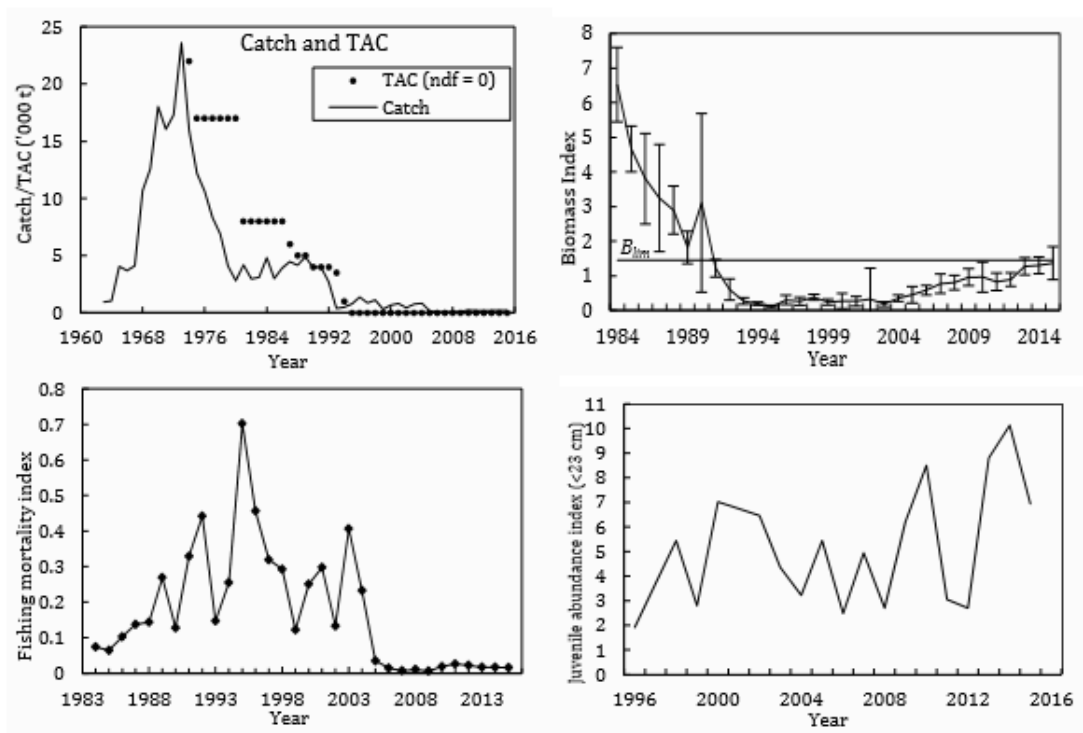


Figure 21. 2J+3KL Witch flounder: Catches and TACs; Biomass index with 95% confidence limits; Fishing mortality and Recruitment indices (NAFO, 2016).

Divisions 3NO

The fishery was reopened to directed fishing in 2015 and is exploited by otter trawl. Prior to the reopening, witch flounder was caught primarily as bycatch in bottom otter trawl fisheries for yellowtail flounder, redfish, skate and Greenland halibut (NAFO, 2019b).

The TAC is set at 1,175 tonnes for 2020.

Last assessment was in June 2019 (NAFO, 2019b) - Stock status: The stock size increased from 1994 to 2013 and then declined from 2013-2015 and has since increased slightly. In 2019 the stock is at 41% B_{msy} (60,000t). There is 20% risk of the stock being below B_{lim} and a 2% risk of F being above F_{lim} (0.063). Except for the growth of the stock following improved recruitment in the late 1990s, it is unclear if the recruitment index is representative.

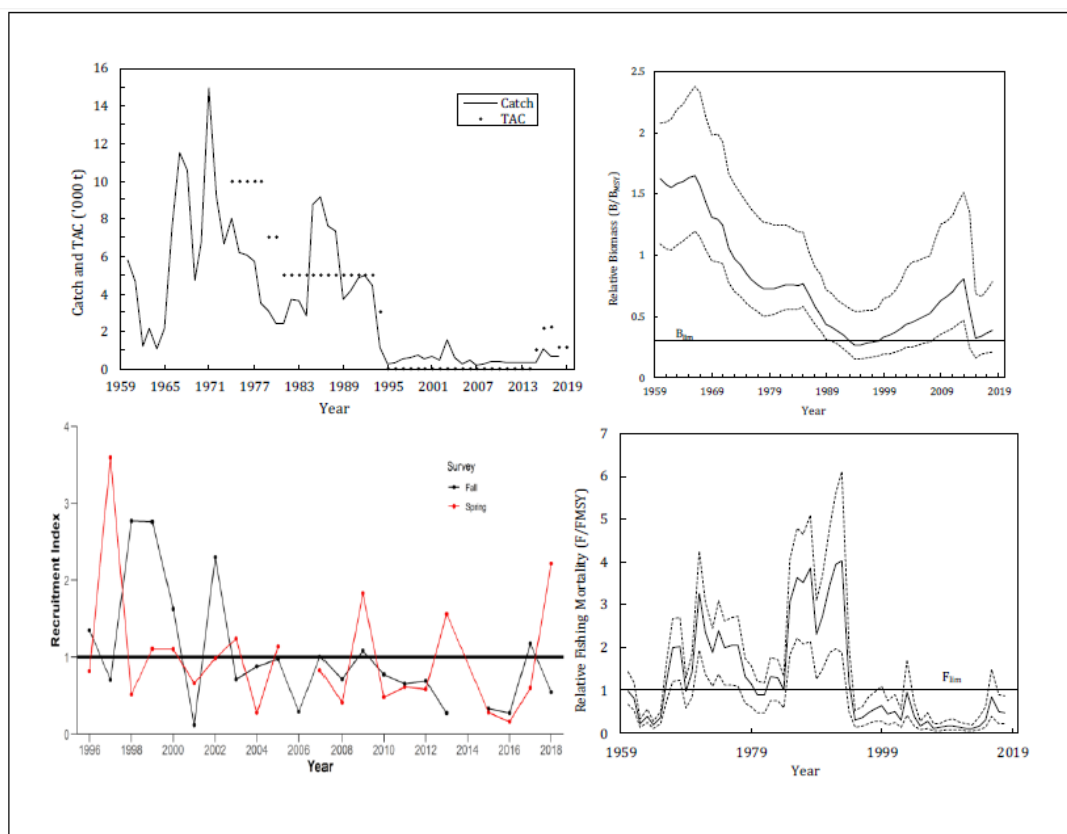


Figure 22. 3NO Witch flounder: Catches and TACs; Biomass; Recruitment and Fishing mortality index. Dashed lines represents the 90% credible intervals (NAFO, 2019b).

Greenland halibut (*Reinhardtius hippoglossoides*)

Description

The *Reinhardtius hippoglossoides* belongs to an order of flat, bilaterally symmetrical fish, the Pleuronectiformes, comprising some seven families and 117 species. The members of this order undergo an amazing transformation during the larval stage. They begin life swimming with the dorsal fin upwards, like any salmon or trout. Gradually, however, one eye migrates across the top of the larva's skull to position itself close to the eye on the other side of the head (Figure 13). There are corresponding modifications to the skull bones, nerves and muscles. The eyeless side, for example, becomes flat while the other side grows slightly rounded. Then, the developing fish turns over and swims on its flat, eyeless side. A few features distinguish the Greenland halibut from other flatfish. Normally, the eyes of flatfish are located on the top, coloured side of the body, and the blind side is white. Most such fish in the north Atlantic are right-sided. That means that individuals of the species lie on the left side as the eye migrates from the left to the right during larval development. In the Greenland halibut, however, the left eye has not completely migrated to the right side but is located on the upper edge of the forehead. Moreover, the blind side is not white, but dark grey, while the other side is nearly black. Furthermore, the fish is not perfectly symmetrical so that some members of the species, those smaller fish that tend to swim in the middle levels of the ocean rather than along the seabed, have been known to swim with the dorsal fin upwards. These special characteristics make the Greenland halibut unusually mobile, and the position of the left eye allows it a greater field of vision than is possessed by most flatfish.



