Eve-Marine Pubert

Study of parasite diversity and otolith shape of plaice (*Pleuronectes platessa*) off the coast of Iceland for stock discrimination.

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Mestrado em Biologia Marinha

Supervisors:

Haseeb Randhawa

Co-supervisor

Karim Erzini

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Declaração de autoria de trabalho

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

Declaration of authorship of work

I declare I am the author of this work, which is original and unpublished. The sources consulted have been duly cited in the text and included in the list of references.

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Abstract

Understanding the stock structure of a commercial species is essential for sustainable management. Failure to do so can lead to the depletion of regional sub-populations, genetic diversity erosion, and ecosystem services loss. Plaice, *Pleuronectes platessa*, are a commercially important species for the Icelandic economy and are found in warm southern and cold northern waters. However, plaice is managed as a single stock. Over the last three decades, the use of parasites as biological markers has proved effective in distinguishing between different fish stocks. The use of otolith shapes for stock discrimination has gained popularity in recent years thanks to advances in image analysis tools and geometric methods. The study on 82 individuals of plaice from different geographical locations and seasons in Iceland showed that the community of parasites and the morphology of otoliths varied significantly between regions and periods, constituting several sub-populations in Icelandic waters. A notable differentiation between Icelandic plaice stocks was observed according to otolith shape and parasitic diversity.

Otolith shape showed significant differences between the four geographical and seasonal groups (North-Summer, North-Winter, South-Summer, and South-Winter). Plaices collected in the southern region during the winter had otoliths with distinct shapes compared with the other groups. The parasite study confirmed the otolith shape results. The South-Winter group was also distinguished by a higher abundance of parasites and a different parasite composition than the other groups.

Hysterothylacium aduncum was a candidate to be a parasite tag due to its constant presence throughout the year and in all regions but with notable variations in abundance and prevalence.

Keywords:

Stock identification Parasite Otoliths shape Fisheries management

Resumo

A compreensão da estrutura das unidades populacionais de uma espécie comercial é essencial para a sua gestão sustentável. Caso contrário, pode levar ao esgotamento de subpopulações regionais, à erosão da diversidade genética e à perda dos serviços ecossistêmicos ligados a dita espécie. Um dos fundamentos da gestão sustentável das pescas é a identificação das unidades populacionais, que é essencial para compreender a sua estrutura. Este conhecimento é inestimável para estratégias de gestão eficazes destinadas a promover práticas de pesca sustentáveis. A solha (*Pleuronectes platessa*), uma espécie de peixe chato, é de importância vital para a economia islandesa. Esta espécie é amplamente pescada em águas islandesas, tanto nas zonas mais quentes do sul como nas águas mais frias do norte. Apesar destas diferenças ambientais acentuadas, a solha islandesa é atualmente gerida como uma única unidade populacional. Nas últimas três décadas, a utilização de parasitas como marcadores biológicos revelou-se eficaz em distinguir as diferentes unidades populacionais de peixes. Em paralelo, a utilização das formas dos otólitos para a discriminação das unidades populacionais ganhou popularidade nos últimos anos, graças aos recentes progressos registados em matéria de ferramentas de análise de imagem e métodos geométricos. Estes dois métodos foram combanidos para determinar a discriminação das unidades populacionais da solha islandesa Pleuronectes platessa, estudando assim a diversidade parasitária e a forma dos otólitos da solha ao largo da costa islândesa. O presente estudo centra-se, por conseguinte, na utilização destes dois métodos, com 82 indivíduos de solha de diferentes localizações geográficas e estações na Islândia. A análise dos dados obtidos mostrou que a comunidade parasitária e a morfologia dos otólitos variam consideravelmente entre regiões e períodos, constituindo várias subpopulações nas águas islandesas. Foi observada uma diferença significativa entre as unidades subpopulacionais de solha islandesa em função da forma dos otólitos e da diversidade de parasitas.

Foram recolhidos ectoparasitas e endoparasitas de todos os órgãos e tecidos da solha amostrados no norte e no sul da Islândia. A prevalência, a intensidade média da infeção e a abundância média foram calculadas para todos os parasitas em cada órgão/tecido de ambas as localidades. As identificações morfológicas dos parasitas foram confirmadas utilizando ferramentas moleculares (código de barras por sequenciação Sanger) e gerando uma biblioteca molecular dos diferentes taxa de parasitas. Esta biblioteca foi utilizada para avaliar o alcance geográfico de outros parasitas e para compreender a sua dinâmica de transmissão. As

comunidades de parasitas foram analisadas utilizando métodos estatísticos multivariados e o sucesso da reclassificação foi avaliado utilizando um teste de permutação para determinar o potencial sucesso desta abordagem. A análise dos otólitos foi efectuada extraindo os otólitos sagitais de cada peixe, aos pares, e fotografando-os com um estereomicroscópio LEICA M165C. As imagens foram limpas e convertidas em imagens binárias utilizando o software Fiji (ImageJ). As imagens resultantes foram usadas para medir o diâmetro máximo de Feret (distância máxima entre dois pontos no otólito) para garantir uma orientação consistente para análise. As formas dos otólitos foram analisadas utilizando o pacote ShapeR do RStudio. Esta análise envolveu a recolha de coeficientes elípticos de Fourier (EFC) para captar os contornos dos otólitos. Os coeficientes foram ajustados até que 98,5% da variância da forma dos otólitos fosse reconstruída. Isto permitiu comparar as diferenças de forma entre as populações estudadas, divididas por estação e região (norte-verão, norte-inverno, sul-verão e sul-inverno). Em seguida, foi efectuada uma permutação do tipo ANCOVA para verificar a significância estatística das diferenças de forma dos otólitos entre os grupos e uma validação cruzada para avaliar o desempenho do modelo de classificação utilizado.

A solha de inverno do sul teve a maior abundância de parasitas, com uma média de 32,93 parasitas por peixe, enquanto a solha de verão do norte teve a menor abundância, com uma média de 0,6 parasitas. Os peixes do norte tendem a ser mais pequenos do que os do sul, especialmente no inverno. Os principais parasitas identificados incluíam nemátodos como o *Anisakis simplex* e o *Hysterothylacium aduncum*, bem como tremátodos como o *Zoogonoides viviparus*. Uma análise de variância (ANOVA) revelou diferenças significativas no tamanho dos peixes entre os grupos de estudo, sendo os peixes do sul maiores no inverno. A relação entre o tamanho dos peixes e a abundância de parasitas foi significativa no norte, mas não no sul. No entanto, o tamanho dos peixes explicou apenas 15,7% da variância da abundância de parasitas no norte. A análise discriminante linear (LDA) mostrou diferenças na composição da comunidade de parasitas entre os grupos, com uma distinção marcada entre os peixes do sul no inverno e os outros grupos.

A forma dos otólitos mostrou diferenças significativas entre os quatro grupos geográficos e sazonais (norte-verão, norte-inverno, sul-verão e sul-inverno). A solha recolhida na região sul durante o inverno apresenta formas de otólitos distintas das dos outros grupos. O estudo feito aos organismos parasitários confirmou os resultados relativos à forma dos otólitos: o grupo do sul do inverno distingue-se igualmente por uma maior abundância de parasitas e por uma

composição parasitária diferente dos outros grupos. As diferenças observadas nos parasitas e na forma dos otólitos sugerem variações geográficas e sazonais nas populações de solha islandesa. A dimensão relativamente pequena das amostras pode ter induzido erros na classificação das populações. Recomenda-se um aumento do número de amostras para melhorar a robustez dos resultados. Alguns parasitas não tinham sido registados anteriormente na solha islandesa, como é o caso de organismos como o *Anisakis simplex*, o *Hysterothylacium aduncum* e o *Zoogonoides viviparus*.

O *H. aduncum*, um abundante nematoda anisakidae nos peixes Norte Atlânticos, é candidato a marcador de parasitas eficaz, devido à sua presença constante ao longo do ano e em todas as regiões, mas com variações notáveis em termos de abundância e prevalência.

No entanto, para que o nosso estudo seja mais robusto, será necessário aumentar o número de amostras, de forma a reduzir a variabilidade dos dados e, por consequencia, melhorar e garantir o vigor dos resultados obtidos. Recomenda-se também a recolha de informações sobre a dieta dos peixes, a fim de compreender melhor a sua cadeia alimentar e o seu comportamento migratório, assim como o alargamento a amostragem a outras regiões do território maritimo islandês (leste e oeste) para obter uma imagem mais completa das populações de solha islandesa.

Palavras-chave:

Identificação das unidades populacionais Parasita Forma dos otólitos

Gestão das pesca

Table of Contents

I- List of Figures

II List of Tables

III. List of Abbreviations

AICc = Akaike Information Criterion corrected

CPUE = Catch Per Unit Effort

DSTs = Data storage tags

EDTA = EthyleneDiamineTetraacetic Acid

EFC = elliptical Fourier coefficients

Ext = exterior

GDP = Gross Domestic Product

GIS = Geographic Information Systems

GLM = generalized linear models

GLMM = Generalized Linear Mixed Model

GSI = Genetic stock identification

ICES = International Council for the Exploration of the Sea

Int = inside

IPCC = The Intergovernmental Panel on Climate Change

LDA = linear discriminant analysis

MFRI = Marine and Freshwater Institute

NS = North Summer

NW = North Winter

PCR = Polymerase Chain Reaction

PSAT = Pop-up Satellite Archival Tags

RFID = Radio Frequency Identification

SAM = Stock Assessment Model in State Space

SD = standard deviation

SNP = Single Nucleotide Polymorphism

SS = South Summer

SW = South Winter

TAE = Tris-Acetate-EDTA

TL = Total Length

VIFs = variance inflation factors

1. Introduction

Overfishing is a significant global threat to the oceans, along with other human impacts such as pollution, deteriorating water quality, and human-induced climate change, particularly in coastal areas worldwide. Harming target species and profoundly altering the structure and functioning of marine food webs even risks eliminating a trophic level, thus weakening ecosystem resilience (Jackson et al., 2001). One of the foundations of sustainable fisheries management is stock identification, which is essential for understanding stock structure. This understanding is invaluable for effective management strategies to rebuild fish stocks and promote sustainable fishing practices. Fish stocks vary over time due to environmental variations (temperature, salinity, currents, nutrients), which affect their reproduction and survival (Brander, 2010). Overfishing reduces populations and stocks beyond their natural renewal (Worm et al., 2009). Climate change and warming waters are altering the distribution of species, pushing them towards colder waters (Pinsky et al., 2013), but sometimes, to maintain optimal environmental conditions, fish also tend to move, which can lead them to migrate northwards when water temperatures rise (Borgerson, 2008). Pollution by heavy metals, plastics, and chemicals is damaging fish health and reproduction (Derraik, 2002). Assessing fish stock accurately and efficiently is essential to ensure sustainable fishing. A good stock assessment will enable efficient biological identification of the fish, which is important for understanding genetic diversity, population structure, and potential gene flow between neighboring populations of the same species. This information is important for conservation efforts, sustainable management practices, and the prediction of population resistance to environmental change (MacKenzie & Abaunza, 1998).

The definition of 'stock' in fisheries has evolved significantly over the past century.
In the early 20th century, Dahl's 1909 perspective viewed a stock primarily as a source of fish, shaped by human activities impacting the population's productivity. He described it as a "harvesting stock," meaning a group of fish defined by their natural attributes and interactions with human fishing practices. This definition focused on how fishing activities modified and grouped natural fish lineages. (Waldman, 2005). At the end of the 20th century, definitions were more theoretical than practical. Larkin (1972) defined a stock as "... a population of organisms which, sharing a common gene pool, is sufficiently discrete to be regarded as a self-perpetuating system that can be managed". Unlike at the beginning of the century, we discuss

stock management here. This definition evolved further with the Magnuson Fishery Conservation and Management Act of 1976, which referred to a species, subspecies, geographical group, or other fish categories that could be managed as a unit (Begg et al., 1999). Most recently, National Oceanic & Atmospheric Administration (NOAA) Fisheries 2012 defined a 'biological' fish stock as a group of fish of the same species inhabiting the same geographic area and interbreeding when mature. This definition emphasizes the biological aspects of stock, focusing on species-specific, geographical, and reproductive characteristics (Headley 2020).

Fisheries management involves the establishment of defined stocks and studies to ensure sustainable fishing and production of fish stocks (Mahé et al., 2019). Understanding the stock structure is essential for establishing strict rules for fisheries management. However, it is essential to strike the right balance between precision and generality when defining stocks. If the definition is too precise, it will not be robust enough to cover all situations, and if it is too general, the definition will be of little use. This dilemma needs to be considered throughout the definition of stocks because they can vary and change, so we need to adapt. If you do not adapt, you could endanger the whole stock and, worst case, condemn it to extirpation (Waldman, 2005).

Conducting stock assessments is complex, even after correct stock identification. Fish are highly mobile, constantly moving to feed, migrate, and spawn, and direct counting is not feasible. These factors can contribute to poor stock assessment and, consequently, to inefficient stock management practices, which can lead to the overexploitation of specific stocks (Ying et al., 2011). To deal with these complexities, scientists use various data collection methods. They rely on fishing reports and deploy research vessels to monitor catch rates, study fish biology, and estimate abundance.

Fisheries stock assessment involves estimating the current and future numbers of fish in the ocean, enabling the setting of sustainable fishing quotas (Cadrin & Dickey-Collas, 2015). It is essential to identify overfishing and adapt future management strategies. Due to factors like oceanic conditions, human activities, and economic conditions, stock assessments need regular updates (Melnychuk et al., 2021).

Stock identification is mandatory for stock assessment (Cadrin et al., 2014). Individual stocks can only be assessed if they are delimited, i.e., if their boundaries are clearly defined to other units of the same species (Waldman, 2005). Stock identification identifies coherent groups of individuals wholly or partially distinct from their fellows in space or time. These groups of individuals must respect several characteristics that are essential for their management: they have their physical life history, including geographically or temporally unique spawning grounds (Dos Santos et al., 2022); they are subject to their natural demographic influences, such as mortality from a particular set of predators (Geraerts et al., 2022) ; their total or partial isolation allows them to adapt their morphological and genetic characteristics to their specific environmental conditions (Schaefer & Fuller, 2022); and the influence of fishing and habitat contamination on their abundance and life cycle (Waldman, 2005, Zhang et al., 2021). However, many populations are still managed as single stocks. This is often due to practical considerations such as resource limitations, which make it difficult to carry out the detailed studies needed to delimit and manage multiple stocks (Cadrin et al., 2014). In addition, historically developed management frameworks based on single-stock management may persist due to economic and political pressures. These pressures may include the need for simple management approaches that are easier to implement and enforce, particularly in regions where data on stock structure is scarce or where there is significant overlap between stocks (Mace, 2004).

Several approaches to stock identification exist. Each provides different information and views of the stocks under consideration, varying regarding analysis, cost, and practicality. Sampling strategy and methodology are therefore essential, as the characteristics of each sample differ with each sampling. This affects the allocated time, place, and type of fishing of caught samples and methods and criteria used to identify each stock.

1. Methods for Stock Identification

1.1/ Acoustic and radio telemetry:

Acoustic and radio telemetry are essential for gathering information on fish and invertebrate species' behavior and migration patterns, and they play a crucial role in stock assessment.

Ultrasonic transmitters emit a sound pulse picked up by a hydrophone equipped with an acoustic receiver (DeCelles & Zemeckis, 2014). Various coding techniques can be used to distinguish between transmitters, such as modulating the transmission frequency, adjusting the tag's pulse rate (number of pulses per second), varying the interpulse interval (time between pulses), or changing the duration of the pulses (DeCelles & Zemeckis, 2014). Radio telemetry uses low-frequency sound transmission in shallow freshwater environments where signals can travel longer distances than acoustic signals. (DeCelles & Zemeckis, 2014) On the other hand, radio telemetry relies on transmitting high-frequency radio signals. However, acoustic telemetry has limitations, and several factors affect the detection of an acoustic beacon. These factors include background noise (e.g., vessel traffic, wave action, snapping shrimp), acoustic shadowing (related to bottom topography and vegetation), and the physical properties of the water (e.g., temperature and turbidity) (Cotton, 2010). In the Atlantic at Placentia Bay, researchers used acoustic telemetry to track the movements of cod fitted with acoustic tags. Using tag detection on tagged fish, the data showed that cod remained faithful to the spawning site during the spawning season and dispersed outside this period (DeCelles & Zemeckis, 2014).

Pop-up Satellite Archival Tags (PSAT)

Pop-up Satellite Archival Tags (PSATs) are used for fish stock identification by providing data on fish's vertical and horizontal movements and environmental behaviour. These devices are attached to fish and record information such as depth and temperature as they move through the ocean. When the tags detach and float to the surface, they send this data to satellites, allowing researchers to track fish migrations and understand their movements between different areas. PSATs aid in stock identification by tracing fish migration routes and determining their spawning or feeding areas (Brunnschweiler, 2013).

1.2/ Age and Growth Analysis:

Throughout the fish's lives, multiple structures will continue to grow parallel with somatic growth, including different calcified formations such as otoliths and scales. These various structures can help compare and identify groups of fish with varying growth histories. Based on these other structures, annual, seasonal, and daily sequential growth marks are deposited, allowing the extraction of chronological information for specific life stages. The use of these calcified structures in stock identification stems from their use in estimating age and growth for stock assessment purposes: variations in growth patterns can indicate different stocks.

Although both otoliths and scales are calcified formations, they show significant differences in structure, composition, and formation process (Panfili et al., 2002). Otoliths begin to form in the embryo, whereas scales form at different times depending on their position on the body; in older fish, scales may thicken or resorb, and growth may cease or become irregular.

Sagittal otoliths, chosen for their large size, have been used to study growth marks. The fish’s age is determined using their otoliths by counting the growth rings on them. To estimate age, the otoliths are photographed and analyzed using manual or automatic methods (Ordoñez et al., 2022). It is also possible to determines fish’s age through their scales, which have microscopic structures that help determine the age. The circuli are lines that form as the fish grows. When growth slows, for example, during the winter months, the circuli become closer together, forming a distinct annulus that marks a year of growth. Counting these lines helps define the age of the fish (Hile, 1936). Another, more effective method of determining the age of elasmobranchs involves examining the growth rings on the inner surfaces of vertebrae. These rings form due to calcium deposits that vary with the seasons, creating bands that can be observed under a microscope. By counting these rings, we can estimate the animal's age (Scacco et al., 2024).

1.3/ Biological Data and Life History Characteristics:

Planktonic dispersal shapes fish populations and forms genotypic, phenotypic, and contingent stocks. Stock definitions hinge on spatial and temporal distinctions in individual groupings during specific life cycle stages. Genotypic stocks imply persistent isolation during spawning from one generation to the next (Hare & Richardson, 2014). The distribution of eggs and early larvae reflects spawning's spatial and temporal patterns. Maturity and variations in reproductive biological cues can help identify distinct populations. Discrete distributions indicate separate spawning areas, while continuous distributions suggest ongoing spawning or a mix following distinct events (Hare & Richardson, 2014). Discrete early life stages are proxies for separate spawning; continuous stages indicate ongoing spawning or a mix of distinct events. Distinct spawning in time or space results in individual stocks, while continuous reproduction creates a single stock. This use of early life stages for stock definition is commonly applied, particularly in defining genotypic stocks (Neilson et al., 1988). A study on North Atlantic haddock analyzed data on the distribution and abundance of haddock eggs, larvae, juveniles, and adults in the Northwest Atlantic, particularly in waters around North America. Data were collected during spring surveys from 1977 to 1988. These surveys identified specific spawning and nursery areas, showing that differences in biological parameters of haddock may indicate distinct stocks (Begg, 2005).

1.4/ Electronic Marking and Tagging:

Radio Frequency Identification (RFID) Tags:

Since the 1980s, new tags have been introduced for individual fish. These markers are called PITs (passive integrated transponders) and use low-frequency radio frequency identification (RFID) technology. (Hall, 2014). The passive integrated transponders are implanted inside the fish in the dorsal pterygiophores, inserted with a tiny 12-gauge needle, allowing many animals to be tagged in the muscle and the body cavity. Externally implanted tags consist of a low-frequency transponder placed in a small glass or plastic shell. As they have no built-in batteries, these tags require detection by a transceiver or 'reader' external to the tagged fish. This reader can be fixed or portable depending on the practicality of recapturing the tagged fish's identification number. (Hall, 2014). A well-known example of a tag is the PTI tags in the Columbia River (Canada & US) (see http://www.ptagis.org/), used to tag over one million juvenile salmon as part of a study to analyze the impact of hydroelectric dams on the movement of juvenile salmon (Hall, 2014).

Visual Tags: External tags with unique markings can be used for visual identification:

The selected fish are tagged at different locations to analyze and retrieve movement data. The fish are captured, tagged, and then released at the exact location. The data collected are used to study their movements and estimate their migration rate. This method shows the population's overall migration rate but not the underlying populations (Schwarz, 2014). More advanced techniques can be divided into two broad categories (with some overlap): methods that assess movements between different stocks measured at specific times and those that assess movements within the same stock in continuous space and time. (Schwarz, 2014)

1.5/ Fatty acid:

Each fish species has its fatty acid profile. However, it is crucial to consider the variability in tissue fatty acid composition in each fish. More than twenty fatty acids are present in concentrations greater than 1% in the lipids of marine animals (Grahl-Nielsen, 2014). The detailed study of fatty acid composition is of particular interest because these components can be influenced by factors such as the age and maturity of the fish, as well as external variables, such as the environment's temperature, pressure, and salinity. However, the most essential parameter to study remains diet. By understanding the diet of a fish community, it is possible to define its ecological environment and trophic interactions (Grahl-Nielsen, 2014). These factors affect neutral triacylglycerides and polar phospholipids, the fatty acids most affected by fish physiology. An in-depth analysis of these components would better understand fish physiology and shed light on their ecosystem's complex dynamics (Grahl-Nielsen, 2014).

Using the composition of fatty acids in their heart tissue, the two Faroese cod stocks, Faroe Bank and Faroe Plateau, showed significant differences between the stocks. Ten fatty acids in total lipids showed significant differences between the stocks. Differences were observed in the phosphatidylcholine and phosphatidylethanolamine fractions, reflecting distinctions between the two populations (Joensen et al., 2000).

1.6/ Genetic Markers:

Microsatellite DNA Analysis:

Microsatellites are short relative DNA sequences of 6 to 10 base pairs in length. These short repetitive sequences can be found in thousands of locations in the genome and have a higher mutation rate than other regions due to replication slippage experienced by the DNA polymerase during replication. These microsatellites are found in non-coding DNA or in areas of the genome that are not subject to selection (Saiki et al., 1988). Once amplified, the alleles can be separated and precisely calibrated on a polyacrylamide gel, showing one or two distinct bands. It is widely used to study variation between individuals in a population (O'Connell et al., 1997). Microsatellite markers are suitable for fisheries and aquaculture management because they quantify genetic variations within the same species population (Abdul Muneer et al., 2009).

