INSTITUTO POLITÉCNICO DE BRAGANÇA
Escola Superior Agrária

A systematic review and meta-analysis of the occurrence of Anisakids in fishery products from European countries

Sabrina El Metennani

Dissertation submited to Escola Superior Agrária de Bragança
to obtain the Degree of Master in Biotechnological Engineering

Supervised by
Ursula Gonzales-Barron

Anne Thébault

Bragança
2024

I dedicate this work to my parents, whose unwavering love and strength have empowered me to pursue my dreams; to my siblings, for their constant encouragement; and to my dear friend Zineb, for being present throughout this journey. To my life partner, Sofiane, whose presence and support have been my anchor, and to the Parisian with the pure heart who made my childhood dream a reality, thank you for standing by me in every situation.

Acknowledgements

I would like to express my sincere gratitude to ***Dr. Ursula Gonzales-Barron*** for the invaluable opportunity to work alongside her and learn from her expertise. Being part of her team has greatly enriched my academic journey.

I extend my heartfelt thanks to ***Dr. Anne Thebault*** for being an exceptional supervisor, providing insightful feedback, and consistently encouraging me to learn and improve.

I am also deeply grateful to ***Dr. Pauline Kooh*** for her invaluable guidance and support, which has significantly contributed to my personal and professional growth.

I am grateful to ***Dr. Mélanie Gay*** for her feedback. I had read many of her papers while working on my report, so it was a privilege to receive her insights on my own work.

Lastly, I would like to express my respect for ***Prof. Vasco Cadavez*** for his constructive suggestions and assistance.

Many thanks to my colleagues from the Food Safety and Quality Analytical Laboratory, particularly ***Yara*** and ***Nathalia***, as well as to all my colleagues from the master's program, for their help and camaraderie since my first day at the Polytechnic Institute of Bragança.

Lastly, I am thankful to each person I met "professors, colleagues, and friends" who has taught me something, as even the smallest gesture can contribute significantly to one's growth.

Sabrina.

The research was funded by the European Food Safety Authority (EFSA) under Grant Agreement “GP/EFSA/BIOHAW/2023/05,” as part of the project “NewPIF – Pathogens in Foods, extension to Vibrio and Anisakids in fisheries.”

Abstract

Anisakids are zoonotic nematodes with a complex life cycle, parasitizing various commercial fish species. These parasites are associated with socioeconomic issues and health risks. However, despite their significance, data related to Anisakids in commercial fishery products in Europe remains incomplete.

The primary aim of this thesis is to conduct a systematic review to answer the question: "What is the occurrence of Anisakids in fishery products from European countries?" The search included studies on Anisakids, seafood, and fishery products from the 2010-2023 period. The databases PubMed, Web of Science, Scopus, and Scielo were searched, in addition to papers retrieved from three published meta-analyses.

Out of the original 274 studies that met the pre-established inclusion criteria, only 103 studies were retained for meta-analysis, as it was restricted to the genus *Anisakis*, the organ sampled fillets, and prevalence in retail fish within Europe. The moderating variables impacting *Anisakis* prevalence showing statistical significance were fishing area, fish host order, sample preparation type, the production mode (wild or aquaculture), sample type and organ sampled (p-values < 0.0001).

Meta-analytical multivariate analysis revealed significant variations in *Anisakis* parasite prevalence across different fishing areas, fish orders, detection methods, and sample preparation types. In Area 27-21 (Atlantic North), the odds of parasite prevalence were significantly higher (OR = 8.06), while the Pacific region also showed elevated prevalence (OR = 3.74) when compared with Area 37 (Mediterranean and Black sea, OR=1). Among fish orders, Salmoniformes exhibited the highest prevalence (OR = 61.14), followed by Gadiformes (OR = 7.44) and Scombriformes (OR = 4.35) in comparison with Clupeiformes Detection using artificial digestion and Press/UV led to higher detection rates when compared to absence of sample preparation. Whole fish demonstrated nearly three times higher prevalence (OR = 2.96) than gutted fish - (reference level). Additionally, the analysis of fillets without belly flaps produced lower prevalence values (OR = 0.21) when compared to fillets with belly flaps, which had the highest contamination levels.

Finally, aquaculture-raised fish exhibited significantly lower pooled prevalence (0.60%) compared to wild-caught fish (18.02%). The widespread presence of *Anisakis* in the edible parts of fish and the associated food safety implications highlight the need for

further investigation into the presence of these parasites in fish currently marketed in Europe.
Keywords: Anisakids, European countries, systematic review, meta-regression.

Resumo

Os Anisakídeos são nematoides zoonóticos com um ciclo de vida complexo, parasitando várias espécies de peixes comerciais. Esses parasitas estão associados a problemas socioeconômicos e riscos à saúde. No entanto, apesar de sua importância, os dados relacionados aos anisakídeos em produtos da pesca comercial na Europa ainda são incompletos.

O objetivo principal desta tese é realizar uma revisão sistemática para responder à pergunta: "Qual é a ocorrência de anisakídeos em produtos da pesca de países europeus?" A pesquisa incluiu estudos sobre anisakídeos, frutos do mar e produtos pesqueiros do período de 2010 a 2023. As bases de dados PubMed, Web of Science, Scopus e Scielo foram consultadas, além de artigos recuperados de três meta-análises publicadas.

Dos 274 estudos originais que atenderam aos critérios de inclusão preestabelecidos, apenas 103 estudos foram retidos para a meta-análise, que foi restrita ao gênero Anisakis, ao órgão amostrado filés e à prevalência em peixes de varejo na Europa. As variáveis moderadoras que impactaram a prevalência de Anisakis e mostraram significância estatística foram a área de pesca, a ordem do hospedeiro peixe, o tipo de preparo da amostra, o modo de produção (selvagem ou aquicultura), o tipo de amostra e o órgão amostrado ($p < 0,0001$).

A análise multivariada meta-analítica revelou variações significativas na prevalência do parasita Anisakis em diferentes áreas de pesca, ordens de peixes, métodos de detecção e tipos de preparo de amostra. Na Área 27-21 (Atlântico Norte), as chances de prevalência do parasita foram significativamente maiores (OR = 8,06), enquanto a região do Pacífico também mostrou prevalência elevada (OR = 3,74) em comparação com a Área 37 (Mediterrâneo e Mar Negro, OR = 1). Entre as ordens de peixes, Salmoniformes apresentou a maior prevalência (OR = 61,14), seguida por Gadiformes (OR = 7,44) e Scombriformes (OR = 4,35) em comparação com Clupeiformes. A detecção usando digestão artificial e Press/UV levou a taxas de detecção mais altas quando comparada à ausência de preparo de amostra. Peixes inteiros demonstraram uma prevalência quase três vezes maior (OR = 2,96) do que peixes eviscerados (nível de referência). Além disso, a análise de filés sem aberturas ventrais produziu valores de prevalência mais baixos (OR = 0,21) em comparação com filés com aberturas ventrais, que apresentaram os níveis de contaminação mais altos. Finalmente, peixes de aquicultura apresentaram prevalência total significativamente menor (0,60%) em comparação com peixes capturados em

ambiente selvagem (18,02%). A ampla presença de Anisakis nas partes comestíveis dos peixes e as implicações para a segurança alimentar ressaltam a necessidade de mais investigações sobre a presença desses parasitas em peixes atualmente comercializados na Europa.
Palavras-chave: Anisakídeos, países europeus, revisão sistemática, meta-regressão.

Table of content

Index of Figures

Index of Tables

Index of Abbreviations

EC: European Commission

EFSA: European Food Safety Authority

EU: European Union

FAO: Food and Agriculture Organization

GA: Gastric Anisakiasis

GAA: Gastroallergic Anisakiasis

IA: Intestinal Anisakiasis

OR: Odds Ratio

PCR: Polymerase Chain Reaction

PIF: Pathogens in Foods Database

PO: Population and Outcome

UV: Ultraviolet

L3: Larvae at 3rd stage

General Introduction

The global consumption of aquatic foods, excluding algae, has seen a significant rise over the past several decades. By 2019, the total consumption had reached approximately 158 million tonnes, a fivefold increase from the 28 million tonnes consumed in 1961. This growth represents an average annual increase of 3.0% since 1961, surpassing the global population growth rate of 1.6% during the same period. This trend has been driven largely by greater availability, consumer preferences changes, technological advancements, and rising incomes.

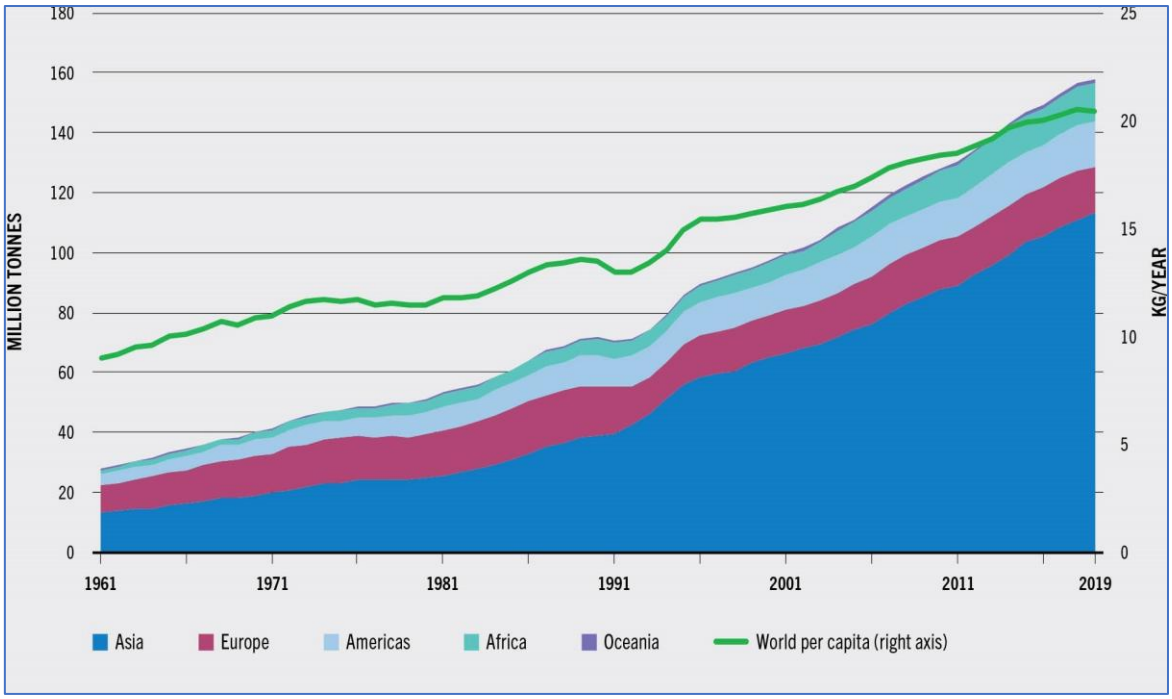


Figure 1. Aquatic food consumption by continent, 1961–2019 (FAO,2022)

In recent years, there has been a notable shift toward raw or marinated fish consumption, a practice that was once limited to regions with traditional raw fish dishes.

However, the growing consumption of raw fish raises important public health concerns. Fish can harbor various parasites that are harmful to humans, including species from the Opisthorchidae, Diphyllobothriidae, and Anisakidae families (Tang et al., 2016). Among these, anisakid nematodes, belonging to the family Anisakidae, present a significant zoonotic threat due to their widespread presence in marine fish products. These parasites complete their lifecycle in marine mammals or piscivorous birds as definitive hosts, while crustaceans act as intermediate hosts, and fish serve as transport hosts. Anisakid larvae

can be found in multiple tissues of numerous fish species, providing a route for human infection (Anderson, 1992).

Before 2010, over 20000 cases of anisakiasis were reported worldwide, with the majority of cases (over 90%) occurring in Japan (EFSA-BIOHAZ, 2010; Baird et al., 2014). Clinically, anisakiasis manifests with symptoms such as severe abdominal pain, nausea, vomiting, and, in some cases, intestinal obstruction. These symptoms can mimic other gastrointestinal conditions, including acute appendicitis and peptic ulcers. Diagnosis often involves patient history, endoscopy, and/or serological testing, with early removal of the parasite critical to preventing chronic infection and the development of gastrointestinal eosinophilic granulomatosis (EFSA-BIOHAZ, 2024).

Given the rise in raw fish consumption and the associated health risks, an improved understanding of factors influencing Anisakis contamination in fish is essential for food safety. Thus, the present study focuses on the prevalence of Anisakis parasites in fish samples and examines key moderating variables, such as fishing areas, preparation methods for detection, and sample type, to assess their impact on Anisakis prevalence. By conducting a meta-analysis of published results, this research aims to provide critical insights for public health authorities, the fishing industry, and consumers, thereby contributing to enhanced food safety measures and the reduction of zoonotic infections.

Moreover, this work is part of the broader European Food Safety Authority-funded project *NewPIF – Pathogens in Foods*, which aims to compile a comprehensive catalogue of published data on parasites in fish and fishery products. Currently, data on fish parasites, including those of public health significance, are scattered across diverse studies and regions. By bringing this information together in one accessible repository, *NewPIF* seeks to support evidence-based risk assessments and policymaking, ultimately enhancing the safety and sustainability of the fish supply chain.

Chapter 1. Literature review: context and objective

1. Anisakid taxonomy

The Anisakidae are a cosmopolitan family of parasitic nematodes found in marine environments but also in freshwater among fish species that inhabit both environments. The taxonomy of the family Anisakidae is classified as shown in Figure 2.

Kingdom: Animalia

Phylum: Nematoda

Order: Ascaridida

Superfamily: Ascaridoidea

- **Family: Anisakidae**

Figure 2. Taxonomic classification of anisakids (Santana-Arocha et al., 2023)

The three main genera of Anisakidae most associated with human infections are:
-*Anisakis* , the most widespread genus, composed of nine known species to date: *Anisakis simplex* (*s.s.*), *Anisakis pegreffii*, *Anisakis berlandi* (formerly *Anisakis simplex* C), *Anisakis typica*, *Anisakis ziphidarum*, *Anisakis physeteris*, *Anisakis brevispiculata*, *Anisakis paggiae*, and *Anisakis nascettii* (formerly *Anisakis* sp.). The two species *A. simplex* (s. s.) and *A. pegreffii* are the most common zoonotic nematodes associated with the consumption of raw or mildly thermally processed seafood (EFSA-BIOHAZ, 2024). The genus *Anisakis* (Figure 03) is known to be the largest and most diverse among the Anisakidae, and it has several distinct species, each with different ecological characteristics (Kuhn et al., 2016).

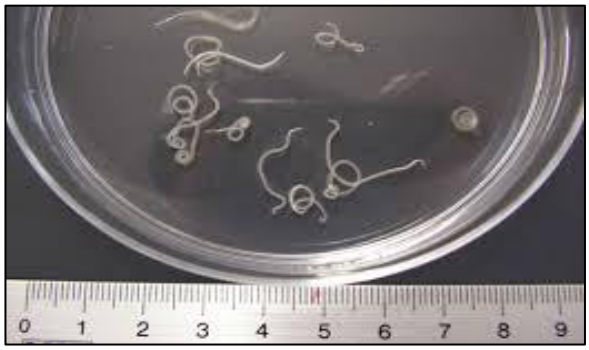


Figure 3. Numerous live Anisakis from Alaska Pollack Theragra chalcogramma (Urawa, S., site D-PAF)

- *Phocanema* (Previously called *Pseudoterranova*), recent taxonomic revisions have proposed the resurrection of the *genus Phocanema,* with *Phocanema decipiens (sensu stricto)* as the type species, to include *Ph. decipiens, Ph. azarasi, Ph. bulbosa, Ph. cattani, and Ph. krabbei,* all parasitizing pinnipeds (Bao et al 2023). This reclassification replaces the former inclusion of these species within the genus *Pseudoterranova,* which was previously considered a cosmopolitan genus with six species: *Pseudoterranova krabbei, Pseudoterranova decipiens (s.s.), Pseudoterranova bulbosa, Pseudoterranova azarasi, Pseudoterranova decipiens E,* and *Pseudoterranova cattani,* reported in various fish hosts including cod and herring (Lunneryd et al., 2015). Under this new classification, *Pseudoterranova* is now restricted to two species infecting kogiid whales*: Ps. kogiae* and *Ps. Ceticola* (Bao et al 2023).

- The genus *Contracaecum* is composed of ten species*: Contracaecum osculatum A, Contracaecum osculatum B, Contracaecum osculatum* (*s.s*), *Contracaecum osculatum D, Contracaecum osculatum E, Contracaecum osculatum baicalensis, Contracaecum radiatum, Contracaecum mirounga, Contracaecum ogmorhini* (*s.s*.), *and Contracaecum margolisi*. The genus Contracaecum has been reported in fish and crustaceans, and it is known to infect a wide host range, including marine mammals (Azevedo et al., 2007)

These three genera present morphological differences that allow them to be distinguished. Five morphological criteria are generally used for easier identification: larval teeth, the opacity of the esophagus, the shape of the ventricle, the presence or absence of an intestinal cecum, and the presence and shape of the mucron, which is an excrescence (Rodríguez et al., 2020). These three genera are zoonotic parasites, meaning they can transmit zoonoses. The molecular studies carried out on Anisakidae have helped to clarify its taxonomy. These studies have provided evidence supporting the idea that Anisakidae, which includes the genera *Anisakis* and *Pseudoterranova,* originated from the same monophyletic group (Amer, 2014). The use of different morphological and molecular tools has played a role in identification and categorization of various anisakids into the families Anisakidae or Raphidascarididae (Aibinu et al., 2019).

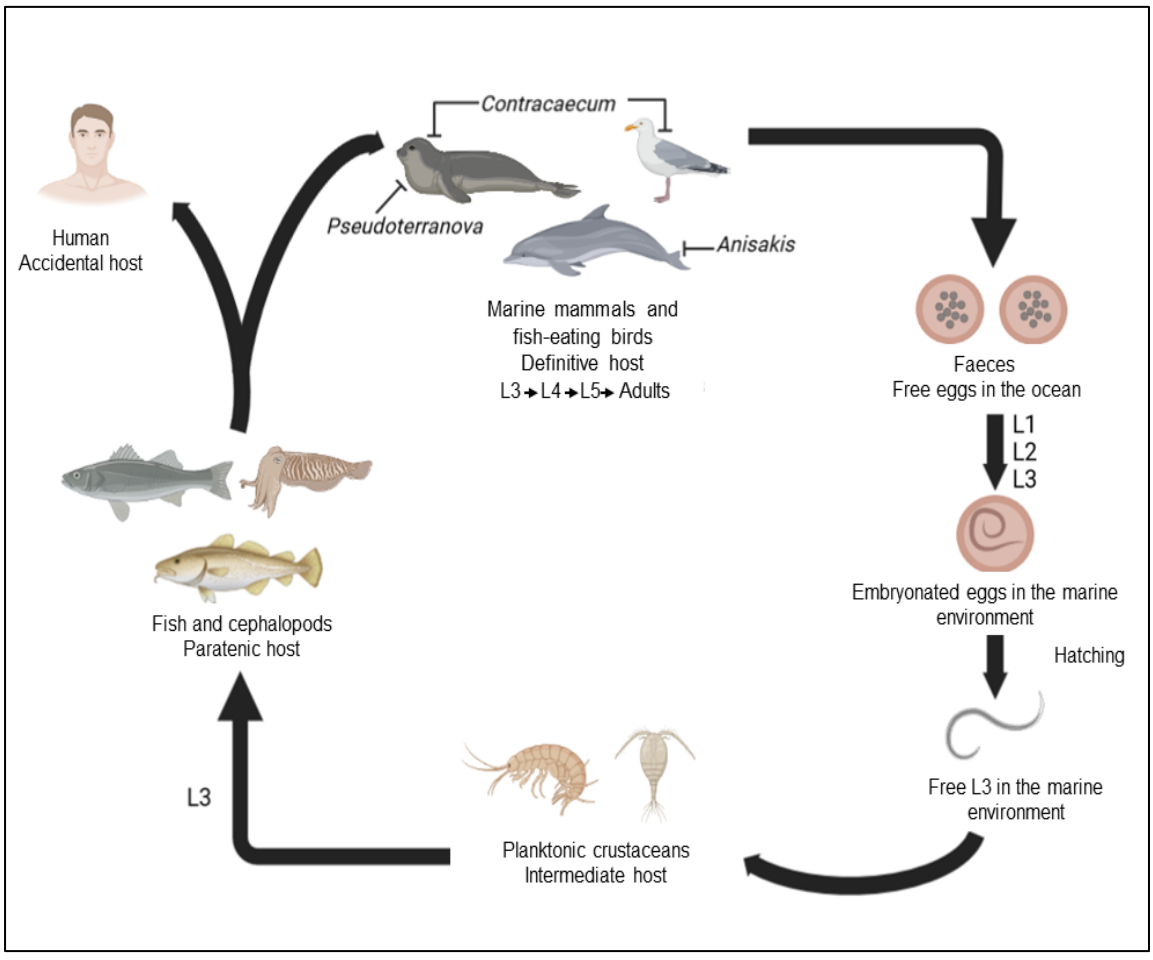
These nematodes are known to invade various fish species, and their distribution is universal, it is also common to find larvae more than one species of Anisakids in the same host (Arcos et al., 2020). Anisakid nematodes from the family Anisakidae are also common parasites of a variety of wild and domestic animals and many species have veterinary, medical and economic importance (Anderson, 1992; Zhao et al., 2016).