Figure 23. Illustration representing Greenland halibut (*Reinhardtius hippoglossoides*).

Distribution

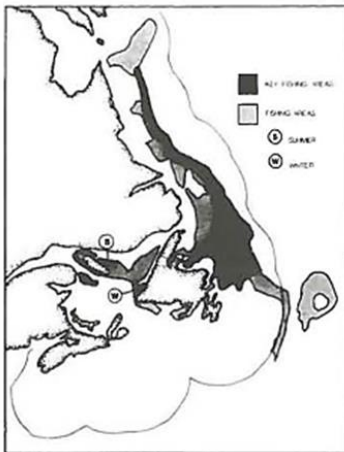


Figure 24. Geographical distribution of Greenland halibut (*Reinhardtius hippoglossoides*) (Bowering, 1993).

Similar species in NAFO: Other flatfishes: American plaice, witch flounder, yellowtail flounder etc.

Most common product presentation: GUH (Guts and head removed), GHT (Guts, head and tail removed) and all kind of fillets.

Fishery

Divisions 3LMNO

Fisheries Commission implemented a harvest control rule (FC Doc. 10/12) to generate annual TACs. The TAC is set at 12,542 tonnes for 2020. The most recent assessment occurred in 2017 when a new Management Strategy was adopted which shall be in force from 2018 to 2023, inclusive.

Subarea 2 and Div. 3K

Due to overlap with the Canadian EEZ no TAC is set at this time.

White hake (*Urophycis tenuis*)

Description

White hake is a groundfish species belonging to the gadoid or cod family of fishes. They have characteristics in common with cod, but in general they are more slender,

soft-bodied fish with a slender caudal peduncle and weak tail (Figure 15). Their eyes are larger than the cod's but the chin barbel is smaller. They also have two dorsal fins, one anal fin and long narrow feeler-like ventral fins. The first dorsal fin has one ray which is filamentous and is as long as the fin proper is high. White hake show considerable variation in colour but are usually muddy or purple brown above with sides sometimes bronzed and the belly a white or yellowish white peppered with small black spots. The dorsal and anal fins are edged with black and the pelvic fins are pale like the belly, but are usually tinged with yellow. The white hake has many characteristics in common with another hake called the red or squirrel hake (*U. chuss*). The two differ in terms of numbers of rows of scales, the length of the filamentous ray on the dorsal fin, the length of the pelvic fins, the gillraker count and the position of the posterior angle of the mouth. There is considerable overlap and variation in some of these characteristics, but there is sufficient difference to consider the two as separate species. It would appear that the white hake is the more numerous of the two species.



Figure 25. Illustration representing white hake (*Urophycis tenuis*).

Distribution

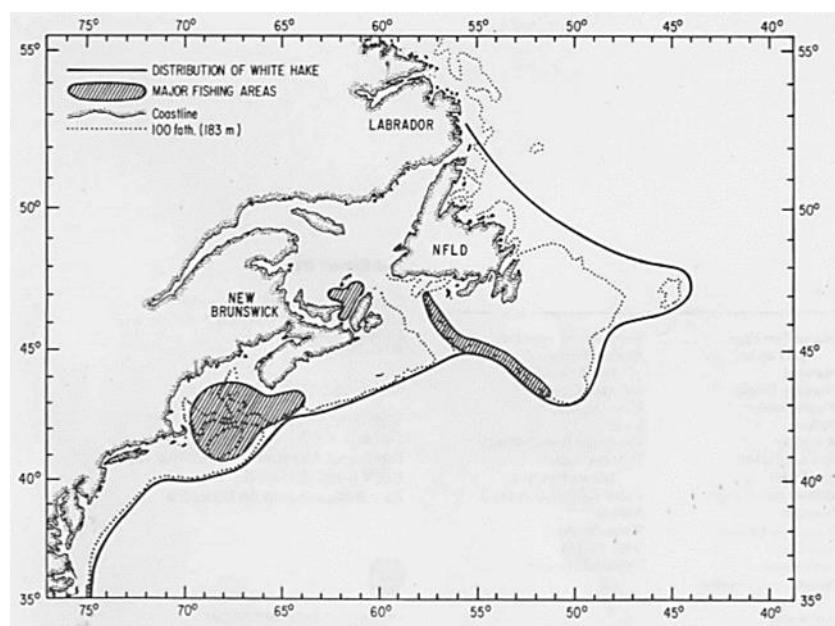


Figure 26. Geographical distribution of white hake (*Urophycis tenuis*) (Bishop, 1993).

Similar species in NAFO: Red hake (*Urophycis chuss*), Longfin hake (*Urophycis chesteri*), cod, haddock (*Melanogrammus aeglefinus*), Pollock (*Pollachius virens*) and others round fishes.

Most common product presentation: GUT (All guts removed), GUH (Guts and head removed), all kind of fillets.

Fishery

White hake are distributed through Divisions 3NO and Subdivision 3Ps though quota is only assigned for Divs. 3NO. Previous studies indicate that fish younger than 1 year, 2+ juveniles, and mature adults distribute at different locations within Div. 3NO and Subdiv. 3Ps so information from the subdivision is factored into assessments of Divs. 3NO.

Divisions 3NO

White hake is caught in directed gillnet, trawl and long-line fisheries. In directed white hake fisheries, Atlantic cod, black dogfish, monkfish and other species are landed as bycatch. In turn, white hake are also caught as bycatch in gillnet, trawl and long-line fisheries directing for other species. The fishery in NAFO division 3NO is regulated by NAFO and in subdivision 3Ps, by Canada (quota initially established in 2018). The fishery is opportunistic when favorable ecosystem conditions allow good recruitment (NAFO, 2019b).

The TAC is set at 1,000 tonnes for 2020.

Last assessment was in June 2019 (NAFO, 2019b) - Stock status: The assessment is considered data limited and is associated with a relatively high uncertainty. Biomass of this stock increased in 1999 and 2000, generated by the large recruitment observed in those years. Subsequently, the biomass index decreased and has since remained variable but lower. No large recruitments have been observed since 2000. Fishing mortality is low.

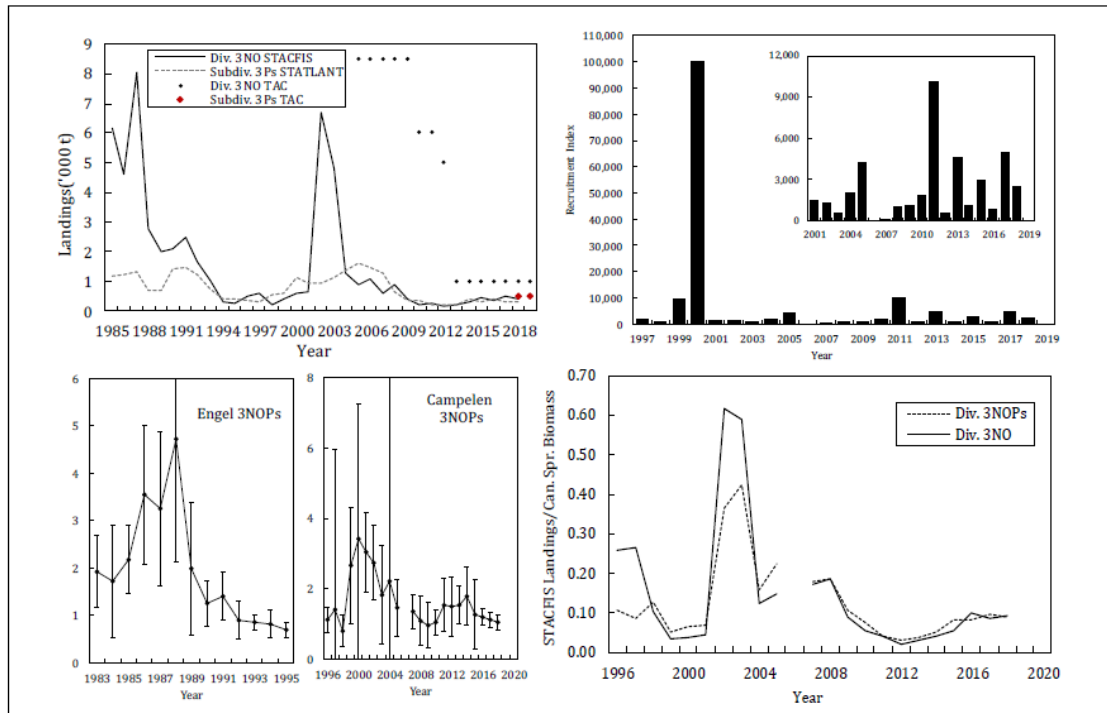


Figure 27. 3NO White hake: Catches and TACs; Recruitment; Survey Biomass index and Fishing mortality index (NAFO, 2019b).