DNA Barcoding :

This approach, characterized by one or more short genetic sequences extracted from a standardized genomic segment used for species identification, aims to rapidly and reliably identify many life forms, such as animals, plants, and microorganisms (Kress & Erickson, 2012). In marine biodiversity assessment, DNA barcoding can also identify individuals at different life stages, incomplete specimens, and cryptic species, i.e., morphologically similar species (Persis et al., 2009, Chakraborty & Ghosh, 2014). It can also be beneficial in identifying mislabeled fishery products and detecting the illegal capture of protected species, thus enabling their protection (Civera, 2003, Basheer et al., 2017, Filonzi et al., 2021).

DNA barcoding is based mainly on the analysis of the mitochondrial COI gene. To do this, there are several steps to follow: DNA extraction, specific amplification of the COI gene by PCR, and then sequencing. The sequences obtained are compared to reference databases to identify species and distinguish populations. The differences between the COI sequences make it possible to identify distinct stocks and analyze genetic relationships through phylogenetic trees or haplotype networks. By comparing these DNA sequences, it is possible to determine whether fish from different regions belong

to distinct stocks, thus indicating a certain degree of genetic isolation (Antoniou & Magoulas, 2014). DNA barcoding involves two basic steps: building a library of barcodes of known species and matching or assigning the barcode sequence of the unknown sample to the barcode library for identification purposes (Hebert et al., 2003). This will allow all living species to be identified by genomics using only one or a few genetic regions. An algorithm will compare the DNA sequences of the unknown species to the database to measure the distance between the sequences (Kress & Erickson, 2012). DNA barcoding typically uses a sequence alignment algorithm to match an unknown sample to a known species by finding the sequence in the database that is closest to the sample sequence (Ratnasingham & Hebert, 2007). Although many markers have been used for DNA barcoding, the COI (cytochrome c oxidase subunit I) gene fragment most effectively identifies 98% of marine and 93% of freshwater fish species (Ward et al., 2005).

SNP (Single Nucleotide Polymorphism) Analysis:

SNPs (Single Nucleotide Polymorphisms) are common genetic variations that substitute a single nucleotide in the DNA. They are generally found in areas of the genome subject to selection and are, therefore, more useful. A large database of population-specific SNP frequencies, combined with targeted amplification of these loci, provides precise resolution of stock origins for many fish species. These SNPs are typically found in regions of the genome that are under selection, making them particularly useful. Genetic stock identification (GSI) SNP protocols rely on amplifying short amplicons by targeted multiplex PCR but sequencing throughput on the Nanopore platform is limited. PCR amplicon concatenation, enabling multiple amplicons to be sequenced in a single read, is a promising approach for increasing sequencing throughput. (Cornelis et al., 2017; Schlecht et al., 2017). Information on these variations can highlight the genetic differences between populations and help find stocks. (Deeg et al., 2022). An SNP study demonstrated that Atlantic herring (*Clupea harengus*) feeding in the Norwegian Sea comes from several stocks. A total of 551 individuals from spawning grounds and 498 from feeding grounds were analyzed. DNA was extracted from fish muscle, fin, or gill tissue. A panel of 120 SNPs was genotyped for each individual. The analyses revealed three main groups: the Faroese herring group, the North Sea Autumn-Spawning Herring group, and the Norwegian Spring-Spawning Herring group (Pampoulie et al., 2024).

1.7/ Geospatial Information Systems (GIS):

Geographic Information Systems (GIS) are technologies that use hardware and software to capture, store, process, analyze, and visualize spatial or geographic data. These data can encompass information on the Earth's physical characteristics, human activities, and phenomena occurring on the planet (Aguilar-Manjarrez & Meaden, 2013). GIS is commonly used for the visualization, quantification, and analysis of spatial data, which in fisheries enables the identification of different fish stocks and their subsequent study by combining this information with other tools such as statistics, modeling, or mapping. Ecological data, particularly fisheries data, generally have a spatial component and lend themselves well to GIS analysis (Eder & Neely, 2013).

A study was conducted on the Pong reservoir in Himachal Pradesh, India, on 20 different fish species. To analyze environmental and ecological parameters such as water temperature, dissolved oxygen, depth, transparency, and sediment characteristics using Geographic Information Systems (GIS), the researchers used the Kriging spatial interpolation method to create thematic maps showing fish distribution and potential breeding areas. They showed that spatial variations in environmental parameters influence the structure of fish communities and their habitats, making it possible to discriminate between fish stocks (Chakraborty et al., 2022).

1.8/ Meristics

Meristics characters consist of various components, often segmented into serially repeated, countable features like the rays of a fin, the number of vertebrae, myomeres in an eel larva, or branchial spines on a gill arch. These characteristics can be external or internal; they need to be defined and quantified, which makes them helpful in comparing specimens (Chase, 2014).

The genesis of these meristic traits is influenced by genetics and environmental conditions during the larval development phase (Swain et al., 2005). These conditions encompass variations in temperature, salinity, dissolved oxygen, CO2, and photoperiod (Hubbs, 1922, Lindsey, 1988). Understanding the impact of these factors on meristic characteristics enables the exploration of stock discrimination. A study on the discrimination of Sebastes mentella stocks in the Irminger Sea, investigated different meristic characters between fish from the oceanic stock (depth 300-450 m) and those from the deep-sea stock (depth 550-800 m), showing variations in the number of rakers on the second dorsal fin (D2) and pectoral fin (P). In addition, gill rakers (horizontal and vertical) also showed marked differences between the two groups. These differences in meristic characters allow us to establish two distinct stocks (Trella et al., 2013).

1.9/ Morphometric Analysis:

Phenotypic stocks involve differences that result from genetic or environmental differences during the period when phenotypic traits develop (Hare & Richardson, 2014). While defining phenotypic stocks may be less efficient and accurate than genetic stocks, morphometric analysis furnishes information about these stocks (Stransky, 2014). These represent groups of individuals sharing similar growth, mortality, and reproduction rates, as indicated. Additionally, morphometric analysis aids in distinguishing species by examining variations in body shape. This natural marking is a complementary analysis to other stock identification methods (Stransky, 2014). Various morphometric characteristics, including eye diameter, pectoral fin length, and the length of the first ray of the pectoral fin, can be considered. Morphometry is a valuable tool that supplements alternative stock identification methods. It can be conducted using cost-effective digital cameras with ample storage capacity, enabling the capture of numerous specimen images during sampling (Stransky, 2014).

1.10/ Otolith Microchemistry:

Otolith microchemistry is a natural marker for the connectivity of target populations using molecular data collected from otoliths; (Miller et al., 2005). This microchemistry can provide many details on environmental history, diet, pollution exposure, and population structure (Prince et al., 1995, Schutkowski et al., 1999, Kierdorf & Kierdorf, 2002, Walther et al., 2010). Variations in otolith elemental composition can indicate the magnitude, frequency, and duration of exposure to environmental stressors (external factors) and aspects of the individual's response to these stressors (internal factors) (Sturrock et al., 2015). Otolith structure and composition are influenced by environmental factors such as metal transport (e.g., protein binding) (Brown & Severin, 2009). They can confound ecological inferences if physiological differences between individuals (e.g., growth rate and reproductive status) induce chemical differences in the otolith (Sturrock et al., 2015). Analyzing otoliths' chemical composition helps identify the geographical origin of fish populations. Still, conversely, it would also help to highlight differences in otolith composition associated with geographical changes between fish populations (Sturrock et al., 2015), such as hypoxia, exposure to or lack of exposure to different contaminants, temperature (which will affect phenotype), and stress hormones. This allows elemental signatures to be measured at specific times in the life cycle, providing insight into the ontogenetic movements of fish stocks (Marui et al., 2001). Using otolith chemistry, it was possible to differentiate the spawning areas of Icelandic cod (*Gadus morhua*). By analysing strontium (Sr), barium (Ba), magnesium (Mg), and calcium (Ca) incorporated into the otoliths, the researchers were able to identify distinct signatures between fish from different spawning areas. This reflects the specific environmental conditions in each area, revealing that cod from different areas formed distinct stocks (Tanner et al., 2016).

1.11/Otoliths shape

The study of otolith shape focuses on phenotypic differences or similarities. Several studies have shown that the shape of the otolith varies from stock to stock (Stransky, 2014). Otoliths differ not only between different species but also within populations of individuals of the same species. Otolith shape is influenced by environmental factors such as water temperature, salinity, food availability, and other habitat variables, and genetic factors, i.e., otolith shapes are determined by the genetic make-up of each individual, and hereditary differences between fish populations can be reflected in otolith structure and morphology. Temperature and salinity can alter how chemical elements are deposited on the otolith, leading to differences in shape. At the same time, the growth and development of the individual (ontogeny) also influence the otolith shape (Nazir & Khan, 2021). This diversity in otolith shape highlights the importance of considering these structures as valuable indicators of stock differentiation. By analyzing phenotypic variation in otoliths, it is possible not only to distinguish between different stocks of the same species but also to understand how specific environmental factors influence the morphology of these structures. (Stransky, 2014) Researchers have studied the otoliths of Patagonian toothfish (*Dissostichus eleginoides*) collected in several geographical regions: the Patagonian Shelf, the South Georgia Islands, and the South Sandwich Islands. They analyzed 459 Patagonian toothfish and 37 Antarctic toothfish (*Dissostichus mawsoni*), photographed otoliths, and characterized their shape using elliptical Fourier coefficients (EFCs) to quantify otolith contours. These shapes were then compared between different geographical regions using multivariate analyses, such as permuted analysis of variance (ANOVA-like) and linear discriminant analysis (LDA). The results enabled us to distinguish three main groups: Patagonian shelf toothfish, South Georgia and South Sandwich Islands toothfish, and Antarctic toothfish (Lee et al., 2018).

1.12/ Parasites as biological tags

A parasite tag is a parasitic organism used to mark and discriminate between fish stocks. It is used as a natural indicator of differences between stocks. A fish only becomes infected with a parasite when it enters the parasite's endemic area, making it a reliable environmental tag (MacKenzie & Abaunza, 2014). A parasite becomes a tag when it meets several conditions: Maintenance of the tag should be done for a reasonable period, no effect of the tag on the tagged organism (invisible to predators, non-toxic, no impact on growth or survival), ability to economically tag large numbers of individuals, relatively rapid and inexpensive detection (Catalano et al., 2014).

The methods used to define a parasite as a biological tag are the taxonomic and morphological identification of parasites found in fish during dissection observation (Baldwin et al., 2012). This method also allows sampling all stages of the parasite life cycle, which helps to increase the corresponding parasite data for a greater number of host individuals collected over a longer period (Catalano et al., 2014).
South African sardines were studied to identify distinct stocks using an encysted digenean parasite as a biological tag. Sardine samples were collected from various ports along the west and south coasts of South Africa. They measured three indices of infection: prevalence, mean intensity of infection, and mean parasite abundance. Sardines from the west coast, assumed to belong to the western stock, had significantly higher parasite loads than those from the south coast, assumed to belong to the southern stock. This was confirmed by statistical models, particularly generalized linear models (GLM), which demonstrated that the differences between stocks were significant, even when seasonal and interannual variations were considered (Izzo et al., 2017) .

1.13/ Statistical Models:

Statistical models are essential for stock discrimination because they predict different possible patterns and evolutions of stocks as a function of their abundance, density, and mortality. This provides a holistic view of the stock structure, and by simulating the model, we can explore the resource's ecological, valuation, and management implications (Kerr & Goethel, 2014). They can also be relevant as they can be used to synthesize stock identification data from sites identified by other techniques, as previously stated with otoliths or genetic data.

These different models can also be used to explore and project hypotheses about the structure and connectivity of other stocks (Secor et al., 2009). This will allow us to understand these structures and their implications better and identify missing information, which plays an essential role in the stability and resilience of a population or species (Hilborn et al., 2003, Kerr et al., 2010).

In the South African sardine (*Sardinops sagax*) stock discrimination study, researchers used generalized linear models (GLM) to analyze the impact of encysted digenean parasites on sardines from putative western and southern stocks. GLMs evaluated the influence of factors such as season, year, fish size, and their interactions on three infection indices: prevalence, mean intensity, and parasite abundance. The results showed that sardines from the western stock had higher parasite loads, confirming the existence of distinct stocks. GLM also revealed that season, year, and fish size played a significant role (Izzo et al., 2017).

2. Methods used in Iceland for stock discrimination

Only a few of these methods have been used in Iceland for stock discrimination.

2.1/ Otolith shape

In Iceland, several studies have defined different stocks using otolith shapes. For example, Jónsson et al., 2021 succeeded in highlighting significant differences in otolith shapes according to the different ecotypes of cod (*Gadus morhua*), which made it possible to demonstrate the effect of growth rate on the discrimination of cod ecotypes based on otoliths. Similarly, Petursdottir et al., 2006, based on the shape of otoliths varying according to various environmental conditions, highlighted significant differences in the growth and shape of otoliths between groups of cod spawning close together. Those sampled near the coast differed from those on the Icelandic bank and continental shelf. These results suggest the existence of different cod stocks, indicating that the large, fast-growing cod spawning in the coastal zone may require special protection because of their importance to the overall productivity of the stock.

2.2/ Otolith chemistry

Otolith chemistry is also widely used in Iceland (Jónsdóttir et al., 2006), which is one such study that used otolith composition to study the stock structure of Icelandic cod in 17 different spawning sites. This study revealed significant differences in the chemical composition of otoliths between the North and South of Iceland and the depth at which the cod are found. Given that this cod stock was managed as a single stock, this research called into question the management of fisheries linked to this specific stock, revealing the importance of this method to be applied more often.

2.3/ Genetic studies

Pampoulie et al. (2022) describe a difference between North-East and South-West Iceland cod. To support this distinction, they opted for a genetic approach based on the study of hemoglobin polymorphism. This study revealed a shift in the frequency of the HbI1 allele from 0.61 in the northeast to 0.09-0.32 in the Southwest. Using the Pan I locus and nine different microsatellite loci, it was demonstrated that cod populations in Iceland are not panmictic, i.e., they are not homogeneously distributed, but that the North-East and South-West make up genetically differentiated grounds. This finding is highlighted by the higher frequency of the Pan IB allele in the South-Western spawning ground than in the North-Eastern region, as well as by differentiations at microsatellite loci. Pampoulie et al. (2006) also identified an increase in the frequency of the Pan IB allele linked to the depth at which the cod are found and confirmed the difference between the coastal and frontal cod ecotypes.

Other genetic studies based on SNPs confirmed the differentiation of cod between the breeding areas of North-East and South-West Iceland (Bonanomi et al., 2015; Therkildsen et al., 2013). Thus, using this molecular tool, (Therkildsen et al. 2013) demonstrated the existence of two distinct populations of Atlantic cod in Icelandic waters, where differentiation is mainly due to selective processes in key genomic regions. The different SNPs used confirmed the differences between coastal and offshore cod populations in South-West Iceland (Pampoulie et al., 2022).

2.4/ Fish tag and spawning studies

Data storage tags (DSTs) have helped provide information on cod migrations in Iceland, notably highlighting the presence of different stocks (Pálsson & Thorsteinsson, 2011). DSTs provide data on depth and temperature for each tagged individual. The results for some individuals are not always similar, though fish are tagged at the same spawning site: some remain in shallow coastal waters (<200 m) all year round, while others head for deeper waters where they feed at thermal fronts. DSTs confirmed the presence of spawning skippers (i.e., mature cod that do not spawn during a spawning season (I. G. Jónsdóttir et al., 2014), and so differences between the population.

2.5/Morphological differences

I. G. Jónsdóttir et al. (2016), using morphology analysis, effectively discriminated the Northern shrimp (*Pandalus borealis*) in four areas of two northwest Icelandic fjords. Other studies adopted an approach based on visual examination of morphology to characterize the components of the cod stock in Iceland. The results revealed morphological indicators in the head, fins, and body of *G. morhua*, which correlated with sex, the genotype of the fish at the pantophysin locus (Pan-I), and the place of capture. Females showed relatively deep body morphology, while the pan-IBB genotype (associated with deep-water feeding behavior) showed more significant variation between fins.

2.6/ Meristic

Using a combination of analyses of otolith shapes and 6 meristic characters: anal fin rays, dorsal fin rays, pelvic fin rays, pectoral fin rays, gill rakers on the upper limb of the first-gill arch, and gill rakers on the lower limb of the first-gill arch, Turan, 2000 has successfully demonstrated the differences between populations of North-East Atlantic herring in Iceland, Norway, and the United Kingdom. Baltic herring, Icelandic herring, Trondheim fjord herring, and the British Isles group show distinct phenotypic divergences, confirming the existence of separate stocks. Genetic and morphometric studies support this distinction, suggesting that fisheries management should consider these stocks independently, as it is unlikely that the others can compensate for the depletion of one stock.

3.The European Plaice (*Pleuronectes platessa)*

The European plaice (*Pleuronectes platessa*) (Fig 1), often called plaice, is a sandy-ground flatfish common in European waters. They regularly migrate between January and March, moving from feeding areas to well-known spawning grounds. For example, around the British Isles in the Southern North Sea and the Irish Sea, this migration occurs across the tongue of slightly warmer and saltier water that flows into the North Sea through the Dover Strait. After the spawning period, they head north to return to their feeding grounds. In Iceland, plaice spawn a little later in the year, between March and June. The spawning grounds are located close to the coast, in areas of strong water movement (Dipper, 2022). Plaice initially feeds on various small benthic organisms, particularly annelids, harpacticoid copepods, amphipods, and small decapods. In the later stages of their life cycle, they will feed mainly on bivalves (mollusks), depending on the availability of the environment (De Raedemaecker et al., 2011).

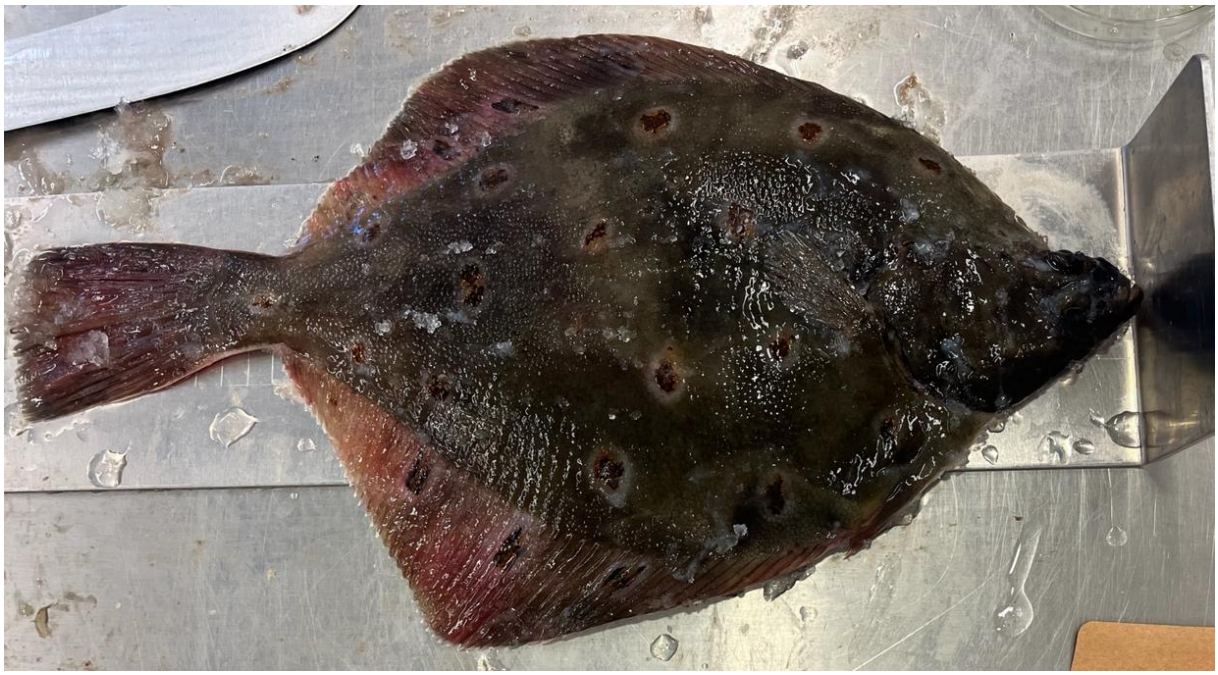

Figure 1: European plaice (*Pleuronectes platessa)* species.

Plaice is most abundant in the southwest and west of Iceland, mainly at depths of 200 m. In plaice, there is sexual dimorphism: females (<55 cm) are larger than males (~45 cm) and reach maturity at 33 cm for males and 38 cm for females. Sexual maturity is defined by size, not age. Spawning occurs at depths of 50 to 100 m. The larvae take between 2 and 3 weeks to hatch and become symmetrical larvae (6 to 8 mm long) with a ventral yolk sac that resorbs after about 8 days. They feed on small diatoms and flagellates and later, as they grow, on larger plankton,

mollusk larvae, and larvaceans. When the larva reaches 10mm (around 4 to 6 weeks after hatching), metamorphosis begins and gradually becomes a flatfish. Its body becomes flatter, and its swimming position is sideways (left side down). The left eye moves to a new dorsal position, which will transform the skull. The swim bladder, present in the planktonic larva, gradually disappears. The upper part becomes pigmented, and adult-like spots appear, while the lower part is depigmented. It is during this transformation that the fish becomes demersal (Dipper, 2022). The number of plaice that have completed metamorphosis varies from year to year.

This may be due to some external factors, such as the strength and direction of the wind, which are responsible for the drift of the larvae, so some larvae cannot reach the sandy areas needed to reach adulthood. For reproduction, the female lays her eggs close to the seabed, which float and are fertilized by the male when they surface, mainly at night. They can lay between 10,000 and 600,000 eggs per season. As they grow, they migrate to deeper waters. Initially, they migrate to depths of less than 20 m, and when they reach around 20 cm in length, they move further out into more open sea (Dipper, 2022).

Plaice fishing is concentrated mainly in the West, Southwest, and Northwest, using bottom and pelagic trawls, with few catches using gillnets and longlines (MFRI Assessment Reports, 2023). Around 70% of plaice have been caught in the West and Northwest recently, with the majority (60-80%) fished at depths of 21 to 80 m. Catches have increased by 1168 tons over the previous year (2021/2020). The catch per unit effort (CPUE), which measures the total weight in trawl where plaice accounts for more than 10% of the total catch, rose from 250 kg/set to around 700 kg/series in 2016. Discards are prohibited by law in Icelandic demersal fisheries. However, the discard rate for small individuals <40cm in demersal seiners was 7% (considered high) and zero in bottom trawls (considered negligible) (MFRI Assessment Reports, 2023).