2. Life cycle

Anisakids typically utilize marine mammals or piscivorous birds as definitive hosts, with planktonic or benthic crustaceans serving as intermediate hosts and fish from marine or freshwater environments as the primary transport hosts. Numerous fish species can carry larval anisakids, in different parts of their bodies, which represent a pathway for human infections. Adults of *A. simplex* are primarily found in the gastrointestinal tract of cetaceans (dolphins, porpoises, and baleen whales), while adults of *Pseudoterranova spp*. inhabit pinnipeds (seals, sea lions, and walruses). Some species of *Contracaecum* reach maturity in pinnipeds, while others mature in fish-eating birds such as cormorants, pelicans, and herons. However, the definitive host range of many anisakid species remains incompletely understood (Anderson, 1992). Additionally, there is some controversy regarding whether alternative transmission routes exist, such as direct infection of fish by ingesting free-swimming larvae or the transfer of larvae from crustaceans, such as krill, to plankton-eating or omnivorous cetaceans, thereby skipping the fish transport host.

After final molting, maturation, and copulation, the female worms shed eggs within the definitive host's feces. These eggs embryonate and hatch in the water, releasing free-swimming third-stage larvae (Køie et al., 1995). The larvae are ingested by crustaceans such as decapods, copepods, or amphipods, within which they grow in the hemocoel. Fish and cephalopod mollusks (squids) become infected by eating planktonic or benthic crustaceans containing third-stage larvae, which bore through the digestive tract wall into the viscera and body cavity, followed by host-induced encapsulation (Anderson, 1992). When an infected fish is consumed by another fish, the encapsulated larvae become digested, thus repeating the larval fish host cycle. This is important from an epidemiological and food safety perspective, as the repeated transfer of larvae between fish within the natural food chain may result in extensive accumulation, particularly in large and older fish, which sometimes harbor hundreds or even thousands of encapsulated larvae (Smith & Wootten, 1978). However, the number of fish host cycles individual larvae may go through without losing infectivity has not yet been investigated. The definitive hosts become infected by eating fish or cephalopods containing the larvae. Humans are considered as accidental hosts of the parasite following consumption of the secondary hosts (Baptista-Fernandes et al., 2017) (Figure 4).

The L3 larval stages are mostly located in the visceral body cavity and outside the internal organs, but they are also found in the musculature of commercially important fish species. The larvae in the visceral body cavity migrate to the musculature *intra vitam* and/or *post-mortem* (EFSA-BIOHAZ, 2024). The post-mortem migration is temperature dependent and described in the species *A. pegreffii* infecting anchovy (*Engraulis encrasicholus*) above 2°C (Cipriani et al., 2016). Storage temperature and time influence the *post-mortem* motility of *A. simplex*. (Cipriani et al., 2024).



3. Methods of detection and identification

To determine the distribution of Anisakidae among various hosts (fish and cephalopods), specific detection and identification techniques have been developed (Seesao, 2015). Two main types of methods are used to detect Anisakidae: non-destructive and destructive methods. Fishermen typically use non-destructive methods that comply with current regulations (Regulation (EC) No. 2074/2005; 2019), as these methods have the advantage

of not affecting the value or quality of the product during inspections. Non-destructive methods involve observing the fillet with the naked eye or using a light table. However, these methods are limited by the nature of the fillets; their thickness, texture, and color can impact the quality of the observation (Levsen et al., 2005).

Destructive methods include the hydraulic press method followed by UV light examination (NF EN ISO 23036-1, 2021) and the artificial digestion method (NF EN ISO 23036-2, 2021). The primary advantage of these methods is that the characteristics of the fillet do not constrain them. A comparison of the detection efficiency of these methods concluded that destructive methods are more thorough and thus allow for better detection of larvae (Gómez-Morales et al., 2018).

For identifying Anisakidae, three main groups of methods are distinguished. The first group includes morphological identification methods using the naked eye or a microscope, which only allow identification up to the genus level for Anisakidae (Smith and Wooten, 1978). To identify larvae at the species level, biochemical and molecular methods are used. Biochemical methods include multilocus enzyme electrophoresis (MEE) (Mattiucci and Nascetti, 2008) and the enzyme-linked immunoassay (ELISA) method (Xu et al., 2010). Molecular methods used for identifying Anisakidae include polymerase chain reaction (PCR) amplification (Mattiucci et al., 2003), restriction fragment length polymorphism (PCR-RFLP) (Espiñeira et al., 2010), Single strand conformation polymorphism (PCR-SSCP) (Gasser et al., 1997), multiplex PCR (Umehara et al., 2008), and real-time PCR (Mossali et al., 2010; Paoletti et al., 2018).

Besides detection and identification, assessing larvae viability is crucial because it relates to the potential for human infection. Although it is not currently possible to confirm that intact and motile larvae can successfully infect humans, future methods may establish whether viable larvae from different sources are capable of human infection. Larvae viability is assessed by measuring their motility, either spontaneous or stimulated with forceps and a needle (EFSA-BIOHAZ, 2010). Assessments occur under various conditions such as natural or UV light (Vidaček et al., 2011), at 37°C or room temperature (Łopieńska-Biernat et al., 2020; Sánchez-Alonso et al., 2020), and at different intervals post-treatment (Onitsuka et al., 2022; Sánchez-Alonso et al., 2020). Methods include counting non-movement times (Lee et al., 2016), video recording (Vidaček et al., 2011), and viability scores (Trabelsi et al., 2019). Most assays, often done on isolated larvae

through manual extraction or artificial digestion, can underestimate mobile larvae, a limitation for designing inactivation protocols (Sánchez-Alonso et al., 2021).

4. Geographical distribution and ecological factors

The nematodes of the family Anisakidae are distributed globally across various fish hosts, yet their prevalence is not uniform. According to Mercken et al. (2020), the prevalence of Anisakidae varies significantly between fish species and fishing regions, with the highest infection rates observed in the Northeast Atlantic. This region's high occurrence of Anisakidae is attributed to factors such as the abundance of cetaceans, which serve as final hosts for some species of the family. This means that the presence and distribution of anisakids larvae are heavily influenced by the geographic preferences of these final hosts and their locations.

According to the the meta-analysis study of Rahmati et al. (2020), spatial geography analysis further identifies high-risk hotspots for *Anisakis spp.* in regions such as the North and Northeast Atlantic, the southwest of the USA, the west of Mexico, the south of Chile, the east of Argentina, Norway, the UK, and the west of Iceland. In contrast, regions like northeast Australia, central and southern Iran, the Caspian Sea, Indonesia, Thailand, northeast Turkey, southern and eastern Saudi Arabia, and Yemen are considered low-risk areas or cold spots for Anisakidae infection.

According to Fiorenza et al. (2020), the northeastern Atlantic FAO region, which includes the Barents, Norwegian, Baltic, North, and Irish Seas, is a significant source of data on *Anisakis spp*., following the Mediterranean and Black Seas. Among the ecological drivers influencing the distribution of Anisakidae, temperature plays a critical role. Temperature, along with salinity and oceanographic conditions, affects the hatching of eggs and the survival and dispersion of the first larval stages of anisakids. Rising temperatures, largely driven by global warming, further exacerbate these effects by altering latitude, oceanographic conditions, circulation patterns, and salinity, all of which have significant impacts on the distribution of Anisakidae (Shamsi, 2019, EFSA-BIOHAZ 2024). The anisakid distribution has been also reported by Ángeles-Hernández et al. (2020) and showed that these parasites can be found worldwide (Figure 05). The geographical albels are in Annex 1.

Figure 5. World map with geographical location of the parasites of the Anisakidae family, collected from different hosts. (Adapted from Ángeles-Hernández et al., 2020). Labels in Annex 1

5. Fish consumption and Zoonotic implications

The global consumption of aquatic foods has increased significantly over the past six decades. In the European Union (EU), the mean per capita annual consumption of fishery products is approximately 24 kg, with around one-quarter of this (about 1.25 million tonnes) supplied by aquaculture. The most consumed fish in the EU in 2021 were tuna (all species combined, mostly wild), Atlantic salmon (mostly farmed), cod (mostly wild), and Alaska pollock (wild). Portugal leads in fish consumption in the EU, with 56.52 kg per capita per year, followed by Spain (43.0 kg), and France, Luxembourg, and Italy, with consumption rates of 32.2 kg, 31.4 kg, and 30.2 kg per capita per year, respectively (EC DG-MARE, 2023).

Over the past two decades, there has been a notable increase in the consumption of raw or marinated fish. Previously, this mode of consumption was confined to regions with traditional dishes featuring raw fish, such as sashimi in Japan, ceviche in Peru and esqueixada in Spain The spread of such diverse culinary cultures has popularized raw fish dishes on a larger scale. For instance, in France, household purchases of ready-to-eat raw fish increased by 390% between 2005 and 2018 (France Agrimer, 2018).

The growing consumption of raw fish poses significant public health concerns. Various parasites that can cause diseases in humans are commonly found in fish flesh. Historically, parasitic infections due to raw fish consumption predominantly affected populations in Southeast Asia, caused by the genera *Clonorchis* and *Opisthorchis*. These small liver flukes are known co-factors in the development of liver cancers and infest over fifteen million people, with more than 200 million at risk of exposure (Tang et al., 2016).

Among these parasites, anisakids, represent a zoonotic risk due to their widespread presence in fishery products. Anisakids can cause anisakidosis in humans, resulting from the ingestion of larvae found in raw or undercooked fish. As global consumption of fishery products continues to rise, particularly in the form of raw dishes, the zoonotic potential of anisakids becomes increasingly significant.

6. Sanitary impact

Anisakis is a parasite that unintentionally hosts humans. The *Anisakis* larvae L3 do not develop to the adult stage, However, in a few cases, fourth-stage larvae were isolated from human patients (Suzuki et al., 2021; EFSA-BIOHAZ, 2024). In general, anisakiasis has a very short lifespan in humans; most of them are destroyed or driven out in a matter of days or weeks without any treatment (Audicana and Kennedy, 2008).

The symptomatic form is described below:
When the larvae are viable and infective, in undercooked or raw infected fish, the L3 of *Anisakis* penetrates the mucosal layers of gastric and/or the intestinal tract within a few hours of being ingested causing tissues damage. This acute gastrointestinal form of *Anisakis* infection is described as gastric anisakiasis (GA) or intestinal anisakiasis (IA). The ulceration is accompanied by an eosinophilic inflammatory response with subsequent granuloma formation surrounding the penetrated larva (EFSA-BIOHAZ, 2024).
Clinical symptoms include severe stomach or abdominal pain, nausea, vomiting and intestinal obstruction, and mimic other common gastrointestinal diseases, such as acute appendicitis, peptic ulcer, etc. Diagnosis requires an anamnestic survey and endoscopy, and/or serological diagnosis. Early removal and identification of the worm is essential to avoid the chronic infection developing into gastrointestinal eosinophilic granulomatosis (EFSA-BIOHAZ, 2024).

The symptoms may also be complicated by a mild to strong allergic response (gastroallergic anisakiasis, GAA). In GAA, allergic symptoms are additional to the acute gastric parasitism, as the live larvae penetrate the gastric mucosa, triggering severe local and systemic allergic reactions mediated by IgE antibodies. This can result in symptoms such as urticaria, angioedema, and potentially anaphylactic shock if not promptly treated. These allergic responses can be complex and varied, emphasizing the need for proper diagnosis and heightened awareness, especially in regions with high fish consumption.

Conversely, for allergic reactions to *A. simplex*, it is unclear whether the parasite is needed to be alive or dead. It is clearly documented that ingestion of viable L3 larvae is required for the induction of allergic manifestations (Mattiucci et al., 2013). However, it is still unknown if an allergic reaction can occur because of exposure to allergens from dead parasites, e.g. present in canned or other heavily processed fishery products (EFSA-BIOHAZ, 2024).

The majority (72%) of human cases of illness caused by above-mentioned species of *Anisakis* are Gastric Anisakiasis (GA), while 26% are Intestinal Anisakiasis (IA) cases, with 2% being extra-gastrointestinal or ectopic anisakiasis (Mattiucci et al., 2018). *A. simplex* (s. s.) has been identified by molecular methods causing invasive gastric and IA (Arai et al., 2014; EFSA-BIOHAZ, 2010; Roca-Geronès et al., 2020). The aetiological agent has not been identified at species level with allergic anisakiasis, in some European countries. *Anisakis pegreffii* causes GA, IA, GAA and ectopic anisakiasis, being identified in cases of invasive anisakiasis (EFSA-BIOHAZ,, 2024)

These allergic responses can be complex and varied, emphasizing the need for proper diagnosis and heightened awareness, especially in regions with high fish consumption. Globally, prior to 2010 (EFSA-BIOHAZ, 2010), there had been over 20000 cases of anisakiasis reported globally, with Japan having the highest prevalence (above 90%) (Baird et al., 2014). According to Yorimitsu et al. (2013), the primary cause of human infection is contaminated sushi and sashimi, which are the national raw fish dishes of Japan. There are an estimated 2000–3000 cases of anisakiasis reported there each year. There have been much more cases of anisakiasis reported as a result of the global adoption of diverse cuisines, advancements in diagnostic tools, and increased awareness of Anisakis and its infection. Additional nations where reports of anisakiasis have been made include the United Kingdom (Qin et al., 2013; Audicana and Kennedy, 2008),

Taiwan (Li et al., 2015), Malaysia (Amir et al., 2016), Korea (Sohn et al., 2015), China (Qin et al., 2013), Spain (Herrador et al., 2018), Italy (Mattiucci et al., 2018; Guardone et al., 2018), France (Audicana and Kennedy, 2008), Germany (Audicana and Kennedy, 2008), Denmark (Andreassen and Jorring, 1970), Norway (Jacobsen and Berland, 1969), Croatia (Mladineo et al., 2016), the United States of America (Kojima et al., 2013), South America (Borges et al., 2012; Eiras et al., 2018), Egypt (Audicana and Kennedy, 2008), South Africa (Audicana and Kennedy, 2008), and Australia (Shamsi and Butcher, 2011), suggesting the occurrence of anisakiasis throughout the world, excluding Antarctica. A recent retrospective epidemiological study of human anisakiasis by Guardone et al. (2018), based on data from Orphanet (Orphanet, 2016), estimated a global incidence of 0.32/100,000 (Aibinu et al., 2019)

Anisakidosis and anisakiasis are underestimated because they are misdiagnosed and there is no compulsory notification at EU level (EFSA-BIOHAZ,2024). An overview of human anisakidosis was also recently provided by Shamsi and Barton (2023).

7. Socioeconomic aspects related to Anisakids in the fish industry

Anisakid nematodes, which commonly infect various marine organisms, are of a major concern due to their potential zoonotic implications and associated economic losses in the seafood industry. For example, these nematodes have been discovered in the abdominal musculature and viscera of certain fish species given them disgusting appearance and this causes local economic problems in fish markets (Llarena-Reino et al., 2015).

Indeed, these economic losses occurring due to rejection of fishery products by fish consumers and sellers may also affect the fishery job market as signaled in Germany under the name of the “nematode crisis”, back in 1987 (Bao et al., 2017). So apart from the concerns regarding food safety, Anisakidae contamination in commercial fish also causes socio-economic issues. Customers are hesitant to purchase contaminated goods and mistake larvae for fish of lower quality, harming the industry's reputation and resulting in financial losses (Bao et al., 2017). Customers' unfavorable opinion of Anisakidae is illustrated by the findings of a willingness-to-pay survey (Bao et al., 2017), which showed that 33% of respondents would refrain from eating fish following an Anisakidae encounter due to concerns about both health risks and appearance. Fish

candling incurs labor costs, while freezing involves both expenses for the freezing process and potential value loss both of which have significant economic impacts. The loss of fishery resources is also in conflict with sustainable management of fisheries, in a context of endangered marine resources.

8. Control and management of Anisakids in European fisheries

8.1. Survival of the parasites to different treatments

According to EFSA scientific opinion (2010, 2024), Anisakidae larvae exhibit varying degrees of sensitivity to chemical and physical treatments (Table 01). These treatments highlight the importance of optimizing conditions for each specific fish product to ensure the effective inactivation of Anisakidae larvae.

The latest EFSA scientific opinion reported , that only freezing, and heat treatment (cooking) are effective against *Anisakis* (EFSA-BIOHAZ 2024): *"to date, freezing and heating are still the most efficient methods to kill Anisakis larvae".*

Another effective strategy reported is the mechanical removal of the parasites from infected organs, ensuring that no larvae remain in the edible parts of the fish. Rapid removal of the viscera (evisceration) after catching, combined with maintaining a temperature of ≤ 2°C, can prevent post-mortem migration of larvae into the flesh during storage, handling, and transportation.

Studies have also shown that the ventral and muscular areas near the visceral cavity, known as the "belly flap," are more heavily infected compared to the dorsal musculature. Thus, removing the belly flap can reduce the presence of anisakid larvae in the fillets of several commercially valuable fish species (EFSA-BIOHAZ, 2024).

Table 1. Summary of the physical and chemical treatments and their effectiveness

Treatment method	Treatment	General conditions	Effectiveness
Physical	Heating	T=60º , time= 10 min (Traditional heating)	Effective against Anisakis L3 larvae
		T=70º , time= 1 min (Traditional heating)	
		T ≥ 74º , time= 15 sec (By microwave)	
	Freezing	-35°C for 15 hours	Effective
		-15°C for 96 hours	Effective
		-18°C for at least 1 day	Effective for cestode larvae (*Diphyllobothrium plerocercoid*)
		-10°C for 5 days	Kills *Clonorchis* and *Opisthorchis metacercariae*
	Drying	T=0º-2º for 3 months (Cod), then 1-12 months of drying	No relevant data about the effectiveness
	Irradiation	Doses up to 3 kGy	Ineffective for *A. simplex* larvae
	Low Voltage Current	Electrical discharge through fish	Effectiveness not adequately proven
	Smoking	Hot smoking (>60°C for 3-8 hours)	Effective
		Cold smoking (<38°C)	Insufficient to kill larvae; must be combined with freezing
Chemical	Salting	NaCl (13%) 5º 24h then dry salting	Effective after 15 days of minimum ripening
		NaCl for 4 days	Showed encouraging results for anchovies
	Marinating	Vinegar + other ingredients (salt/sugar/lemon juice..etc)	Effectiveness not adequately proven
	Natural compounds (curcuma longa), essential oil, plant extracts	New approaches of inactivation that are still being studied.	