Thorny skate (*Amblyraja radiata*)

Description

Commercial catches of skates comprise a mix of skate species (*Raja radiata*, *Bathyraja spinicauda*, *Raja hyperborean*, *Raja senta*, etc). Thus the catch of skate refers to *Raja* spp. However, thorny skate dominates, comprising about 95 % of the skate taken in the catches.

The bodies of skates are strongly flattened dorsoventrally (generally disc-like), with a well-developed tail which usually has two small dorsal (upper) fins (Figure 17). These are either separated by a very narrow gap or joined at their bases. The mouth is situated on the ventral (lower) surface. The highly developed pectoral (front) fins are attached to the sides of the head, lending them a wing-like appearance. A pair of well-developed spiracles (breathing holes) are usually present, located on top of the head. Breathing is accomplished by inhaling through the spiracles and expelling the water through the gills. The males, when mature, usually have a well developed pair of claspers, or mating organs, which are situated at the apex of the pelvic (rear) fins and tail. Depending on the species, these claspers can be rather long and slender, or relatively short and sturdy with

a club-like thickening (as in thorny skate). The claspers have a cartilaginous hatched (covered) blade-shaped structure called a "knife" which is mostly hidden underneath in the posterior ventral (rear) lobe opening. This knife can be very sharp and care should be taken in handling. The males also develop several rows of alar (spawning) spines on the dorsal surface of the pectoral fins. These spines, when mature, are very sharp and hook-like at the tip. Depending on size or stage of maturity of the skate, there may be 2 to 5 rows with 4 to 10 spines per row. These spines develop during the maturing period and are used to hold the female during mating. The thorny skate (*Raja radiata*) can be distinguished by a row of large thorns on the dorsal (upper) surface along the spine extending from the scapular (front shoulder) region behind the first dorsal fin — with one sometimes between the first and second dorsal fin. There are not more than a total of 19 thorns, or a total of 10 behind the axils of pelvic fins. In addition, there are generally two or three large thorns on each shoulder; one thorn in front and one behind each eye; and one close to the inner end of each breathing hole. Some smaller thorns are scattered on the snout (nose), pectoral fins and tail. The base of the thorns on the dorsal surface is usually star - shaped. The thorny skate is usually brown on the dorsal surface with some darker spots; the under surface is generally white or slightly sooty. It may grow to approximately 100 to 110 cm.



Figure 28. Illustration representing thorny skate (*Amblyraja radiata*).

Distribution



Figure 29. Geographical distribution of thorny skate (*Amblyraja radiata*) (McKone and LeGrow, 1983).

Similar species in NAFO: Other skates (*Raja radiata*, *Bathyraja spinicauda*, *Raja hyperborean*, *Raja senta*, etc). However, thorny skate dominates, comprising about 95 % of the skate taken in the catches.

Most common product presentation: WNG (Wings only), and wings without skin.

Fishery

Thorny skate are distributed within Divisions 3LNO and Subdivision 3Ps. Skates in all divisions are managed as a single stock. Subdivision 3Ps is presently managed as a separate unit by Canada and France in their respective EEZ's while 3LNO is managed by NAFO. As bycatch, it is primarily caught in the cod and redfish fisheries.

Divisions 3LNO

Thorny Skate is caught in directed gillnet, trawl and long-line fisheries. In directed Thorny Skate fisheries, Atlantic Cod, Monkfish, American Plaice and other species are landed as bycatch. In turn, Thorny Skate are also caught as bycatch in gillnet, trawl and long-line fisheries directing for other species. The fishery in NAFO division 3LNO is regulated by quota and mesh size (280mm).

The TAC is set at 7,000 tonnes for 2020.

Last assessment was in June 2018 (NAFO, 2018b) - Stock status: The stock is currently above B_{lim} . The probability that the current biomass is above B_{lim} is >95%. Total survey biomass in Divs 3LNOPs has remained stable since 2007. Recruitment in 2017 was above average. Fishing mortality is currently low.

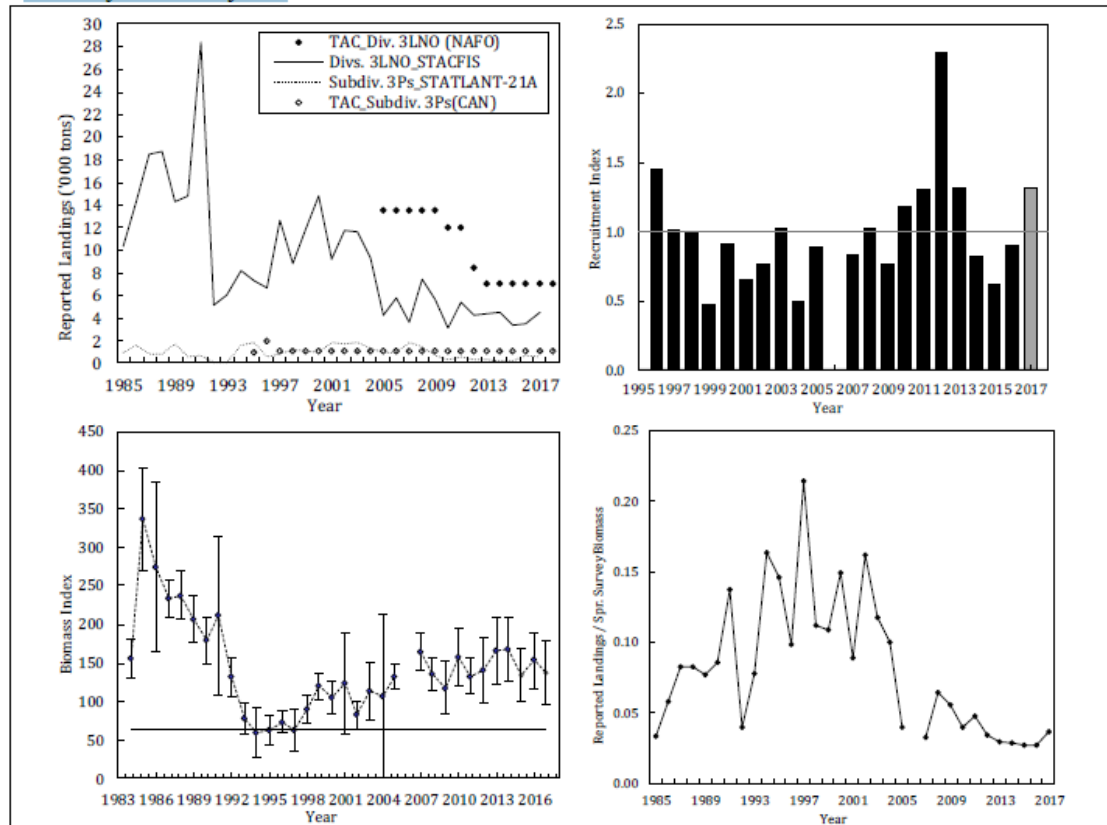


Figure 30. 3NO Thorny skate: Catches and TACs; Recruitment; Biomass; and Fishing mortality (NAFO, 2018b).