In December 2019, ICES (International Council for the Exploration of the Sea) agreed with Iceland to include plaice in its assessment process, and 2022 decided to use a SAM (Stock Assessment Model in State Space). This new SAM model is a statistical catch-at-age model based on commercial data 1979, the Icelandic spring groundfish survey from 1985, and estimated annual recruitment at three years old (MFRI Assessment Reports, 2023). All surveys have shown that plaice is currently stable in Iceland. Nevertheless, regulations have been put in place to protect spawning plaice. Specific spawning grounds in the West and South-West of Iceland are closed to fishing during the spawning period in April.

Climate change is causing temperatures to rise and altering oceanographic conditions globally, especially in the North Atlantic Ocean, affecting ocean currents. Statistical models have predicted increases in atmospheric and terrestrial temperatures over this century (Arnason, 2007), with global temperatures expected to rise between 1.5 and 6 degrees Celsius by 2100 (Arnason, 2007). The Intergovernmental Panel on Climate Change (IPCC) warns of a more significant rise in temperatures in Arctic regions, resulting in a drastic reduction in ice cover. This will lead to increased absorption of solar radiation by the surface, resulting in a rise in water temperatures (Borgerson, 2008) and a salinity change with the melting ice or evaporation, which changes the water's salt and can impact water (S. Jónsson & Valdimarsson, 2005). Iceland, whose currents are influenced by the Arctic, will directly feel the consequences of these temperature changes. The fishing industry is vital to the Icelandic economy, accounting for 8-9% of employment, around 10% of GDP, and over 40% of total exports (Statistics Iceland, 2005, Arnason, 2007). Consequently, global warming will considerably impact Iceland's fish stocks and, by extension, the country's economy, which is heavily dependent on it (Arnason, 2007). The effect of global warming on Arctic and sub-Arctic currents calls for close monitoring of fish stocks and adaptation of their management in response to these changes. That is why well-defined fish stocks and good management are essential. The plaice is of growing importance for Icelandic fisheries, as its CPUE in trawl and demersal seine fisheries has gradually increased from 120 kg/hour in 2000 to nearly 250 kg/hour in 2014, with minor fluctuations around this value since then (MFRI Assessment Reports, 2023).

The seabed topography around Iceland, Greenland, and Jan Mayen significantly influences regional currents, leading to varied water temperatures and salinity levels. In Iceland's South and West, warm and salty Atlantic currents prevail. Near North-West Iceland, the Irminger Current divides, with one branch flowing towards Greenland and the other, the North Iceland Irminger Current, moving towards Iceland's North and East. The East Greenland Current, colder and polar, flows South, meeting another current North of Iceland to form the East Iceland Current, which, mixed with warmer Atlantic waters, has less polar characteristics. This current flows East and South-East, eventually reaching the Western Norwegian Sea. The presence of warm, salty waters from currents near Jan Mayen makes the Iceland Sea's conditions milder than polar currents. These diverse currents lead to significant hydrographic changes, especially around North Iceland, where Atlantic waters provide crucial nutrients and maintain favorable conditions for marine life production (Fig 2) (Vilhjálmsson, 2002).

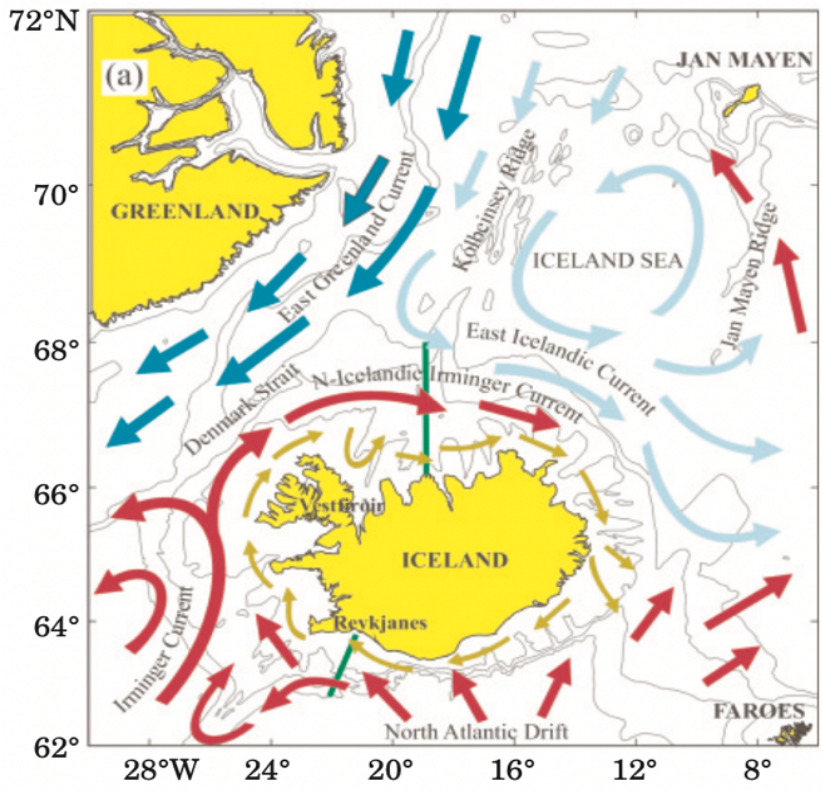

Figure 2 (Vilhjálmsson, 2002) :_Map representing the current in Iceland, Greenland, and Jan Mayen region. Red: warm Atlantic currents; Blue: low salinity Polar currents; Light Blue: Arctic current/waters; Yellow: Icelandic coastal currents

4.Parasites and otolith shape for plaice's stock discrimination:

This thesis focused on parasites as non-invasive natural markers (Pascual & Hochberg, 1996) and otolith shape. This is because the geographic range of a host species overlaps with the endemic areas of some of its parasites. A host fish is only infected by a given parasite when it enters its endemic zone (Williams et al., 1992). Nevertheless, for a parasite to be considered a tag, it must meet several criteria. The parasite must vary significantly in infection according to the regions studied; live for a long time in the host, the duration depending on the study; be easy to detect and identify to avoid wasting time; be easily detected, site-specific parasites being ideal; not alter host behavior through pathological effects (Tharaldsen, 1993).

Knowing which parameters influence the study area is essential to finding the proper parasite as a tag. Several factors can affect the distribution or diversity of parasites, including biotic parameters such as temperature, currents, and salinity (Bommarito et al., 2022). Changes in these parameters can influence the presence and prevalence of parasites.

The shape of otoliths reflects both environmental and genetic factors. Unlike external morphological characteristics, otolith shape is relatively stable and less affected by short-term conditions such as feeding or reproduction. Regional variations in otolith shape make distinguishing fish from different geographical areas possible, making it a reliable indicator for identifying stocks (Nazir & Khan, 2021) .

Although there is no specific parasitic marking method for assessing plaice stock discrimination in Iceland, other techniques have been explored. Wickins and Macfarlane (1973) analyzed the infection of 257 plaice from the southern North Sea, five types of endoparasitic helminths, and one ectoparasitic copepod, observing significant variations in prevalence and intensity for *Cucullanus heterochrous* nematodes and Anisakidae larvae according to breeding areas, suggesting their usefulness as biological indicators. In addition, Van Banning et al. (1978) proposed the myxosporean parasite *Myxobolus aeglefini* as a marker for plaice in the North-East (Mackenzie & Hemmingsen, 2015). Despite this, numerous parasites have been identified in plaice, but only two have been reported in Iceland (Table 1), both being external parasites found in the gills. No parasite tags in plaice have yet been identified in Iceland, and no environmental parameter information is available.

Table 1 : table of parasites found in plaice (*Pleuronectes platessa*) classified according to their class, name, organ in which they were found and country in which they are found.

Taxonomie (class)	Name of Parasite	Organ	Location	References
Acanthocephala	*Corynosoma strumosum*	Visceral cavity	Proleptus obtusus	(Mackenzie & Gibson 1970)
Cestoda	*Tetraphyllidean larvae*	Intestine and rectum	Scotland	(Mackenzie & Gibson, 1970)
	Pseudophyllidean larvae	Visceral cavity	Scotland	(Mackenzie & Gibson, 1970)
	Bothriocephalus scorpii	Intestine	Scotland	(Mackenzie & Gibson, 1970)
	Trypanorhynch Larvae	Visceral cavity and gut wall	Scotland	(Mackenzie & Gibson, 1970)
Ciliata	*Trichodina Borealis*	Gills	Scotland	(Mackenzie & Gibson, 1970)
	peritrichous ciliates	Gills	France	(Haensly et al., 1982)
	Scyphidia Adunconucleata	Gills	Scotland	(Mackenzie & Gibson, 1970)
Cnidaire	*Glugea stephuni*	Stomach and intestine	Scotland	(McVicar, 1975)
	Sphaerospora platessae	Cartilage	Ireland	(Mackenzie & Gibson, 1970)
Copepode	*Lenaeocera branchialis*	Gill	Germany	(Scholz & Waller, 1992)

Taxonomie (class)	Name of Parasite	Organ	Location	References
	Acanthochondria A. cornuta	Gill	North sea/Faroes/Iceland	(Kabata, 1959)
	Lepeophtheirus pectoralis	Gill	Germany	(Wickins & Macfarlane, 1973)
Crustacea	*Chalimus larvae*	Fins	Scotland	(Mackenzie & Gibson, 1970)
	Trypanosoma platessae	Blood	England/Ireland	(Cottrell, 1977)
Digenea	*Cryptocotyle lingua metacercariae(Metacercariae)*	Flesh	Scotland	(Mackenzie & Gibson, 1970)
	stephanostomum baccatum metacercariae (Metacercariae)	Flesh and Viscera	Scotland	(Mackenzie & Gibson, 1970)
	Rhipidocotyle sp. Metacercariae (Metacercariae)	Gill	Scotland	(Mackenzie & Gibson, 1970)
	Derogenes varicus	Stomach	Scotland,Germany	(Mackenzie & Gibson, 1970)
	Hemiurus communis	Stomach	Scotland	(Mackenzie & Gibson, 1970)
	Lecithaster gibbosus	Intestine	Scotland	(Mackenzie & Gibson, 1970)
	Podocotyle atomon	Intestine and rectum	Scotland	(Mackenzie & Gibson, 1970)
	Podocotyle sp.	Intestine and rectum	Scotland	(Mackenzie & Gibson, 1970)

Taxonomie (class)	Name of Parasite	Organ	Location	References
	Plagioporus varius	Intestine	Scotland	(Mackenzie & Gibson, 1970)
	Cryptocotyle lingua (Metacercariae)	Muscle and connective tissue	South England	(Cotrell, 1977)
	Rhipidocotyle johnstonei (Metacercariae)	Muscle and connective tissue	South England	(Cotrell, 1977)
	encysted helminth larva	Liver	France	(Haensly et al., 1982)
	Zoogonoides viviparus	Rectum	Scotland,Germany	(Mackenzie & Gibson, 1970)
Monogenea	*Gyrodactylus unicopula*	Gills and Fins	Scotland	(Mackenzie & Gibson, 1970)
	Gyrodactylus unicopula	Skin	Norway	(OÎesen & Amin, 2011)
Myxosporea	*Myxobolus platessae*	Cartilage	Iceland	(Mackenzie & Gibson, 1970)
	Myxobolus aeglefini	Gill and Skull	easternpart of the North Sea and Norway	(Mellergaard & Nielsen, 1983)
	Myxobolus aeglefini	Cartilage	Denmark	(Nielsen et al., 2002)
	Trilosporoides platessae	Gallbladder	Denmark	(Køie, 2005)
Nematoda	*Contracaecum aduncum*	Stomach and intertine	Scotland	(Mackenzie & Gibson, 1970)

Taxonomie (class)	Name of Parasite	Organ	Location	References
	C. aduncum larvae	Visceral cavity	Scotland	(Mackenzie & Gibson, 1970)
	Cucullanus heterochrous	Intestine and rectum	Scotland	(Mackenzie & Gibson, 1970)
	Proleptus obtusus	Intestin	England	(Harris & Cottrell, 1976)
	CucuNanus heterochrous *Capillaria wickinsi*	Intestin Intestin	Germany Germany	(Wickins & Macfarlane, 1973)
	Phocanema decipiens	muscle and visceral	Iceland	(Hauksson & Ólafsdóttir, 1995)
	Anisakidae larvae	Intestin	Germany	(Wickins & Macfarlane, 1973)
Protozoa	*?*	Stomach (mucosa and submucosa)	France	(Haensly et al., 1982)
	?	Lumina of the renal tubules	France	(Haensly et al., 1982)
	Ichthyobodo necator	Skin and gill	Scotland	(Haensly et al., 1982)
Sporozoa	*Sphaerospora irregularis*	Urinary ducts	Scotland	(Mackenzie & Gibson, 1970)

Therefore, this study aims to take a multidisciplinary approach to assess whether parasites and otoliths can be markers for stock discrimination for *P. platessa* in Icelandic water, considering seasonal variations (winter, summer) and location (North, South). Each parasite's abundance, prevalence, intensity, and otolith shape of every fish will be considered. The anticipated outcome is that abundance will vary across different seasons and regions, potentially necessitating various tags to differentiate among the fish stocks.

2. Materials and methods

2.1/ Samples

A total of 82 individual plaice from seven sites in Northern Iceland and along the Reykjanes Peninsula on the southern coast of Iceland were sampled (Figure 2.1). These specimens were obtained from seven different sampling sites and at various depths. Four sampling sites were in North Iceland, where fish were collected three times in the winter (February) and once in the summer (August). In the South, three sites were sampled, with collections occurring twice in the winter (March)and once in the summer (October) (Table 2.1). The fish were classified based on their geographical location and the season when they were collected, resulting in four distinct groups: the North Winter group with 27 specimens, the South Winter group containing 14 specimens, the North Summer group with 20 specimens, and the South Summer group which included 21 specimens. As the months making up the winter samples were not the same, and August and October were not part of summer, we performed a chi-square test to determine whether there was a difference between the sexes of the fish and a Mann-Whitney test to see if the parasite abundance varied between these two months.

Table 2.1: Detailed record of plaice sampling by date, number, region, and season in North and South Iceland with geographical and depth data.

Sampling Date	Sampling numbers	Region	Season	Latitude	Longitude	Depth (m)
10.02.2023	10	North	Winter	N66°06′33	W18°33′31	80
20.02.2023	7	South	Winter	N66°03′33	W17°34′41	125
01.03.2023	7	South	Winter	N63°57′57	W22°48′20	77
20.03.2023	17	North	Winter	N65°42′44	W20°26′03	53
03.08.2023	20	North	Summer	N65°49'36	W20°30'43	124
06.10.2023	21	South	Summer	N64°05'58	N64°05'58	18

Initially, our study was conceived with only two primary groups: North and South plaice during winter. However, unforeseen challenges in the form of inclement weather and storm warnings at sea resulted in an insufficient number of samples, compromising the accuracy of our research. Consequently, we decided to introduce two additional groups: northern and southern plaice during the summer season. This ensured a larger sample size and incorporated our study's seasonal parameters. Fresh ungutted fish from the North were delivered overnight on ice to the

Reykjavík Fish Market, where we went to pick them up, and the one from the South arrived in Sandgerði. The latter were transported to Reykjavík on ice.

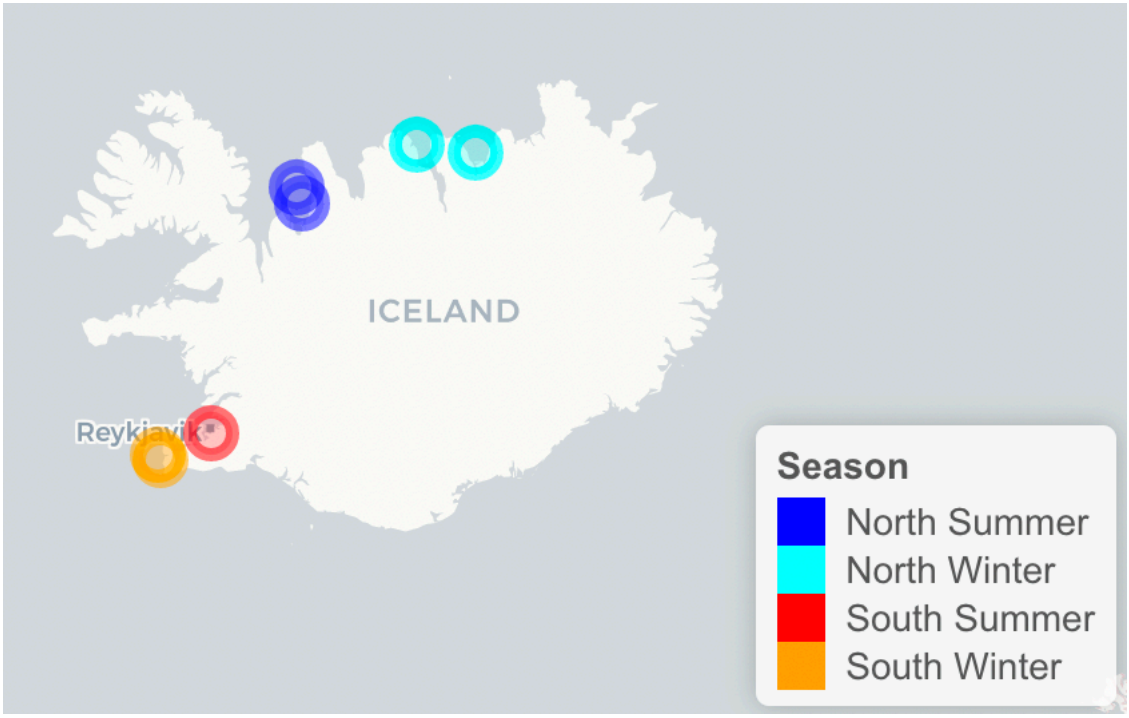

Figure 2.1 : map of Iceland showing the different capture sites for each study group: North Summer N = 20, North Winter N = 27, South Summer N = 21, and South Winter N = 14.

2.2/ Necropsy

Each fish was measured (total length [TL]) to the nearest cm. Parasites on the fins and skin were recovered through external examination. Necropsies were conducted at the University of Iceland (Faculty of Life and Environmental Sciences) to recover the parasites (external and internal) and determine their distribution and abundance in the various organs. All parasites recovered were collected and preserved in Eppendorf tubes with 96% alcohol to maintain their condition for later molecular analyses.

Specific tissues, including the eyes, gills, and brain, were extracted promptly and observed under a microscope in saline water prepared in the laboratory by mixing 1L of distilled water with 8 g of sea salt for immediate inspection. The saline solution preserved the osmoregulatory balance for parasites, ensuring optimal tissue integrity during observation. Additionally, otoliths were removed and individually labeled for otolith shape analysis. A filament of the gills was cut and stored in separate tubes per sample containing 96% ethanol for molecular analysis (not conducted as part of this study). Various organs, including the heart, stomach, intestine, gonads, mesenteries, gall bladder, liver, and spleen, were sampled and labeled corresponding to the fish individual and organ. These organ samples were placed in labeled bags and stored in a freezer at -30°C for future examination (Figure 2.2). Before freezing, a visual inspection was conducted to determine the sex and maturity of the fish based on the gonads (the presence of sperm means it is a mature male, the presence of eggs means mature female, no gonad visualization means immature male, and no eggs but the presence of gonads mean immature female). This freezing approach enabled efficient organ sampling while minimizing potential harm to parasites. Freezing the organs was chosen because processing them takes too long (around 45 minutes for the entire necropsy and inspection of all the organs), risking damage to both the organs and the parasites.

Figure 2.2 : Various organs of *Pleuronectes platessa* are labeled and ready to be frozen for later inspection.

Organs were observed submerged in saline water. Subsequent microscopic examination enabled detailed observation and analysis of organ samples for parasite abundance from each respective organ. Based on morphology, each parasite was classified into different types, e.g., nematodes, cestodes, copepods, etc. Parasite morphotypes from the other organs were isolated separately and stored in 1.5 ml Eppendorf tubes filled with 96% ethanol to confirm their identification using molecular techniques.

2.3/ Molecular analysis

2.3.1/ DNA extraction

Genomic DNA was extracted from each parasite morphotype from the different organs, aiming for three extractions per morphotype per organ when feasible. Individual samples of smaller parasites, like trematode metacercariae, were placed in distinct tubes for DNA extraction. In the case of nematodes, a small section (disc, approximately 1 mm thick) from the parasite's central part was extracted. The remaining tissue was preserved in 96% ethanol and kept as a paragenophore (Pleijel et al., 2008). A total of 36 distinct parasite forms were processed; the parasites were labeled based on the morphological identification conducted earlier (for instance, "nematode type 1"), indicating the specific organ in which they were not discovered and whether they were located on the inside or outside of each respective organ. Red, white, and glowing dots were noted for their presence or absence; they weren't subjected to molecular analysis. While copepods were counted and identified, they were not included in the molecular work.

Before starting, the laboratory bench was carefully cleaned with distilled water. Then, it was sanitized using a solution of 96% ethanol to avoid potential sources of DNA contamination. The parasite-handling part of the tools was immersed in ethanol and placed in the flame of a Bunsen burner to burn off potential contaminants before starting and between each extraction. Parasites were prepared as described previously, based on their identification. The portion removed from the parasite was placed into a new, empty Eppendorf tube for drying, allowing the ethanol (in which the parasites were preserved) to evaporate. The DNA extraction mix comprised 10 µl of Fish Buffer (Devlin et al., 2004), one µl of Tween 20 at a concentration of 20%, two µl of double distilled water, and two µl of Proteinase K. The Tween helps lyse cells by breaking down the cell membrane and nuclear envelope, thus allowing for the release of DNA from the nucleus. Proteinase K facilitates protein breakdown for better access to cells and breaks down histone proteins to free DNA. Each tube was flicked and spun before incubation. The tubes were placed in a hot block set to 65°C. Over the next two hours, the tubes were flicked and spun every 20 minutes. After this, the temperature was raised to 95°C for ten minutes to deactivate the Proteinase-K. The tubes were left at room temperature for 30 minutes and then stored in a freezer at -30 °C.

2.3.2/ PCR

As for the DNA extraction, the laboratory bench was carefully cleaned with distilled water and 96% EtOH to prevent contamination of the samples. The frozen DNA templates were thawed, followed by a flick and spin to ensure homogeneity. Containers or racks for each DNA template were kept separate to maintain the bench's cleanliness and avoid cross-contamination. Like the DNA templates, the frozen PCR mix and primers were thawed, spun to ensure uniformity, and then placed on ice to maintain their temperature stability.