8.2. Regulatory framework related to the parasites in fishery products

The regulatory framework related to the fishery products is established in part D of Annex III, Section VIII, Chapter III to Regulation (EC) No 853/2004 as follows :

1. Food business operators placing on the market the following fishery products derived from finfish or cephalopod molluscs:

a- fishery products intended to be consumed raw; or

b- marinated, salted and any other treated fishery products, if the treatment is insufficient to kill the viable parasite, must ensure that the raw material or finished product undergo a freezing treatment in order to kill viable parasites that may be a risk to the health of the consumer.

2. Food business operators need not carry out the freezing treatment set out in point 1 for fishery products:

a- that have undergone or are intended to undergo before consumption a heat treatment that kills the viable parasite. In the case of parasites other than trematodes, the product is heated to a core temperature of 60 °C or more for at least one minute

b- that have been preserved as frozen fishery products for a sufficiently long period to kill the viable parasites;

c- from wild catches, provided that:

(i) there are epidemiological data available indicating that the fishing grounds of origin do not present a health hazard with regard to the presence of parasites; and

(ii) the competent authority so authorises;

d- derived from fish farming, cultured from embryos and have been fed exclusively on a diet that cannot contain viable parasites that present a health hazard, and one of the following requirements is complied with:

(i) have been exclusively reared in an environment that is free from viable parasites; or

(ii) the food business operator verifies through procedures, approved by the competent authority, that the fishery products do not represent a health hazard with regard to the presence of viable parasites.

A food business operator must ensure that fishery products originate from fishing grounds or farms that comply with the specific conditions outlined in the regulatory framework before placing them on the market. This includes ensuring that products not subjected to

freezing or any treatment capable of eliminating viable parasites posing health risks are appropriately documented. Any additional information accompanying the fishery products or included in the commercial documents may fulfill this requirement. Based on the regulatory framework, samples subjected to freezing are generally assumed to be free from parasites. Furthermore, aquaculture species at the distribution stage are expected to have minimal contamination or be completely free from contamination.

9. Meta-analysis as a powerful statistical synthesis tool

9.1. Definition and objectives of meta-analysis

A meta-analysis is a statistical process that integrates findings from several independent investigations, such as opinion surveys, experimental trials, and causal models (Glass, 1976). The main goal of a meta-analysis is to provide a more accurate estimate of the effect of an intervention or treatment by increasing statistical power. Since individual studies often differ in terms of populations, designs, and various other factors, it has been proposed that combining their results can yield an estimate with greater generalizability compared to using a single study alone (Sutton et al., 2001).

Meta-analysis can also be utilized to get insight into the causes of heterogeneity or discrepancies between the primary research's findings. Accordingly, meta-analysis examines not only the studies' reported results but also every facet of the research designs that gave rise to them, including theoretical frameworks, operational definitions of variables, population samples, methods for gathering data, statistical analysis, and, most importantly, how potential confounding variables were handled to offer a different interpretation of the reported results (Noble, 2006).

9.2. The process of systematic review and meta-analysis

A systematic review is the first step in conducting a meta-analysis. Its main objective is to generate a summary of all available research, later evaluated for quality, relevance, and further reporting findings. The aim of the relevance screening process is to identify the publications that could directly contribute to answering the meta-analysis query or questions (Gonzales-Barron, 2011).

After studies validation and approval the data extraction step follows. Both numerical and non-numeric data should be included in the data extraction from the primary investigations in order to give the information required for synthesizing and summarizing

the findings. Each primary study should yield data that can be extracted, including research characteristics pertaining to the population, intervention, outcome, and study design, as well as the findings. As a final, the statistical analysis of the primary studies' outcomes, the meta-analysis, can be performed (Sargeant et al., 2005).

9.3. Previous meta-analysis and EFSA scientific opinions on Anisakids in fisheries

The description of the distribution of zoonotic Anisakidae genera has been the subject of many studies. In 2020, three publications (Fiorenza et al., 2020; Mercken et al., 2020; Rahmati et al., 2020) presenting data on the Anisakidae analyzed this distribution using meta-analysis. This approach consists of a statistical analysis of data from publications selected following a systematic review of the literature. Each of these three meta-analyses had different objectives.

The aim of the study by Fiorenza et al. (2020) was to monitor and compare changes in the prevalence of the genera *Anisakis* and *Pseudoterranova* over time. A meta-regression analysis showed that the abundance of *Anisakis* species (average number of worms per fish) increased significantly over a 53-year period from 1962 to 2015, while *Pseudoterranova* species showed no significant change over 37 years, from 1978 to 2015. When standardizing the results to the 1978-2015 period for comparison, *Anisakis* species abundance increased 283 times, while *Pseudoterranova* species remained unchanged. This rise in *Anisakis* could affect human health, marine mammals, and fisheries profitability. Mercken et al.'s meta-analysis from 2020 focused on the three zoonotic genera' frequency in the waters of nations importing fish for the Belgian market. The study found widespread presence of Anisakidae in commercial fish imported into Belgium (Mercken et al., 2020). Overall results showed a widespread occurrence of Anisakidae with a high variability in prevalence between fish species and fishing sea. Cod (*Gadus morhua*), Atlantic salmon (*Salmo salar*) and wild seabass (*Dicentrarchus labrax*) have pool mean prevalences of 33%, 5% and 16% respectively (wild and aquaculture). Order of fish were also studied for risk factors investigation (i.e., Gadiformes (cod) or clupeiforms (herring)). Fish caught in the Northeast Atlantic had the highest rate of infection (68%) and those from the Mediterranean Sea the lowest. Higher prevalences were found when inspecting the viscera (mean prevalence 59%) compared to the muscle (29%) and with destructive techniques such as enzymatic digestion or UV press (46%) compared to candling, the routine method (23%). Farmed fish were found to

be the least infected (2%) but were still not Anisakidae free (Merken, 2020). The aim of the study by Rahmati et al. (2020) was to draw up a map correlating the prevalence of the *Anisakis* genus with the geographical regions with a high potential for allergic anisakidosis. It identified five fish families commonly infected with *Anisakis* spp. (Which are Lophiidae ,Trichiuridae, Merlucciidae and Gadidae) and highlighted hot spot areas for allergic anisakiasis, with the highest rates in Portugal and Norway, emphasizing the need for allergologists to consider allergic anisakiasis as a public health issue in high-risk countries.

The EFSA BIOHAZ panel (2010) has concluded that there is inadequate data on allergic reactions to fishery product parasites, with *Anisakis simplex* being the primary concern. Allergy to *A. simplex* can occur via infection or exposure to allergens, with live larvae posing a higher risk. Effective prevention involves controlling *A. simplex* infection using freezing or heating treatments, though alternative methods' efficacy is uncertain. Wild-caught fish and inadequately monitored farmed fish are at risk of containing parasites, Farmed Atlantic salmon reared in controlled environments is the one exhibiting a negligible risk.

EFSA (2024) examined parasite prevalence in EU/EFTA farmed fish, with particular attention to species such as European seabass and Atlantic salmon. Market-quality farmed fish such as Atlantic salmon were found to be free of parasites, however some parasites are a problem in freshwater and marine habitats.

The European Food Risk Assessment Fellowship Program (EU-FORA) in Portugal launched a work program called "Food safety of fish and zoonoses: fish consumption and microbiological risk assessment and perception, from fisherman to final consumers in Portugal". This program, which was organized by the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) in Porto, sought to learn more about the attitudes and risk perceptions of the Portuguese people about fish contamination with *Anisakis* spp. Additionally, it looked at the population's risk of anisakiosis and evaluated their understanding of preventive measures (Golden et al., 2022).

10. Objectives of this work

The main objective of this study was to comprehensively assess the occurrence of anisakids in fishery products from European countries through the conduction of a systematic review and meta-analysis. After identification of relevant studies from academic databases, the quality and methodological rigor of the studies was evaluated. Data extraction was performed to synthesize the levels of prevalence of Anisakids in fishery products; and to determine the most relevant moderating factors driving the observed outcomes. The meta-analytical assessment was divided into two stages:

a. After the description of the dataset, a first statistical analysis was carried out to provide an overall understanding of the factors (methods, organs, form of the product, among others) that can explain the heterogeneity in the prevalence outcomes for the different order of fish species. The results were compared to those already described in previous meta-analyses and EFSA reports (EFSA-BIOHAZ, 2010).

b. Prevalence outcomes were meta-analyzed by fish order and by different FAO fishing areas for the main commercial orders consumed in the European countries: Scombriformes (tuna (mostly wild)), Salmoniformes (Atlantic salmon (mostly farmed)), Gadiformes (cod and Alaska Pollock (mostly wild))) Clupeiformes (herring, anchovies, sardine, sprat

Chapter 2. Material and methods

1. Systematic Review

1.1. Review question

The definition of the review question followed a PO (population and outcome) question structure (EFSA-BIOHAZ, 2010). In this case, the review question refers to the occurrence (prevalence and/or intensity/abundance) of Anisakids larvae in fishery products produced and/or commercialized in European countries. This descriptive question has a simple PO structure with two distinct components, described as follows:

Population: Fishery products produced and/or available for consumption in Europe for human consumption.

Outcome: Occurrence of at least one of the following genus of parasites in fishery products, namely *Anisakis, Pseudoterranova, Phocanema, Contracaecum*, expressed as either prevalence or concentration (intensity or abundance) within the specific population.

1.2. Conduction of the searches in the databases

A specific search is conducted for studies related to the research question "What is the occurrence of Anisakids in fishery products from European countries? " Therefore, the search terms consisted of a logical connection of generic terms, the list of Anisakidae, and a list of seafoods and fishery products (Kooh *et al.*, 2023; see Annex 2). . The searches retained sources in English, French, Portuguese and Spanish; whereas the publication period was restricted from 2010 to 2023. Proper search strings were prepared for the included databases Web of Science, PubMed, Scopus, and Scielo. All search strings were duly tested. The bulk of references were downloaded in Bib or Nbib format, converted to RIS format, and uploaded into a systematic review software (DistillerSR Version 2.35).

To initiate the review process, a deduplication procedure is implemented through the utilization of Distiller SR. This step is crucial for eliminating duplicate entries obtained from various databases, ensuring the integrity of the dataset. Following deduplication, the title and abstract screening is conducted on the Distiller SR platform. This involves systematically assessing each study against predefined inclusion and exclusion criteria outlined in the respective forms.

1.3. Determination of inclusion/exclusion criteria

For a study to be included in the systematic review, it should comply with criteria related to study characteristics and criteria related to report characteristics.

The eligibility criteria determined are the following :

- Study design and setting: The collection of occurrence data necessitates observational studies, including cross-sectional or longitudinal designs, where a systematic, randomized sampling approach is employed with a minimum of three food units. This sampling can be achieved through either a simple or stratified design.
- Population: The focus is on fishery products and composite foods containing fishery products, obtained at various stages, such as primary production, processing facilities, retail outlets, or restaurants. The specific food chain stage from which samples are taken must be explicitly specified.
- Outcomes of interest: The study must report on the prevalence and/or enumeration of various parasitic genera, including *Anisakis, Pseudoterranova, Phocanema, Contracaecum* that are the most potential to cause human zoonotic disease. Within fishery products or composite foods containing them. For prevalence, the study should present the sample size and the number of positive samples. For enumeration, it should include the sample size along with a measure of concentration (intensity/abundance).
- Method: The parasitological analytical method employed must be indicated, or a reference to the method should be provided. Additionally, the sample size should exceed three units.
- Criteria related to report characteristics: The full text should be in English, Spanish, French, or Portuguese. The study should be a primary research study.

1.4. Screening and selection of studies

A full-text screening is conducted to determine the studies that meet all specified inclusion criteria. This thorough screening process considers a range of more specific criteria to ensure that the selected studies align closely with the research objectives and methodological requirements.this process was ensured by answering the following question for each record.

1. Is the full text available? Yes/No
2. Is the article in English, Spanish, French, or Portuguese? Yes/No
3. What is the publication type? Primary research study / Review or systematic review / Other
4. Is occurrence data provided in the study for Anisakidae in fishery products and composite foods containing fishery products? Yes/No
5. Does the occurrence data of any of the aforementioned pathogens in foods originate from observational studies? Yes/No
6. Does the study specify the food chain stage where samples were taken? (Foods, as finished product or during production/processing, must be sampled from primary production, processing facilities, retail, or restaurant establishments)
7. Is the microbiological method used indicated, or a reference provided, and is the sample size higher than 3 units? Yes/No
8. Were the fishery products sampled from a European country? Yes/No
9. Were specimens grown in aquaculture systems?
10. For the concerned pathogen in food, does the study present results on: Prevalence / Enumeration / Both / None of them
11. If on prevalence, does the study provide sample size and the number of positive samples?
12. If on enumeration, does the study provide sample size and measure of concentration (or intensity/abundance)?

1.5. Assessing the methodological quality

Each eligible primary research study will undergo a rigorous quality assessment to evaluate the potential for bias. The results of a study will be signaled as having potential for bias if there is any suspicious of:

- **Selection bias**

Situations where proper randomization of food units is doubted, such as when sampling occurs within a monitoring program or surveillance post-outbreak.

- **Aggregation bias or Reporting bias**

Combining prevalence or enumeration results for distinct food classes within the same category (results for various fish species merged as an example) or for samples from different food chain stages.

- **Detection bias**

Detection and/or quantification of fish parasites not conducted using approved or known microbiological methods, or inadequately described methods. Lack of explicit indication of the analytical sample amount, with assumptions drawn from previous publications, provided ranges, or manufacturer's instructions.

- **Quality of reporting**

Lack of concordance between results in the text and those in tables, with preference given to tabular data in case of discrepancies.

Unclear calculation of mean counts, indicating a poor quality of reporting.

Results derived from studies exhibiting at least one of these potential validity threats will be flagged as potentially biased.

2. Data extraction

2.1. Data extraction from primary research studies

From each article selected the extraction process encompasses three key categories of information: General framing of the study, study characteristics relevant for explaining heterogeneity in outcomes, and outcome measures, detection, and/or quantification as determined by Gonzales-Barron et al. (2022).The data extraction is facilitated through an Excel formatwith the subsequent step involving the automatic transfer of extracted data to the PIF database (Gonzales-Barron et al., 2021)

2.2. General framing data

Study ID: a unique identifier for each study.

Study type: classification of the study as a survey or comparison study.

Country of publication: the country where the study is published.

Study duration: the period over which the study was conducted.

2.3. Food characteristics data

Country of sampling: the location where the food samples were obtained.

Country of origin: source or production location of the sampled food.

Country of consumption: country where the food is intended for consumption.

Packaged status: indicates if the sampled food is packaged or not; and if it is packaged what is the type of package used.

Sampling stage: the specific stage in the food production or processing chain where sampling occurred; for instance, primary production (commercial or research fishing for example, manufacturing facilities or distribution level (retail or restaurants).

Production method : method employed in producing the sampled food (if the fish species is from wild or aquaculture production system).

Fishing or harvesting area of origin: geographic location where the fishing or harvesting occurred. According to FAO fishing areas, subareas, etc.

Geographical label: any additional label or designation related to the geographical origin of the food.

Temperature at sampling: the temperature recorded at the time of sample collection (Ambient , Frozen or NA)

Name of the fish species: According to WORMS database classification to harmonize the names obtained from the papers

Organ sampled: if the fish is sampled as whole or gutted unit and which part is analyzed: fillets, visceral organs or the whole unit.

More data related to the food like RTE status, food label, etc

Methods used for detection (naked eye, microscopy, molecular), enumeration, and infectivity essays are also to be considered (See Annex 3 for detailed methods).

2.4. Outcome data

Prevalence results: data related to the prevalence of parasites in the sampled food.

Enumeration results: information intensity or abundance of parasites in the sampled food

$$Prevalence(\%) = \frac{Number\ of\ infected\ fish}{Total\ number\ of\ fish\ examined} \times 100$$

$$Intensity = \frac{Total\ number\ of\ parasites}{Total\ of\ infected\ fish}$$

$$Abundance = \frac{Total\ number\ of\ parasites}{Total\ number\ of\ fish\ examined}$$

The data extraction process was carried out using a spreadsheet, ensuring a systematic and organized approach to collecting relevant information. The subsequent transfer of this extracted data to the PIF database (Gonzales-Barron et al., 2021) will enhance data management and facilitate subsequent analyses. The analysis was restricted to the genus *Anisakis,* and edible samples (fillets) commercialized in European countries only. Cooked and frozen fillets were removed. We assume equal pathogenicity of *Anisakis* species for humans.

3. Statistical method and strategy

A meta-analysis is a statistical approach that summarizes quantitative findings from multiple studies investigating the same research topic, and the same value of interest. Meta-analysis provides a robust and gain of power in estimating an overall result across the studies. The main pitfall is the heterogeneity commonly found among the outcomes. In the present study, prevalence data (or proportions) were analyzed. Each study included in a meta-analysis of proportions contributes a specific number of "successes" and a corresponding total sample size. The prevalence is referred to as the "effect size" (ES) of the meta-analysis.

3.1. Logit transformation of the proportion

In order to summarize proportion data, a variable transformation must be opted. Thus, prevalence outcomes underwent logit transformation, which produces values that can be assumed to follow a normal distribution (Wang, 2023).. All meta-analyses were therefore conducted on the logit transformed scale. Subsequently, the logits were converted back in proportions (or prevalences) for reporting and interpretation purposes. The procedure for calculating the logit and back transformation are as following:

If **P** is the proportion, **S=** number of positive, **N** =total sample size;

$$\boldsymbol{P} = S/_N$$

$$\boldsymbol{logit(P)} = \ln\left(\frac{P}{1-p}\right) = \ln\left(\frac{S}{N-S}\right) \quad \text{Eq (1)}$$

The back transformation is given as follows,

$$\boldsymbol{P} = \frac{exp^{logit(P)}}{1+exp^{logit(P)}} \quad \text{Eq (2)}$$

3.2. Weighting by inverse variance

In meta-analysis, samples of of different size should not have the same contribution to the pooled outcome. Effect sizes were therefore weighted by the inverse of their sampling variances, giving greater weight to larger studies and allowing their effect sizes to have a greater impact on the overall mean (Wang, 2023).

The variance of the logit estimate of a proportion is:

$$\boldsymbol{Var_{logit(P)}} = \frac{1}{N \times P} + \frac{1}{N(1-P)} \quad \text{Eq (3)}$$

The relative weight of each sample is given by the above relationship:

$$\boldsymbol{W_i} = \frac{1}{Var_{logit(P)}} = NP(1-P) \quad \text{Eq (4)}$$

This implies that when the event of interest is extremely rare (i.e. p = 0) or extremely common (i.e., p = 1), the logits and their sampling variances become undefined.

In practice, the common solution is to add an arbitrary constant of 0.5 to correct np and n(1-p) for all studies (Wang, 2023).