Capelin (*Mallotus villosus*)

Description

Once thought to be different species, the Atlantic and Pacific capelin are now considered to be the same species, *Mallotus villosus*. The specific name comes from the Latin, "hairy", and it is interesting to note that the common name of capelin in Norway is "lodde" from the word lodden, meaning hairy. This name may have derived from the spawning ridges of the male. During spawning, two pairs of ridges develop, a prominent dorsal pair running the length of the body above the lateral line and a smaller ventral

pair extending from the pectoral fin back to the pelvic fin (Figure 19). These ridges are formed from elongation of scales that project outward to form soft (hairy) ridges. Besides the spawning ridges, the males also have larger fins which project from the body at this time. Males are larger than females at sexual maturity and this together with the larger fins and spawning ridges gives the males a distinctly more robust appearance. These external sexual characteristics in the males begin to develop at least four to five weeks before the spawning season. During the rest of the year the sexes are almost indistinguishable. Mature specimens are relatively small, approximately 13-20 cm. The colour ranges from olive to bottle green above the lateral line and silver below. The fish are elongate and slender and there are two fins on the back, a larger dorsal fin in the middle and a smaller adipose fin just in front of the tail (Carscadden, 1981).



Figure 31. Illustration representing capelin (*Mallotus villosus*).

Distribution

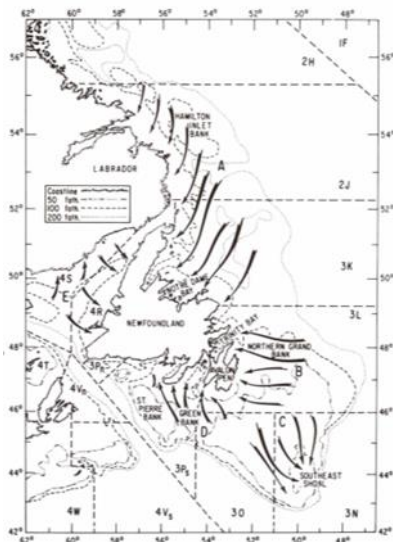


Figure 32. Geographical distribution of capelin (*Mallotus villosus*) (Carscadden, 1981).

Similar species in NAFO: Sand lances/eels (*Ammodytes*), American smelt (*Osmerus mordax*)

Most common product presentation: No reference is found

Fishery

Divisions 3NO

Capelin was caught in a directed trawl fishery. There is low bycatch in other trawl fisheries. The directed fishery was closed in 1992 and the closure has continued. No catches have been reported for this stock from 1993 except 1 t of Spanish catch in 2014 and 5 t Estonian catch in 2016. In 2017, 11 t of discards were reported (NAFO, 2018b).

No directed fishing was advised through 2019- 2021. Unless an acoustic survey is available, the Scientific Council will be unable to produce more specific advice.

Last assessment was in June 2018 (NAFO, 2018b) - Stock status: Acoustic surveys series terminated in 1994 indicated a stock at a low level. Although biomass indices have increased in recent years, bottom trawl surveys are not considered a satisfactory basis for a stock assessment of a pelagic species.

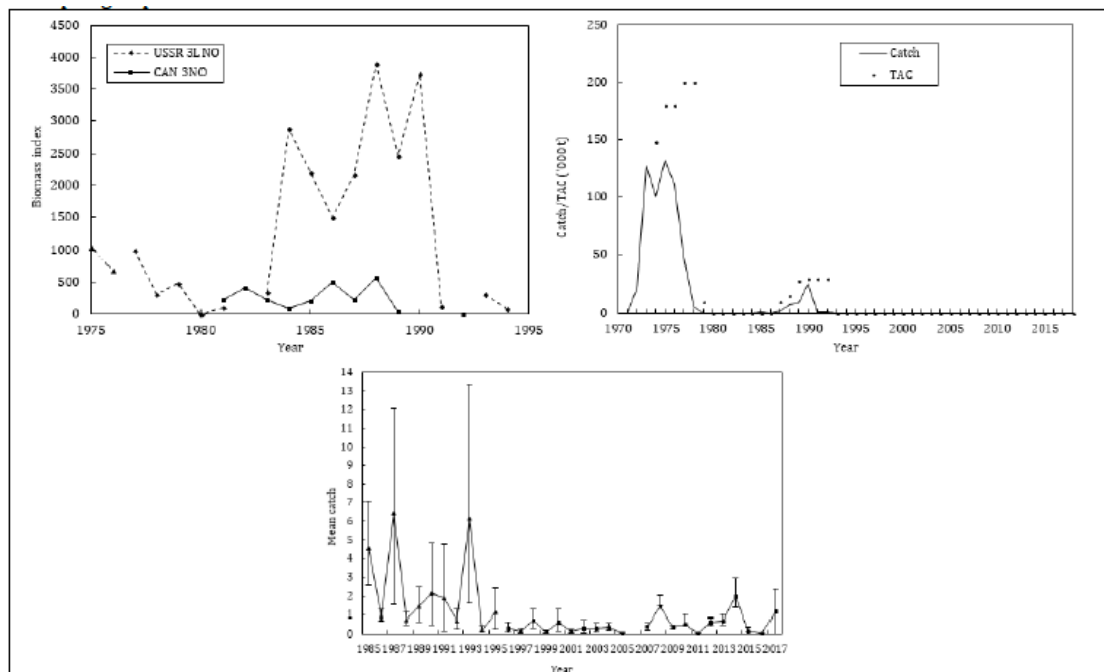


Figure 33. 3NO Capelin: Biomass index; Fishing mortality and mean catch from Canadian spring surveys (NAFO, 2018b).

Shrimp (*Pandalus borealis*)

Description

Shrimp belong to a class of animals known as the crustaceans which includes lobsters, crabs, and crayfish. They possess a hard outer shell (exoskeleton), have jointed legs and, since most are aquatic, breath through gills (Figure 21). The northern shrimp is pale scarlet, has a pair of large compound eyes and attains total lengths of 15 to 16 cm. The shell covering the head and thorax (carapace) is modified into a long, curved, sabre-like structure called a rostrum which has numerous spines on both edges.

Many shrimps, including the northern shrimp, are good swimmers. Appendages on the tail (abdomen), called pleapods, act like paddles and enable the animals to move with remarkable agility, both horizontally and vertically, over considerable distances. Sudden flexing of the tail also allows rapid movement over short distances as an emergency mechanism of escape.



Figure 34. Illustration representing shrimp (*Pandalus borealis*).

Similar species in NAFO: other shrimps.

Most common product presentation: WHL (No processing).

Fishery

Directed fishing for Northern shrimp must follow the guidance of Article 9, Article 13 (mesh size 40mm) and Annex IIB (Authorized Topside Chafers/Shrimp Toggle Chains) of the NAFO Conservation and Enforcement Measures.

Divisions 3LNO

The fishery, until 2014, was a directed bottom trawl fishery and there is little or no bycatch of shrimp in other trawl fisheries. A moratorium (no directed fishery) has

remained in place since it was declared in 2015. The fishery in Div. 3LNO is regulated by quota.

Last assessment was in September 2019 (NAFO, 2019c) - Stock status: Currently the risk of the stock being below B_{lim} is greater than 95%. There is no indication of improved recruitment.