Each PCR (25 µl total volume) consisted of 12.5 µl of MyTaq 2x Master Mix (containing $MgCl_2$, dNTPs, and Taq polymerase) (New England Biolabs), 0.35 µl of forward Primer and 0.35 µl of reverse Primer of 50 nM/µL concentration, and 11.3 µl of double-distilled water was added to adjust the final volume to 25 µl per PCR (assuming 0.5 µl of DNA template being added). Primer combinations were specific to target fragments, such as 28S rDNA. These conserved regions are utilized in phylogenetic studies (Telford et al., 2015), including 28S rDNA for certain groups, COI (cytochrome oxidase I) as a mitochondrial DNA marker frequently employed for species barcoding due to its interspecific variability (Hebert et al., 2003), and ITS (Internal Transcribed Spacer). Those barcoding were used for taxa-specific identification thanks to specific DNA sequences (Table 2.2).

Each PCR set included positive and negative controls. The positive control confirms that the PCR can amplify the target sequence and that all reagents work correctly. If the PCR does not produce the expected results with the positive control, this indicates a problem with the reagents, extraction, or protocol. The negative control is used to detect cross-contamination or the presence of non-specific DNA/RNA. The absence of amplification in the negative control means that any signal detected in the samples is specific to the target of interest and not due to contamination.
After adding the DNA template, all PCR tubes were placed into the thermal cycler, and the appropriate PCR program was initiated to commence the amplification process (Table 2.3).

Table 2.2 : Primer targets and target sizes for the different parasite functional groups for the PCR.

Parasites	Primers	Target	Target size
Monogeneans, Cestodes and Unknowns	T01N (forward) (5'-GAT GAC CCG CTG AAT TTA AG-3') and T13N (reverse) (5'-GCA CCT GAG TTG TTA CAC ACT-3') (Harper & Saunders, 2001)	5’ end of 28s rDNA	1400-1900 base pairs
Trematodes	BD3 (Forward) (5'-GTCGTAACAAGGTTTCCGTA-3') and 536 (Reverse) (5'-CAGCAGCCGCGGTAATTCCAGCT-3') (García-Varela & Nadler, 2005)	5’ end of 28s rDNA	1200 – 1700 base pairs
Nematodes	93 (Forward)(5'-GTCGTAACAAGGTTTCCGTA-3') and 94 (Reverse)(5’-CAGCAGCCGCGGTAATTCCAGCT-3’) (Nadler et al., 2005)	Internal transcribed spacer	1000 base pairs
Acanthocephalans	LCO1490 (forward) (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (reverse) (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al., 1994)	Cytochrome oxidase subunit 1	750 base pairs

Table 2.3 : Thermo-cycler protocols for the different parasite groups for the PCR.

Parasite	Denaturation	cycle	Denaturation	Annealing	Extension	Final extension
Monogeneans, Cestodes, Unknowns (Harper & Saunders, 2001)	4min at 94°C	38	30 sec at 94°C	30 sec at 50°C	2 min at 72°C	7 min at 72°C
Trematodes (García-Varela & Nadler, 2005)	1min at 94°C	35	60 sec at 94°C	60 sec at 50°C	90 sec at 72°C	10 min at 72°C
Nematodes (Nadler et al., 2005)	4min at 94°C	25	30 sec at 94°C	30 sec at 45°C	60 sec at 72°C	7 min at 72°C
Acanthocephalans (Folmer et al., 1994)	3min at 94°C	35	60 sec at 94°C	60 sec at 40°C	60 sec at 72°C	7 min at 72°C

Once the PCR program ran, the PCR samples were removed from the thermal cycler and placed in the fridge for electrophoresis gel, PCR purification, and sequencing.

2.3.3/ Electrophoresis Gel

A 1.5% agarose electrophoresis gel was run to confirm the PCR succeeded (Figure 2.3). The gel consists of 100 ml of TAE buffer (Tris base, acetic acid, EDTA) at 1x concentration in a 150-250 ml Erlenmeyer flask to which is added 1.5 g of agarose powder along with 5 µl of EtBr (ethidium bromide) to visualize DNA migration. The gel is placed in an electrophoresis tank filled with 1x TAE buffer, which acts as a conductor for the electric current and maintains the pH during electrophoresis. The anode and cathode have been positioned to facilitate DNA migration towards the positive charge, with the positive anode (red node) at the bottom and the negative cathode (black node) at the top. Since DNA is negatively charged due to its phosphate backbone, it naturally moves towards the positively charged anode under the influence of an electric field (Roberts & Dryden, 2013). A 100 bp or 1 kb DNA ladder (New England Biolabs) was placed in the first lane of the gel to provide molecular weight markers for comparison. A mixture containing 1 µl loading dye, 3 µl distilled water (dH_2O), and 2 µl PCR product was added in each subsequent well. The loading dye helps visualize samples during loading and migration and helps DNA/PCR products sink into the well and not float at the surface. The PCR product is the DNA segment of interest that needs to be separated and analyzed. The last two wells are reserved for the positive and negative controls. The analysis takes between 30 and 45 minutes under 80 volts, after which the gel is observed under UV light and photographed.

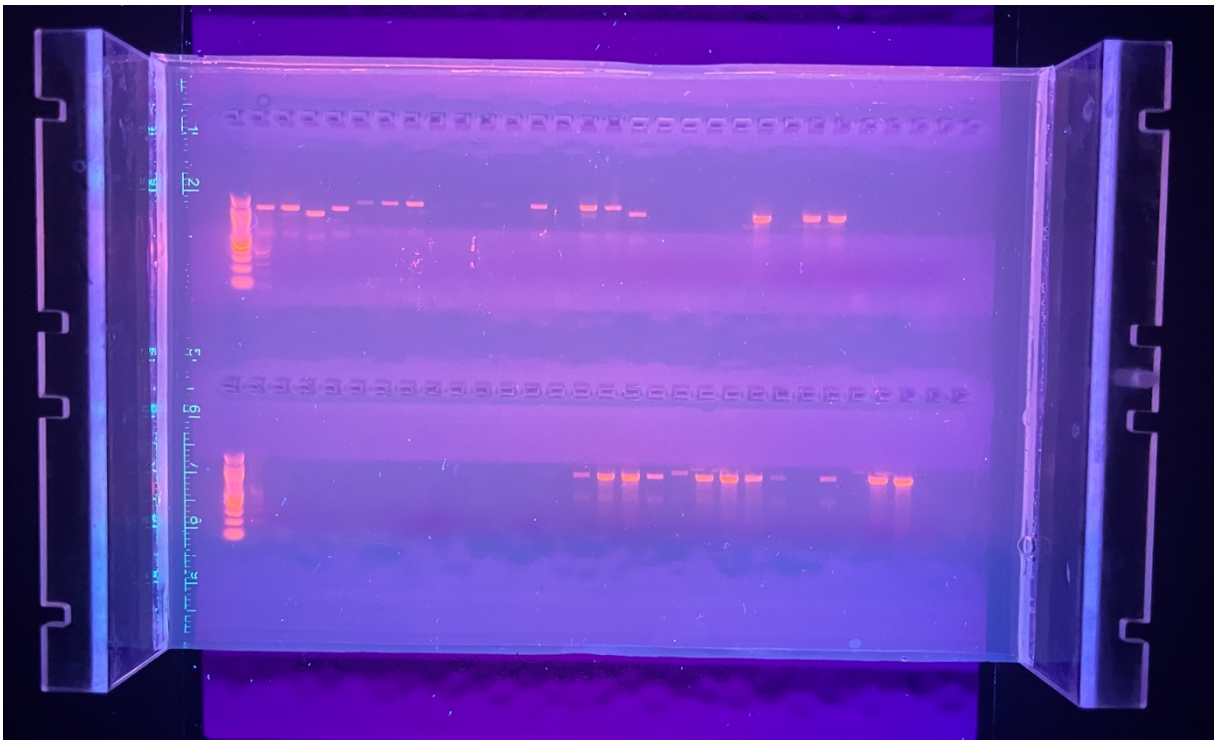

Figure 2.3 : Electrophoresis Gel result[HR1] s with 100 bp ladder of the parasites samples from EM1 to EM36 with positive and negative control (first and second column).

2.3.4/ ExoSap

For each successful PCR, the PCR product was purified using ExoSAP (Applied Biosystems), a combination of Exonuclease I and Shrimp Alkaline Phosphatase enzymes. ExoSAP is adept at cleaning PCR products by eliminating surplus primers and unused nucleotides. This enzymatic cleanup method enhances the precision and yield of PCR reactions and offers a quicker alternative to traditional purification techniques like gel purification, spin columns, or beads.Ten µl of each successfully amplified PCR product was transferred to a tube labeled with a strip. The Exo-Sap was removed from the freezer and centrifuged, then placed on ice to thaw. Two µl of Exo-SAP mixture was added to each tube containing PCR-positive samples. The thermal cycler was set to the "Exo-Sap" protocol, which purifies PCR products by removing DNA primers and unincorporated nucleotides. This protocol uses two enzymes: exonuclease I (Exo) to degrade single-stranded DNA primers and Arctic shrimp alkaline phosphatase (SAP) to dephosphorylate unincorporated nucleotides, preventing their further use (Werle et al., 1994). After the reaction, samples were prepared for direct sequencing by Haseeb Randhawa or stored at -20 °C for later use. Initial PCR results can be used to assess the amount of template

required for the preparation of the sequencing reaction. Purified PCR products were sent to Microsynth (Germany) for direct sequencing (Sanger sequencing) using the PCR primers.
My supervisor manually edited the sequences using Sequencher 5.4.6 (Gene Code Corporation) and blasted them using BlastN. The results were then included in an Excel spreadsheet with their identification name, sequence length, sequence coverage, and % similarity to other sequences in GenBank.

2.4/ Data Analyses

After molecular identification of morphologically identified parasites, abundance was determined by calculating the ratio of observed parasites to the number of fish inspected. This was repeated for each parasite species/morphotype. Each parasite's mean Intensity of infection was defined by dividing the total parasite count by the number of hosts infected with each parasite species/morphotype. We determined each parasite/morphotype prevalence as the fraction of hosts infected out of the total fish population examined (Bush et al., 1997).

2.4.1/ ANOVA

To assess the potential influences of sex (mature female, mature male, immature), region (south, north), and season (winter, summer) on fish size, we performed a three-factor ANOVA. This statistical method was also used to examine these three factors' interactions. To confirm that the ANOVA was suitable for our dataset, we first checked the underlying assumptions required for the analysis. The Shapiro-Wilk test was employed to assess the normality of the ANOVA residuals, and Levene's test was used to ensure the homogeneity of variances between groups. Once these hypotheses had been confirmed, we carried out post hoc Tukey tests designed to identify specific differences between the levels of the factors if the ANOVA revealed significant effects.

2.4.2 / Generalized Linear Mixed Model (GLMM)

In our study, we used a generalized linear mixed model (GLMM) to investigate the influence of fish length, season, and region on parasite abundance, treating these factors as fixed effects due to their constant impact on the data set. To account for the potential non-independence of our data, we incorporated fish identity as a random effect, allowing us to capture individual variation between fish. We tested the need for interactive terms, particularly between length, season, and region, to see if the combined effects of these variables significantly influenced parasite abundance beyond their individual effects by considering interactions that could be ecologically significant. Four models were run to test this: a model including only a random effect for individuals ("Fish"), without any fixed predictor, a model adding fixed effects for region and seasons, the third model includes an interaction between region and seasons, in addition to the random effect for individual fish, and the last model is an extension of the previous model, adding fish length as an additional fixed covariate, and their AICc were compared. The model with the lowest AICc was selected. During model formulation, we performed collinearity diagnostics to ensure that our fixed effects did not exhibit multicollinearity, which could skew our results. This involved calculating variance inflation factors (VIFs), and as a result, the gender variable was excluded from the final model due to its strong collinearity with the length variable. We performed diagnostic checks to confirm that our data met the GLMM assumptions, including residual analysis for normality and homoscedasticity. This ensured that our model was a good fit for the data. The selection of the best-supported model was based on an information-theoretic approach, using Akaike's information criterion corrected for small sample sizes (AICc). We compared three candidate models: GLMM, ANOVA, and Linear regression, and we selected the one with the smallest AICc, the GLMM model.

2.4.3/ Linear Discriminant Analysis (LDA)

Linear Discriminant Analysis (LDA) was employed to determine the most effective linear combinations of variables for calculating discriminant functions to distinctly separate classes defined by seasons and regions in our study. The effectiveness of LDA was assessed by comparing the classes predicted by the model, corresponding to the four different study groups. This comparison enabled us to evaluate the discriminating power of the parasites to the groupings (season and region). Parasite data were selected based on their morphological identification based on data post-molecular identification. Two separate LDA analyses were carried out: the first included all data, and the second considered only parasites having infected at least 5% of the host population.

2.5/ Otolith shape

Once the sagittal otoliths were extracted in pairs for each fish, they were stored and labeled with the code of the fish they corresponded to. They were then photographed in a particular way. The right sagittal otolith was photographed with the *sulcus acoustics* facing downwards and the dorsal side pointing toward the top of the image (Jónsson et al., 2021). Otoliths were photographed with a LEICA M165C stereo microscope at Iceland's Marine and Freshwater Research Institute using x1 magnification (Figure 2.4).

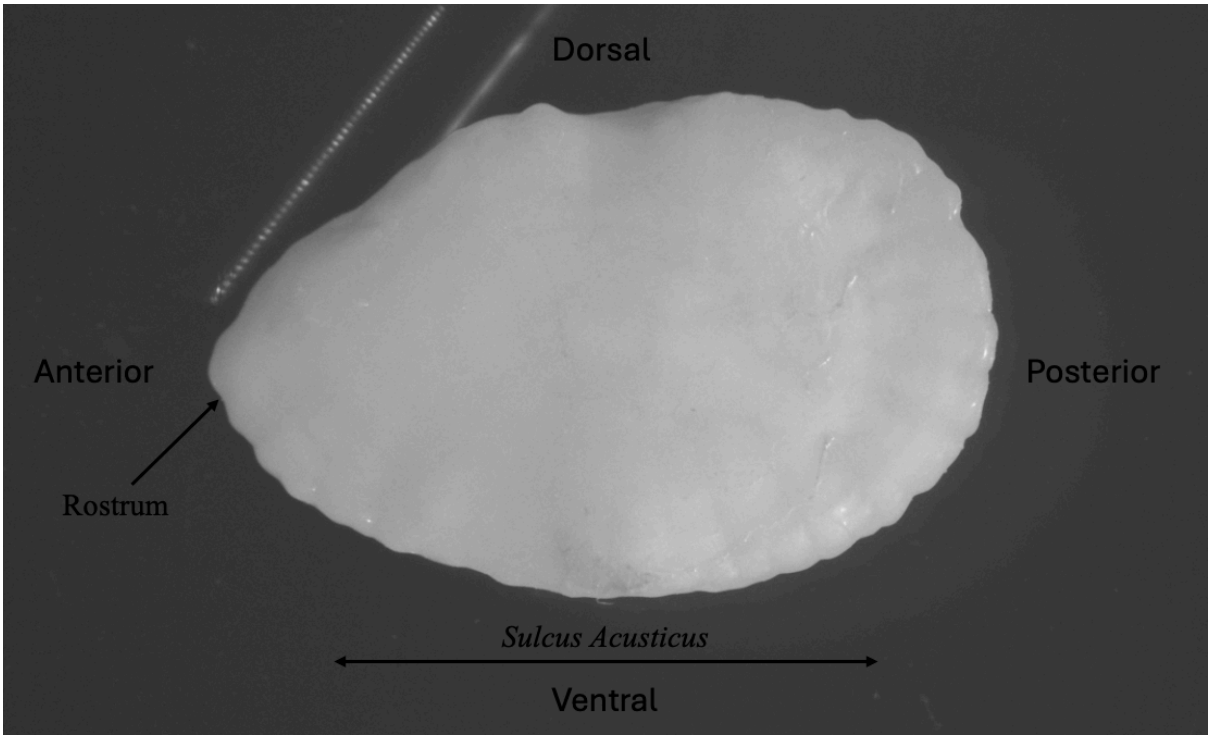

Figure 2.4: Picture of the right otolith of the PL41 fish taken with the LEICA M165C stereo microscope.

Then, with the Fiji software, (*Fiji: ImageJ*) the pictures were cleaned (removing dirt from the photographs to avoid distorting the measurements) and converted into 8-bit binary images, creating a black shape on a white background (Figure 2.5). The otolith's maximum Feret diameter (the maximum distance between any two points on the otolith's edge) was measured. For pairs of otoliths with cracked rights, the left otolith was photographed and modified in Fiji to have the same orientation as the others.

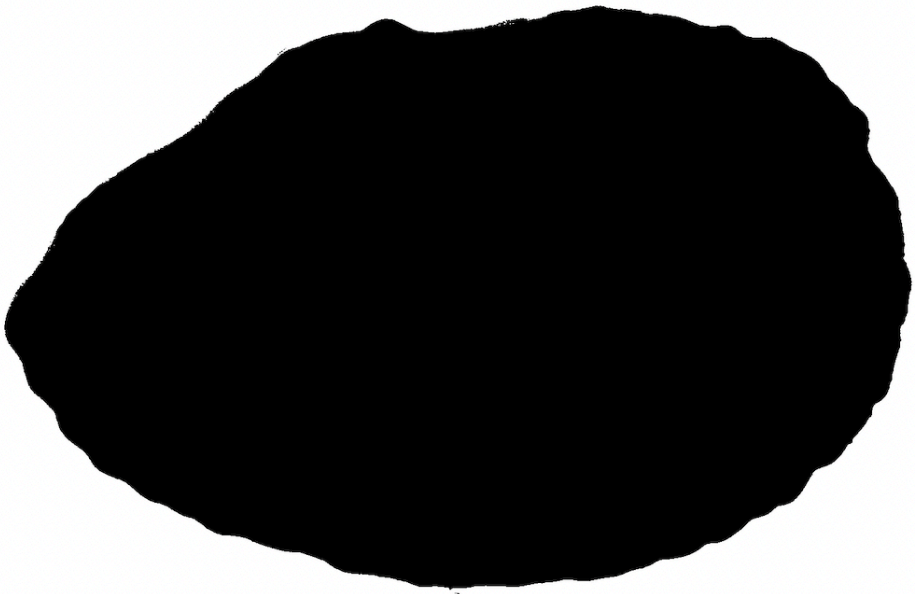

Figure 2.5: The Fiji software converted PL41 otolith’s picture into 8-bit binary images.

The shape of the otoliths was then analyzed using the package ShapeR (Libungan & Pálsson, 2015) in R studio.
An Excel file in CSV format was created with several pieces of information per fish: in which country the fish was caught (Iceland), which population (North Winter, North Summer, South Winter, and South Summer); the length of the ferret was calculated using ImageJ, the name of the file in which the images were stored, the number associated with the fish and the calibration (198), which were used to link the biological information of each fish to the otolith contour. The "length_cm" column must exist to remove the effect of allometric growth on otolith shape (articles 15 and 16 of the otolith article).

To calculate the differences or similarities between the otoliths of the different study groups, we obtained the elliptical Fourier coefficients (EFC). There are two ways of calculating these coefficients: wavelet levels or Fourier harmonics. In our case, I chose wavelet because it is a more recent method with more scientific articles about it. Wavelet outline analyses were carried out to adjust the wavelet levels for each otolith contour until 98.5% of the variance in otolith shape could be reconstructed.

Using the wavelet values calculated previously, the mean shapes of the otoliths in the four groups were calculated and visually constructed to give a figure showing similarities and differences.

To finish, an ANCOVA-like permutation was carried out using the vegan package (*Vegandevs/Vegan*, 2012/2024) to see if the variances of the different otolith groups had a statistical difference in the shape of the otoliths in each group studied. Cross-validation was then carried out using the red package (Cardoso & Branco, 2023) to test the performance of our model.

3. Results

3.1/Descriptive results

Sampling was conducted in February and March to form the winter group. In summer, sampling was carried out in August and October, and after a chi-square test with a p-value > 0.05 to see if there was a difference between the sexes in these two months (no difference here), we considered these two groups to form the summer group. However, there was a difference in parasite abundance with a Mann-Whitney test with a p-value < 0.001.

Our 81 samples were sexually identified. In the North, 47 plaices were sampled. Winter samples comprised 27 individuals with an average length of 35.44 cm (± 5.17 cm) and a parasite abundance of 15.52 (± 3.39). Summer individuals comprised 20 individuals, averaging 40.21 cm (± 4.46 cm) and a parasite abundance of 0.6 (± 0.18). Of these 47 Northern fish, eight were females averaging 42.00 cm (± 4.24 cm) in length and a parasite abundance of 2.75 (± 1.07); 21 were males averaging 39.24 cm (± 4.97 cm) in length and a parasite abundance of 2.91 (± 0.60). Eighteen individuals were immature, with an average length of 33.30 cm (± 3.27 cm) and a parasite abundance of 21.17 (± 4.29) (Table 3.1).

In the South, there were 34 plaices sampled. These winter samples comprised 13 samples with an average length of 43.15 cm (± 4.24 cm) and a parasite abundance of 32.93 (± 8.82). Summer Southern samples had 21 individuals with an average length of 39.16 cm (± 6.57 cm) and a parasite abundance of 30.33 (± 7.75) (Table 3.2). Of these Southern plaices, ten were females, averaging a length of 40.60 cm (± 3.63 cm) and a parasite abundance of 35.30 (± 7.68). There were 19 males, with an average length of 42.21 cm (± 6.65 cm) and an average parasite abundance of 31.68 (± 9.69). There were five immature individuals in the South, averaging a length of 34.20 cm (± 3.56 cm) and a parasite abundance of 21.80 (± 6.28) parasites (Table 3.3).

In all, the 18 collected females had an average length of 41.22 cm (± 3.86 cm) and a parasite abundance of 20.83 (± 5.49), whereas the 40 collected males averaged 40.65 cm (± 5.95 cm) with an average of 18.42 (± 6.41) parasites. The 23 immature individuals averaged a length of 33.57 cm (± 3.27 cm) and a parasite abundance of 21.30 (± 4.58) (Table 3.4).
In summer, 41 fish were sampled: ten females, 26 males, and five immatures. Females had an average length of 39.60 cm (± 3.27 cm) and a parasite abundance of 26.40 (± 7.47). Males had an average length of 40.58 cm (± 6.13 cm) and a parasite abundance of 10.62 (± 4.95). Immature individuals had an average length of 34.20 cm (± 3.56 cm) and a parasite abundance of 21.80 (± 6.28) (Table 3.5). In winter, 40 fish comprised eight females, 14 males, and 18 immatures. Females averaged 43.25 cm (± 3.73 cm) and had an average parasite abundance of 13.00 (± 2.14). Males averaged 40.70 cm (± 5.82 cm) and had an average parasite abundance of 27.71 (± 9.11). The immature individuals averaged 33.39 cm (± 3.27 cm) and had an average abundance of 21.06 (± 4.29) parasites (Table 3.6).