3.3. Effect size or Pooled prevalence estimate

The weighted average proportion (i.e., the summary proportion or pool effect size ES_P) can be computed as follows (Wang, 2023):

$$\boldsymbol{ES_p} = \frac{\sum W_i P_i}{\sum W_i} \quad \text{Eq(5)}$$

and the sampling error by the following equation,

$$\boldsymbol{SE} = \sqrt{\sum W_i} \qquad \text{Eq (6)}$$

The confidence interval of the pooled outcome is given by:

$$\boldsymbol{P_l} = P - Z_{(1-\alpha)}(SE)$$
$$\boldsymbol{P_u} = P + Z_{(1-\alpha)}(SE) \quad \text{Eq (7)}$$

where $Z_{(1-\alpha)}$=1.96 when α=0.05 (confidence interval at 95%)

For coverting the estimate to original scale of the proportion, the pooled estimate and confidence interval values areback-transformed as shown in Equation 2.

$$W_i logit(Pi) = \mu + \varepsilon_i,\ \varepsilon \sim N(0, SD) \quad \text{Eq (8)}$$

3.4. Fixed, random and mixed model and algorithm of estimation

Different issues can arise from the approach described in Equation 8 (Viechtbauer, 2010) The estimate cannot be extrapolated to the "population" of potential samples. Heterogeneity can be too large and confidence estimate is too narrow in relation with this heterogeneity. Last, if data are not independent (coming from the same study), this formula does not allow to take it into consideration.

The next step is then to add a random effect to the model (random model). Here, the random effect is attributed to the intercept (most simple approach) and follow a normal distribution. Equation 9 derived from Equation 8 becomes:

$$\boldsymbol{w_i logit(P_i)} = \propto X + u_i + \varepsilon_i, u \sim N(0, \tau), \varepsilon \sim N(0, SD) \quad \text{Eq (9)}$$

Nb: the weight w_i is estimated by $w_i = 1/(\tau^2 + sd^2)$,

The samples *i* included in the meta-analysis are assumed to be a random sample from a larger population of samples. The population of samples is a hypothetical population of an essentially infinite set of samples comprising all of the samples that have been conducted, that could have been conducted, or that may be conducted in the future.

In order to explain the heterogeneity between outcomes, different variables can be added as fixed effects to test whether they moderate or drive the pooled outcome. The main idea

is that if the moderator is significant, the meta-analysis can be stratified by the different levels of this factor, in order to produce pooled estimates on homogeneous sets of data. In the model below, a moderator X has been added to the meta-analysis, with its respectiveα slope or coefficient;

$$\boldsymbol{w_i logit(P_i)} = \propto X + w + \varepsilon, u \sim N(0, \tau), w \sim N(0, sd), \varepsilon \sim N(0, SD) \quad \text{Eq (10)}$$

Equation 11 is the equation of a **mixed model combining random and fixed effects (X)**. This is also the equation of **meta-regression** described below. More complex random effects can also be used (in relation with slope or multi-level approach) but are not considered here. To estimate the parameters of the different models described above, different algorithm can be used such as Maximum likelihood (ML) or Restricted Maximum likelihood (REML). The REML method is considered more robust and is widely used for giving pool estimates confidence intervals. However, for comparison of models with fixed effect using AIC criteria, the models are fitted with Maximum Likelihood (ML).

3.5. Meta-regression

Meta-regression can be made by adding fixed effects to the model, when the estimate is indicating significant remaining heterogeneity. For estimating what kind of subgroup can be interesting, we can test if some explanative variables (i.e., moderators) can explain a part of the remaining heterogeneity.

Moderators can be qualitative or quantitative. When they are qualitative, a reference level must be defined first (in general lowest or upper level, with a large amount of data). A coefficient can be estimated from the data for each level of the qualitative variable. In contrast, a quantitative moderator has only one slope or coefficient

Since the outcome data for analysis are logit transformed, the model expresses the log of the odds of the probability of being positive as a linear function of explanatory variables, whose coefficients are interpretable as the odds ratio (OR).

- Exclusion of fixed intercept in the model: For models including moderators, an omnibus test of all the model coefficients can be conducted without a fixed intercept. In this situation, all coefficients are compared with zero. If at least one coefficient is significantly different from zero, the Omnibus test QM is significant (below 0.05).

- Inclusion of fixed intercept. This intercept represents now the reference level. If it is included, the omnibus test compares the difference between the coefficients and the defined reference level. If QM is significant, it indicates that one coefficient is significantly different from others. At least one level is increasing or decreasing significantly the prevalence.

  The assessment of the QM statistics inform of the decision of performing a subgroup analysis.

3.6. Multivariate analysis and model selection

Different moderators can be added to the model (multivariate analysis). To better explore which factors better explain the data, and due to the number of factors to explore, the AIC criterion was chosed using Maximum Likelihood fitting.
The Akaike Information Criterion (AIC) is defined as:

$$\mathbf{AIC} = -2 \text{ maximum log likelihood} + 2p$$

where p is the number of parameters. The AIC criterion penalizes the number of parameters to estimate (coefficients) to avoid over-fitting.
The quality of fitting of the model to the data and comparison between models can be done by comparing their AIC the one having the lowest AIC is the best models and also (Burnham & Anderson, 2002). For each candidate model, we examined the Akaike weights (W), which are relative model likelihoods normalized over the likelihoods of all possible sub-models. A weight can be interpreted as the probability for a candidate of being the best model given the data and the set of possible sub-models (Wagenmakers & Farrell, 2004). We chose the final model based on the number of parameters, following the principle of parsimony. Thus the model with the fewest parameters was seleted from the set of “best models”; and, furthermore, when two models had the same number of parameters, model with the highest Akaike weight was selected.

3.7. Subgroup analysis , Heterogeneity and publication bias controls

The final best model was evaluated by classical meta-regression analysis. After assessing what kind of fixed effects moderators explained heterogeneity, homogeneous

subgroups were constituted (Equation 9 and 10). However, for transparency post-checks controls are needed and described below.

- **Forest plot**

A forest plot shows the results of the individual studies (i.e., the estimated effects or observed outcomes) together with their (usually 95%) confidence intervals at raw or transformed scales. A four-sided polygon, sometimes called a summary 'diamond', is added to the bottom of the plot, showing the pooled estimate based on the model (with the center of the polygon corresponding to the estimate and the left/right edges indicating the confidence interval limits a 95%, with or without random effect). Whenever the confidence interval of the pooled estimate is not crossing the confidence interval of a particular study (sample), it is an indicator of heterogeneity (Gordon,2024).

- **Funnel plot**

Funnel plots are useful diagnostic tools for assessing the presence of heterogeneity and potential publication bias in meta-analyses (Rothstein et al., 2005). For models without moderators, the observed outcomes are plotted on the horizontal axis, while the vertical axis represents their corresponding standard errors (i.e., the square root of the sampling variances). A vertical line marks the overall effect estimate, surrounded by a pseudo-confidence interval region defined by $\pm 1.96 \cdot SE$, where SE is the standard error value on the vertical axis. In models involving moderators, the residuals replace the observed outcomes on the horizontal axis, and a vertical line at zero serves as a reference with the same pseudo-confidence interval (Viechtbauer, 2010).

A symmetric funnel plot around the vertical line suggests no publication bias. However, asymmetry, particularly at the plot's base where standard errors are higher, may indicate bias. Asymmetry at the base of the plot, with elevated standard errors, can suggest heterogeneity. Furthermore, data points outside the pseudo-confidence intervals may indicate the presence of heterogeneity. Pronounced asymmetry at the bottom of the plot, with more data concentrated on one side, may indicate publication bias and the potential for an overestimated effect size. Specific statistical tests of publication bias can provide further confirmation but are not detailed here (Viechtbauer, 2010).

- **I^2 and Cochran's Q test for heterogeneity**

Heterogeneity can be quantified by dividing it into two distinct components: the between-study variance, which arises from the true variation among a body of studies, and the within-study variance, resulting from the sampling error.

The between-study heterogeneity is characterized by the variance of the true effect size underlying the data,τ^2, a statistic called tau-squared. Under the assumption of normality, 95% of the true effects are expected to fall within$\pm 1.96 \times \tau$ of the point estimate of the summary effect size. Thus, τ^2 reflects the total amount of systematic differences in effects across studies.

I^2 or intraclass correlation indicates the ratio of the observed heterogeneity, representing the true between-study variance, to the total observed heterogeneity (i.e., the sum of between- and within-study variance). As a result, it facilitates the comparison of heterogeneity estimates across meta-analyses, regardless of the original scale used in the meta-analyses themselves. I^2 can take values from 0% to 100%.

Higgins et al. (2003) propose the following interpretation for I^2: I^2 = 0%: No heterogeneity; 25%: Low heterogeneity; 50%: Moderate heterogeneity; 75%: Substantial heterogeneity; 90%: Very high heterogeneity; 100%: Extreme heterogeneity.

The presence of between-study heterogeneity is generally examined using also a $\chi 2$ test with a statistic Q (Cochran, 1954). The null hypothesis indicates that all studies share the same true effect (Hedges & Olkin, 1985 in Wang 2023). The Q-test and its p-value serve as a test of significance to address the null hypothesis:

H0: $\tau^2 = 0$. If the value of the Q-statistic is above the critical $\chi 2$ value, we reject the null hypothesis and conclude that the effect sizes are heterogeneous. Under such circumstances, a random-effects model should be the next step to take. If Q does not exceed this value, then we fail to reject the null hypothesis (Wang,2023). For meta-regression Q is QE test.

I^2 and Q test have their own limitations (Wang, 2023). We will use a random effect model by default. If the residual τ^2 estimate is 0, the model is equivalent to a fixed effect model.

- **Sensitivity analysis**

An extreme value can have an impact on the pooled estimate. We can detect an outlier by different methods such as Cook distance or forest plot. In case this particular value is extreme but not linked to any particular bias, this fact can be mentioned. By contrary, if this value is linked to a biased or low quality study, then it can be removed from the meta-analysis.

3.8. Overall Strategy of analysis and practical considerations

A random effect was set for the level of study (publication). Due to the large amount of potential moderators and the unbalanced nature of the data retrieved, no interaction was considered. The analysis was limited to the genus: Anisakis, type of sample: fillet, and sampling stage: retail level in European countries.

3.8.1. Descriptive analysis and data treatment

- Inclusion criteria : Meta-regression needs a minimum of samples to be considered as robust. For inclusion in the analysis, 10 dataset publications /order of fish were set as minimum, and for each order of fish, at least 5 outcomes in 2 different FAO areas. For each factor considered, and in general, at least 10 datasets in 2 different categories were set. Empty values (NA) are not admitted for any moderators concerned, except FAO areas for retail stage, and the gutted/eviscerated field.
- For samples at the retail stage, only data sampled in European countries were kept. Analysis at whole fish level was not performed because the prevalence in whole fish are only reported if organs are not reported or are negative, and whole fish positive.
- The frequency of NA was first investigated in a given factor, applying a threshold of greater than 20% for factor removal. In addition, factor levels could be grouped with others, if the level was below 10% of sample size. In this way, the final dataset for exploration was obtained.

3.8.2. Multivariate analysis

For investigating potential moderator variables, a non-nested model of the type,

Fishing_area + Order_host + Essay_sample_preparation_Type + Sampling_stage + Wild_or_Aquaculture + Point_of_sampling + Type_of_sample + Organ_sampled + Year of sampling , was used. The model was fitted using ML algorithm and candidate models compared using AIC.

3.8.3. Meta-analyses adjusted to the three topmost important fish orders

Taking into account the previous results, meta-analytical statistics such as I^2, QM, Q/QE, and AIC were stored for each meta-regression or meta-analysis model. At subgroup level, CI, and forest and funnel plot were built using the REML-based solution.

All meta-analytical modelling was carried out using the R software (R Core Team, 2023). For all analyses, after adjusting the complete model, all possible sub-models were compared using the MuMin (Bartoń, 2024) package, and the metafor package for meta-analysis (Viechtbauer, 2010) The ggplot2 and dplyr packages were used for graphics and data summarization.

Chapter 3: Results

The literature search performed in October 2023 resulted in the following number of references: 307 records from Web of Science, 305 records from Scopus, 188 records from PubMed, and 24 records from Scielo. Additionally, 185, 60 and 51 papers were retained from the meta-analysis publications of Rahmati et al (2020), Fiorenza et al. (2020) and Marcken et al. (2020), respectively.

Thus, the initial dataset contained 274 unique study IDs, representing the number of papers from which data was extracted, and 3647 rows corresponding to the total number of entries. For the current study, the data was further restricted based on the following criteria:

- **Genus**: Only the genus *Anisakis* was included, excluding *Phocanema* and *Contracaecum*.
- **Organ sampled**: The analysis focused on fillets, specifically "Fillets", "Fillets-Belly flap", and "Fillets-Fillet without belly flap."
- **Sampling stage**: Data was restricted to primary production from global sources, as these fish might be consumed in Europe. For the retail stage, only European countries were included.
- **Sample condition**: Frozen and cooked samples were excluded under the assumption that parasites would be absent from these samples.
- **Prevalence results** : only prevalence results were considered.

After applying these filters, the final dataset contained 461 entries and 103 unique study IDs. The systematic review process is summarized in the PRISMA chart shown in Figure

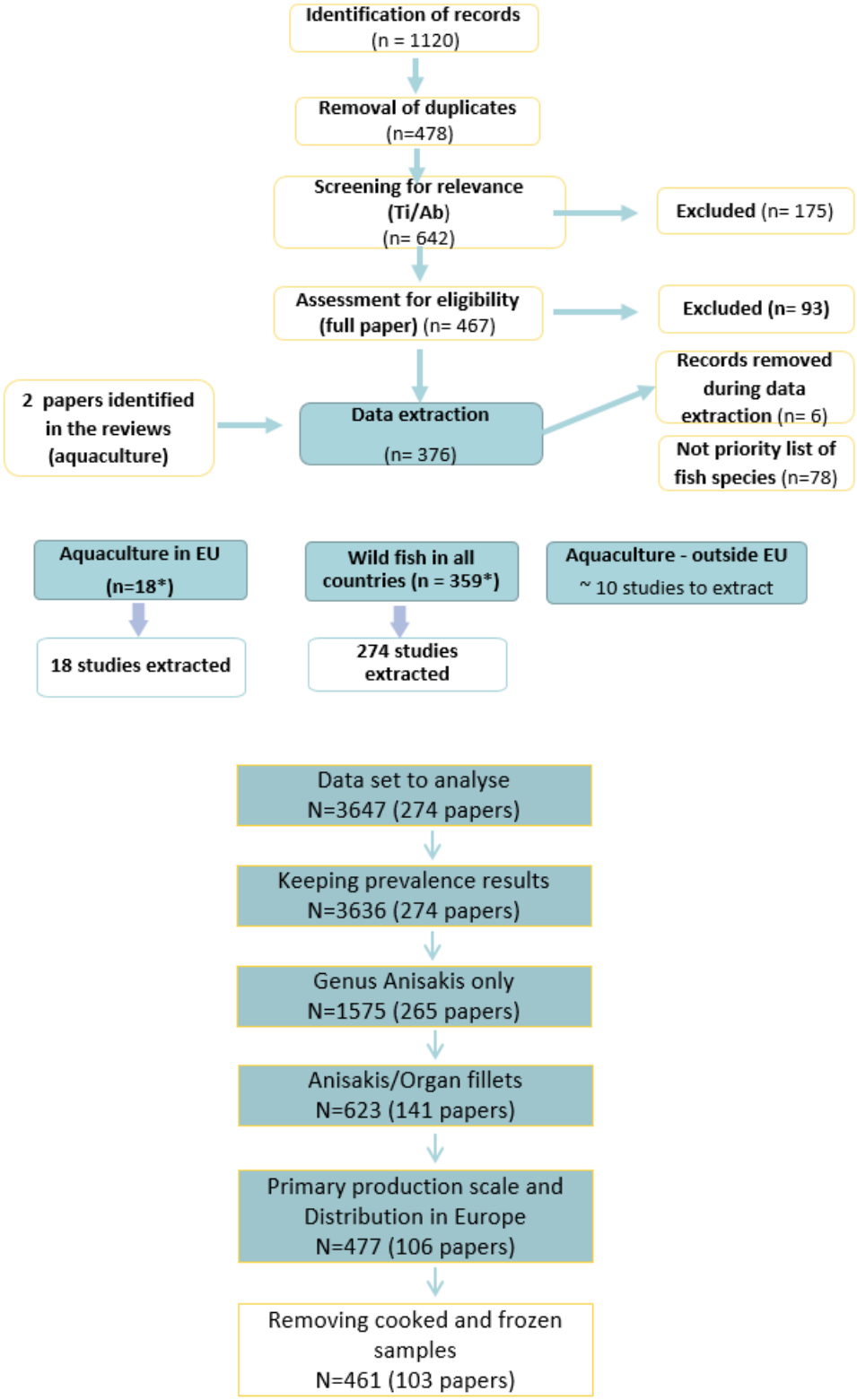


Figure 6. PRISMA chart for Anisakids 2010-2020

In this meta-analysis, nine factors were identified as potential drivers of the prevalence of parasites across studies. For each factor, a hypothesis was developed based on previous research or legislative regulations. The nine factors are as follows:

- **Year of sampling**: Contamination levels are expected to increase at the primary production level due to climate change. However, at the retail stage in Europe, contamination levels are expected to decrease after the implementation of regulations in 2004.

- **FAO Areas**: Previous meta-analyses indicate that FAO Area 37 (Mediterranean and Black Sea) tends to have the lowest contamination prevalence.

- **Fish Order (Species)**: Contamination levels vary across fish species. For example, Mercken's et al meta-analysis reported that Cod *(Gadus morhua)* exhibited high contamination levels.

- **Method of parasite detection/preparation**: Destructive methods, such as press/UV and digestion techniques, are considered more efficient for detecting parasites than visual examinations without preparation.

- **Type of sample**: Whole fish units are expected to have higher contamination levels compared to gutted fish, as evisceration prevents the migration of parasites into the fillets.

- **Organ sampled**: Of the three types of fillets considered, belly flaps are expected to show the highest contamination levels.

- **Production method**: Aquaculture fish are expected to have lower contamination levels than wild-caught fish, as feeding systems in aquaculture are controlled.

- **Sampling stage**: Lower contamination levels are anticipated at the retail stage due to regulatory controls established for fish distribution.

- **Point of sampling**: Commercially sourced fish are expected to have lower contamination levels than research-caught fish, given differences in fish size and health status.

Other factors, such as fish size and sex, could have been considered, but these were either difficult to test or not provided by the authors.

1. Description of the dataset

The descriptive phase allows us to comprehensively understand the content of each factor in the included studies, and to identify differences and proportions. This helps group or exclude certain categories, leading to greater accuracy in the results.

1.1. Year of sampling

Table 2 shows the unbalancedness of the data in relation to the year of sampling, as some years are linked to higher number of while others contain very few studies. The level NA informs of a considerable amount of data that did not indicate year of sampling.

Table 2. Number of records and percentual frequency

Year	n	Frequency (%)
2001	1	0.22
2003	5	1.08
2004	19	4.12
2006	18	3.90
2007	14	3.04
2008	3	0.65
2009	16	3.47
2010	6	1.30
2011	7	1.52
2012	92	19.96
2013	89	19.31
2014	5	1.08
2015	17	3.69
2016	30	6.51
2017	41	8.89
2018	26	5.64
2019	19	4.12
2020	4	0.87
2021	1	0.22
NA	48	10.41
Total	461	100

1.2. FAO Areas

Table 3 shows how the records are distributed according to the FAO areas.