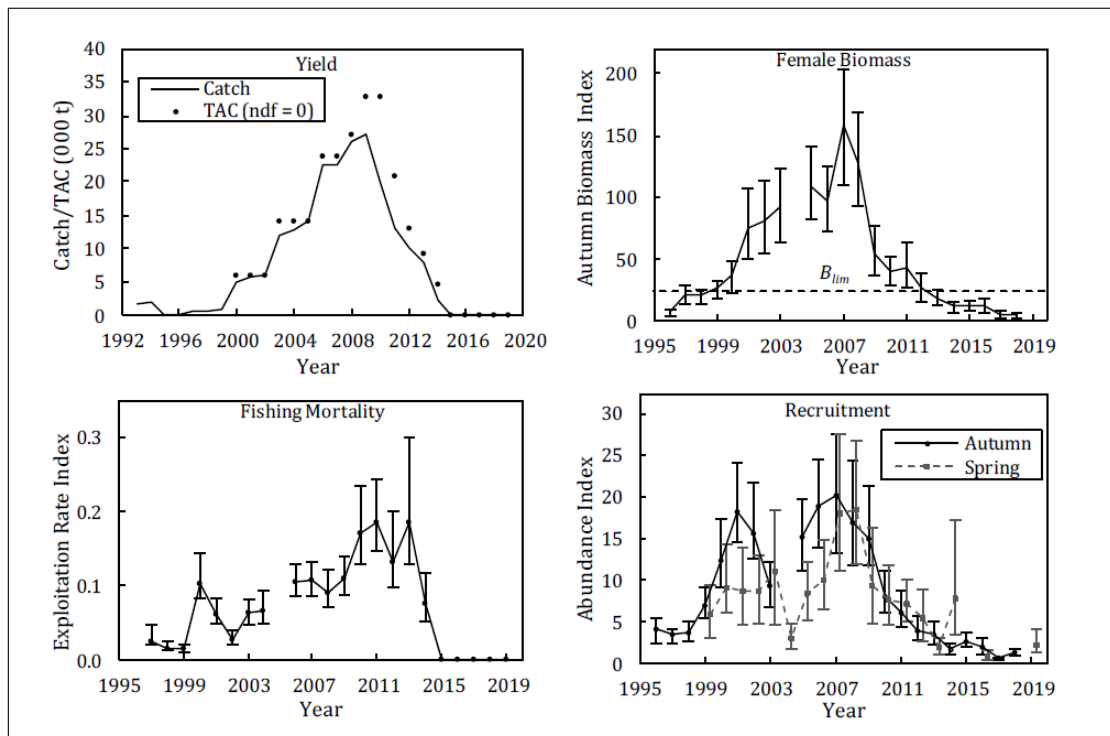


Figure 35. 3LNO Shrimp: Catches and TACs; Survey Female Biomass, Exploitation rate and Recruitment indices. Error bars indicate 95% confidence intervals (NAFO, 2019c).

Division 3M

This fishery is effort-regulated. The effort allocations were reduced by 50% in 2010 and a moratorium was imposed in 2011, this fishery will reopen in 2020. Bycatches are not quantified but it is known that small redfish is caught in this fishery when the recruitment of redfish is high.

The TAC is set for 2020 at 2,640 days (109 vessels).

Last assessment was in September 2019 (NAFO, 2019c) - Stock status: The stock has shown signs of improvement since 2014, and in 2019, the stock has a very low probability of being below B_{lim} . Although recruitment has been weak in the last decade, the recruitment index (age 2) has been increasing since the lowest observed in 2014.

The Scientific Council advised that the catch in 2020 should not exceed the 2009 level (5,448 tonnes).

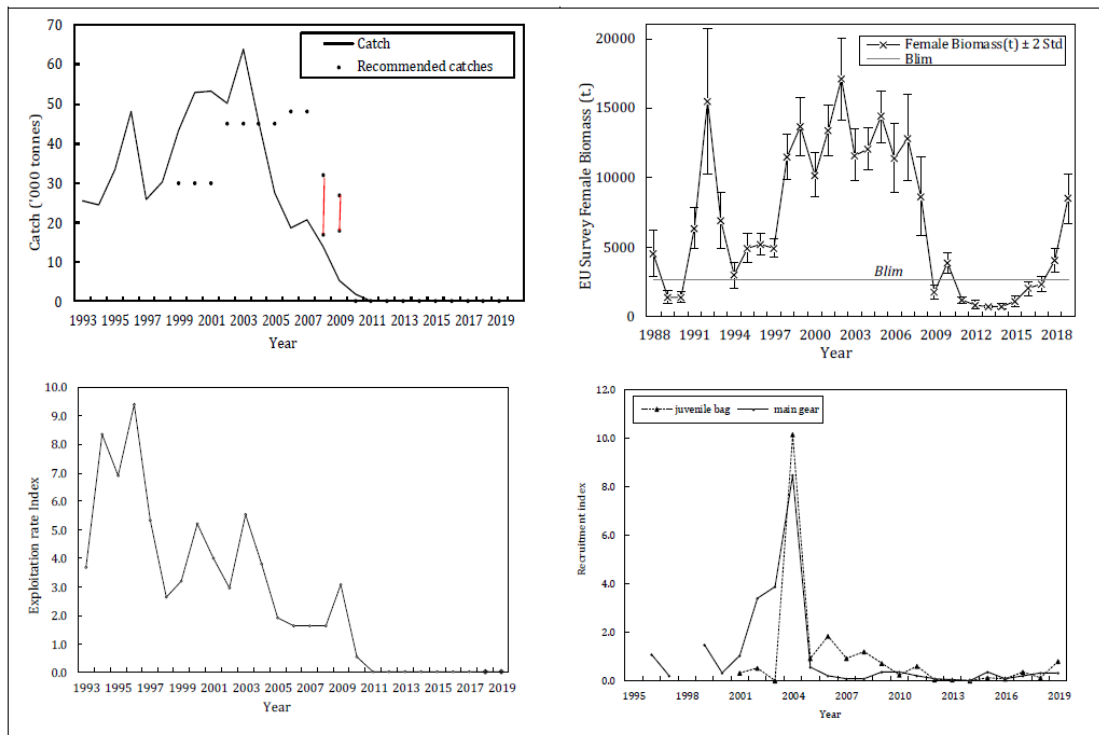


Figure 36. 3M Shrimp: Catches and TACs; Survey Female Biomass, Exploitation rate and Recruitment indices. Error bars indicate 2 std. err (NAFO, 2019c).

Splendid alfonsino (*Beryx splendens*)

Description

The Berycidae (alfonsinos) family are a member of the order Beryciformes. The family includes ten species in two genera, *Beryx* and *Centroberyx*. Generally, the common name “alfonsino” is used to refer to splendid alfonsino, *Beryx splendens* (Lowe 1934). There is also another species of alfonsino that, in some regions, is important in the

fisheries and is variously referred to as longfinned beryx, red bream or broad alfonsino, *B. decadactylus*.

Anon (2009) reports alfonsino to have 4 dorsal spines, 13–16 soft dorsal rays, 4 anal spines, and 26-30 soft anal rays (Figure 22). The first infraorbital bone has a spine projecting laterally on its anterior end and the lateral line extends to caudal fin. In young fishes, the second dorsal ray is elongated.

Beryx splendens can be distinguished from *B. decadactylus* in having a shallower body and fewer dorsal-fin soft rays (13–15 dorsal-fin soft rays for *B. splendens* versus 16–20 for *B. decadactylus*). *B. splendens* is distinguished from other redfishes and the closely related roughies (Trachichthyidae) by having only four dorsal-fin spines.

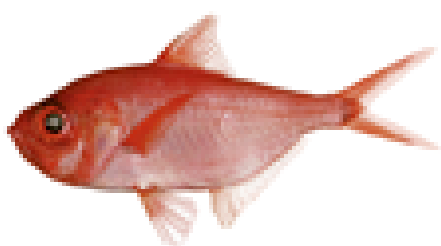


Figure 37. Illustration representing splendid alfonsino (*Beryx splendens*).

Distribution

Alfonsino (*B. splendens*) is widely distributed and can be found in the deep waters of the Atlantic, Indian and Pacific oceans. In the North Atlantic, Alfonsino is an oceanic demersal species which form distinct aggregations, at 300–950 m depth, on top of seamounts. Alfonsino is distributed over a wide area which may be composed of several populations. Stock structure is unknown. Until more complete data on stock structure is obtained it is considered that separate populations live on each seamount.

Similar species in NAFO: redfishes (*Sebastes spp.*)

Most common product presentation: JAP (Transversal cut removing all parts from head to belly), GUH (Guts and head removed), WHL (No processing)

Fishery

Division 6G

Commercial aggregations of alfoncino on the Corner Rise have been found on three seamounts. Two of them named “Kükenthal” (known also as “Perspektivnaya”) and “C-3” (“Vybornaya”) are located in the NAFO Regulatory Area. One more bank named “Milne Edwards” (“Rezervnaya”) is located in the Central Western Atlantic. Russian vessels fished in this area in different periods between 1976 and 1999 using pelagic trawls. There is no statistics on the Russian fishery on separate seamounts. Based on the information collected in the 2004 Spanish experimental survey in Corner Rise, a directed commercial fishery had been conducted since 2005 by Spanish vessels. Since 2006 virtually all the effort has been made in the Kükenthal seamount with pelagic trawl gear (NAFO, 2019b).