Table 3.1: Summary of the results by sex (Female, Male, Immature) in the North, including mean parasite and SD, number of parasite richness (i.e. the variety of parasite species found in the fish), total sample size per sex, mean fish length (total length [TL] in cm) per study group (with standard deviation [SD]), and number of infected organs by parasites per sample. The total sample size is 47 *Pleuronectes platessa.*

Sex	Total samples	Mean (TL) (cm) and (SD)	Mean Parasite Abundance And SD	Parasite Richness	Infected organs
Female	8	42.00 (± 4.24)	2.75 (± 1.07)	4	5
Male	21	39.24 (± 4.97)	2.91 (± 0.60)	6	8
Immature	18	33.39 (± 3.27)	21.17 (± 4.29)	5	6

Table 3.2: Summary of results by sample: region (North and South) and season (Winter and Summer), including abundance of parasites, Parasite richness, number of female, male, and immature fish, sample size, mean fish length (total length [TL] in cm) per study group (with standard deviation [SD]), and number of infected organs by parasites per sample. The total sample size is 82 *Pleuronectes platessa.*

Region	Seasons	No of Females	No of Males	No of Immature	Total samples	Mean (TL) (cm) and (SD)	Mean Parasite Abundance And SD	Parasite Richness	Infected organs
North	Winter	3	6	18	27	35.44 (± 5.17)	15.52 (±3.39)	6	7
South	Winter	5	8	1	13	43.15 (± 4.24)	32.93 (±8.82)	7	7
North	Summer	5	15	0	20	40.20 (± 4.46)	0.6 (±0.18)	1	3
South	Summer	5	7	9	20	39.16 (± 6.57)	30.33 (±7.75)	3	3

Table 3.3: Summary of the results by sex (Female, Male, Immature) in the South, including mean parasite abundance and standard deviation (SD), parasite richness, total sample size per sex, mean fish length (total length [TL] in cm) per study group (with SD), and number of infected organs by parasites per sample. The total sample size is 34 *Pleuronectes platessa.*

Sex	Total samples	Mean (TL) (cm) and (SD)	Mean Parasite Abundance and SD	Parasite Richness	Infected organs
Female	10	40.60 (± 3.63)	35.30 (± 7.68)	5	5
Male	19	42.21 (± 6.65)	31.68 (± 9.69)	5	8
Immature	5	34.20 (± 3.56)	21.80 (± 6.28)	2	2

Table 3.4: Summary of results by sex (Female, Male, Immature), including mean parasite abundance and standard deviation (SD), Parasite richness, total sample size per sex, mean fish length (total length [TL] in cm) per study group (with SD), and number of infected organs by parasites per sample. The total sample size is 82 *Pleuronectes platessa.*

Sex	Total samples	Mean (TL) (cm) and (SD)	Mean Parasite Abundance and SD	Parasite Richness	Infected organs
Female	18	41.22 (±3.86)	20.83 (± 5.49)	4	6
Male	40	40.65 (± 5.95)	18.42 (± 6.41)	7	11
Immature	23	33.57 (± 3.27)	21.30 (± 4.58)	4	7

Table 3.5: Summary of the results by sex (Female, Male, Immature) during the summer season, including mean parasite and SD, number of parasite diversity, total sample size per sexe, mean fish length (total length [TL] in cm) per study group (with standard deviation [SD]), and number of infected organs by parasites per sample. The total sample size is 41 *Pleuronectes platessa.*

Sex	Total samples	Mean (TL) (cm) and (SD)	Mean Parasite Abundance and SD	Parasite Richness	Infected organs
Female	10	39.60 (±3.27)	26.40 (± 7.47)	4	3
Male	26	40.58 (±6.13)	10.62 (± 4.95)	4	3
Immature	5	34.20 (±3.56)	21.80 (± 6.28)	2	2

Table 3.6: Summary of the results by sex (Female, Male, Immature) during the winter season, including mean parasite and SD, number of parasite diversity, total sample size per sexe, mean fish length (total length [TL] in cm) per study group (with standard deviation [SD]), and number of infected organs by parasites per sample. The total sample size is 40 *Pleuronectes platessa.*

Sex	Total samples	Mean (TL) (cm) and (SD)	Mean Parasite Abundance and SD	Parasite Richness	Infected organs
Female	8	43.25 (± 3.73)	13.00 (± 2.14)	5	5
Male	14	40.79 (± 5.82)	27.71 (± 9.11)	7	11
Immature	18	33.39 (± 3.27)	21.06 (± 4.29)	5	7

An ANOVA revealed a significant difference in fish size according to the study group ($p < 0.001$). The Shapiro-Wilk test for normality of residuals gave a p-value < 0.001, indicating that the residuals did not deviate significantly from a normal distribution. Levene's test for homogeneity of variances gave a p-value of 0.344, suggesting homogeneity between groups. Tukey's post hoc tests revealed a significant difference in fish size between North_Winter and North_Summer (adjusted $p < 0.05$) and between South_Winter and North_Winter (adjusted $p < 0.001$). The average size of fish from the North_Winter was 35.44 cm (± 5.17), and from the North_Summer was 40.20 (± 4.46) and from the South_Winter 43.15 (± 4.24) (Table 3.2)

During summer in the North, fish were generally around 35 cm in TL, with most sizes concentrated between 30 and 40 cm. A notable peak occurs at 38 cm, with around 8% of the sampled fish. Although a few fish can reach up to 50 cm, size variability is less pronounced than in other seasons. In winter, fish sizes range from 30 to 45 cm in the north, with high frequencies observed at 35, 37, and 42 cm, each around 5%. In the south, fish show a main size peak of around 40 cm in summer, with frequencies around 3 and 4%. Sizes are mainly clustered around this peak, with less frequent variations at the extremes of the measurement scale. In winter, fish in the South tend to be larger, with sizes ranging from 35 to 50 cm, and the highest frequency peaks at around 8% for sizes between 40 and 45 cm. Summer fish in the South are, on average, larger than those in the North, with a peak of around 40 cm compared to 35 cm in the North. The differences become even more pronounced in winter. Southern fish show larger sizes, reaching up to 50 cm, and a wider distribution of sizes than northern fish (Figure 3.1).

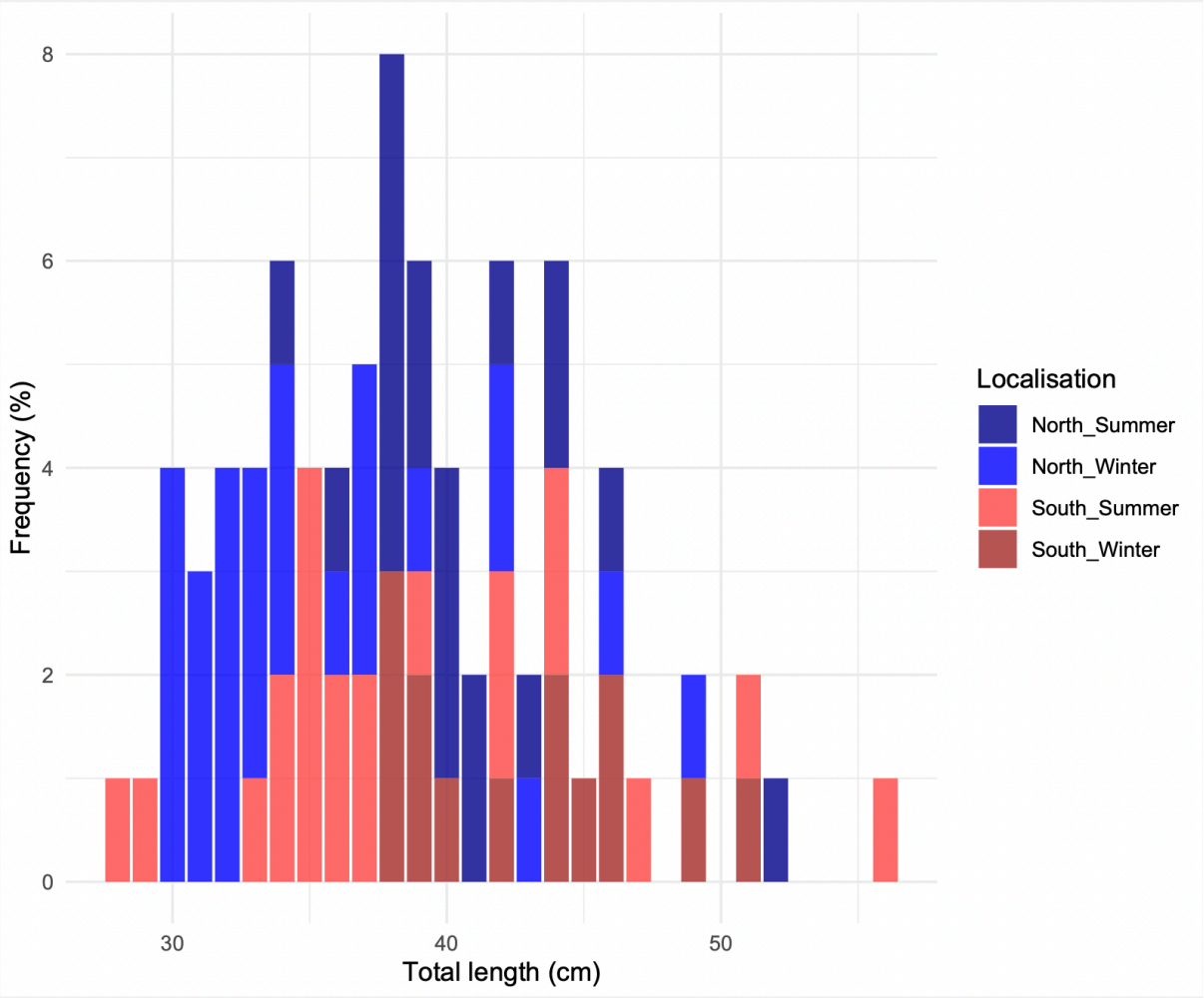

Figure 3.1: Length frequency (%) per total fish length class in cm per groups sampled: North_Summer (Dark blue) n =20, North_Winter (Light blue) n =27, South_Summer (Light Red) n =21, and South_Winter (Dark red) n =14.

An ANOVA revealed a significant difference in fish size according to sex ($p < 0.001$). The Shapiro-Wilk test for normality of residuals gave a p-value of 0.086, indicating that the residuals did not deviate significantly from a normal distribution. Levene's test for homogeneity of variances gave a p-value of 0.283, suggesting homogeneity between groups. Tukey's post hoc tests for sex revealed a significant difference in fish size between immature fish and females (adjusted $p < 0.001$), as well as between immature fish and males (adjusted $p < 0.001$), but not between males and females (adjusted $p = 0.942$). The average size of male and female fish was 40.65 (± 5.95) and 41.22 cm (± 3.86), respectively, and the average immature size was 33.57 cm (± 3.27) (table 3.4).

In the north, females show a high initial frequency of 4% at 30 cm of length, gradually decreasing to around 1% at 50 cm. Immature fish are mainly concentrated around 40 cm in TL, with a maximum frequency of around 3%. As for males, their frequency increases from 1% at 35 cm to a peak of almost 5% at 45 cm before decreasing to around 1% at 50 cm (Figure 3.2A). In the south, females show a stable frequency in size, varying from 1 to 2% from 30 to 50 cm, with peaks around 40 cm. Immature fishs shows a high frequency of around 40 cm, reaching around 4%. Males have a similar distribution to females, with a slightly higher peak of 3% at 40 cm (Figure 3.2B). During the summer, females maintain a uniform frequency of around 1-2% from 30 to 50 cm. Immatures peak at around 4% at 35 cm. Males increase their frequency to around 4% at 45 cm (Figure 3.2C). In winter, females peak at 4% at 35 cm and decrease to around 2% at 45 cm. Immatures show a high frequency of 4% at 30 cm, which decreases rapidly with increasing size. Males show a distribution centered around 35 cm, with a peak of 4% and a decrease to around 1% at 50 cm (Figure 3.2D).

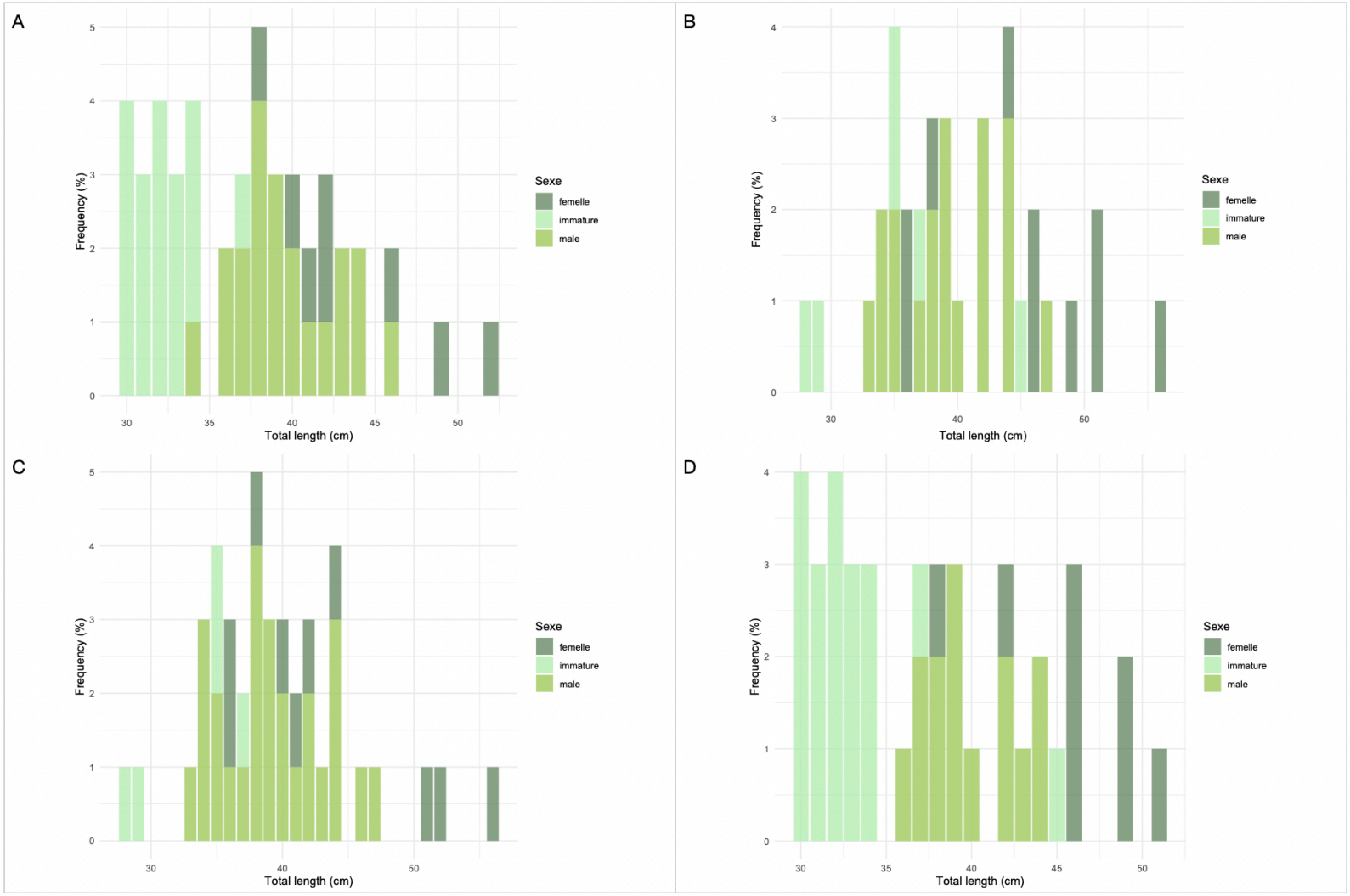

Figure 3.2: Length frequency (%) per total fish length in cm per sexe female (dark green), immature (light green), and male (medium green), of samples in north (A) female n = 8, male = 21 immature n = 18, south (B) female n = 10, male = 19 immature n = 5, summer (C), female n = 10, male n = 26 immature n = 5 and winter (D) female = 8, male n = 14, immature n = 18.

In the South region, the variation of the parasite abundance as a function of the TL is not statistically significant ($p = 0.410$), and fish size explains only 2.1% of the variance in parasite abundance ($R^2 = 0.021$). Similarly, in summer, the relationship was not statistically significant (p-value = 0.399), and fish size explained only 1.8% of the variance ($R^2 = 0.018$). In Winter, the relationship is also not statistically significant ($p = 0.829$), with an R^2 of just 0.1% ($R^2 = 0.001$). In the North region, on the other hand, the relationship is statistically significant ($p = 0.006$), and fish size explains 15.7% of the variance in parasite abundance
($R^2 = 0.157$). There is wide variability in the log of parasite abundance for similar fish sizes. In each graph, no clear trend or correlation indicates increased or decreased parasite abundance with increasing fish size. (Fig 3.3)

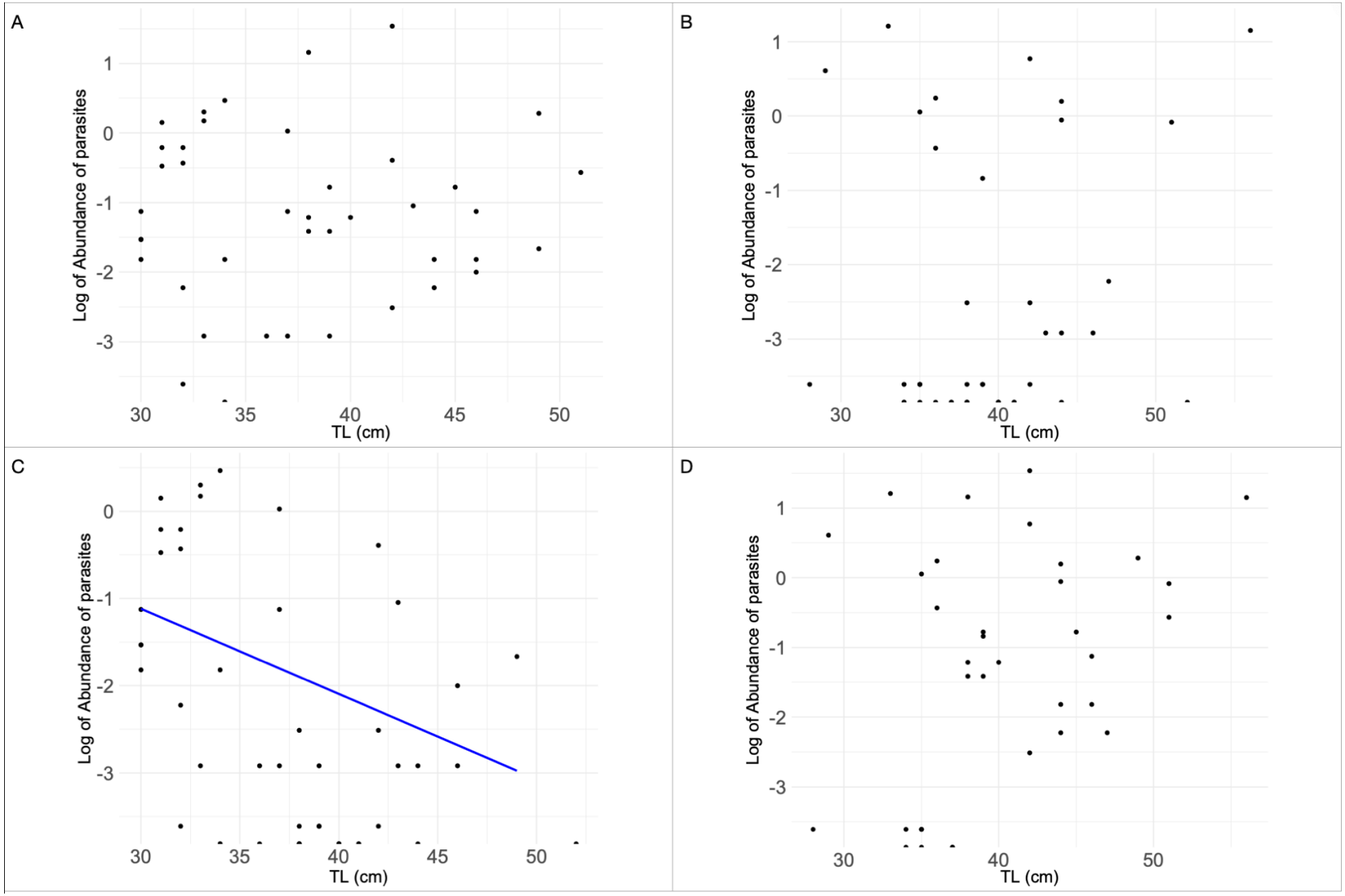

Figure 3.3: Relationship between log10 of parasite abundance and total fish length (TL) in cm: A during Winter (n=41), B during Summer (n=41), C in the North (n=47), and D in the South (n=35) .

3.2/Molecular results

This study found parasites belonging to at least four families: two nematode families and two trematode families.

We could sequence 11 parasites: 10 nematodes and 1 trematode found in the intestines. One of the nematodes is probably of the same species as *Anisakis simplex*, with a sequence length of 976 bp and a similarity of 99.9% with the one found in *Physeter catodon* host in Spain: Genbank accession number (GAN) ON622795. One of the nematodes is probably the same species as *Hysterothylacium aduncum*, with a sequence length of 1,046 bp and a similarity percentage of 99.9 with the ones found in the *Zoarces viviparus* host in the Denmark North Sea GAN JX845135, the host *Gadus morhua* in the Danmark Baltic Sea GAN KU306719 and the host *Sprattus sprattus* in the Denmark Baltic Sea GAN KU306720. One of the nematodes was from the same family as *H. aduncum,* with a sequence length of 1,033 bp and a percent similarity of 95% with those found in the hosts: *Z. viviparus* in the Danish North Sea JX845135, *G. morhua* in the Danish Baltic Sea KU306719 and *S. sprattus* in the Danish Baltic Sea KU306720. Seven of the nematodes are probably affiliated with the same family as *Dichelyne* cf *pleuronectidis*, i.e., the Cucullanidae, with incomplete sequence lengths for three of them: 886 bp, 503 bp, and 893 bp, and four of them with complete sequence lengths: 1,046 bp, 1,097 bp, 1,060 bp, and 1,023 bp. All have percent similarity below 92%, so they probably are also from the same family of the nematode *D.* cf *pleuronectidis* with GAN MT791107, MT791109, and MT791110 recovered from Australasian snapper *Chrysophrys auratus* in Australia. The trematode found in the inner gut is probably from the same family as *Z. viviparu*s, order Plagiorchiida, with a sequence length of 1,767 bp and 71% similarity to GenBank GAN accession numbers of pæarasites recovered from hosts *Callionymus lyra* in the UK (GAN AY222271), and *Buccinum undatum* in the Russian White Sea, Keret archipelago (GAN OP956067).