Table 3. Number of records and percentual frequency by FAO fishing areas

Fishing area	n	Frequency (%)
Area 37: Mediterranean and Black sea	185	40.13
Area 27: Atlantic, Northeast	138	29.93
NA	44	9.54
Area 61: Pacific, Northwest	35	7.59
Area 87: Pacific, Southeast	16	3.47
Area 57: Indian Ocean, Eastern	14	3.04
Area 41: Atlantic, Southwest	10	2.17
Area 67: Pacific, Northeast	8	1.74
Area 51: Indian Ocean, Western	7	1.52
Area 34: Atlantic, Eastern Central	2	0.43
Area 21: Northwest Atlantic	1	0.22
Area 71: Pacific, Western central	1	0.22
Total	461	100

Some categories displayed a low number of records, so we proceeded to merge them, resulting in the recategorization (Table 4).

Table 4. Number of records and percentual frequency by FAO fishing areas after merging

Fishing area	n	Frequency (%)
Area 37: Mediterranean and Black sea	185	40.13
Area 27-21 Atlantic North	139	30.15
Area 61-67-71-87 Pacific	60	13.02
Other Fishing Areas	44	9.54
Area 51-57 Indian Ocean	21	4.56
Area 34-41 Atlantic Other	12	2.6
Total	461	100

The reference level for this category was chosen to be the Mediterranean and black sea area 37 as it has the highest number of records and has been proven by previous studies to have the lowest prevalence levels of contamination. The process of grouping/merging was further done for each factor to have more harmonized categories, only modified tables are presented.

1.3. Fish Order

For the fish order, only levels having more than 3 records were kept, the order Clupeiformes was chosen as the reference level as many authors have reported it is the order having less contamination across the 4 most consumed in Europe (Table 5).

Table 5. Number of records and percentual frequency by fish order

Host order	n	Frequency (%)
Gadiformes	129	28.35
Clupeiformes	85	18.68
Scombriformes	68	14.95
Eupercaria incertae sedis	54	11.87
Carangiformes	27	5.93
Salmoniformes	22	4.84
Pleuronectiformes	15	3.30
Perciformes	10	2.20
Siluriformes	8	1.76
Lophiiformes	7	1.54
Mulliformes	7	1.54
Cypriniformes	5	1.10
Aulopiformes	4	0.88
Mugiliformes	4	0.88
Osmeriformes	4	0.88
Beloniformes	3	0.66
Cichliformes	3	0.66
Total	455	100

1.4. Sample preparation

The sample preparation methods include both destructive and non-destructive methods. Therefore, the reference level chosen is 'no preparation', classified under the non-destructive methods and which is also expected to yield the lowest prevalence results (Table 6).

Table 6. Number of records and percentual frequency by essay sample preparation type

Essay sample preparation Type	n	Frequency (%)
Press/UV	159	34.95
Artificial Digestion	154	33.85
No preparation	134	29.45
Other	8	1.76
Total	455	100

1.5. Type of sample

At the sampling point (Table 7), the fish can be obtained as a whole unit, gutted fish, or fillets only. The reference level set was gutted fish, as it is expected to have lower contamination levels compared to the whole unit.

Table 7. Number of records and percentual frequency by type of sample

Type of sample	n	Frequency (%)
Whole fish	402	88.4
Gutted fish	37	8.13
Fillets	16	3.52
Total	455	100

1.6. Organ sampled

The organ sampled chosen as a reference level was the belly flap (n=30) even though it is not the category having the highest number of records (Table 8), but this category is suspected as the most contaminated one.

Table 8. Number of records and percentual frequency by organ sampled

Organ sampled	n	Frequency (%)
Fillets	404	88.8
Fillets-Belly flap	30	6.51
Fillets-Fillet without belly flap	21	4.62
Total	455	100

1.7. Production method

The production method chosen as the reference level is wild-caught fish, due to its significantly higher number of records and its expected higher prevalence levels compared to aquaculture (Table 9).

Table 9. Number or records and percentual frequency according to the production level

Wild or Aquaculture	n	Frequency (%)
Wild	413	90.76
Aquaculture	38	8.24
NA	4	0.87
Total	461	100

1.8. Sampling stage

Primary production was chosen as the reference level as it has the highest number of records as fish species at this stage are not subjected to any treatment (Table 10).

Table 10. Number of records and percentual frequency according to the sampling stage

Sampling stage	n	Frequency (%)
Primary Production	396	87
Distribution	59	13
Total	455	100

1.9. Point of sampling

Many categories were grouped into two main categories: commercial and non-commercial (Other) with 'commercial' chosen as the reference level (Table 11).

Table 11. Number of records and percentual frequency according to the point of sampling

Sampling point	n	Frequency (%)
Commercial	284	62.42
Other	171	37.58
Total	455	100

2. Univariate analysis

The univariate meta-regression models analyzed various factors influencing the prevalence of *Anisakis* parasites, each revealing significant findings:

- **Fishing Area**: The model, which included 455 observations, indicated significant differences in parasite prevalence across different fishing areas. Despite this, the residual heterogeneity remained high ($\tau^2 = 4.29$, $I^2 = 97.13\%$), suggesting substantial unexplained variability. The test for moderators showed a highly significant effect of fishing area on prevalence ($QM(6) = 679.81$, $p < 0.0001$).
- **Fish Host Order**: Significant differences were observed among fish host orders in relation to *Anisakis* prevalence. High residual heterogeneity persisted ($\tau^2 = 4.10$, $I^2 = 96.95\%$), indicating that other factors may also influence prevalence. The test for moderators confirmed a significant impact of fish host order on parasite prevalence ($QM(17) = 736.11$, $p < 0.0001$).

- **Sample Preparation Type**: The effect of sample preparation type on *Anisakis* prevalence was significant. However, substantial residual heterogeneity remained ($\tau^2 = 5.02$, $I^2 = 97.54\%$), pointing to incomplete explanation of variability by preparation methods. The test for moderators was highly significant ($QM(4) = 554.47$, $p < 0.0001$), highlighting the influence of sample preparation on detection rates.
- **Sample Type**: Analysis of sample type (whole fish, gutted fish, fillets) showed significant effects on parasite prevalence. High residual heterogeneity ($\tau^2 = 5.57$, $I^2 = 97.79\%$) suggested that variability in prevalence was not fully explained by sample type alone. The test for moderators was significant ($QM(3) = 478.62$, $p < 0.0001$).
- **Organ Sampled**: The impact of the organ sampled (e.g., fillets, belly flap) on *Anisakis* prevalence was significant, but high residual heterogeneity ($\tau^2 = 5.29$, $I^2 = 97.66\%$) indicated additional sources of variability. The test for moderators confirmed a significant effect of the organ sampled on detection rates ($QM(3) = 521.64$, $p < 0.0001$).
- **Production Method**: The production method also significantly affected parasite prevalence. Despite this, high residual heterogeneity ($\tau^2 = 5.20$, $I^2 = 97.66\%$) remained, suggesting that the variability is not entirely explained by production methods alone. The test for moderators was significant ($QM(3) = 526.66$, $p < 0.0001$).
- **Sampling Stage**: The analysis revealed significant effects of sampling stage on *Anisakis* prevalence, though residual heterogeneity remained substantial ($\tau^2 = 5.59$, $I^2 = 97.79\%$). The test for moderators was highly significant ($QM(2) = 474.20$, $p < 0.0001$), indicating the importance of sampling stage in influencing detection rates.
- **Point of Sampling**: Finally, the point of sampling significantly impacted *Anisakis* prevalence. High residual heterogeneity ($\tau^2 = 5.55$, $I^2 = 97.77\%$) suggested that other factors may also play a role. The test for moderators was significant ($QM(2) = 480.13$, $p < 0.0001$), emphasizing the influence of sampling point on parasite detection.

These analyses collectively highlight the significant role of various factors in explaining the variability in *Anisakis* prevalence, though substantial unexplained heterogeneity remains.

3. Choice of the model

To identify the optimal multivariate meta-regression model for analyzing the prevalence of *Anisakis* parasites, a comprehensive model including all factors (Fishing area, Fish host order, Sample preparation type, Sampling stage, Wild or Aquaculture, Point of sampling, Sample type, and Organ sampled) was initially specified. The best-fit model was found to be the one comprising 6 factors: Fishing area, Fish host order, Sample preparation type, Wild or Aquaculture, Sample type, and Organ sampled, as it presented the lowest AIC value (1919.5). This model should be preferred for its balance between goodness-of-fit and model complexity.

4. Multivariate analysis

4.1. FAO Areas

The results of the multivariate analysis related to the Fishing areas are as follows (Table 12):

- Fishing area: Area 27-21 Atlantic North: This area showed a very high odds ratio (OR = 8.0638, 95% CI: 4.9564 to 13.1195), suggesting that the odds of *Anisakis* parasite prevalence are substantially higher compared to the reference area.

- Fishing area: Area 34-41 Atlantic Other: The odds ratio (OR = 0.2435, 95% CI: 0.0298 to 1.9889) is less than 1.0, but not statistically significant (p = 0.1874), indicating that this area does not significantly differ from the reference area in terms of prevalence.

- Fishing area: Area 51-57 Indian Ocean: The odds ratio (OR = 0.8803, 95% CI: 0.2792 to 2.7757) is close to 1.0 and not statistically significant (p = 0.8277), suggesting no substantial difference in prevalence compared to the reference area.

- Fishing area: Area 61-67-71-87 Pacific: This area has a significant odds ratio (OR = 3.7442, 95% CI: 1.8263 to 7.6764), indicating higher odds of *Anisakis* parasite prevalence compared to the reference area.

- Fishing area: Other Fishing Areas: The odds ratio (OR = 1.7022, 95% CI: 0.7557 to 3.8346) is greater than 1.0 but not statistically significant (p = 0.1992), suggesting that the prevalence in these other fishing areas is not significantly different from the reference area.

Table 12. Odds ratio estimates and confidence intervals for FAO regions

Categories of fishing area	OR	Lower Bound (2.5 pct)	Upper Bound (97.5 pct)
Fishing_area: Area 27-21 Atlantic North	8.06	4.95	13.11
Fishing_area: Area 34-41 Atlantic Other	0.24	0.03	1.98
Fishing_area: Area 51-57 Indian Ocean	0.88	0.28	2.77
Fishing_area: Area 61-67-71-87 Pacific	3.74	1.83	7.68
Fishing_area: Other Fishing Areas	1.70	0.75	3.83
Area 37: Mediterranean and Black sea	1.00	-	-

4.2. Fish order

Among the four most consumed orders of fish in European countries, Clupeiformes, which had been selected as the reference group, had the lowest prevalence levels. For Gadiformes, the odds ratio (OR) of 7.44 indicated a notably high prevalence of Anisakis parasites compared to the reference group. Same applies to Salmoniformes which exhibited an exceptionally high OR of 61.14, suggesting a very pronounced prevalence. At the end, Scombriformes also showed a significant increase in parasite prevalence, with an OR of 4.35. Table 13 summarizes the results for all the fish orders recovered in our study.

Table 13. Odds ratio estimates and confidence intervals for fish orders

Categories of fish order	OR	Lower Bound (2.5 pct)	Upper Bound (97.5 pct)
Aulopiformes	2.21	0.26	18.59
Beloniformes	1.64	0.13	20.47
Carangiformes	3.65	1.54	8.63
Cichliformes	0.19	0.01	2.80
Cypriniformes	1.77	0.22	14.41
Eupercaria incertae sedis	18.16	6.47	50.94
Gadiformes	7.44	4.18	13.26
Lophiiformes	6.26	1.48	26.49
Mugiliformes	8.77	0.6	128.7
Mulliformes	1.53	0.33	7.21
Osmeriformes	0.66	0.12	3.68
Perciformes	4.69	1.27	17.3
Pleuronectiformes	0.88	0.3	2.57
Salmoniformes	61.14	19.2	194.7
Scombriformes	4.35	2.24	8.44
Siluriformes	17.05	2.99	97.29
Clupeiformes	1.00	-	-

4.3. Sample preparation

Table 14 compiles the results regarding the sample preparation method used for detection.. Employing the Artificial digestion method, the odds of detecting contamination in a fish sample was found to be 7.81 times greater than the absence of preparation. Furthermore, using the Press/UV method would lead to a higher probability of detecting Anisakis in a contaminated sample(OR = 6.11) in comparison to the no preparation method.

Table 14.Odds ratio and confidence interval estimates according to sample preparation

Categories of sample preparation method	OR	Lower Bound (2.5 pct)	Upper Bound (97.5 pct)
Artificial Digestion	7.81	4.57	13.35
Other	1.20	0.07	21.37
Press/UV	6.11	3.5	10.67
No preparation	1.00	-	-

4.4. Type of sample

The reference level chosen was the gutted fish, in this case assuming it is the one having the lowest contamination levels. For whole fish, the odds ratio (OR) of 2.96 suggested that whole fish has nearly a three-fold higher prevalence of *Anisakis* parasites than the gutted fish. The fillets also displayed an OR of 2.62, indicating a higher prevalence of *Anisakis* parasites compared to the reference category. The results are summarized in Table 15.

Table 15. Odds ratio and confidence interval estimates for type of sample

Categories of type of samples	OR	Lower Bound (2.5 pct)	Upper Bound (97.5 pct)
Whole fish	2.96	1.42	6.17
Fillets	2.62	0.87	7.95
Gutted fish	1.00	-	-

4.5. Organ sampled

The odds ratio (OR) of 0.34 for fillets and 0.21 for fillets without the belly flap both indicated a significantly lower prevalence of *Anisakis* parasites compared to the reference organ, the belly flap, which is the one normally having the highest contamination. Results are presented in Table 16.

Table 16. Odds ratio and confidence interval estimates for organ sampled

Categories of organs	OR	Lower Bound (2.5 pct)	Upper Bound (97.5 pct)
Fillets	0.34	0.16	0.74
Fillets-Fillet without belly flap	0.21	0.08	0.57
Belly flap	1.0	-	-

4.6. Production method

This meta-analysis confirmed the low prevalence of *Anisakis* parasites in fish raised through aquaculture compared to the reference category, wild fish. This suggested a significantly lower probability of detection of suchparasites in aquaculture due to their highly controlled settings (Table 17)

Table 17.Odds ratio and confidence intervals for production methods

Categories of production method	OR	Lower bound (2.5 pct)	Upper Bound (97.5 pct)
Aquaculture	0.014	0.005	0.042
NA	0.84	0.021	32.93
Wild	1.00	-	-

5. Meta-analysis test on homogenous categories

As explained in the Methodology section, after exploration of the moderating variables, meta-analysis was then conducted on more specific and homogenous categories. It was performed for three major fish orders commonly consumed in Europe: Gadiformes, Clupeiformes, and Scombriformes. The order Salmoniformes was not included due to the low number of available records. The analysis was also restricted to whole fish and fillets as the sampled organs, with no preparation done prior to parasite detection for wild caught fish. Additionally, two main FAO areas were included: the North Atlantic and the Mediterranean.

5.1. Gadiformes

The selected subset for the meta-analysis included:

- **Fishing Area: Area 27-21 (Atlantic North)**

The predicted overall prevalence of *Anisakis* parasites is 70.66% (CI95%: 52.84%,83.8%) indicating some uncertainty around the estimate but a high prevalence overall.

In the random-effects model (k = 30) using the REML method, the estimated tau², which reflects total heterogeneity, was 4.23 (SE = 1.19), and the square root of tau² (tau) was 2.06. This suggests substantial heterogeneity in the data, further supported by an I^2 value of 98.91%, indicating that nearly all variability in the effect sizes is due to heterogeneity between studies rather than sampling error.

With regards to the publication bias, the funnel plot (Figure 10, left) did not display pronounced asymmetry, but a slight imbalance, with more studies clustered in the top section of the funnel compared to the bottom. This suggests that larger studies with higher precision contributed more to the overall analysis, while smaller studies might be underrepresented.

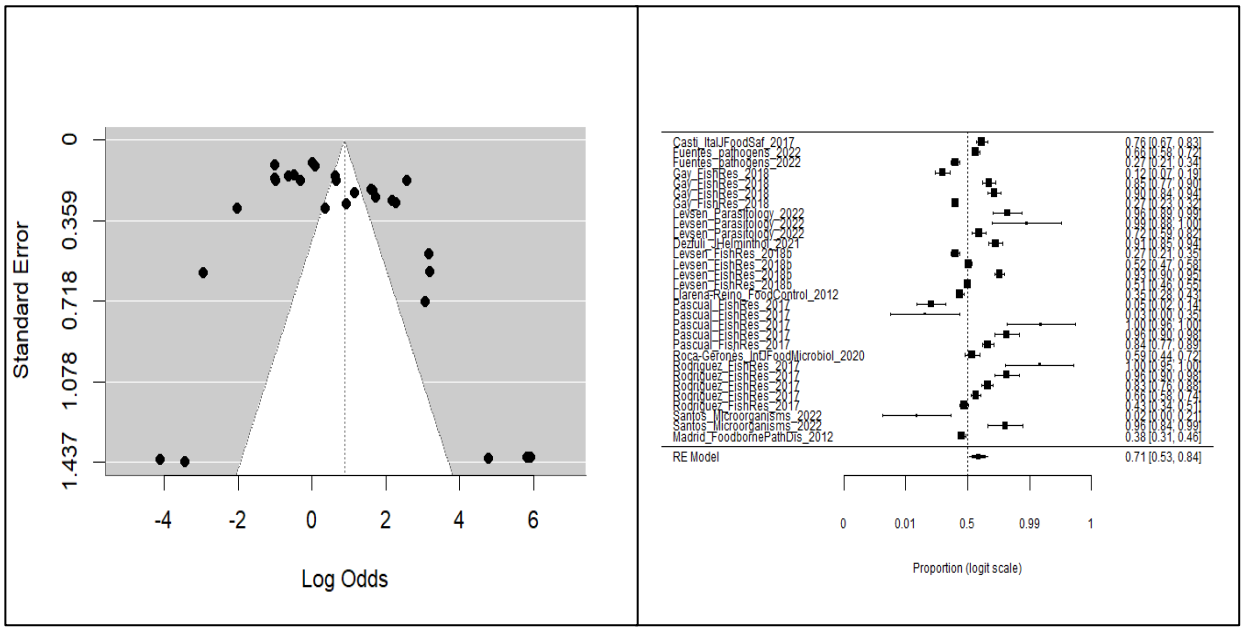


Figure 7. Funnel plot and Forest plot for Gadiformes FAO 27

An additional publication bias test was performed using the full model with the total number of units tested as a moderator. Such formal test for publication bias yielded a p-value of 0.0456, which is just below the conventional threshold for statistical significance ($p < 0.05$). This indicates that there is a potential presence of publication bias, although the evidence is not conclusive. However, the forest plot (Figure 10, right) showed some variations in individual studies estimates which would suggest the presence of heterogeneity in the dataset.

- **Fishing Area: Area 37 Mediterranean**

The model predicted an overall prevalence of Anisakis parasites of 0.91% (95% CI: 0.45% - 1.85%), highlighting a low prevalence with some uncertainty around the estimate.

In the random-effects model (k = 34) using the REML method, the estimated tau^2 was 3.28 (SE = 1.09), with the square root of tau^2 (tau) being 1.81. This implied a high level of heterogeneity among the studies, corroborated by an I^2 value of 93.76%, suggesting that a significant portion of the variability in the effect sizes is attributable to between-study heterogeneity rather than sampling error.