No analytical or survey based assessment were possible. The only data available at present are the catch and effort time series. Despite the difficulties of interpreting the CPUE as an indicator of stock status and knowing that this species is easily overexploited and can only sustain low rates of exploitation, the sharp decline in CPUE to the lowest observed (92% lower than in 2017) and catches in the last year indicate an apparent overfishing situation and that the stock may be depleted. The SC advises in June 2019 to close the fishery until biomass increases to exploitable levels (NAFO, 2019c).

The fishery was closed in 2020.

Last assessment was in June 2019 (NAFO, 2019b) - Stock status: Appears to be depleted.

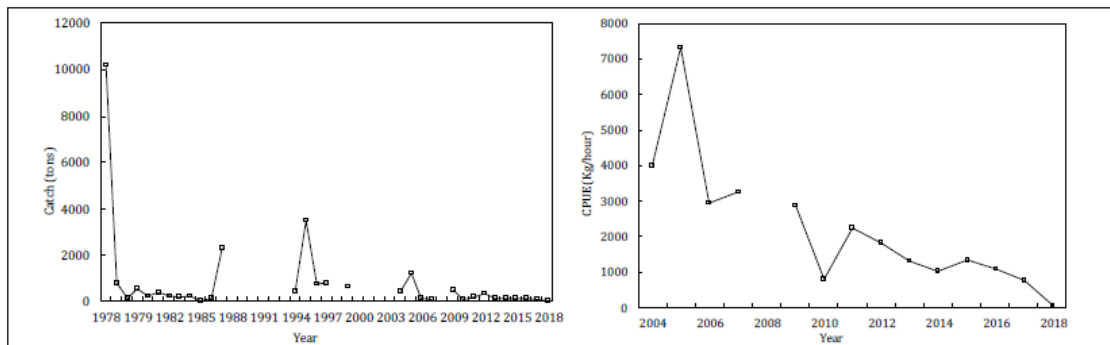


Figure 38. 6G Splendid alfonsino: Catch and CPUE (NAFO, 2019b).

Short-finned Squid (*Illex illecebrosus*)

Description

Squid belong to the Class Cephalopoda of the Phylum Mollusca. There are approximately 650 recognized species of cephalopods alive today and more than 10,000 fossil forms. Cephalopod translates literally into "head footed" which explains why squid, as well as the nautilus, cuttlefish and octopus among others, with their arms and tentacles attached directly to their heads, are so named.

People are often surprised to learn that the rapidly-swimming squid (Figure 23), with no external shell, is related to molluscs such as clams, oysters and snails. In fact, squid have a small internal shell called a pen which extends along the back of the body and acts as a support to the soft, muscular body.

Short-finned squid reach a body or "mantle" length of more than 30 cm and a total length of more than 60 cm. The cigar-shaped body has two triangular fins at the rear, and a funnel and distinct head with eight sucker-equipped arms and two tentacles, on the other end. They have large, well-developed eyes and a strong parrot-like beak. Squid use their fins for swimming in much the same way fish do, and the funnel for extremely rapid "jet" propulsion forward or backward. The squid's capacity for sustained swimming allows it to migrate long distances as well as to move vertically through hundreds of metres of water in its daily feeding.

The short-finned squid generally has a mixed, iridescent coloration of milky white and rusty brown. The colour changes rapidly as the squid expands or contracts the colour cells in its skin as a camouflage or in response to attack (Rowell, 1989).



Figure 39. Illustration representing short-finned squid (*Illex illecebrosus*).

Distribution



Figure 40. Geographical distribution of short-finned squid (*Illex illecebrosus*) (Rowell, 1989).

Similar species in NAFO: Other Cephalopods.

Most common product presentation: TUB (Tube only), WHL (No processing).

Fishery

Subareas 3 + 4

Prior to the mid-1980s, international bottom trawl and midwater trawl fleets participated in directed fisheries in Subareas 3, 4 and 5+6. Since 1999, there has been no directed fishery in Subarea 4, but some squid is taken as bycatch in the Canadian small-mesh bottom trawl fishery for silver hake. Directed fisheries currently consist of a Canadian inshore jig fishery in Subarea 3 and a small-mesh bottom trawl fishery in Subareas 5+6. In 2018, at least one vessel conducted a directed trawl fishery in 3O. There is no bycatch in the jig fishery. There are separate management regulations applied by NAFO, USA and Canada (NAFO, 2019d).

Fishing may not occur from 1 January to 30 June in Subareas 3 +4 in accordance with Article 11, NAFO CEM.

The TAC is set at 34,000 tonnes for 2020.

Last assessment was in September 2019 (NAFO, 2019d) - Stock status: Trends in fishery and research vessel survey data indicate that a period of high productivity (1976-1981) occurred in Subareas 3+4 between two low productivity periods (1970-1975 and 1982-2017). During 2018, the Div. 4VWX survey was not completed. However, the Div. 4VWX biomass index and mean body size during 2019 indicate that the stock may be moving towards a high productivity period.

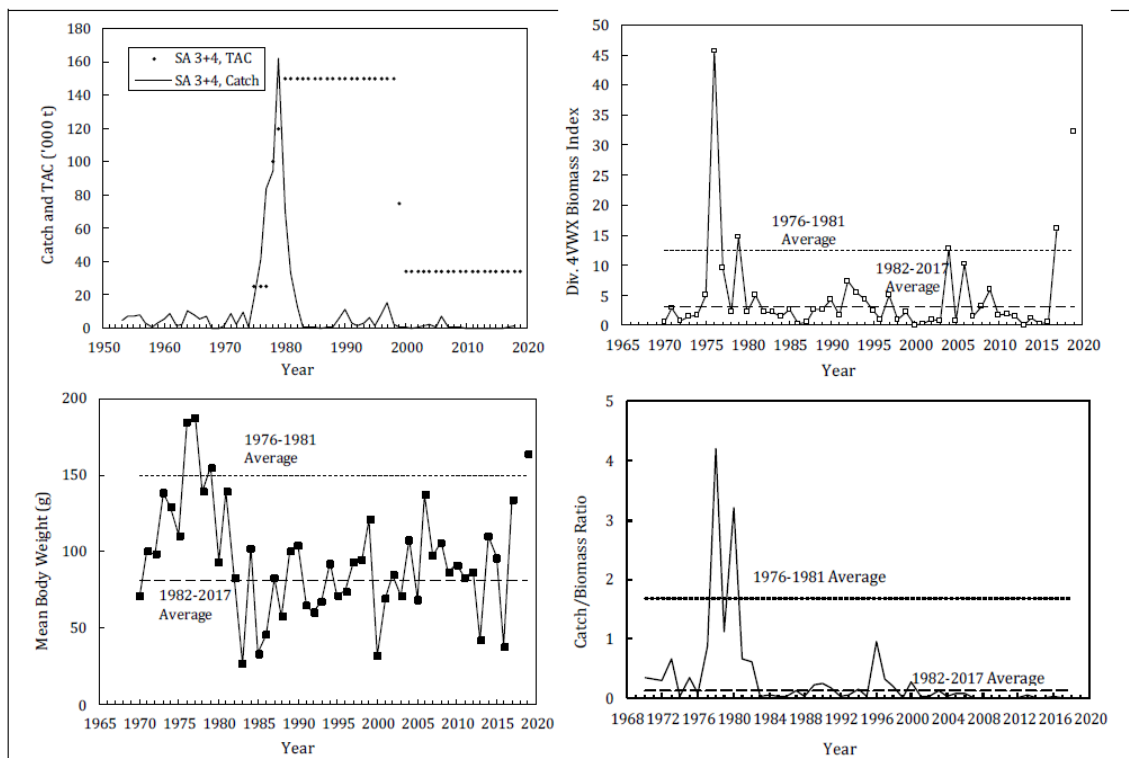


Figure 41. SA 3 + 4 Short-finned Squid: Catches and TACs; Biomass; Mean Body Weight and Fishing mortality (NAFO, 2019d).