For the outer intestine, the single nematode found is probably of the same species as *A. simplex* with an incomplete sequence length of 976 bp and a similarity of 99.80% with the one recovered from of *P. catodon* host in Spain: GAN ON622795.

The liver contained 33 nematodes. Twenty of these are probably the nematode *A. simplex*, all with an incomplete sequence length of between 904 bp and 985 bp and similarity of 99 to 100% with the GenBank accession number recovered from the host *P. catodon* found in Spain GAN ON622795. Eleven of the 33 nematodes are probably *H. aduncum*, with 909 to 1055 bp incomplete sequence lengths. They have a similarity between 99 and 100% with the ones recovered from the hosts of *Z. viviparus* found in the North Sea in Denmark: GAN JX845135; *G. morhua* found in the Baltic Sea in Denmark: GAN KU306719 and *S. s*prattus found in the Baltic Sea in Denmark: GAN KU306720. One of the 33 nematodes found in the liver is probably the same species as *Contracaecum osculatum*, with an incomplete sequence length of 987 bp and 99% similarity recovered from the host *Halichoerus grypus* found in the Baltic Sea at Bothnian Bay: GAN AF411203. The last of the 33 nematodes in the liver is probably *Phocanema decipiens*, with an incomplete sequence length of 921 bp and 98% similarity with the Genbank accession numbers recovered from the host *G. morhua* in Norway GAN JQ673262 and *G. morhua* also in Norway GAN JQ673263.

We identified a metacercaria in the eyes, probably from the same family as *Apatemon gracilis.* It has a full-length sequence of 1,760 bp and a 99.76% similarity percentage with the parasite recovered from the host *Radix auricularia* found in Japan GAN LC599500.

In the mesenteries, eight nematodes were identified, with six of them probably *A. simplex* with incomplete fragment lengths of 843 to 989 bp and a percentage of 100% similar to the those recovered from the hosts *Scomber japonicus* found in Egypt GAN MT355320; *Scomber scombrus* GAN MN871437 and *P. catodon* identified in Spain GAN ON622795. The other two nematodes located in the mesenteries are probably *H. aduncum* with an incomplete sequence length of 921 bp and 909 bp, respectively, and both 100% similar to the ones from the hosts *Z. viviparus* found in the North Sea in Denmark GAN JX845135; *G. morhua* found in the Baltic Sea in Denmark GAN KU306719 and *Sp. sprattus* found in the Baltic Sea in Denmark: GAN KU306720.

Two nematodes inside the stomach probably have an affinity with the Cucullanidae family. They have sequence lengths of 882 and 1,057 bp, respectively, and a similarity of 91.94% with the Genbank identification numbers MT791107, MT791109, and MT791110, from the Australasian snapper *C. auratus* in Australia.

A nematode, probably *H. aduncum*, was identified from the outside of the stomach. It has a sequence length of 990 bp and a similarity percentage of 99% with the GenBank identification numbers recovered from the hosts *Z. viviparus* found in the North Sea in Denmark: GAN JX845135; *G. morhua* found in the Baltic Sea in Denmark: GAN KU306719; and *Sp. sprattus* found in the Baltic Sea in Denmark: GAN KU306720.

In the heart, a nematode was identified that has a probable affinity with *the H. aduncum* family with a sequence length of 634 bp and a similarity percentage of 98.52% with the nematode from GAN KT767161 in the Northern Wadden Sea.

3.3/ Parasite community

The graph (Fig 3.4) shows that the horizontal axis (TL) ranges from around 30 to 50 cm. The vertical axis (Species Richness) measures the specific richness of parasites, expressed as the number of different parasite species detected in each fish, with values ranging from 0 to around nine species. Species richness as a function of total fish size reveals a non-significant relationship ($p = 0.152$) and a low coefficient of determination ($R^2 = 0.026$). This means that total fish size explains only 2.6% of the variance in species richness. Observing the points on the graph reveals a wide dispersion without indicating any clear pattern.

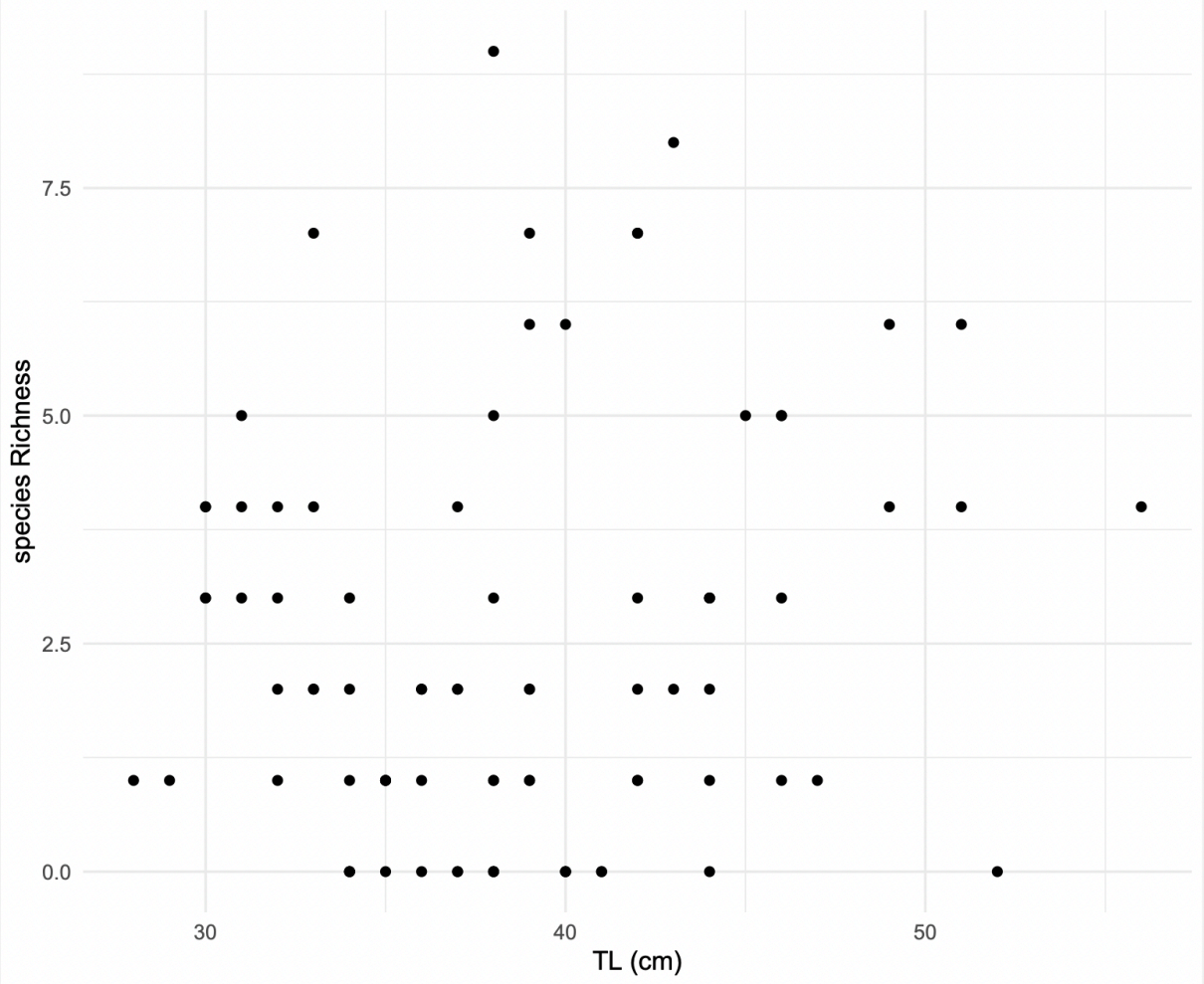

Figure 3.4: Relationship between parasite species richness and total fish length (cm) of all the samples (N=82).

In the south, winter samples, the most abundant parasite was the trematode *Rhipidocotyle* sp. in the gills, with a mean abundance of 20.86, a prevalence of 43%, and an intensity of 48.67 per infected host. However, the nematode *A. simplex* in the liver also has a high prevalence of 57% (Table 3.7).
In the northern winter samples, the most abundant parasite was the trematode *Z. viviparous* in the intestine, with a mean abundance of 11.00, 56% prevalence, and an intensity of infection of 19.80 (Table 3.8).

In the South, during summer, the most abundant parasite was *Z. viviparous* in the intestine, with a mean abundance of 27.52, a prevalence of 52.38%, and an intensity of infection of 52.55. However, the nematode in the liver *A. simplex,* also has a a mean abundance of 0.33, a high prevalence of 19.05%, and an intensity of infection of 1.75 (Table 3.9).

In the north summer, the parasite with the highest abundance was the cucullanid nematode in the intestine, with a mean abundance of 0.30, 20% prevalence, and an intensity of infection of 1.40 (Table 3.10).

Table 3.7: Parasite community from the group South Winter of *Pleuronectes platessa* (N = 14) for each organ. Values correspond to mean Abundance (Prevalence %) [mean Intensity of infection]. The parasite(s) may be located outside (ext) or inside (int) of the organ.

Species	Gills	Eyes	Stomach_Int	Stomach_Ext	Brain	Intestin_Int	Intestin_Ext	Heart	Liver	Mesenteries	Filet
NEMATODA											
Phocanema decipiens									0.07 (7%) [1.00]		
Contracaecum osculatum				0.07 (7%) [1.00]					0.14 (7%) [2.00]		
Hysterothylacium aduncum						2.14 (1%) [3.00]					
C*ucullanidae*									0.43 (57%) [2.21]	0.14 (7%) [2.00]	
Anisakis simplex											
METACERCARIAE											
Meta.Gen.sp	0.07 (7%) [1.00]				0.07 (7%) [1.00]	0.07 (7%) [0.07]			0.07 (7%) [1.00]	0.21 (14%) [1.50]	0.07 (0.07) [1.00]
TREMATODE											
Apatemon sp											
Rhipidocotyle sp	20.86 (43%) [48.67]										
Zoogonoides viviparus						2.21 (36%) [6.20]					
Trema.Gen.sp			0.21 (14%) [1.50]		0.07 (7%) [1.00]						

Table 3.8 : Parasite community from the group North Winter of *Pleuronectes platessa* (N = 27) for each organ. Values correspond to Abundance (Prevalence %) [Intensity]. The parasite(s) may be located outside (ext) or interior (int) of the organ.

Species	Gills	Eyes	Stomach_Int	Stomach_Ext	Brain	Intestin_Int	Intestin_Ext	Heart	Liver	Mesenteries	Extern
NEMATODA											
Phocanema decipiens									0.04 (4%) [1.00]		
Contracaecum osculatum											
Hysterothylacium aduncum								0.04 (4%) [1.00]	0.48 (22%) [1.29]	0.07 (4%) [1.00]	
Cucullanidae						2.30 (7%) [4.13]					
Anisakis simplex									0.15 (4%) [4.00]	0.22 (15%) [1.50]	
TREMATODE											
Meta.Gen.sp	0.19 (4%) [5.00]					0.04 (4%) [1.00]				0.04 (4%) [1.00]	0.04 (4%) [1.00]
Apatemon sp		0.04 (4%) [1.00]									
Rhipidocotyle sp	1.00 (41%) [2.45]										
Zoogonoides viviparus						11.00 (56%) [19.80]					
Trema.Gen.sp						0.07 (7%) [1.00]		0.04 (4%) [1.00]			

Table 3.9 : Parasite community from the group South Summer of *Pleuronectes platessa* (N = 21) for each organ. Values correspond to Abundance (Prevalence %) [Intensity]. The parasite(s) may be located outside (ext) or interior (int) of the organ.

Species	Gills	Eyes	Stomach_Int	Stomach_Ext	Brain	Intestin_Int	Intestin_Ext	Heart	Liver	Mesenteries	Extern
NEMATODA											
Phocanema decipiens											
Contracaecum osculatum											
Hysterothylacium aduncum									0.05 (9%) [1.00]		
Cucullanidae						0.05 (5%) [1.00]					
Anisakis simplex									0.33 (19%) [1.75]	0.14 (5%) [3.00]	
TREMATODE											
Meta.Gen.sp											
Apatemon sp											
Rhipidocotyle sp	0.14 (4%) [3.00]										
Zoogonoides viviparus						27.52 (52%) [52.55]					
Trema.Gen.sp											

Table 3.10 : Parasite community from the group North Summer of *Pleuronectes platessa* (N = 20) for each organ. Values correspond to Abundance (Prevalence %) [Intensity]. The parasite(s) may be located outside (ext) or interior (int) of the organ.

Species	Gills	Eyes	Stomach_Int	Stomach_Ext	Brain	Intestin_Int	Intestin_Ext	Heart	Liver	Mesenteries	Extern	Filet
NEMATODA												
Phocanema decipiens												
Contracaecum osculatum												
Hysterothylacium aduncum									0.05 (5%) [1.00]			
Cucullanidae						0.30 (20%) [1.50]						
Anisakis_simplex												
TREMATODE												
Meta.Gen.sp												
Apatemon sp												
Rhipidocotyle sp												
Zoogonoides viviparus												
Trema.Gen.sp												

The four parasites that stand out among all the groups are *Rhipidocotyle* sp. in the gills, *A. simplex* in the liver, and both *Z. viviparus* and the cucullanid nematode in the intestine. When we look at the average parasitic infection for all samples, these four parasites have the infections and ranges (abundance, prevalence and intensity) but also a large standard deviation. *Rhipidocotyle* sp. in the gills has a mean abundance of 3.93 but a standard deviation of 20.89. *Anisakis simplex* in the liver has a mean abundance of 0.89 and a standard deviation of 2.16. *Zoogonoides viviparus* in the intestine has a mean abundance of 11.05 and a standard deviation of 22.97. The cucullanid nematode has an abundance of 1.21 and a standard deviation of 2.44 (Table 3.11).

Table 3.11: Average parasitic infections for each parasite identified by organ, standard deviation and (range) of *Pleuronectes platessa* (N = 82)

Species	Gills	Extern	Brain	Mensentere	Liver	Intestin_Int	Intestin_Ext	Stomach_Int	Stomach_Ext	Filer	Heart	Gonads
NEMATODA												
Rhipidocotyle sp	3.93 ± 20.89 (0-3)											
Phocanema decipiens					0.01 ± 0.11 (0-1)							
Contracaecum osculatum					0.01 ± 0.11 (0-1)							
Hysterothylacium aduncum				0.04 ± 0.25 (0-2)	0.24 ± 0.73 (0-2)	0.04 ± 0.25 (0-2)			0.05 ± 0.27 (0-2)		0.01 ± 0.11 (0-2)	
Cucullanidae						1.21 ± 2.44 (0-1)		0.04 ± 0.25 (0-1)				
Anisaki _simplex				0.13 ± 0.54 (0-3)	0.89 ± 2.16 (0-3)		0.09 ± 0.43 (0-3)					0.02 ± 0.22 (0-3)
TREMATODE												
Apatemon gracilis												
Meta.Gen.sp	0.07 ±0.56 (0-3)	0.01± 0.11 (0-1)	0.01 ±0.11 (0-3)	0.04 ± 0.19 (0-3)	0.01 ± 0.11 (0-3)	0.02 ± 0.16 (0-3)				0.01 ± 0.11 (0-3)		
Zoogonoides viviparus						11.05 ± 22.97 (0-1)						
Trema.Gen.sp			0.01 ± 0.11 (0-1)			0.39 ± 3.31 (0-1)		0.04 ± 0.25 (0-1)			0.01 ± 0.11 (0-1)	
COPEPODE												
Cop.Gen.sp	0.35 ± 0.99 (0-1)	0.02 ± 0.22 (0-1)										
CYSTACANTH				0.01 ± 0.11 (0-1)	0.04 ± 0.19 (0-1)							
Cyst.Gen.sp												
UNKNOW												
unknow.Gen.sp	0.01 ± 0.11 (0-1)		0.01 ± 0.11 (0-1)	0.01 ± 0.11 (0-1)								

3.4/LDA

Linear discriminant analysis (LDA) revealed variations in parasite community composition among the four study groups. The LD1 axe represents 88.67% of the variance, and the LD2 axe represents 9.4%. The South Winter group showed the most significant differences, characterized by metacercaria in the brain, copepods externally, and trematodes in the stomach. There was a noticeable overlap between the North Summer, North Winter, and South Summer groups, suggesting marked similarities in parasite diversity and abundance. The group North Winter shows differences in parasite community along the LD2 axis. The parasites that make the differences between the groups are the cucullanid nematode in the stomach, metacercaria in the intestine, unknown in the mesenteries, *H. aduncum* in the intestine, *A. gracilis* in the eyes, acanthocephalan cystacanths in the mesenteries, and metacercariae in the gills. (Fig 3.5)

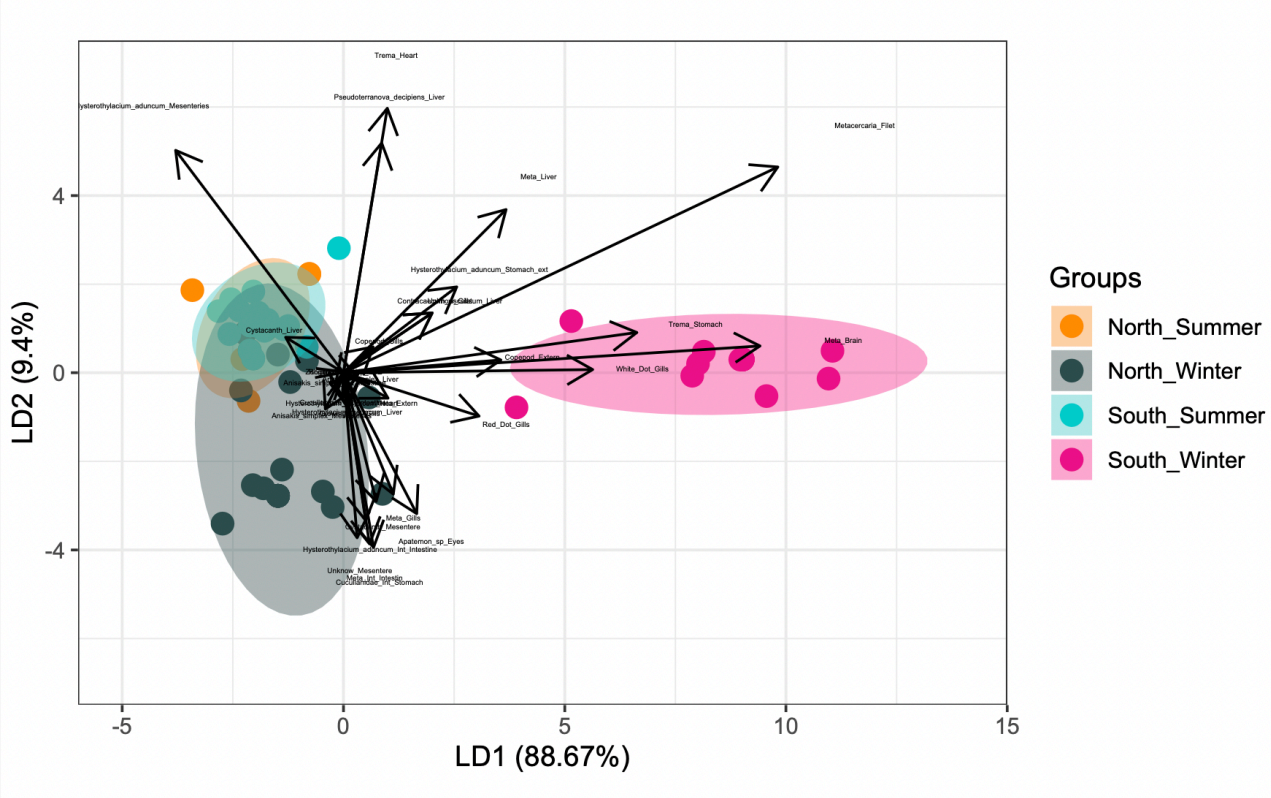

Figure 3.5: Bi-plot of Linear discriminant analysis for the parasites of the Icelandic plaice (*Pleuronectes platessa*) by sampling region and season. The ellipses correspond to data groups and illustrate the distribution and concentration of points associated with each group. The larger the ellipse, the greater the variability within the group. They help visualize how groups overlap or differ from one another. Arrows represent the vectors of variables (in this case, parasite species) in discriminant space. The direction of each arrow indicates how a variable contributes to group separation along the discriminant axes (LD1 and LD2). The length of the arrows reflects the relative importance of each variable in group differentiation: the longer the arrow, the bigger the impact of the variable on group separation.

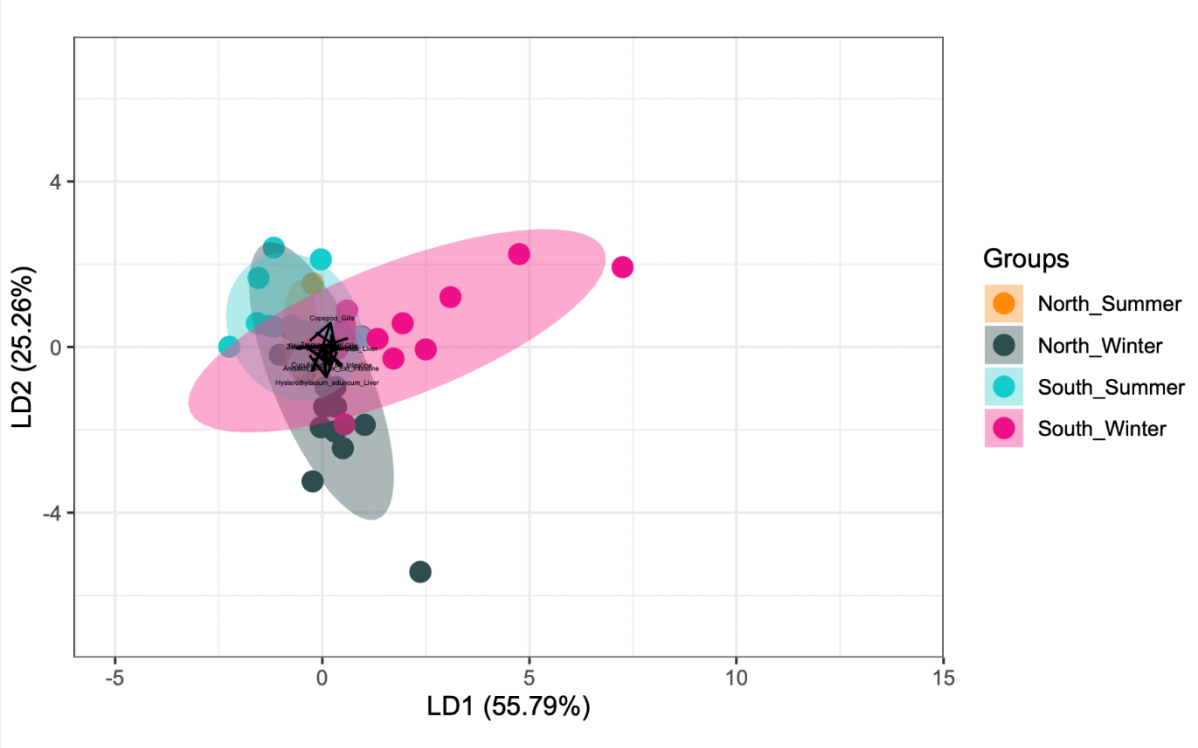

Figure 3.6: Bi-plot of Linear discriminant analysis scores for the parasites of the Icelandic plaice (*Pleuronectes platessa*) by sampling region of the most abundant parasites (present at least 5% in each fish). The ellipses correspond to data groups and illustrate the distribution and concentration of points associated with each group. The larger the ellipse, the greater the variability within the group. They help visualize how groups overlap or differ from one another. Arrows represent the vectors of variables (in this case, parasite species) in discriminant space. The direction of each arrow indicates how a variable contributes to group separation along the discriminant axes (LD1 and LD2). The length of the arrows reflects the relative importance of each variable in group differentiation: the longer the arrow, the bigger the impact of the variable on group separation.