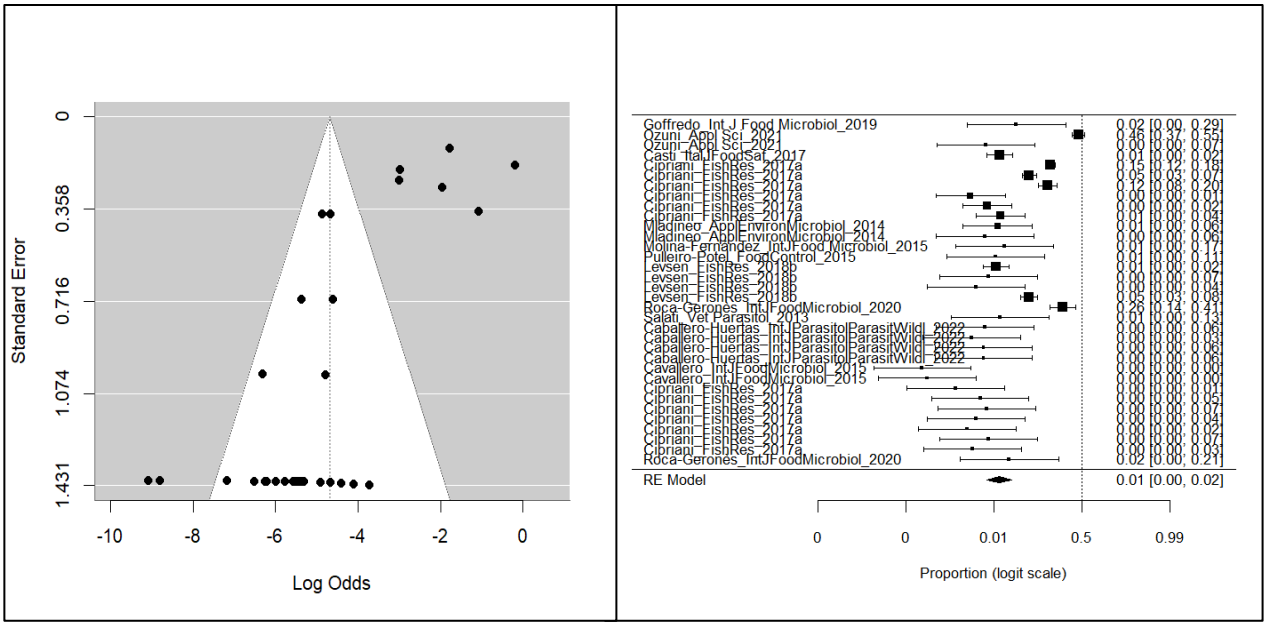


Figure 8. Funnel plot and Forest plot for Gadiformes FAO 37

The formal test for publication bias (previously described) revealed a p-value of $9.34x10^{-18}$, strongly indicating the presence of publication bias. This conclusion is supported by the funnel plot, which shows asymmetry, particularly with more studies clustered on the right-hand side of the funnel. The absence of studies in the lower left corner suggests smaller studies with negative or non-significant results may be missing, indicating that such studies are less likely to be published; whereas larger, more precise studies appear symmetrically distributed (Figure 11, left).

5.2. Clupeiformes

- **Fishing Area: Area 27-21 (Atlantic North)**

The model predicted an overall prevalence of Anisakis parasites of 15.45% (95% C: 8.25% - 27.06%), indicating a substantial prevalence with considerable uncertainty around the estimate.

In the random-effects model (k = 14) using the REML method, the estimated $tau^2$was 1.49 (SE = 0.69), with the square root of tau^2 (tau) being 1.22. This suggested a high level of heterogeneity among the studies, as evidenced by an I^2 value of 98.58%, evidencing therefore that most of the variability in the effect sizes is due to heterogeneity rather than sampling error.

A publication bias test was conducted using a model with the total number of units tested as a moderator. The test yielded a p-value of 0.0037, indicating a potential presence of publication bias.

- **Fishing Area: Area 37 Mediterranean**

The model estimated an overall prevalence of Anisakis parasites of 0.91% (95% CI: 0.45% - 1.85%), indicating a very low prevalence with considerable uncertainty around the estimate.

In the random-effects model (k = 34) using the REML method, the estimated tau^2 was 3.28 (SE = 1.09), with the square root of tau^2 (tau) being 1.81. Once again, the level of heterogeneity among the studies was high, as reflected by an I^2 value of 93.76%, suggesting that the majority of the variability in effect sizes is due to between-study heterogeneity rather than sampling error.

The formal publication bias test yielded a p-value of $9.34x10^{-18}$, indicating a significant presence of publication bias, which should be considered when interpreting the results.

5.3. Scombriformes

- **Fishing Area: Area 27-21 (Atlantic North)**

The model estimated an overall prevalence of Anisakis parasites of 31.25% (95% CI: 22.77% - 41.21%), indicating a relatively high prevalence with a broad confidence interval suggesting some uncertainty around the estimate.

In the random-effects model (k = 10) using the REML method, the estimated tau^2 was 0.43 (SE = 0.23), with the square root of tau^2 (tau) being 0.65. This can be interpreted as a high degree of heterogeneity among the studies, as indicated by an I^2 value of 93.37%, meaning that most of the variability in effect sizes is due to between-study heterogeneity rather than sampling error.

The test for heterogeneity revealed a Q statistic of 135.42 (df = 9), with a p-value < .0001, confirming substantial heterogeneity across studies.

- **Fishing Area: Area 37 Mediterranean**

The model estimated an overall prevalence of Anisakis parasites of 10.52% (95% CI: 3.80% - 25.90%), reflecting a moderate prevalence with considerable uncertainty around the estimate.In the random-effects model (k = 11) using the REML method, the estimated $tau^2$was 2.89 (SE = 1.50), with the square root of tau^2 (tau) being 1.70. Furthermore, the I^2 value of 96.89% also suggested that nearly all of the variability in effect sizes was due to differences between studies, rather than sampling error.

The test for heterogeneity yielded a Q statistic of 233.49 (df = 10), with a p-value < .0001, confirming thus substantial heterogeneity across studies.

Chapter 4: Discussion

In the initial dataset, 274 unique study IDs were identified, representing the total number of papers from which data was extracted, yielding 3647 entries. After applying further selection criteria, the final dataset for this study consisted of 461 entries from 103 unique studies. For comparison, Rahmati et al. (2020) included data from 264 publications from 2000 to 2020 in their systematic review.

In Marcken et al. (2020), a total of 519 articles were initially identified, with 83 articles retained after exclusion based on abstract and full-text screening, resulting in 432 datasets. Of these, 62 and 22 studies were used to calculate prevalence and intensity, respectively, with 21 studies identifying larvae to species level and reporting absolute numbers. Additionally, 18 studies compared farmed versus wild fish.

Fiorenza et al. (2020) analyzed data from 123 manuscripts and 755 data points, summarizing findings from 56778 fish and 446615 anisakid nematodes over nearly four decades.

The variation in study count and data volume across studies reflects differences in inclusion criteria, the search engines utilized, and the fact that all papers from these three meta-analyses were assessed for eligibility in our research and also due to the differences in the objectives of the research. When analysis was restricted to the genus *Anisakis* in fillet samples (in European countries), the significant factors identified were fishing area, fish host order, sample preparation type, method of production, sample type, and organ sampled.

1. Comparison with previous findings

The Mediterranean area and Black sea (Area 37) was chosen as the reference level in the analysis, with prevalence levels in fillets from the Atlantic regions found to be 8 times higher. This aligns closely with the findings of Mercken et al. (2020), despite differences in the datasets and specifications used. The Mediterranean Sea exhibited the lowest prevalence, with an overall rate of 14% (95% CI: 9–28%). Meanwhile, the Northeast Atlantic Ocean, which includes the Barents Sea, Norwegian Sea, Icelandic Waters, and the North Sea, showed a significantly higher prevalence, with a combined overall rate of

68% (95% CI: 55–79%), the highest pooled prevalence observed in this study (Mercken et al. (2020).

Rahmati et al. (2020) reported prevalence levels of *Anisakis spp*. larvae in fish from the families Lophiidae (86.9%), Trichiuridae (77.1%), Merlucciidae (67.8%), and Gadidae (56.8%). A possible explanation for his findings was that *Anisakis spp.* larvae are not host-specific and can transfer between fish species until they reach their definitive hosts. As fish from the most infected families in this study age, they may accumulate more *Anisakis* larvae due to prolonged exposure and feeding on infected prey. For example, many of the preferred prey species of these fish, such as *Engraulis* and *Sardina*, have been reported to harbor *Anisakis* larvae in the Mediterranean region (Gushchin & Corten, 2016; Gazzonis et al., 2017; Serracca et al., 2014). In addition to the findings of Mercken et al. (2020), the Scombroidei and Gadiformes showed the highest prevalence rates of 65% (95% CI: 48–78%) and 56% (95% CI: 46–66%), respectively.

Our results are in agreement with these findings. We selected Clupeiformes as the reference group, assuming it would have the lowest prevalence, and the results confirmed significantly higher prevalence levels in other groups, particularly for Gadiformes (which includes Gadidae and Merlucciidae) and Scombriformes (Trichiuridae). Higher prevalence rates were also observed in other fish orders, such as Salmoniformes and Lophiiformes (Lophiidae).

Regarding sample preparation for detection, the non-destructive method "no preparation" was chosen as the reference level. Our results indicated that the prevalence rates were 7 and 6 times higher for artificial digestion and Press/UV methods, respectively, both of which are destructive. This is in agreement with the findings from Mercken et al. (2020), who reported a prevalence of around 46% (95% CI: 36–57%) using destructive methods, compared to only 23% (95% CI: 16–33%) with visual inspection without preparation.

Destructive methods tend to allow a better observation of parasites, as shown by Gomez-Morales et al. (2018), who demonstrated the superior observation quality these methods afford. Levsen et al. (2005) further supported this notion, indicating that non-destructive methods exhibit a lower observation quality. This significant impact of destructive methods on prevalence likely stems from their ability to detect smaller and often overlooked parasites.

However, despite the superior sensitivity of destructive methods, they come with notable limitations. These include sampling bias due to the typically smaller sample sizes employed, resource constraints that make their application impractical for large

populations, and the loss of variability, which may result in either missing rare occurrences or overrepresenting clusters of parasites.

In our dataset analysis, 413 records were from wild-caught fish and 38 from aquaculture systems. The results show highly significant differences between the two systems, with aquaculture fish exhibiting a low prevalence level of 0.014 compared to wild-caught fish, which were used as the reference group. This finding aligns with Mercken et al. (2020), who also reported that wild-caught fish had a higher contamination rate than aquaculture fish. Similarly, the EFSA (2024) emphasized that the risk of anisakiasis for consumers is considered very low when consuming fish from aquaculture (Crotta et al., 2016; EFSA-BIOHAZ, 2024). Fish specimens in aquaculture systems are kept in net enclosures with small mesh sizes and fed a nematode-free artificial diet, significantly reducing the likelihood of Anisakidae transmission (Wootten et al., 2010). Thus, Anisakids infections in farmed fish have been documented, at very low occurence levels (Angot and Brasseur, 1993; Cammilleri et al., 2018; Torres et al., 2010).

2. Subgroup analysis

The meta-analysis by subgroup provides pooled estimates of prevalence levels that can be compared with other studies conducted under similar conditions or involving the same fish orders. However, no closely comparable studies were identified within the subgroups analyzed here. Levsen (2022), for instance, investigated the species diversity and spatial distribution of anisakid nematodes in Arctic stocks of Atlantic cod, saithe, and haddock, which belong to the Gadiformes family in the Atlantic region. In that study, the prevalence of Anisakis in Arctic cod and haddock exceeded 70%, aligning with the exceptionally high prevalence levels observed for the Gadiformes family in the Atlantic area in this meta-analysis.

Regarding the sample type and the organ sampled, whole fish displayed twice the percentage levels of contamination to gutted fish. Organ sampled fillets and fillets without belly flap had 0.34 and 0.21 times lower contamination levels compared to be the most contaminated. These results aligns with the strategies of parasite removals reported in EFSA 2024 scientific opinion as one of the most reliable methods to prevent parasites from being present in the edible parts of fish is the mechanical removal of parasites from infected areas. Prompt gutting can stop anisakids from migrating into the fish's muscle tissue, while filleting and trimming can further reduce the parasite load in the flesh.

Several studies have shown that removing most belly flaps through trimming can significantly decrease the presence of anisakid larvae in the fillets of various commercially valuable fish species. For instance, a recent study by Levsen et al. (2022) found that trimming the belly flaps reduced the occurrence of *A. simplex* larvae in saithe (*Pollachius virens*) fillets by 86%, and by over 90% in NE Arctic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). Additionally, this method reduced the presence of *Ph. decipiens* (s. l.) in NE Arctic cod fillets by at least 45% (Levsen et al., 2022). However , since the immediate cleaning may most likely be performed at the sea, the removed contaminated viscera can be thrown back into the sea and eaten by other species. The prevalence of infection may be then boosted through this practice (McCelland et al. 1990; Aibui et 2011).

3. Limitations of the current study

Despite the valuable insights gained from this study, several limitations should be acknowledged, as they may have influenced the interpretation of our results.

First, even though we selected a model that incorporates six factors potentially influencing *Anisakis* prevalence, each factor was tested independently, without exploring potential interactions between them. This approach, while useful for isolating the effect of individual variables, may have overlooked the complex relationships that exist between these factors. For example, the interaction between fish type and sampling stage could lead to variations in parasite detection, as the prevalence of *Anisakis* may differ depending on whether the fish is freshly caught or processed. Similarly, the production method (wild-caught vs. farmed) could interact with geographic location, given that environmental conditions, such as water temperature or pollution levels, vary regionally and may influence parasite prevalence differently in wild and farmed fish.

By not examining these potential interactions, we may have missed important synergies or antagonisms between variables, which could have led to different outcomes in the meta-regression analysis. Additionally, factors such as fish weight, length, and sex were not included due to the lack of consistent data reporting across studies, which limited data harmonization and completeness.

We also chose not to analyze lower taxonomic categories, such as individual fish species, as doing so would have significantly reduced the number of records available for analysis.

Similarly, analyzing smaller fishing subareas would have resulted in a loss of data due to the high occurrence of missing values (NAs) in these cases.

Recent studies have highlighted significant temporal trends in *Anisakis spp*. abundance. Fiorenza et al. (2020) conducted a meta-regression analysis, revealing a substantial increase in *Anisakis* spp. abundance (average number of worms per fish) over a 53-year period, from 1962 to 2015. In contrast, *Pseudoterranova* spp. showed no significant change in abundance over a shorter 37-year period, from 1978 to 2015. Additionally, Fiorenza et al. (2020) detected a significant rise in *Anisakis spp*. abundance within the prey species of marine mammals over a 36-year period, while *Pseudoterranova* spp. abundance remained stable during the same period.

Mastick et al (2024) similarly reported no change in *Pseudoterranova* spp. abundance over the years, reinforcing these findings. This contrast in trends between the two species raises interesting questions regarding ecological and environmental factors driving the proliferation of *Anisakis* spp.

In our analysis, the factor "year" was excluded from the first level of the meta-regression due to the high number of NAs (42 records approximately); however, it could have been a highly significant variable. Notably, a legislative law was introduced in Regulation (EC) No 853/2004, requiring food operators to implement control measures and visual inspections of fish intended for human consumption. This legislative change likely influenced the detection and reporting of *Anisakis* spp. over time. Had we considered this law, we could have analyzed trends before and after its implementation, potentially revealing important shifts in *Anisakis* spp. prevalence that coincide with changes in food safety regulations.

Conclusions and perspectives

In this study, we demonstrated the significant variability in the prevalence of *Anisakis* species across different fishing areas and fish orders (species). The widespread presence of anisakid larvae has critical food safety implications for all countries that consume fish (particularly in raw state). Our findings also align with previous studies, confirming lower prevalence levels in Mediterranean areas compared to the Atlantic, where many European countries share borders and offer a diverse range of fish species in their markets, increasing the likelihood that infected fish could reach consumers.

The Gadiformes family exhibited particularly high prevalence rates, including cod, a widely consumed species in Portugal and Europe. Although farmed fish is generally considered safer for consumption, it is not entirely risk-free.

This systematic review and meta-analysis has provided preliminary data on potential prevalence across European countries without requiring extensive, time-consuming laboratory analyses. However, it is essential to note that additional data are currently being gathered to complete the dataset. Furthermore, while this meta-analysis offers valuable insights, it cannot substitute a comprehensive exposure risk assessment study with homogeneous sampling across all European countries.

The Anisakidae family primarily consists of three genera that pose a threat to human health: *Anisakis*, *Contracaecum*, and *Phocanema* (previously known as *Pseudoterranova*). Future analyses should focus on the latter two genera, as they include several species capable of affecting humans. Our initial analysis was limited to fillet samples, but future steps could involve examining prevalence in the viscera and analyzing studies that report results for whole fish.

Having a complete dataset covering all studies on Anisakid parasites, other factors can also be explored, such as comparing prevalence in freshwater fish versus marine fish, and estimating pooled prevalences by fish species. Additionally, factors excluded from our best-fit model, such as the sampling stage, could be reintegrated for further analysis.

This work opens up interesting avenues for future research. One of them is to conduct a meta-analysis on parasite intensity and abundance, since these measurements are directly linked to human risk exposure, especially if parasite quantities are determined by fillet weight. However, this will require further data on the average fillet weight for each fish species.

Tailored study plans could be developed based on our meta-analysis findings, targeting the most contaminated regions and species, rather than conducting broad laboratory experiments on all fish available in the market.