This thorough description helped in the understanding of the specific reality of the fisheries and fleets operating in the targeted NAFO RA.

2.2.4. SUB-TASK 2.3 – ELABORATION OF MANUAL OF PROCEDURES

The Sub-Task 2.3, entitled “Elaboration of Manual of Procedures”, was initiated in mid-August after the delivery of the Interim Report. The timely accomplishment of the chronologically previous Sub-Tasks (2.1 and 2.2) was a decisive contribution for the accomplishment of Sub-Task 2.3. Indeed, the structure and several main key pillars of the IMP have already been brought to a state of development that makes possible the successful fulfilment of this Sub-Task’s objectives within the stipulated timeframe.

The IMP structure can already be sketched to be built along the following main themes:

- I - Introduction and Objectives;
- II - Definitions;
- III - Types of Sample;
- IV - Kit for the Collection of Samples;
- V - Procedures for Sampling, Collection, Registration, Transport, and Delivery;
- VI – Laboratory Analysis;
- VII - Costs;
- VIII - Forms;
- IX - Certification of the Fishery Inspectors;
- X – Versions;
- XI – Legal References;
- XII – Annexes.

In the section I – Introduction and Objectives, the regulatory framework that encompasses the development and establishment of an International Manual of Procedures is presented. Within the frame of NAFO, the European regulatory framework was used as the reference in structuring the aims and scope of the document.

Moreover, on the basis of the performed Literature Review (Task 1) as explained in section 2.1.3., genomics and DNA analyses were concluded to be preferential methods of molecular analysis reaching a quality standard that enables their application in food authenticity testing. In particular, it is mentioned in section I of IMP that the DNA sequences present in the individuals of each species, although different from each other,

share patterns that allow, without ambiguity, to distinguish each of the individuals belonging to it from any representatives of other species. This makes possible the specific classification of any individual through the analysis of the respective DNA sequences, the study of which is viable in almost any biological vestige that belongs to it, since the same DNA is found in all its organic parts. Hence, several methods of DNA analysis shall be possible, including DNA barcoding, that is, to identify a species by extracting a short DNA sequence from a specific gene or genes and comparing it with a reference library of such DNA sequences.

Moreover, it was considered that the European Fisheries Control Agency (EFCA) is the most adequate entity for providing suitable training to the Inspectors, comprising overall approach to fishing vessels operating in NAFO RA, random selection of lots, and sampling procedures and techniques. Since EFCA organizes training workshops for NAFO Inspectors on a yearly basis, it was also advised that the updated IMP version should be distributed among them and feed-back on the IMP should be expected every year. In addition, EFCA was deemed to be a better (or at least complementary) forum to discuss potential refinements of the IMP, as results of ongoing genetic analyses can be shared with EFCA on a practical routine basis.

The scope and main goals of the IMP are also presented and briefly discussed. The addressees of the IMP are also mentioned. Likewise, the concept of the Chain of Custody and its procedures are outlined and appraised.

In the section II – Definitions, the concepts necessary for the scope of the IMP are defined. Namely, definitions are given for: Box; Chain of Custody; Fishery Products; Lot; Presentation/Product Presentation; Shipment; and Transformation. The definitions were revised and trimmed to their core concepts.

Regarding section III – Types of Sample, it is emphasized that to carry out a test by DNA analysis requires sample collection of biological material by removing biological material from part of the fishery product and that this part may be fins, teeth, scales, muscle, skin, blood or viscera. Moreover, it was added a detailed list of possible fish

species that may be sampled in the NAFO RA, which, in turn, was built on the basis of the Descriptive Analysis of the Fishing Fleets operating in the NAFO RA.

Concerning section IV – Kit for the Collection of Samples, it is explained in what consists the kit for muscle sampling, being also mentioned further items beyond the kit itself. The kit items comprise: a numbered safety casing; three bottles; one non-reusable ampoule containing ethanol; disposable tip forceps; disposable scalpel; two pairs of non-powdered disposable vinyl gloves; absorbent cloth/tissue; and a 15 cm x 10 cm disposable polypropylene board. It is also mentioned that each kit is used for the collection of a single sample and that each EU member country as well as extra-EU countries (being EU and the extra-EU countries NAFO contracting parties) will engage the respective Unity of Inspection and Control for the management of the Kits.

With respect to section V - Procedures for Sampling, Collection, Registration, Transport, and Delivery, a detailed array of procedures is given for the various main steps of the global action.

For sampling, the number of samples of the lot to collect is given as a function of a formula that uses as input the probability of species variability in a given fishery products' lot, which, in turn, is a function of the fisheries knowledge in the NAFO RA, as seen in section 2.2.3.2. (Sub-Task 2.2 – Preparation of a Descriptive Analysis of Fishing Fleets). For a clearer understanding of the formula, its application was exemplified.

$$n_0 = Z^2 \times p \times q/e^2$$

Where,

n_0 – Sampling dimension;

Z^2 - Abscissa of the normal curve that cuts off an area α at the tail ($1 - \alpha$ equals the desired confidence level, for instance, 95 %);

e - Intended level of precision;

p - Estimated proportion of an attribute that is present in the population (species

identity);

q – Corresponds to 1-p.

The value for Z is found in statistical tables which contain the area under the normal curve. On the other hand, the value of p may be estimated on the basis of the probability of finding a fish box from a different species in the fish lot of a given targeted species. On the basis of previous knowledge of the fish species and fishing fleets highlighted in the Descriptive Analysis of the Fishing Fleets operating in the NAFO RA, it is possible to assess p to be in a wide range (particular case-studies for different fish species and taking into account fish preparation processes, for instance, in fillets, are being prepared for inclusion in the IMP).

However, Inspectors should adjust p to the empirical experience as they feed-back the species identification results into the formula, thereby calculating specific sampling dimensions for each species. For instance, if it is observed a probability of 1:275 of finding a fish box from a different species in the fish lot of a given targeted species, that is, p is 0.00364, for a confidence level of 95 % and e of 0.05, n₀ is approximately 6.

For the case when the size of the population to be sampled is not large (< 10,000 boxes) and in accordance to STECF suggestion, minimum sampling size (n₀) was adjusted with the finite population correction as follows:

$$n_{0\text{corrected}} = N \times n_0 / (n_0 + N - 1)$$

where N denotes the population size (i.e. number of boxes in a lot).

Moreover, whenever minimum sampling size is estimated to be less than 3 boxes, a minimum sample of size equal to 3 boxes was established.

Finally, the consortium agreed that representative genetic sampling throughout the whole NAFO RA was out of the scope of this contract, as inspectors have their own risk assessments that define the inspection strategies to target specific lots.

In the case of collection, the minimum portion required for DNA analysis is given. It is also referred that if the size or characteristics of the product to be sampled are not sufficient to make up the recommended portion, in particular for bivalve molluscs or crustaceans, whole specimens or parts of specimens, such as crab legs, should be collected. Moreover, a list of special aspects that must be taken into account in the collection step are expressed and described.

The registration of collection is also the object of a detailed description. Indeed, in order to ensure the chain of custody, the entire process must be documented through the collection record and the identity of the individuals who had access or handled the sample(s), promoting trustworthiness, integrity, and sample (evidence) traceability until use of the sample as evidence. The specific regulatory framework is presented.

A new sub-section entitled “Preventing Cross-Contamination of Samples” was added. It lists a set of good practices that minimize the risk of sample cross-contamination.

Likewise, the transport and delivery are associated to a specific set of procedures. Namely, the maximum period for delivery of the sample in the laboratory must be five working days after sample collection. The Form of transport and delivery of the sample must always be filled out at the time of delivery of the collection in the laboratory or whenever the evidence(s) (sample(s)) is handled or transported by a person other than the Inspector who made the collection. In the case that it is necessary to carry the sample through transport companies, a copy of the transport documents must be attached to the Form of transport and delivery of the sample.