The LD1 axe represents 55.79% of the variance, and the LD2 axe represents 25.26%. There was a noticeable overlap between the four groups, North Summer, North Winter, South Summer, and South Winter, suggesting marked similarities in parasite diversity and abundance with a larger ellipse on the LD1 axis for the South Winter group. The arrows are very close and overlapping, which could indicate that the associated variables do not have very distinct contributions or are highly correlated. However, the North Winter shows differences in the parasite community along the LD1 axis (Fig 3.6).

3.5/GLMM

Several factors influence the dependent variable, parasite abundance, in the generalized linear mixed model (GLMM). The intercept, with a negative coefficient of -2.78, suggests that the baseline value of parasite abundance is lower when the other variables (region, seasons, and size) are zero, showing a statistically significant result ($p = 0.024$). The analysis shows that the winter season is associated with a significant increase in abundance ($p < 0.001$. Similarly, the southern region is associated with a significant increase in abundance ($p < 0.001$). The interaction between South and Winter variables shows that the combined effect is less pronounced than the sum of the individual effects, suggesting a moderating impact of this specific combination ($p < 0.001$). On the other hand, the effect of length on abundance is not statistically significant ($p = 0.267$), indicating that variations in fish size do not have a detectable impact in this model. (Table 3.12)

Table 3.12: Generalized linear mixed model (GLMM) result to show the influence of the variable fish length, season, and region on parasite abundance with the estimate, standard error, z-value, and p-value.

Variable	Estimate	Standard Error	z-value	p-value
Intercept	-2.78391	1.23458	2.255	0.0241
Seasons:Winter	3.36297	0.69788	4.819	<0.0001
Region:South	3.44198	0.69470	4.955	<0.0001
RegionSouth:SeasonsWinter	-3.09418	0.86261	3.587	0.0003
Length	0.03501	0.03155	1.109	0.2672

3.6/ Otolith Shape

Otoliths from the four different study groups showed little visual morphological difference (Fig. 3.7). However, to confirm this difference, we used the first five coefficients of the 109 elliptical Fourier coefficients (EFC) per otolith that were generated during the shape analysis with RStudio. These first five wavelets were chosen because they represented 98.5% of the otolith variation that could be explained (Fig. 3.8).

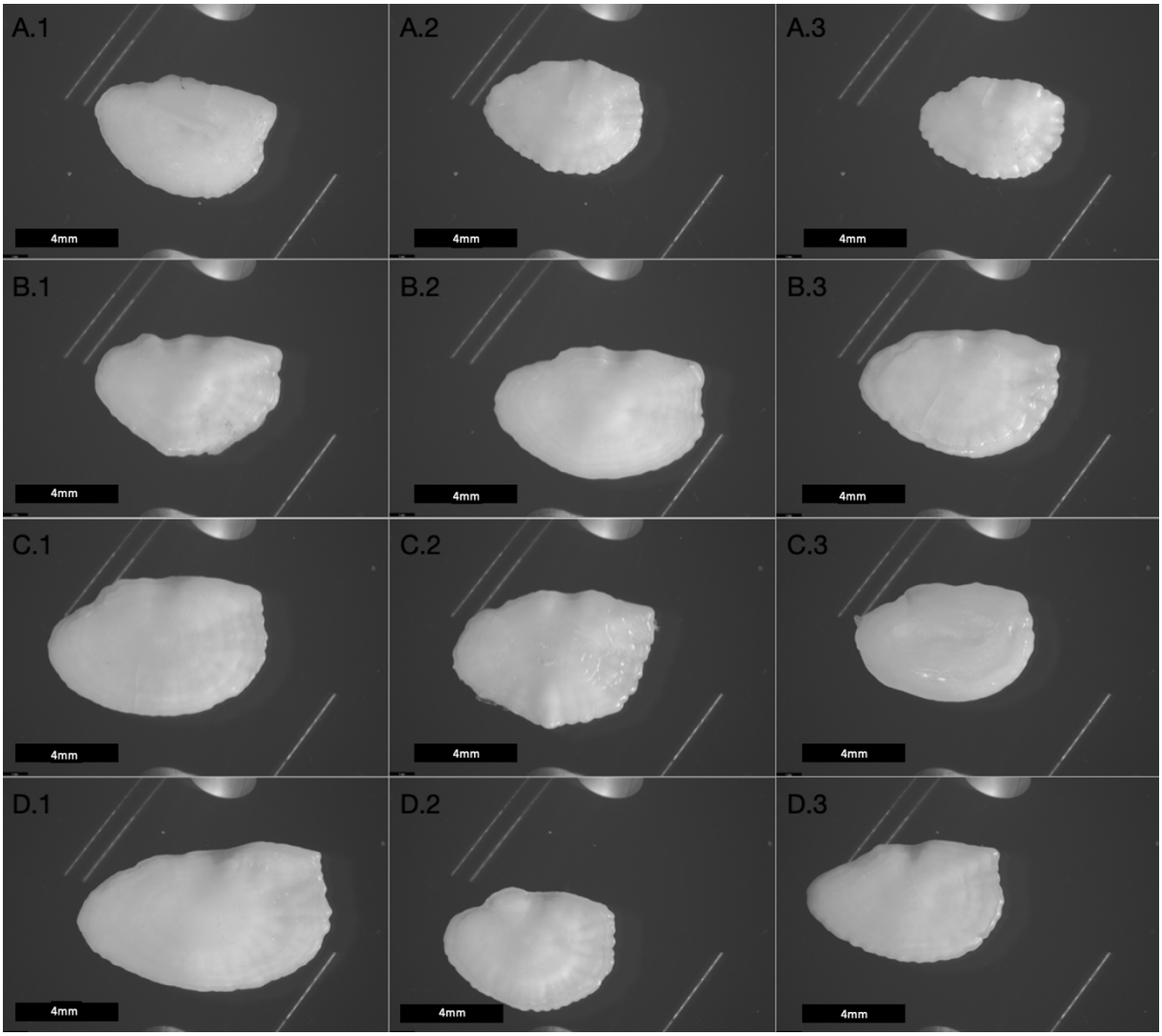

Fig 3.7: photos taken of whole otoliths of Iceland plaice in (A) North Winter with A.1 = PL01 (37cm), A.2 = PL02 (34cm), A.3 = PL03 (32cm), (B) South Winter with B.1 = PL12 (44cm), B.2 = PL13 (46cm), B.3 = PL15 (40cm), (C) North Summer with C.1 = PL42 (44cm), C.2 = PL44 (34cm), C.3 = PL45 (40cm), and (D) South Summer with D.1 = PL63 (47cm), D.2 = PL64 (33cm) and D.3 = PL65 (39cm).

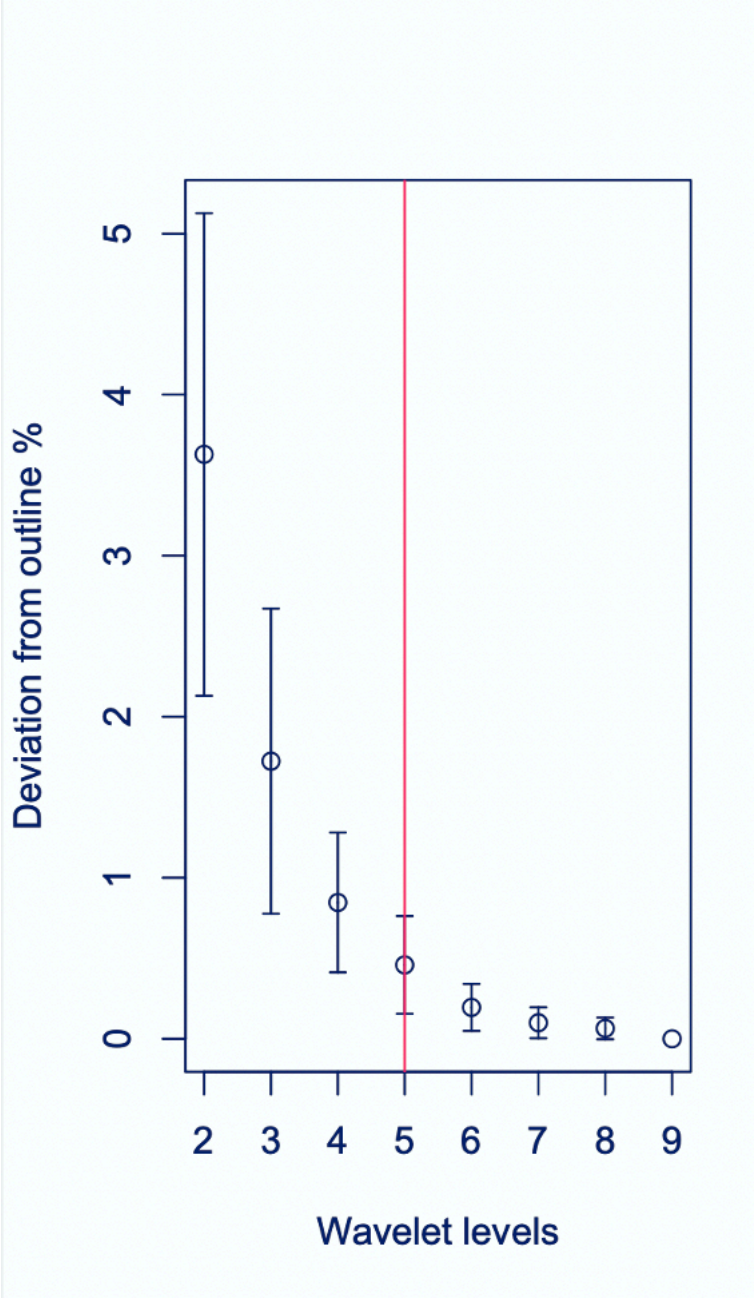

Fig 3.8: Quality of the Wavelet outline reconstruction. The red vertical lines show the number of levels of Wavelet needed for a 98.5% reconstruction accuracy.

We reconstructed otolith contours using the average of elliptic Fourier harmonics over the four sampled regions. Each group studied corresponded to a different color (Fig. 3.9). A graph shows the wavelet coefficients' mean and standard deviation (sd) for all otoliths combined, the proportion of variance between groups, and the intraclass correlation. This graph allowed us to identify the differences at the otolith level. We observed variability in otolith shape. Differences were particularly noticeable in the North Winter group compared with the other study groups at the 90- to 0-degree level (Fig. 3.9). However, the mean confirms these differences and standard deviation (SD) of the wavelet coefficients for all otoliths combined, with the most significant differences identified around degrees 0 and 360 and the minor differences at degree 140 (Fig. 3.10).

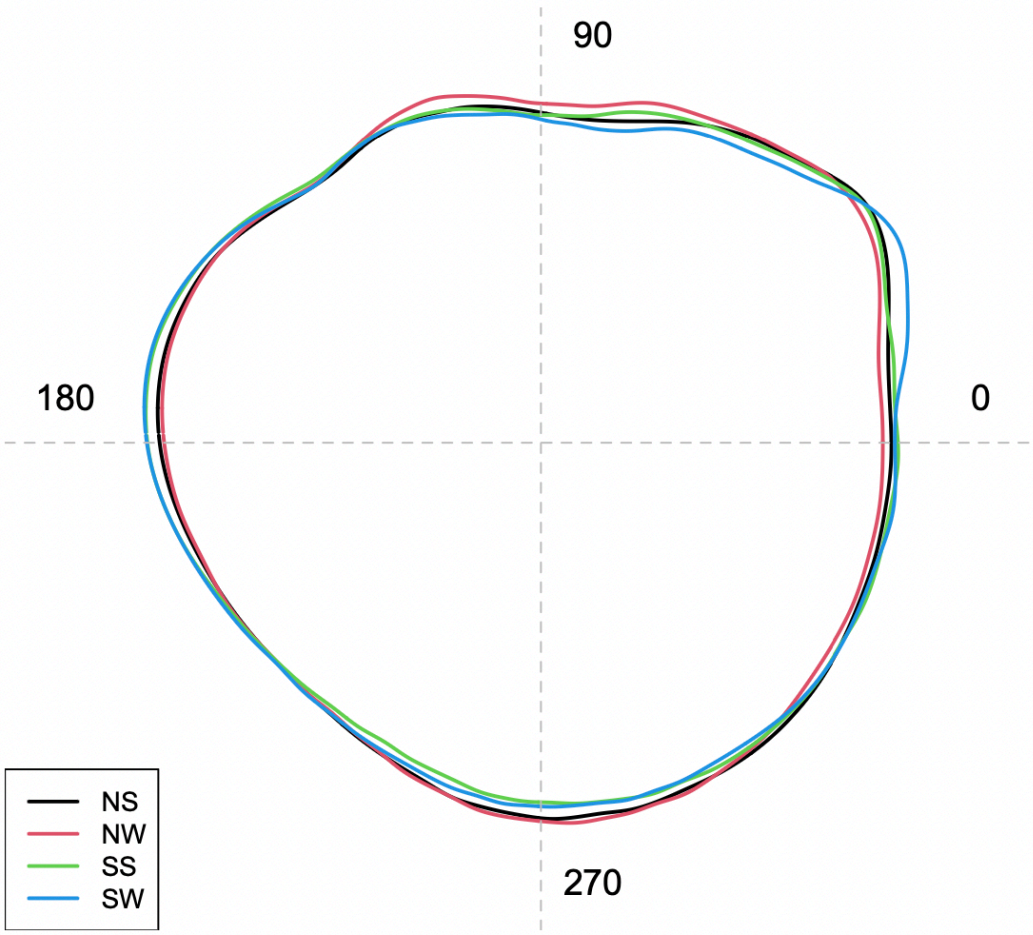

Fig 3.9: Mean otolith shape based on Wavelet reconstruction for four discrete plaice populations from Iceland: NS (North Summer in black), NW (North Winter in red), SS (South Summer in green, and SW (South Winter in blue). Numbers represent angles in degrees (°) based on polar coordinates (see Fig 6.10). The centroid of the otolith (center of the cross) is the center point of the polar coordinates.

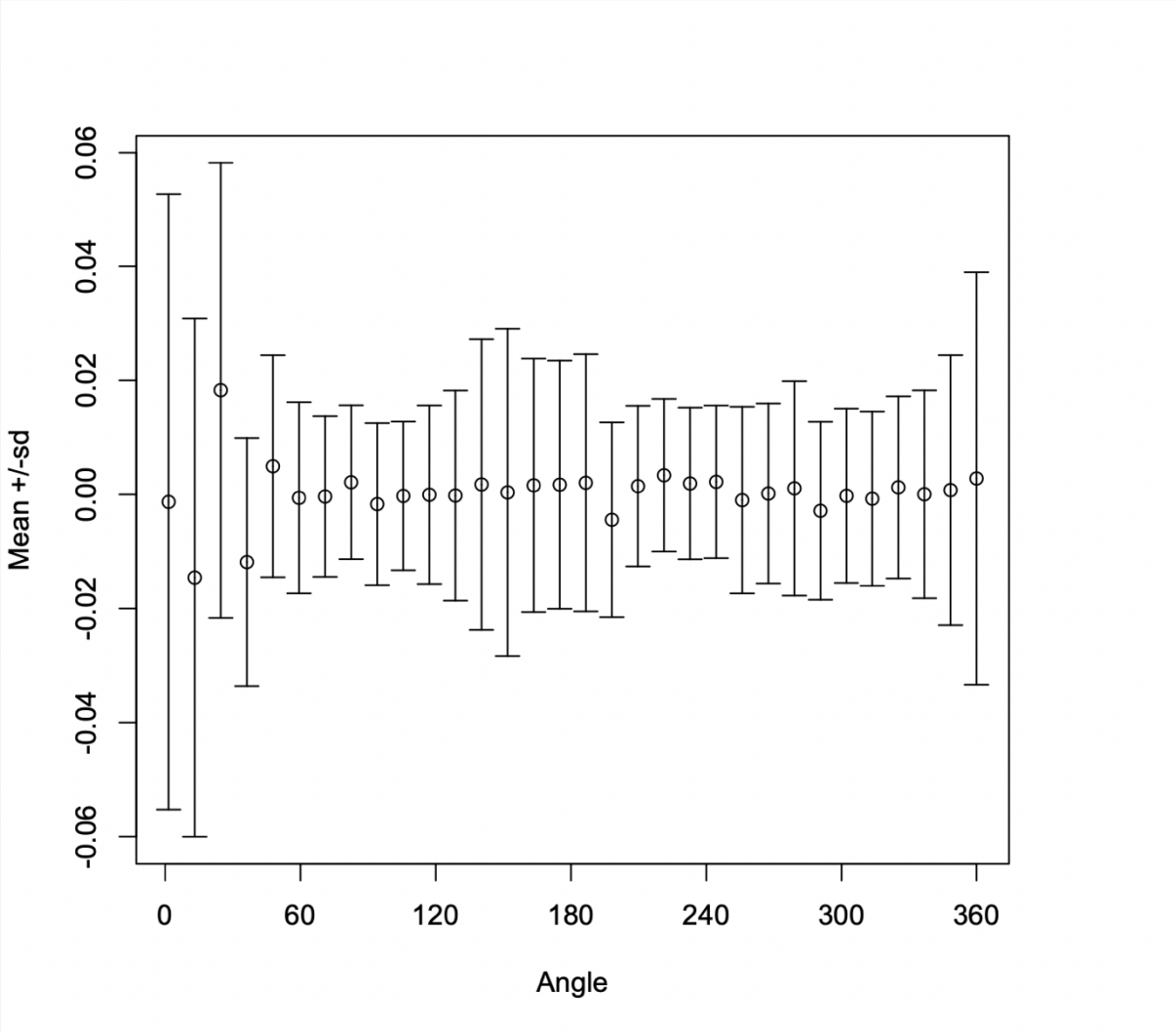

Fig 3.10: Mean and standard deviation (sd) of the Wavelet coefficients for all combined otoliths and the proportion of variance among groups or the intraclass correlation. The horizontal axis shows the angle in degrees (°) based on polar coordinates (see also Fig 6.9), where the centroid of the otolith is the center point of the polar coordinates.

The results of the ANOVA permutation test validated the hypothesis that there was a significant ($F = 2.809$; $p < 0.001$) difference in otolith shape between the four samples: North Summer, South Summer, North Winter, and South Winter.

Cross-validation was used to evaluate the linear discriminant analysis (LDA) model. The classification error of 0.598 means that, on average, around 59.76% of the predictions made by the LDA model are incorrect. In other words, the model correctly classified observations around 40.24% of the time.

4. Discussion

This thesis aimed to assess whether parasites and otoliths could serve as biological markers for stock discrimination of Icelandic plaice *Pleuronectes platessa* between northern and southern Iceland and between winter and summer seasons. No previous studies have been conducted to find differences in the Icelandic plaice population through otolith and parasite studies. To answer the question of which parasites make up the fauna of plaice parasitic communities, the 82 specimens collected were examined for ectoparasites and endoparasites and the shape of their otoliths. The results showed a separation of parasitic communities and otolith shape between Icelandic winter plaice individuals in the south compared with the three other populations studied: north in winter, north in summer and south in summer.

In Iceland, a study has already shown that using microsatellites and mitochondrial DNA shows significant differences between *P. platessa* collected in the south-west (Reykjanes Peninsula) and north-west (Westfjords), indicating genetic differentiation and fish fidelity to their specific spawning and feeding grounds (Solmundsson et al., 2005). The southern plaice in our study were collected from the Reykjanes peninsula, and all samples collected in this area in a given season are similar in month and location. In our samples, parasites' abundance and species richness are different in the north and south, specifically in the south in winter where a different parasite community and otolith form is found than in the north, which could show a difference between these two fish populations.

Otolith shape is determined by various environmental, genetic, and physiological factors, as these variables can change from one population to another, which will be reflected in the shape of otoliths (Nazir and Khan, 2021). Temperature influences otolith growth and chemical composition by affecting mineral solubility and precipitation (Morrongiello et al., 2012). Temperature affects the deposition rate of materials, resulting in faster growth and affecting the shape of the otolith (Morrongiello et al., 2012). We know that the north and south of Iceland are influenced by two different currents: to the north, the cold Greenland current, and to the south, the warm Atlantic current, which could affect the shape of otoliths in these different regions (Burke et al., 2008). The seas around Iceland generally have higher summer and lower winter water temperatures, influenced by the warm North Atlantic Current to the south and the cold East Greenland Current to the north. This dynamic creates significant temperature differences between northern and southern Iceland, directly influencing fish size and growth (Johannessen et al., 1994). As Greenland water is colder and has lower salinity, fish exposed to this water mass may grow more slowly, impacting the shape of otoliths. In Iceland, cod from the inshore spawning area, characterized by colder, less saltine waters, showed slower growth rates and distinct otolith shapes from those of cod from warmer, saltier offshore areas (shoal and shelf) as shallow waters are warmer than deep waters (Petursdottir et al., 2006). Different growth rates can lead to variations in otolith shape. Rapid growth rates can alter the concentration of ions in the endolymph, thus impacting otolith structure (Jónsson et al., 2021). In Alaskan waters, the growth rates of juvenile Pacific cod (Gadus macrocephalus) were manipulated by controlling food intake and temperature variations. Using sagittal otoliths, the researchers measured elements such as manganese (Mn) and strontium (Sr). The results revealed that rapid growth rates increased manganese incorporation into otoliths. In contrast, strontium incorporation was reduced. As growth rates impact the deposition of different elements on otoliths, which in turn influence otolith shape, these different growth rates directly influence mineral deposition and, consequently, otolith shape (Miller and Hurst, 2020).