References

- Aibinu, I. E., Smooker, P. M., & Lopata, A. L. (2019). Anisakis nematodes in fish and shellfish- from infection to allergies. *International Journal for Parasitology. Parasites and Wildlife, 9*, 384–393. https://doi.org/10.1016/j.ijppaw.2019.04.007
- Amer, O. S. A. (2014). The impact of fish parasites on human health: Review article. *Journal of the Egyptian Society of Parasitology*, *44*(1), 249–274. https://doi.org/10.12816/0006465
- Amir, A., Ngui, R., Wan Ismail, W. H., Wong, K. T., Ong, J. S. K., Lim, Y. A. L., Lau, Y.-L., & Mahmud, R. (2016). Case report: Anisakiasis causing acute dysentery in Malaysia. *American Journal of Tropical Medicine and Hygiene*, *95*(2), 410-412-410–412. https://doi.org/10.4269/ajtmh.16-0007
- Anderson, R. C. (1992). Nematode parasites of vertebrates: Their development and transmission. 2nd edition. Introduction. *Nematode Parasites of Vertebrates:* 1–16. https://doi.org/10.1079/9780851994215.0001
- Andreassen, J., & Jorring, K. (1970). Anisakinosis in Denmark. Infection with nematode larvae from marine fishes. *Nordisk Medicin*, *84*(47), 1492–1495.
- Ángeles-Hernández, J. C., Gómez-de Anda, F. R., Reyes-Rodríguez, N. E., Vega-Sánchez, V., García-Reyna, P. B., Campos-Montiel, R. G., Calderón-Apodaca, N. L., Salgado-Miranda, C., & Zepeda-Velázquez, A. P. (2020). Genera and species of the Anisakidae family and their geographical distribution. *Animals : An Open Access Journal from MDPI*, *10*(12), 2374. https://doi.org/10.3390/ani10122374
- Angot, V., & Brasseur, P. (1993). Épidémiologie et prophylaxie 014 de l'anisakiase enquête sur l'infestation de 3 espèces de consommation courante en France. *Journal de Pédiatrie et de Puériculture*, *6*(4), 240–242. https://doi.org/10.1016/S0987-7983(05)80501-8
- Arai, T., Akao, N., Seki, T., Kumagai, T., Ishikawa, H., Ohta, N., Hirata, N., Nakaji, S., Yamauchi, K., Hirai, M., Shiratori, T., Kobayashi, M., Fujii, H., Ishii, E., Naito, M., Saitoh, S., Yamaguchi, T., Shibata, N., Shimo, M., & Tokiwa, T. (2014). Molecular genotyping of Anisakis larvae in middle eastern japan and endoscopic evidence for preferential penetration of normal over atrophic mucosa. *Plos One*, *9*(2). https://doi.org/10.1371/journal.pone.0089188

- Arcos, S. C., Robertson, L., Ciordia, S., Sánchez-Alonso, I., Careche, M., Carballeda-Sanguiao, N., Gonzalez-Muñoz, M., & Navas, A. (2020). Quantitative proteomics comparison of total expressed proteomes of Anisakis simplex sensu stricto, A. pegreffii, and their hybrid genotype. *Genes*, 11(8), Article 8. https://doi.org/10.3390/genes11080913
- Audicana, M. T., & Kennedy, M. W. (2008). Anisakis simplex: From obscure infectious worm to inducer of immune hypersensitivity. *Clinical Microbiology Reviews*, *21*(2), 360–379. Scopus. https://doi.org/10.1128/CMR.00012-07
- Azevedo, R. K. D., Abdallah, V. D., & Luque, J. L. (2007). Ecologia da comunidade de metazoários parasitos do acará Geophagus brasiliensis (Quoy e Gaimard, 1824) (Perciformes: Cichlidae) do rio Guandu, Estado do Rio de Janeiro, Brasil. *Acta Scientiarum. Biological Sciences*, *28*(4), 403–411. https://doi.org/10.4025/actascibiolsci.v28i4.406
- Baird, F. J., Gasser, R. B., Jabbar, A., & Lopata, A. L. (2014). Foodborne anisakiasis and allergy. *Molecular and Cellular Probes*, *28*(4), 167-174-167–174. https://doi.org/10.1016/j.mcp.2014.02.003
- Bao, M., Giulietti, L., Levsen, A., & Karlsbakk, E. (2023). Resurrection of genus Phocanema Myers, 1959, as a genus independent from Pseudoterranova Mozgovoĭ, 1953, for nematode species (Anisakidae) parasitic in pinnipeds and cetaceans, respectively. *Parasitology International*, 97, 102794. https://doi.org/10.1016/j.parint.2023.102794
- Bao, M., Pierce, G. J., Pascual, S., González-Muñoz, M., Mattiucci, S., Mladineo, I., Cipriani, P., Bušelić, I., & Strachan, N. J. C. (2017). Assessing the risk of an emerging zoonosis of worldwide concern: Anisakiasis. *Scientific Reports*, *7*(1), 43699. https://doi.org/10.1038/srep43699
- Baptista-Fernandes, T., Rodrigues, M., Castro, I., Paixão, P., Pinto-Marques, P., Roque, L., Belo, S., Ferreira, P. M., Mansinho, K., & Toscano, C. (2017). Human gastric hyperinfection by Anisakis simplex: A severe and unusual presentation and a brief review. *International Journal of Infectious Diseases*, *64*, 38–41. https://doi.org/10.1016/j.ijid.2017.08.012
- Borges, J. N., Cunha, L. F. G., Santos, H. L. C., Monteiro-Neto, C., & Santos, C. P. (2012). Morphological and molecular diagnosis of Anisakid nematode larvae

from cutlassfish (trichiurus lepturus) off the coast of Rio de Janeiro, Brazil. *PLoS One*, *7*(7), 40447–40447. https://doi.org/10.1371/journal.pone.0040447

- Burnham & Anderson. (2002). *Model Selection and Multimodel Inference*.
- Cammilleri, G., Costa, A., Graci, S., Buscemi, M. D., Collura, R., Vella, A., Pulvirenti, A., Cicero, A., Giangrosso, G., Schembri, P., & Ferrantelli, V. (2018). Presence of Anisakis pegreffii in farmed Sea bass (Dicentrarchus labrax L.) commercialized in southern Italy: A first report. *Veterinary Parasitology*, *259*, 13-16-13–16. https://doi.org/10.1016/j.vetpar.2018.06.021
- Cipriani, P., Acerra, V., Bellisario, B., Sbaraglia, G. L., Cheleschi, R., Nascetti, G., & Mattiucci, S. (2016). Larval migration of the zoonotic parasite Anisakis pegreffii (Nematoda: Anisakidae) in European anchovy, Engraulis encrasicolus: Implications to seafood safety. *Food Control*, *59*, 148-157-148–157. https://doi.org/10.1016/j.foodcont.2015.04.043
- Cipriani, P., Giulietti, L., Bao, M., Palomba, M., Mattiucci, S., & Levsen, A. (2024). *Post-mortem* tissue migration of Anisakis simplex (s.s.) larvae (Nematoda: Anisakidae) in three commercially harvested fish species from the Northeast Atlantic: The role of storage time and temperature. *Food Control*, *157*, 110162. https://doi.org/10.1016/j.foodcont.2023.110162
- Cochran, W. G. (1954). The combination of estimates from different experiments. *Biometrics*, *10*(1), 101–129. https://doi.org/10.2307/3001666
- Crotta, M., Ferrari, N., & Guitian, J. (2016). Qualitative risk assessment of introduction of Anisakid larvae in Atlantic salmon (Salmo salar) farms and commercialization of products infected with viable nematodes. *Food Control*, *69*, 275–284. https://doi.org/10.1016/j.foodcont.2016.04.058
- Eiras, J. C., Pavanelli, G. C., Takemoto, R. M., & Nawa, Y. (2018). Fish-borne nematodiases in South America: Neglected emerging diseases. *Journal of Helminthology*,*92*(6). https://doi.org/10.1017/S0022149X17001006
- Espiñeira, M., Vieites, J. M., & Santaclara, F. J. (2010). Species authentication of octopus, cuttlefish, bobtail and bottle squids (families Octopodidae, Sepiidae and Sepiolidae) by FINS methodology in seafoods. *Food Chemistry*, *121*(2), 527–532. https://doi.org/10.1016/j.foodchem.2009.12.042

- EC DG- MARE (European Commission: Directorate-General for Maritime Affairs and Fisheries. (2023). *The EU fish market – 2023 edition*. Publications Office of the European Union. https://doi.org/10.2771/38507
- FAO. (2022). Consumption of aquatic foods. https://doi.org/10.4060/cc0461en
- Fiorenza, E. A., Wendt, C. A., Dobkowski, K. A., King, T. L., Pappaionou, M., Rabinowitz, P., Samhouri, J. F., & Wood, C. L. (2020). It's a wormy world: Meta-analysis reveals several decades of change in the global abundance of the parasitic nematodes Anisakis spp. and Pseudoterranova spp. in marine fishes and invertebrates. *Global Change Biology*, *26*(5). https://doi.org/10.1111/gcb.15048
- Gasser, R. B., & Monti, J. R. (1997). Identification of parasitic nematodes by PCR-SSCP of ITS-2 rDNA. *Molecular and Cellular Probes*, *11*(3), 201–209. https://doi.org/10.1006/mcpr.1997.0106
- Gazzonis, A. L., Cavallero, S., Zanzani, S. A., Olivieri, E., Malandra, R., Ranghieri, V., D'Amelio, S., & Manfredi, M. T. (2017). Anisakis sp. and Hysterothylacium sp. larvae in Anchovies (Engraulis encrasicolus) and Chub mackerel (*Scomber colias*) in the Mediterranean Sea: Molecular identification and risk factors. *Food Control*, *80*. https://doi.org/10.1016/j.foodcont.2017.05.004
- Glass, G.V.,1976. Primary, secondary and meta-analysis of research. *Educational Research*. 5 (1, 3), 3-8
- Golden, O., Caldeira, A. J. R., & Santos, M. J. (2022). Raw fish consumption in Portugal: A survey on trends in consumption and consumer characteristics. *Food Control*, *135*, 108810. https://doi.org/10.1016/j.foodcont.2022.108810
- Gómez-Morales, M. A., Castro, C. M., Lalle, M., Fernández, R., Pezzotti, P., Abollo, E., & Pozio, E. (2018). UV-press method versus artificial digestion method to detect Anisakidae L3 in fish fillets: Comparative study and suitability for the industry. *Fisheries Research*, *202*, 22–28. https://doi.org/10.1016/j.fishres.2016.12.011
- Gonzales-Barron U, Butler F. The use of meta-analytical tools in risk assessment for food safety. *Food Microbiol.* 2011 Jun;28(4):823-7. https://doi.org/10.1016/j.fm.2010.04.007
- Gonzales-Barron, U., Cadavez, V., Thebault, A., & Kooh, P. (2021). The Pathogens-in-Foods Database (PIF) (Version 1; https://pif.esa.ipb.pt/). Zenodo. https://doi.org/10.5281/zenodo.8186278

- Gonzales-Barron, U., Cadavez, V., Faria, A. S., Guillier, L., Thebault, A., & Kooh, P. (2022). Review Protocol for the "Pathogens in Foods" Database: Prevalence and Concentration of Main Biological Hazards in Food Matrices (Version 2). Zenodo. https://doi.org/10.5281/zenodo.7850017
- Gordon M, Lumley T (2024). *forestplot: Advanced Forest Plot Using 'grid' Graphics*. R package version 3.1.5, https://github.com/gforge/forestplot
- Guardone, L., Armani, A., Nucera, D., Costanzo, F., Mattiucci, S., & Bruschi, F. (2018). Human anisakiasis in Italy: A retrospective epidemiological study over two decades. *Parasite*, *25*, 41. https://doi.org/10.1051/parasite/2018034
- Gushchin, A. V., & Corten, A. (2016). Feeding of pelagic fish in waters of Mauritania: 2. Representatives of Families Carangidae, Scombridae, Pomatomidae, and Trichiuridae. *Journal of Ichthyology*, *56*(1), 98–106. https://doi.org/10.1134/S0032945216010045
- Hazards (EFSA, BIOHAZ), E. P. on B. (2010). Scientific Opinion on risk assessment of parasites in fishery products. EFSA Journal, 8(4), 1543. https://doi.org/10.2903/j.efsa.2010.1543
- Hazards (EFSA, BIOHAZ), E. P. on B., Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bover-Cid, S., Chemaly, M., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Nonno, R., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Buchmann, K., Careche, M., Bolton, D. (2024). Re-evaluation of certain aspects of the EFSA Scientific Opinion of April 2010 on risk assessment of parasites in fishery products, based on new scientific data. Part 1: ToRs1–3. *EFSA Journal*, *22*(4), e8719. https://doi.org/10.2903/j.efsa.2024.8719
- Herrador, Z., Daschner, Á., Perteguer, M. J., & Benito, A. (2019). Epidemiological scenario of anisakidosis in Spain based on associated hospitalizations: The tip of the iceberg. *Clinical Infectious Diseases*, *69*(1), 69–76. https://doi.org/10.1093/cid/ciy853
- Higgins, J. P. T., Thompson, S. G., Deeks, J. J., & Altman, D. G. (2003). Measuring inconsistency in meta-analyses. *British Medical Journal*, *327*(7414), 557–560.
- Jacobsen, K. B., & Berland, B. (1969). Fish nematodes as a cause of acute and chronic gastroenteritis with tissue eosinophilia. *Nordisk Medicin*, *82*(36), 1104–1111.

- Køie, M., & Fagerholm, H. P. (1995). The life cycle of Contracaecum osculatum (Rudolphi, 1802) sensu stricto (Nematoda, Ascaridoidea, Anisakidae) in view of experimental infections. *Parasitology Research*, *81*(6), 481–489. https://doi.org/10.1007/BF00931790
- Kooh, P., Gonzales-Barron, U., Cadavez, V., Talleu, R., El Metennani, S., Gay, M., & Thébault, A. (2023). *Systematic Review Protocol for the "Pathogens in Foods" Database: Occurrence of parasites of public health importance in fishery products*. https://zenodo.org/records/10270810
- Kojima, G., Usuki, S., Mizokami, K., Tanabe, M., & Machi, J. (2013). Intestinal anisakiasis as a rare cause of small bowel obstruction. American Journal of Emergency Medicine, 31(9), 1422.e1-1422.e2-1422.e1-1422.e2. https://doi.org/10.1016/j.ajem.2013.05.015
- Kuhn, T., Cunze, S., Kochmann, J., & Klimpel, S. (2016). Environmental variables and definitive host distribution: A habitat suitability modelling for endohelminth parasites in the marine realm. *Scientific Reports*, *6*. https://doi.org/10.1038/srep30246
- Lee, W. J., Seo, D. J., Oh, H., Jeon, S. B., Jung, D., & Choi, C. (2016). Simultaneous detection and prevalence of allergens in anisakis species isolated from marine fishes. *Journal of Food Protection*, *79*(5), 789-794-789–794. https://doi.org/10.4315/0362-028X.JFP-15-539
- Levsen, A., Cipriani, P., Palomba, M., Giulietti, L., Storesund, J. E., & Bao, M. (2022). Anisakid parasites (Nematoda: Anisakidae) in 3 commercially important gadid fish species from the southern Barents Sea, with emphasis on key infection drivers and spatial distribution within the hosts. Parasitology, 149(14), 1942–1957. https://doi.org/10.1017/S0031182022001305
- Levsen, A., Lunestad, B. T., & Berland, B. (2005). Low detection efficiency of candling as a commonly recommended inspection method for nematode larvae in the flesh of pelagic fish. *Journal of Food Protection*, *68*(4), 828–832. https://doi.org/10.4315/0362-028x-68.4.828
- Li, S.-W., Shiao, S.-H., Weng, S.-C., Liu, T.-H., Su, K.-E., & Chen, C.-C. (2015). A case of human infection with *Anisakis simplex* in Taiwan. *Gastrointestinal Endoscopy*, *82*(4), 757–758. https://doi.org/10.1016/j.gie.2015.03.1983

- Llarena-Reino, M., Abollo, E., Regueira, M., Rodríguez, H., & Pascual, S. (2015). Horizon scanning for management of emerging parasitic infections in fishery products. *Food Control*, *49*. https://doi.org/10.1016/j.foodcont.2013.09.005
- Łopieńska-Biernat, E., Stryiński, R., Polak, I., Pawlikowski, B., Pawlak, J., & Podolska, M. (2020). Effect of freezing on the metabolic status of L3 larvae of Anisakis simplex s. S. *Infection, Genetics and Evolution*, *82*. https://doi.org/10.1016/j.meegid.2020.104312
- Lunneryd, S.-G., Boström, M. K., & Aspholm, P. E. (2015). Sealworm (Pseudoterranova decipiens) infection in Grey seals (Halichoerus grypus), cod (Gadus morhua) and shorthorn sculpin (Myoxocephalus scorpius) in the Baltic Sea. *Parasitology Research*, *114*(1), 257–264. https://doi.org/10.1007/s00436-014-4187-z
- Mastick, N. C., Fiorenza, E., & Wood, C. L. (2024). Meta-analysis suggests that, for marine mammals, the risk of parasitism by Anisakids changed between 1978 and 2015. *Ecosphere*, *15*(3), e4781. https://doi.org/10.1002/ecs2.4781
- Mattiucci, S., Cianchi, R., Nascetti, G., Paggi, L., Sardella, N., Timi, J., Webb, S. C., Bastida, R., Rodríguez, D., & Bullini, L. (2003). Genetic evidence for two sibling species within Contracaecum ogmorhini Johnston & Mawson, 1941 (Nematoda: Anisakidae) from otariid seals of boreal and austral regions. *Systematic Parasitology*, *54*(1). https://doi.org/10.1023/A:1022145926409
- Mattiucci, S., Fazii, P., De Rosa, A., Paoletti, M., Megna, A. S., Glielmo, A., De Angelis, M., Costa, A., Meucci, C., Calvaruso, V., Sorrentini, I., Palma, G., Bruschi, F., & Nascetti, G. (2013). Anisakiasis and gastroallergic reactions associated with Anisakis pegreffii infection, Italy. *Emerging Infectious Diseases*, *19*(3), 496-499-496–499. https://doi.org/10.3201/eid1903.121017
- Mattiucci, S., Giulietti, L., Paoletti, M., Cipriani, P., Gay, M., Levsen, A., Klapper, R., Karl, H., Bao, M., Pierce, G. J., & Nascetti, G. (2018). Population genetic structure of the parasite Anisakis simplex (s. S.) collected in Clupea harengus L. from North East Atlantic fishing grounds. *Fisheries Research*, *202*, 103-111-103–111. https://doi.org/10.1016/j.fishres.2017.08.002
- Mattiucci, S., & Nascetti, G. (2008). Advances and trends in the molecular systematics of Anisakid nematodes, with implications for their evolutionary

ecology and host-parasite co-evolutionary processes. *Advances in Parasitology*, *66*, 47–148. https://doi.org/10.1016/S0065-308X(08)00202-9

- McClelland. (1990). Larval anisakine nematodes in various fish species from Sable Island Bank and vicinity. *Population Biology Of Sealworm, 83-118.*
- Mercken, E., Van Damme, I., Serradell, A., & Gabriël, S. (2020). Presence of Anisakidae in commercial fish species imported into the Belgian food markets: A systematic review and meta-analyses. *International Journal of Food Microbiology*, *318*. https://doi.org/10.1016/j.ijfoodmicro.2019.108456
- Mladineo, I., Popovic, M., Drmic-Hofman, I., & Poljak, V. (2016). A case report of Anisakis pegreffii (Nematoda, Anisakidae) identified from archival paraffin sections of a Croatian patient. *Bmc Infectious Diseases*, *16*. https://doi.org/10.1186/s12879-016-1401-x
- Mossali, C., Palermo, S., Capra, E., Piccolo, G., Botti, S., Bandi, C., D'Amelio, S., & Giuffra, E. (2010). Sensitive detection and quantification of nisakid parasite residues in food products. *Foodborne Pathogens and Disease*, *7*(4), 391-397-391–397. https://doi.org/10.1089/fpd.2009.0428
- Noble, J.H., 2006. Meta-analysis: methods, strengths, weaknesses and political uses. *Journal of Evaluation in Clinical Practice* .147 (1), 7-20.
- Onitsuka, C., Nakamura, K., Wang, D., Matsuda, M., Tanaka, R., Inoue, Y., Kuroda, R., Noda, T., Negoro, K., Negoro, T., & Namihira, T. (2022). Inactivation of anisakis larva using pulsed power technology and quality evaluation of horse mackerel meat treated with pulsed power. *Fisheries Science*, *88*(2), 337–344. Scopus. https://doi.org/10.1007/s12562-022-01593-2
- Orphanet. (2016). *Prevalence and incidence of rare diseases. Available at: https://www.orphadata.com/*
- Paoletti, M., Mattiucci, S., Colantoni, A., Levsen, A., Gay, M., & Nascetti, G. (2018). Species-specific Real Time-PCR primers/probe systems to identify fish parasites of the genera *Anisakis*, *Pseudoterranova* and *Hysterothylacium* (Nematoda: Ascaridoidea). *Fisheries Research*, *202*, 38–48. https://doi.org/10.1016/j.fishres.2017.07.015
- Qin, Y., Zhao, Y., Ren, Y., Zheng, L., Dai, X., Li, Y., Mao, W., & Cui, Y. (2013). Anisakiasis in China: The First Clinical Case Report. *Foodborne Pathogens and Disease*, *10*(5), 472–474. https://doi.org/10.1089/fpd.2012.1325