In a new section VI – Laboratory Analysis, a detailed description of the possible DNA analysis methods that may be applied to fish samples is presented. This is largely based on the Literature Review (Task 1). It presents the most usual DNA analysis methodologies, their advantages and drawbacks as well as which criteria must be used to choose a particular methodology.

Afterwards, a detailed description of DNA analysis procedures is given. It comprises the procedures for the sample pre-treatment, the sample preparation and extraction (both for genomic and mitochondrial DNA), and the DNA amplification by Polymerase Chain

Reaction. The procedures for performing DNA barcoding and other identification approaches through DNA after PCR are presented in the IMP (Deliverable D2.1).

Moreover, in future, it would be useful to develop reliable, real time barcoding and species identification applications (e.g. on smartphones) that could allow pre-scanning of samples by inspectors, so as to reduce the number of final genetic analyses to be conducted in labs.

In section VII – Costs, the calculation of the cost of the whole procedure is explained.

For section VIII – Forms, it is highlighted that the models of the Forms for collection of samples of the fishery products and for the transport and delivery of samples of fishery products are an integral part of the IMP and are presented in the section XII – Annexes.

Concerning section IX - Certification of the Fishery Inspectors, the institutional and regulatory aspects that encompass this subject are presented.

Regarding section X – Versions, the rules and nomenclature of the various versions of the IMP are defined.

For section XI – Legal References, it is emphasized that the references to the legal diplomas of the NAFO contracting parties (in the case of EU, commissioning each member state) are done in the respective updated versions.

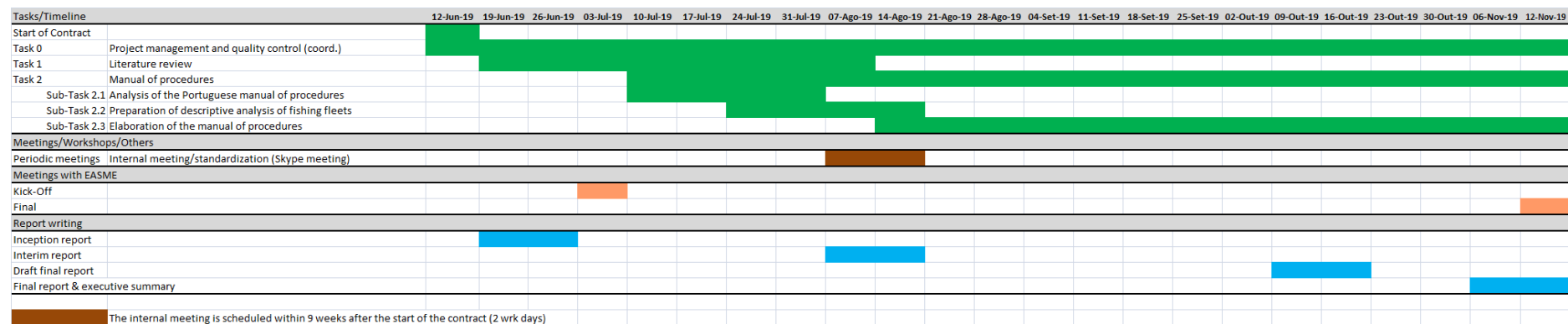
Finally, in section XII – Annexes, the case-studies regarding estimation of sample dimension (number of boxes to be sampled per lot), the statistical tables for the calculation of sample dimension, the Form for the collection of samples of the fishery products, and the Form for the transport and delivery of samples of the fishery products are fully presented.

The IMP document has been completed and has reached its definite version, being deliverable D2.1.

3. TIMETABLE FOR THE IMPLEMENTATION

The schedule of the work to be developed during the project is detailed in Table 9 and follows the tender specifications with exception of the extension of Task 1 deadline. The contract started on 12th June 2019 and its planned duration is 5 months, i.e., 22 weeks. Its end will be on the 12th November 2019.

Table 9. Tasks and activities chronogram.



Executive Agency for Small and Medium-sized Enterprises (EASME); European Maritime and Fisheries Fund (EMFF)

EASME/EMFF/2016/008 Provision of Scientific Advice for fisheries beyond EU Waters

"Study to produce an International Manual of Procedures (IMP) to be used in the NAFO Regulatory Area to guide the collection of samples from fisheries products for genetic analysis"

4. LIST OF DELIVERABLES

Table 10 provides a summary and the timing of the deliverables to be submitted to EASME and DG-MARE during the course of this project. There was no alteration in the Deliverables list.

Table 10. Deliverables list, dissemination level and due date.

Deliverable no.	Deliverable name	Dissemination level	Del. date
D.01	Inception Report	EASME/DG MARE	26 th June 2019
D.02	Interim Report	EASME/DG MARE	11 th August 2019
D0.3	Draft Final Report	EASME/DG MARE	23 rd October 2019
D0.4	Final Report and Executive Summary	EASME/DG MARE	12 th November 2019
D1.1	Review Document	EASME/DG MARE	11 th August 2019
D2.1	Manual of procedures	EASME/DG MARE	12 th November 2019

5. LIST OF MEETINGS

Table 11 shows the list of meetings that are envisaged during the study. Likewise, no departure from planning.

Table 11. Meetings list, location, participants and tentative dates.

Meeting	Meeting name	Location	Participation	Date
M0.1	Kick off meeting	Brussels	EASME/DG MARE + Project Coordinator (IPMA) + FWC Coordinator (AZTI)	4 th July 2019
M0.2	Meeting for standardization of EU manual protocols ¹	Skype meeting	IPMA + AZTI + IEO	14 th August 2019
M0.3	Interim meeting	Skype meeting	EASME/DG MARE + Project Coordinator (IPMA) + FWC Coordinator	28 th August 2019
M0.3	Final meeting	Brussels	EASME/DG MARE + Project Coordinator (IPMA) + FWC Coordinator (AZTI)	12 th November 2019

¹ Proposed additional meeting. Not specifically requested in the ToRs.

6. LIST OF MILESTONES

The detailed list of Project Milestones is listed in Table 12.

Table 12. List of milestones, nature, due date and mean of verification.

Mil. no.	Milestone name	Task no.	Nature ²	Exp. date	Means of verification ³
MS 1.1	Establishment of the guidelines and criteria to assist in the conduction of the literature review	1	R	19 th June 2019	Inception Report ⁴
MS 1.2	Review of the data and information gleaned from the literature review	1	R	7 th August 2019	Progress Report
MS 2.1	Review of significant precedents (Portuguese manual of procedures)	2	R	31 st July 2019	Progress Report
MS 2.2	Establishment of guidelines for the descriptive analysis of the fishing fleets	2	R	14 th August 2019	Progress Report
MS 2.3	Critical review and assessment of existing data collected under current protocols against previous DCF and SFPA requirements	2	R	14 th August 2019	Progress Report
MS 2.4	Assessment of potentially needed changes to data reporting protocols under latest DCF and SFPA requirements	2	R	14 th August 2019	Progress Report
MS 2.5	Based on MS 2.1 to 2.4, summary of inputs to be considered for the development of manual of procedures in Sub-Task 2.3	2	R	14 th August 2019	Progress Report
MS 2.6	Establishment of the guidelines to be followed in the elaboration of the manual of procedures	3	O (manual)	21 st August 2019	Draft Final Report ⁵
MS 2.7	Preparation of a draft of the manual of procedures	3	O (manual)	25 th September 2019	Draft Final Report
MS 2.8	Manual of procedures for the collection of samples from fisheries products for genetic analysis	2	O (manual)	12 th November 2019	Draft Final Report

¹ Nature of the Deliverable/Milestone: **M** = meeting; **R** = Report, **O** = Other (specify)

³ Refer to indicators as appropriate.

⁴ First Interim draft Report should be submitted on Week 9.

⁵ Draft Final Report should be submitted on Week 16.

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