Genetic variations, such as growth rate regulation and genetic divergence between different fish populations, can result in morphological differences in otoliths; genetically distinct herring populations from the Irish Sea and Celtic Sea have otolith shapes that vary significantly due to differences in growth rates influenced by genetic and environmental factors. The otoliths of Irish Sea herring, which breed in autumn, tend to be more elongated and have a different curvature to those of Celtic Sea herring, which breed in winter (Burke et al., 2008). Using otolith contour reconstructions based on the mean of elliptical Fourier harmonics across the four sampled regions (Fig. 3.8), these analyses revealed a difference in the shape of the otoliths

in the north winter group compared to the three other groups studied. However, the classification error of our study is 59.76%, which is relatively high, indicating that the LDA model for otoliths fails to discriminate well between the four different plaices populations studied. The results suggest that the current LDA model could better classify our data.

However, (Fowler et al., 1995) through controlled laboratory experiments, they found that higher temperatures and salinity levels generally increased the strontium/calcium ratio in the otoliths of juvenile Atlantic whitefish (Micropogonias undulatus), affecting the internal structure of the otoliths by modifying the growth of successive layers and thus their shape. They also noted that these variations were not the same at all life stages, indicating that the different ontogenetic stages of fish indirectly influence otolith shape (Campana, 1999).

As mentioned above, otolith growth is influenced by multiple factors. As fish age, these factors increasingly influence otolith shape. Having fish of the same age can minimize these variations and improve the understanding of age-specific growth patterns. Nevertheless, as fish size does not always reflect internal changes in otolith structure, which are more directly related to age, having samples of different sizes has much less impact on the results than not having samples of the same age (Mapp et al., 2017). In our study, fish were not selected based on age and size, which may confound the results. Having the same number of juveniles and adults in each study group is important for more revealing results.

Reproductive stages can also affect the transport of ions and the flow of elements to otoliths, impacting their shape. As otoliths develop through the precipitation of calcium carbonate (in the form of aragonite) from endolymphatic fluid in the inner ear, reproductive stages can alter the balance of ions, such as calcium and carbonate, due to hormonal and metabolic variations. This can influence the rate and mode of deposition of otolith layers, affecting their shape and structure (Morales-Nin, 2000). That is why it is important to locate plaice spawning grounds in the southwest of the island, where our winter group was fished in the same area, i.e., the Reykjanes peninsula (MFRI Assessment Reports, 2023).

The diet also impacts the composition of the fluid contained in the membranous labyrinth of the inner ear: the endolymph, which affects the biomineralization of otoliths (Darnaude et al., 2014). A sample of 42 plaice was collected in the eastern channel in and around the Pas de Calais Strait (France) to see if diet impacted otolith shape. Stomach contents were identified, and it was found that the taxonomic composition of prey correlated with variation in otolith shape. They analyzed different prey categories, such as Annelids, Carids, Bivalves, and Gastropods. They found that these prey categories could affect the otolith's overall shape (such as ellipticity) and finer details. This may be due either to the amount of protein present in the

prey, which influences the synthesis of the otolith organic matrix and otolith growth, or to the protein composition of the prey, particularly in essential amino acids, which could directly affect the crystalline structure of the precipitated calcium carbonate ($CaCO_3$), and thus the shape of the otolith (Mille et al., 2016).

We suggest that not all parasites present in Icelandic plaice have been discovered or identified. Looking at the literature, only three two parasites have been reported to date in Iceland in the plaice: the copepod *Acanthochondria cornuta* in the gill (Kabata, 1959), the Myxosporea *Myxobolus platessae* found in the cartilage (Karlsbakk et al., 2017) and *P.decipiens* in the muscles and the visceral (Hauksson and Ólafsdóttir, 1995). *Myxobolus aeglefini*, a myxosporidian parasite, has been highlighted as a potential tag for plaice in the eastern North Sea, particularly in the Skagerak-Kattegat region of Norway. The parasite infects the head and gill cartilage of plaice, and its geographical distribution has been suggested as helpful in differentiating the different plaice stocks in this region (Mellergaard & Nielsen, 1984.). There are notably two other parasites known to be biological tags for plaice in the waters of the North Sea where plaice have been recorded. The nematode *Cucullanus heterochrousa* was noted for its different prevalence and intensity of infection among plaice from various spawning grounds in the southern North Sea, making it another candidate as a biological marker for stock identification (Wickins and Macfarlane, 1973). We found a parasite from the same family, which could represent a potential tag. *Phocanema* decipiens, which we also found in our samples, has previously been used as a biological tag to differentiate stocks of spinymouth rockfish (*Sebastes mentella*) in the North Atlantic. Its prevalence and abundance were analyzed in different fishing areas at depths greater than 500 m, from the Labrador coast to the Faroe Islands, above 500 m, from the Grand Banks to the Faroe Islands, and around the Icelandic coast. The parasite was mainly found in the intestines of fish. Its geographical distribution and prevalence varied among regions, reflecting differences in fish stocks (Klapper et al., 2016). Nematode Anisakidae larvae, including species such as *Anisakis simplex*, have been identified as potential markers due to the variability of their infection levels between different plaice populations (Mackenzie and Hemmingsen, 2015) We found in our North winter, south winter, and south summer samples several *A.simplex* parasites that represent a potential for a biological tag.

Hysterothylacium aduncum is a parasite commonly found in various flatfish (Pleuronectiformes) (Klimpel and Rückert, 2005) in and around the North Sea. In Iceland, this parasite has been detected in cod (Palsson et al., 1985), eel (Kristmundsson and Helgason, 2007), Atlantic Wolffish in south Iceland (Elfarsson, 2023, Bray, 1987), Haddock in north Iceland (Guðmundsson, 2023), and redfish (Bakay et al., 2024). Eels inhabiting the marine and fresh waters of southern Iceland showed the presence of the parasite, mainly in the intestine (Kristmundsson and Helgason, 2007). In H. *aduncum*, adults and larvae were found in various organs, mainly in northern Icelandic cod (Palsson et al., 1985). In our study, the parasite was found more frequently in places from the north (10 individuals) than those from the south (4 individuals). The crustaceans Euphausiacea and Mysidacea are recognized as the first intermediate hosts of *Hysterothylacium* (*S*mith, 1971). In the case of Icelandic cod, the final host becomes infected by the prey (Palsson et al., 1985). The crustaceans Euphausiacea and Mysidacea are first eaten by capelin, then ingested by cod, qualifying them as intermediate hosts and cod as final hosts. Plaice feed exclusively on benthic organisms, including certain crustaceans. By consuming these crustaceans and having found only juvenile parasites in our samples, plaice could be one of the intermediate hosts or paratenic of *H. aduncum*. *Hysterothylacium aduncum* eggs, ingested by crustaceans and consumed by the secondary host or final predator, show that larvae smaller than 0.2 cm do not survive in fish (Køie, 1993). However, larvae measuring between 0.2 and 0.3 cm in length can survive, and those over 0.3 cm reach maturity in the final host, generally localizing in the intestine (Klimpel and Rückert, 2005).

The parasite *H. aduncum* represents a biological marker for the identification of stocks of Atlantic horse mackerel *Trachurus trachurus L*. Samples from the North Sea showed a much higher prevalence and intensity of *H. aduncum* than those from neighboring areas, indicating limited mixing and supporting the distinction of this population. This differentiation makes it possible to identify distinct stocks (MacKenzie et al., 2008). This parasite can represent a potential biological tag for our studies.

In Iceland, the metacercaria of *Apatemon gracilis* is a parasite frequently found in freshwater, particularly in lakes; it was found in Lake Myvatn in the North of Iceland (Faltýnková et al., 2023) in a Gasteropoda *Gyraulus parvus*, also in Þingvallavatn in sticklebacks fish (*Gasterosteus aculeatus*) in the eyes (Sæmundsdóttir, 2023) and the eyes of arctic charr From Thingvalla-vatn lake and Sel-vatn lake (Kristmundsson and Richter, 2009). The parasite *A. gracilis* was only found once in plaice sampled in the North during winter for our study. This area, crucial for the nesting of many avian species, saw the first census of this parasite in 2002 (Faltýnková et al., 2023). Mainly observed in summer and autumn, *A. gracilis* remains uncommon in marine fish. The life cycle of *A. gracilis* involves several intermediate hosts: the first is typically a pulmonate snail (Blair, 1977, Blasco-Costa et al., 2016, Soldánová et al., 2017). The second is a fish; the final host is a fish-eating bird. To integrate efficiently into this ecological cycle, *A. gracilis* has adapted to be consumed by predators, which facilitates its passage to the next stage of development. Morphological studies have shown that these parasites have enlarged tails to avoid small predators, thus reducing their ingestion by crustaceans (Mironova et al., 2019). Once ingested by fish, the latter enables the parasite to progress to its metacercarial stage. *Apatemon gracilis* is a freshwater parasite found in plaice, a saltwater fish. Although plaice is primarily a marine species, it has been reported that they occasionally enter estuarine and even freshwater areas to use cold-water nurseries, such as the Valosen estuary and the Storfjord in northern Norway, indicating that they settle and grow in these estuarine habitats during the early stages of their lives (Filipe et al., 2015). This could explain the presence of this parasite, as the plaice seems to have been infected by *A. gracilis* early in its life in freshwater and then moved out to sea. The *A. gracilis* parasite mainly affects fish vision through its metacercarial stage, which can encyst in various parts of the fish's body, including the eyes.

Metacercariae can physically obstruct vision by encysting in ocular tissues, leading to opacification or other forms of visual impairment similar to cataracts. This obstruction disrupts the normal functioning of the eye. It affects the fish's ability to see clearly, considerably affecting its ability to detect predators and, therefore, to survive for long periods in the ocean, hunt for food, and orientate itself in its environment (Bell et al., 2002). It involves the fish's vision and pushes them toward the surface, where birds more easily capture them (Karvonen et al., 2013). This parasite is not known to be a biological tag in stock discrimination and in the instance, the plaice likely acts as a dead end host.

A parasite of the Cucullanidae family has been found in plaice. The parasite *C. heterochrous* has been found in North Sea plaice, notably in Norway (Urskog, 2014), Germany, and England, in the intestines and stomachs (Wickins and Macfarlane, 1973). This nematode has also been found in fish in Iceland. *Cucullanus heterochrous* is mainly found in plaice and winter plaice (Pleuronectiformes), feeding on the host's stomach and intestinal contents (Køie, 2000). The North-East Atlantic is home to two species of Cucullanidae: *C. cirratus*, which generally infects gadids, and *C. heterochrous*, which typically infects flatfish (Køie, 2000).

The life cycle of *C. heterochrous* begins with the emergence of fish eggs (Pleuronectiformes) into the water, where they transform into embryos. Embryos pass through three stages of development: the first (L1), the second (L2), and the third (L3), with the latter being the hatching stage. The embryo does not hatch until a transported/intermediate host eats it. Polychaetes appear to act as a proper intermediate host in the North Atlantic (Køie, 2000) and are also eaten by plaice (Doornbos and Twisk, 1984). The cycle is complete when a suitable host fish (flatfish) eats the polychaete - in our case, plaice is a good candidate - and the larvae can then develop to a fourth (L4) and fifth stage, the sexually mature stage (L5). Thus, the *C. heterochrous* found in flatfish are in their last larval stage or adult (Køie, 2000) .

As mentioned above, the parasite *C. heterochrous* is a good candidate for being a biological tag of plaice in stock discrimination. Indeed, one study examined how marked differences in the prevalence and intensity of infection of plaice intestines by *C. heterochrous* were observed between plaice from different spawning grounds in the southern North Sea. These differences suggest that *C. heterochrous* could help distinguish different plaice stocks according to their exposure to specific environmental conditions and the intermediate hosts associated with the parasite (Mackenzie and Hemmingsen, 2015). However, in our study, infection levels were similar in all four sample groups, so the parasite of the Cucullanidae family does not represent a good biological tag.

The nematodes *Anisakis simplex* and *P. decipiens* were also found in our sample in the liver, intestine, and mesenteries for *A. simplex* and the liver for *P. decipiens.* In Iceland, *A. simplex* has already been found in cod (in the liver) (Severin et al., 2020) , in the body cavity, flesh, gonads, heart, intestine, liver, mesenteries, pyloric caeca, spleen, the stomach of haddock (Guðmundsson, 2023) , in the musculature and visceral organs of beaked of the redfish (Klapper et al., 2015), saithe fillets, and the gills viscera (Højgaard, 1997) , in the body cavity of the capelin (Palsson and Beverley-Burton, 2011), in the body cavity, flesh, gallbladder, gonads, intestine, liver, mesenteries, spleen, stomach of the wolf fish (Elfarsson, 2023). *Phocanema decipiens* was found in Iceland in the muscle and visceral of the plaice (Hauksson and Ólafsdóttir, 1995), the haddock body cavity (Guðmundsson, 2023), the cod's muscles and body cavity (Grainger, 1959) , wolf fish's muscle tissue, and flesh (Elfarsson, 2023), the body tissues of saithe (Højgaard, 1997) larval form in the intestinal wall (Kristmundsson and Helgason, 2007).

Marine mammals, the final host, release their eggs into the water via excrement, hatching them into L2 stage larvae and floating freely in the water (Nagasawa, 1990). Crustaceans, mainly euphausiids (krill), ingest them and develop into L3-stage larvae in these hosts (Klimpel and Palm, 2011, Lunneryd et al., 2015). Stage L3 larvae infect fish and cephalopods when they consume the infected crustaceans. Fish and cephalopods act as paratenic hosts, transmitting the larvae throughout the marine food chain (Nagasawa, 1990). Marine mammals in Iceland, mainly common (*Phoca vitulina* L.) and grey seals (*Halichoerus grypus*), will prey upon fish and cephalopods, allowing the development of the larvae into adult stages, completing the life cycle by being final hosts (Ólafsdóttir and Hauksson, 1998, Hauksson and Ólafsdóttir, 1995) (Zuo et al., 2018). In winter, these parasites are found in the plaice from the island's north, close to harbor and grey seal colonies (Hauksson, 2007, Granquist, 2022). This was also the case in the Southern winter samples for the nematode *Contracaecum osculatum*, known to have similar life cycles and reportedly found in similar species at similar life stages (Ólafsdóttir and Hauksson, 1998, Klimpel and Palm, 2011, Zuo et al., 2018, Køie and Fagerholm, 1995). This would explain the presence of these parasites in Icelandic plaice, since a host fish is only infected by a given parasite when it enters the parasite's endemic zone. This would define the Southern sampling zones as an endemic region for *C. osculatum* and the Northern sampling zones as an endemic region for *A. simplex* and *P. decipiens,* given their presence in several species in different life stages in these zones. This also means the Icelandic plaice population can be defined as an intermediate or paratenic host for these nematode species.

Anisakis simplex is a useful biological marker for stock discrimination of various fish species, including herring and horse mackerel. *Anisakis simplex* larvae are commonly found in fish migrating between different salinity regions, such as the Baltic and North Sea. The prevalence and abundance of *A. simplex* larvae differ considerably from one fish stock to another due to their distinct migration routes and feeding areas. These differences make it possible to use the presence and intensity of *A. simplex* infections as a marker to distinguish different fish populations according to their ecological and geographical characteristics (Mackenzie and Hemmingsen, 2015). *Phocanema decipiens* proved to be a marker for distinguishing between Balsfjord and Barents Sea cod populations. The prevalence of *P. decipiens* was much higher in Barents Sea cod than in Balsfjord cod, suggesting a distinct ecological or geographical separation between these stocks (Hemmingsen et al., 1991).

The trematode *Zoogonoides viviparus* was found in Iceland for the first time in our study but is known to be abundant in North Atlantic flatfish (Schmidt et al., 2003) .
Eggs are excreted by the final host (the fish) into the water, where they hatch into miracidia larvae (Atopkin et al., 2024). The larvae infect a gastropod mollusk. In this mollusk, the larvae transform into sporocysts and produce cercariae. The cercariae leave the mollusk and infect a secondary intermediate host, often polychaetes or other marine invertebrates, where they transform into metacercariae (Kremnev et al., 2023). The metacercariae are ingested by a fish, where they transform into adults. The adults reproduce and release eggs, completing the life cycle and making the plaice the definitive host. As plaice feed mainly on benthic organisms, they will likely ingest an infected polychaete and become infected (Kremnev et al., 2023). This parasite is not known to be a biological tag in stock discrimination.

The LDA results (Figs 3.5 and 3.6) show that the differences between the groups are based on the rare parasites. In both cases, the north winter group is separated from the other three groups: north winter, north summer, and south summer However, during the LDA, when we calculate using only the most common parasites (those present in at least 5% of each fish), the differences become much less pronounced. The presence and abundance of rare parasite species can vary considerably between host populations, mainly when populations are geographically distant. This variability makes rare parasites particularly useful for distinguishing between different fish stocks (Poulin and Kamiya, 2015). Parasites that do not have a high dispersal capacity, which is often linked to the movement of their hosts, can be good tags because the similarity of their community decreases rapidly due to their distance (MacKenzie and Abaunza, 1998). The rarity of some parasite species increases their discriminatory power in differentiating stocks; these species are more likely to be specific to specific regions or populations. LDA analyses represent identification tools for these rare parasites (MacKenzie, 2002). These parasites are associated with particular host populations or environmental conditions, which can help distinguish stocks and identify fish populations' ecological niches or migratory behaviors (MacKenzie and Abaunza, 1998). However, the presence of these parasites in samples can cause variability and noise, making their results less reliable if the sample size is small (Sindermann, 1982). Common parasites are widely distributed, which allows for more robust datasets that are less prone to sampling errors than with rare parasites. Common parasites are ideal for large-scale studies with general trends over large geographic areas (MacKenzie and Abaunza, 1998). However, due to their widespread presence, these parasites can mask subtle differences between closely related populations, thus reducing their effectiveness for fine-scale ecological differentiation (Marcogliese and Jacobson, 2015).

Sampling for the summer group was carried out in August for the north and south of the island; it was also carried out in October due to the unavailability of fishing boats to go out to sea and my availability to carry out all the sampling. Including the October samples in the summer group could lead to observations of parasite abundance that do not accurately reflect typical summer conditions, as according to our ANOVA analysis, the sex distributions between fish caught in August and October were statistically similar, but their parasite abundance is statistically different. The ANOVA showed a significant difference in mean parasite abundance per fish between August and October. With an abundance of 0.6 in August and a higher abundance of 33 parasites per fish in October. This difference could be explained by water temperature. Parasites are more abundant in warmer water (Palm, 2011). In October, sampling was done in the south of the island, whereas in August, it was done in the north. These two regions are influenced by two currents: the Greenland current in the north, which brings cold water, and the Atlantic current in the south, which brings warm water. In Iceland, the general trend is towards lower temperatures and changes in water salinity and currents during the transition from summer to autumn. These changes can alter the availability of nutrients, affecting phytoplankton production and, subsequently, the entire food web and, thus, parasitic abundance (Marcogliese and Jacobson, 2015).

During the summer, plaices are most active in shallow water, where they feed and develop, except for the plaices in our north summer sample (August), which were caught at a depth of 124m. Fish living in deeper waters, such as plaice caught at 124m, have reduced exposure to parasites present in shallow waters, which impacts parasite diversity and intensity. Environmental conditions are different at depth than at the surface: lower temperatures, greater pressures, less light and oxygen, and less access to nutrients at depth can limit the development and transmission of certain parasites (Marcogliese, 2023, Lauria et al., 2011).

This summer is crucial for building up energy reserves for the spawning season.

When temperatures start to drop in autumn, plaice begin to migrate to deeper, warmer waters. This migration is linked to their spawning behavior, generally occurring where the water remains relatively warmer than in surrounding areas. The transition from summer to autumn is accompanied by a change in water temperature, which directly influences the behavior of plaice, mainly their feeding and migratory habits. Colder waters in autumn could reduce their metabolic rate and adapt their feeding behavior to conserve the energy they need to survive and reproduce during the colder months (MFRI Assessment Reports, 2023). Parasite abundance shows a large standard deviation, indicating that infestation levels vary significantly among the individuals or samples studied. This suggests that some individuals in the population may have many parasites while others have few, which could show the specificity of these parasites to each of their study groups.

The ANOVA shows a significant difference between the average size of fish in the north in summer and winter ($p < 0.05$). It also indicates an essential difference between mean fish size in the south in winter and the north in winter ($p < 0.001$). In the north, mean fish size is higher in summer (40.20 cm) than in winter (33.44 cm). Similarly, the average fish size is higher in the south in winter (43.15 cm) than in the north in winter (33.44 cm). The group with the largest average fish size also has the highest average parasite abundance, with south winter having an average size of 43.15 cm and a parasite abundance of 32.93 cm. Environmental conditions can explain the differences in fish size between these regions and seasons. Higher temperatures in summer and southern Iceland create more favorable conditions for the growth of juvenile fish. Young fish develop best in warmer waters, between 7 and 15 degrees Celsius, as these temperatures optimize their metabolism and growth (Fonds et al., 1992, van der Sleen et al., 2018). Larger fish tend to have higher parasite richness and abundance. As the fish grows, its body surface area and internal volume increase, allowing more parasites to live inside. Larger fish live longer and are therefore exposed to a greater variety of environments and prey, with the possibility of being infected by more parasites (Bagge et al., 2004). But the species richness of parasites per total fish length (cm) (Fig 3.4) of all the samples shows that total fish size explains only 2.6% of the variance in species richness, indicating a deficient explanatory capacity and also no evident trend suggesting that larger fish systematically host more parasite species.

5. Conclusion

Using a multi-disciplinary approach to otolith shape and parasite identification, we found differences in the Icelandic plaice population. Indeed, the southern winter plaice group differs from the other groups studied in otolith shape and the abundance and presence of parasites. Since otolith shapes and parasites are influenced by their environment, this group of plaice lives in different environments. It does not have the same ecosystem as the other three groups: South in summer, North in summer, and North in winter. As the southern and northern regions are influenced by two different currents (the cold Greenland current for the north and the warm Atlantic current for the south (Vilhjálmsson, 2002)), these two regions don't have the same oceanographic conditions, notably in terms of temperature, density, and salinity, which was reflected in our study. A potential tag would be *H. aduncum*.

This parasite is frequently found in flatfish (Klimpel and Palm, 2011), and its life cycle is known and simple. This parasite is present in the four groups studied with different abundances and prevalences (table 3.7, 3.8, 3.9, and 3.10), showing its infection is constant throughout the year. It has a greater abundance and prevalence in the south in winter, with otolith shapes different from other groups in the south in winter. It has no negative impact on the host and does not cause death. To deepen our research, we would need to take more samples - around a hundred from each group – with more sampling groups around Iceland, including east and west Iceland, to get more relevant results. Our results (parasites and otoliths) demonstrate that an increased sample size might reduce variability in our sampled populations, increase the likelihood of detecting a biological signal to discriminate between these plaice sub-populations, and improve model robustness. During dissections, it would also be a good idea to take stomach contents to identify each group's diet more precisely, enabling us to decipher their food chain better and, therefore, better conservation.

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