- Rahmati, A. R., Kiani, B., Afshari, A., Moghaddas, E., Williams, M., & Shamsi, S. (2020). World-wide prevalence of Anisakis larvae in fish and its relationship to human allergic anisakiasis: A systematic review. *Parasitology Research*, *119*(11), 3585-3594-3585–3594. https://doi.org/10.1007/s00436-020-06892-0
- Roca-Geronès, X., Alcover, M. M., Godínez-González, C., González-Moreno, O., Masachs, M., Fisa, R., & Montoliu, I. (2020). First molecular diagnosis of clinical cases of gastric anisakiosis in Spain. *Genes*, *11*(4). https://doi.org/10.3390/genes11040452
- Rodríguez, N. E. R., Sánchez, V. V., Anda, F. R. G. D., Reyna, P. B. G., Rosa, L. G. D. L., & Zepeda-Velázquez, A. P. (2020). Species of Anisakidae nematodes and Clinostomum spp. Infecting lisa Mugil curema (Mugilidae) intended for human consumption in Mexico. *Revista Brasileira de Parasitologia Veterinária*, *29*(1), e017819. https://doi.org/10.1590/s1984-29612020002
- Rothstein, H. (2007). Publication bias as a threat to the validity of meta-analytic results. *Journal of Experimental Criminology*, 4, 61–81. https://doi.org/10.1007/s11292-007-9046-9
- Sánchez-Alonso, I., Carballeda-Sangiao, N., González-Muñoz, M., Navas, A., Arcos, S. C., Mendizábal, A., Cuesta, F., & Careche, M. (2020). Freezing kinetic parameters influence allergenic and infective potential of *Anisakis simplex* L3 present in fish muscle. *Food Control*, *118*, 107373. https://doi.org/10.1016/j.foodcont.2020.107373
- Sánchez-Alonso, I., Carballeda-Sangiao, N., Rodríguez, S., Tejada, M., Navas, A., Arcos, S. C., González-Muñoz, M., & Careche, M. (2021). Anisakis simplex (s.l.) resistance to the action of gastric enzymes depends upon previous treatments applied to infected fish mince and affects antigen release. *Journal of the Science of Food and Agriculture*, *101*(9), 3908–3916. https://doi.org/10.1002/jsfa.11031
- Santana-Arocha, Z., Jurado-Ruzafa, A., & Foronda, P. (2023). *Estudio de la prevalencia de anisákidos zoonóticos en Sardina pilchardus (sardina de ley), Sardinella aurita (alacha), Sardinella maderensis (machuelo), Scomber colias (caballa) y Trachurus picturatus (chicharro).*
- Seesao, Y. (2015). *Caractérisation des Anisakidae dans les poissons marins: Développement d'une méthode d'identification par séquençage à haut-débit et*

étude de prévalence [Phdthesis, Université du Droit et de la Santé - Lille II]. https://theses.hal.science/tel-01412878

- Serracca, L., Battistini, R., Rossini, I., Carducci, A., Verani, M., Prearo, M., Tomei, L., Montis, G., & Ercolini, C. (2014). Food safety considerations in relation to Anisakis pegreffii in Anchovies (Engraulis encrasicolus) and Sardines (Sardina pilchardus) fished off the Ligurian Coast (Cinque Terre National Park, NW Mediterranean). *International Journal of Food Microbiology*, 190C, 79–83. https://doi.org/10.1016/j.ijfoodmicro.2014.08.025
- Sargeant, J.M., Amezcua, M., Rajic, A., Waddell, L., 2005. A guide to conducting systematic reviews in agri-food public health. Available at: Food Safety Research and Response Network, Canada http://www.fsrrn.net/UserFiles/File/ conductingsysreviewsenglish[1].pdf.
- Shamsi, S., & Barton, D. P. (2023). A critical review of anisakidosis cases occurring globally. *Parasitology Research*, *122*(8), 1733–1745. https://doi.org/10.1007/s00436-023-07881-9
- Shamsi, S., & Butcher, A. R. (2011). First report of human anisakidosis in Australia. *Medical Journal of Australia*, *194*(4). https://www.mja.com.au/journal/2011/194/4/first-report-human-anisakidosis-australia
- Shamsi, S., Stoddart, A., Smales, L., & Wassens, S. (2019). Occurrence of Contracaecum bancrofti larvae in fish in the Murray-Darling Basin. *Journal Of Helminthology*,(93)574-579-574–579. https://doi.org/10.1017/S0022149X1800055X
- Smith, J. W., & Wootten, R. (1978). Anisakis and anisakiasis. *Advances in Parasitology*, *16*, 93–163. https://doi.org/10.1016/s0065-308x(08)60573-4
- Sohn, W.-M., Na, B.-K., Kim, T. H., & Park, T.-J. (2015). Anisakiasis: Report of 15 gastric cases caused by Anisakis type I larvae and a brief review of Korean anisakiasis cases. *Korean Journal of Parasitology*, *53*(4), 465-470-465–470. https://doi.org/10.3347/kjp.2015.53.4.465
- Sutton, A.J., Abram, K.R., Jones, D.R., 2001. An illustrated guide to the methods of meta-analysis. *Journal Of Evaluation In Clinical Practice*. 7 (2), 135-148.

- Suzuki, J., Murata, R., & Kodo, Y. (2021). Current Status of Anisakiasis and Anisakis Larvae in Tokyo, Japan. *Food Safety*, *9*(4), 89–100. https://doi.org/10.14252/foodsafetyfscj.D-21-00004
- Tang, Z.-L., Huang, Y., & Yu, X.-B. (2016). Current status and perspectives of Clonorchis sinensis and clonorchiasis: Epidemiology, pathogenesis, omics, prevention and control. *Infectious Diseases of Poverty*, *5*(1), 71. https://doi.org/10.1186/s40249-016-0166-1
- *The state of World fisheries and aquaculture 2024*. (n.d.). https://doi.org/10.4060/cd0683en
- *The fisheries and aquaculture sector in France , FranceAgriMer 2018.* https://www.franceagrimer.fr/fam/content/download/61053/document/a4-CC%20p%C3%AAche%202019%20eng-CORRIGE.pdf?version=8
- Torres, P., Quintanilla, J. C., Rozas, M., Miranda, P., Ibarra, R., Martín, M. F. S., Raddatz, B., Wolter, M., Villegas, A., Canobra, C., Hausdorf, M., & Silva, R. (2010). Endohelminth parasites from salmonids in intensive culture from southern Chile. *Journal Of Parasitology*, *96*(3), 669-670-669–670. https://doi.org/10.1645/GE-2211.1
- Trabelsi, N., Nalbone, L., Marotta, S. M., Taamali, A., Abaza, L., & Giarratana, F. (2019). Effectiveness of five flavored Tunisian olive oils on Anisakis larvae type 1: Application of cinnamon and rosemary oil in industrial anchovy marinating process. *Journal of the Science of Food and Agriculture*, *99*(10), 4808–4815. https://doi.org/10.1002/jsfa.9736
- Urawa, S. « Database of parasite in Fish or sellfish. D-PAF.Anisakis simplex. » https://fishparasite.fs.a.u-tokyo.ac.jp/index-eng.html
- Umehara, A., Kawakami, Y., Araki, J., & Uchida, A. (2008). Multiplex PCR for the identification of *Anisakis simplex* sensu stricto, *Anisakis pegreffii* and the other anisakid nematodes. *Parasitology International*, *57*(1), 49–53. https://doi.org/10.1016/j.parint.2007.08.003
- Vidaček, S., Heras, C. D. L., Solas, M. T., García, M. L., Mendizábal, A., & Tejada, M. (2011). Viability and Antigenicity of *Anisakis simplex* after Conventional and Microwave Heating at Fixed Temperatures. *Journal of Food Protection*, *74*(12), 2119–2126. https://doi.org/10.4315/0362-028X.JFP-11-108

- Wagenmakers, E.-J., & Farrell, S. (2004). AIC model selection using Akaike weights. *Psychonomic Bulletin & Review*, *11*(1), 192–196. https://doi.org/10.3758/BF03206482
- Wang, H., Dai, Y., Huang, S., Rong, S., Qi, Y., & Li, B. (2023). A new perspective on special effective interventions for metabolic syndrome risk factors: A systematic review and meta-analysis. *Frontiers in Public Health*, *11*, 1133614. https://doi.org/10.3389/fpubh.2023.1133614
- Wootten, R., Yoon, G., & Bron, J. (2010). *A Survey of Anisakid Nematodes in Scottish Wild Atlantic Salmon*.
- Xu, X., Sui, J., Cao, L., & Lin, H. (2010). Direct competitive enzyme-linked immunosorbent assay (ELISA) for rapid screening of anisakid larvae in seafood. *Journal of the Science of Food and Agriculture*, *90*(5), 877–881. https://doi.org/10.1002/jsfa.3898
- Yorimitsu, N., Hiraoka, A., Utsunomiya, H., Imai, Y., Tatsukawa, H., Tazuya, N., Yamago, H., Shimizu, Y., Hidaka, S., Tanihira, T., Hasebe, A., Miyamoto, Y., Ninomiya, T., Abe, M., Hiasa, Y., Matsuura, B., Onji, M., & Michitaka, K. (2013). Colonic intussusception caused by Anisakiasis: A case report and review of the literature. *Internal Medicine*, *52*(2), 223–226. https://doi.org/10.2169/internalmedicine.52.8629
- Zhao, W.-T., Lü, L., Chen, H.-X., Yang, Y., Zhang, L.-P., & Li, L. (2016). Ascaridoid parasites infecting in the frequently consumed marine fishes in the coastal area of China: A preliminary investigation. *Parasitology International*, *65*(2), 87–98. https://doi.org/10.1016/j.parint.2015.11.002
- (N.d.). Retrieved September 10, 2024, from https://fishparasite.fs.a.u-tokyo.ac.jp/Anisakis%20simplex/Anisakis-simplex-eng.html

Softwares:

- Bartoń K (2024). *MuMIn: Multi-Model Inference*. R package version 1.48.4, https://CRAN.R-project.org/package=MuMIn.
- DistillerSR. Version 2.35. DistillerSR Inc.; 2023. Accessed May-June 2023. https://www.distillersr.com/.
- R Core Team (2023). _R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org.

- Viechtbauer W (2010). “Conducting meta-analyses in R with the metafor package”. Journal of Statistical Software, 36(3), 148. doi:10.18637/jss.v036.i03.

Annexes

Annex 1: Geographical labels for world map with geographical location of the parasites of the Anisakidae family, collected from different hosts.

Map	Localization/Continent	Map	Localization/Continent	Map	Localization/Continent
1	Australia/Oceania	18	North of Morocco/Africa	35	Coast of Argentina/ South America
2	Victoria Australia/ Oceania	19	Taiwanese waters/Asia	36	Aegean Sea, Turkey/ Europe and Asia
3	St. Paul Island, Alaska/North America	20	Senegal/Africa	37	Argentina, Sea of Patagonia/ South America
4	Japanese waters/Asia	21	Mediterranean coast, Spain/Europe and Africa	38	Alexandria City, Mediterranean Sea, Egypt/Africa
5	Water Australia/Oceania	22	Brazilian waters/South America	39	Minas Gerais/ South America
6	Mediterranean sea/ Europe, Africa and Asia	23	Baltic Sea/Europe	40	South east Chilean coast/South America
7	Atlantic Ocean	24	Hudson Bay and Hudson Strait, Canada/North America	41	Hurghada City, Gulf of Suez, Red Sea, Egypt/Africa
8	South Africa/African	25	Caspian Sea/between Europe and Asia	42	Tyrrhenian coast of southern Italy/Europe
9	Macquarie Island, Pacific Ocean southwest/ Asia and Oceania	26	Rio de Janeiro, Brazil/South America	43	Faeroe Islands/ Europe
10	New Caledonia/Oceania	27	Indonesia/Asia	44	Halfway Island, Australia/Oceania
11	Philippine archipelago/Asia	28	Madeiran waters Portugal/African	45	Hawaii/North America
12	Northern Adriatic Sea/Europe	29	Chile/South America	46	Louisiana, USA/ North America
13	Zhoushan, Zhejiang, China/Asia	30	Colombia/ South America	47	Twynams Paar, Ceylon, South Australia and Queensland/Oceania
14	Republic of Korea/Asia	31	Southern New South Wales, Australia/Oceania	48	Natal, northern, Brazil/South America
15	Yellow Sea/Asia	32	Heron Island, Queensland. Australia/Oceania		
16	Sicily and Messina/Italy/Europe	33	Greek waters/Europe		
17	China Sea/Asia	34	Northern Gulf of Mexico/Central America		

Annex 2: Categorisation system of methods for fish parasites (based on Anisakids)

Sample Preparation

Type of preparation essay	Preparation essay and references
Press/UV	1. NF EN ISO 23036-1 (2021) 2. Karl et al., 1993 3. Other
Artificial digestion	1. NF EN ISO 23036-2 (2021) 2. CODEX STAN 244-2004 3. World Health Organisation, 2003 4. Other
Incubation at 15°C overnight	1. Shamsi et al. 2016
DNA extraction	1. REDExtraction-N-Amp tissue PCR Kit (Mossali et al., 2010) 2. Wizard Genomic DNA Clean-Up System (Lopez and Pardo, 2010) 3. Wizard Genomic DNA Purification Kit (Lopez and Pardo, 2010 ; Valentini et al., 2006 and Mattiucci et al., 2003) 4. Modified Wizard Genomic DNA Purification Kit (Lopez and Pardo, 2010) 5. QIAmp DNA Blood Mini Kit (Lopez and Pardo, 2010) 6. QIAamp DNA Mini Kit (Umehara et al.,2008) 7. DNeasy blood and tissue kit (Paoletti et al., 2018) 8. NaCl method (Lopez and Pardo, 2010) 9. Phenol chloroform method (Lopez and Pardo, 2010) 10. method of Sodium Dodecyl Sulphate(SDS)/proteinase K treatment (Fang et al., 2011 and Hu et al., 2001) 11. The DNA was extracted from 300 mg of fish muscle tissues and mixtures fish-parasite (Espiñeira et al. 2010) 12. Other DNA extraction method
No preparation	
Other	Specify :

Sample Detection (EssayDet)

Type of detection essay	Detection essay and references	Cutoff/ molecular target (and references)
Naked eye	1. Gay et al. 2019	
Candling table	1. Levsen et al. 2005 2. Gay et al. 2019	
Quantitative PCR (qPCR)/ real time PCR (rtPCR)		Genes used for qPCR : 1. ITS-1 (Mossali et al., 2010) 2. ITS-2 (Fang et al., 2011) 3. ARNr 18S (Mossali et al., 2010) 4. COX2 (used for processed products) (Lopez and Pardo, 2010) 5. Other
Automatic nematode identification system	1. Sivertsen et al. 2012	
Other	Specify:	

Sample Enumeration (Essay Enum)

Type of enumeration essay	Enumeration essay and references	Cutoff/ molecular target (and references)
Naked eye		
Candling table		
Molecular	1. Quantitative PCR (qPCR)/ real time PCR (rtPCR) 2. Quantitative fluorescence PCR (Fang et al. 2011) 3. Other	Genes used for qPCR : 1. ITS-1 (Mossali et al., 2010) 2. ITS-2 (Fang et al., 2011) 3. ARNr 18S (Mossali et al., 2010) 4. COX2 (used for processed products) (Lopez and Pardo, 2010) 5. Other
Other	1. Specify:	

Sample Infectivity (Essay Infectivity)

Type of infectivity essay	Infectivity essay and references
Staining	1. Malachite green staining and microscopic examination (Podolska et al., 2019) 2. Exposure of Anisakidae to different intensity of the fluorescent emission was rated in arbitrary units as maximum, medium, slight, or no fluorescence (Vidacek et al., 2010)
Immunology	1. Dot blot using rabbit anti-A. simplex crude extract polyclonal antibody and rabbit anti-recombinant (r)Ani s 4 polyclonal antisera (Carballeda-Sangiao et al., 2016) 2. Anti Ani s 4 and IgE western blotting (Rodriguez-Mahillo et al., 2007)
Microscopy	1. Optical microscopy observation (Sanchez-Alonso et al., 2021) 2. Scanning electron microscopy (SEM) observation. (Tejada et al., 2006) 3. Environmental scanning electron microscopy (ESEM) observation. (Tejada et al., 2006)
Metabolism	1. Assessment of the oxygen consumption rate (OCR) of Anisakis larvae during various mitochondrial respiration states (Sanchez-Alonso et al., 2019) 2. Observation of mobility by flexion stimulation with forceps and needle (EFSA-BIOHAZ, 2010) 3. Agar penetration test (Arizono et al., 2012)
RT-PCR	1. Łopieńska-Biernat et al.,2020
Other	Specify :

Sample Identification

Type of identification essay	Identification essay and references	Cutoff/ molecular target (and references)
Morphological identification	1. Naked eye 2. Microscopy (NF EN ISO 23036-2 (2021)) 3. Other *Allows to identify up to the genus of the anisakids According to the criteria of Berland (1961), Huang & Bussieras (1988) and Möller (1989*	

Biochemical methods	1. MEE (Multilocus Enzyme Electrophoresis)/ MEE/ isoenzyme typing (Mattiucci and Nascetti, 2008) 2. ELISA (enzyme-linked immunosorbent assay) -> antigene Ani s 7 (Xu et al., 2010) 3. Other	
Molecular method	1. PCR -> Specify the percentage of individuals whose PCR has to be carried out	Genes used for PCR: 1. mtDNA cytb (mitochondrial gene encoding cytochrome b) (Mattiucci et al., 2003) 2. COX 1 (code for mitochondrial cytochrome oxidase 1) (Blouin, 2002) 3. COX 2 (code for mitochondrial cytochrome oxidase 2) (Valentini et al., 2006) 4. ssrRNA (small subunit ribosomal RNA) (Hu et al., 2001) 5. IsrRNA (large subunit ribosomal RNA) (Hu et al., 2001) 6. ITS-1, Internal transcribed spacer 1 7. ITS-2, Internal transcribed spacer 2 8. Other ribosomal DNA sequence

Annex 3: Search terms for the Systematic review

General terms (Title-Keywords-Abstract):

"microbial quality" OR "microbial safety" OR "microbiological quality" OR "microbiological safety" OR analyses OR analysis OR concentration OR contamination OR count* OR detection OR enumeration OR incidence OR investigation OR occurrence OR presence OR prevalence OR sampling OR survey* OR abundance OR intensity

Fish parasites (Title-Keywords-Abstract):

Anisakids: Anisakidae OR anisakid* OR Anisakis OR Pseudoterranova OR Phocanema OR Contracaecum OR Phocanema OR "A. simplex" OR "A. pegreffii" OR "A. berlandi" OR "A. typica" OR "A. ziphidarum" OR "A. physeteris" OR "A. brevispiculata" OR "A. paggiae" OR "A. nascettii" OR "P. krabbei" OR "P. decipiens" OR "P. bulbosa" OR "P. azarasi" OR "P. cattani" OR "C. osculatum" OR "C. radiatum" OR "C. mirounga" OR "C. ogmorhini" OR "C. margolisi" OR "Ph. decipiens" OR "Ph. azarasi" OR "Ph. cattani" OR "Ph. krabbei" OR "Ph. bulbosa"

Foods (Title-Keywords-Abstract):

Seafood: seafood OR seafoods OR "sea food" OR "sea foods" OR crustacean* OR shellfish OR bivalve* OR mollusc* OR mollusk* OR fish* OR finfish* OR "fishery product*" OR "marine gasteropod*" OR cephalopod OR cephalopods OR crustacean OR crustaceans OR echinoderm OR echinoderms OR "sea urchin" OR "sea urchins" OR holoturid* OR tunicate OR tunicates OR urchin* OR crab* OR prawn* OR shrimp* OR lobster* OR "crayfish" OR crabfish OR crawfish OR langoustine OR scampi OR "clam" OR "clams" OR "carpet shell*" OR scallop* OR Pecten OR oyster* OR cockle OR cockles OR mussel OR mussels OR mytilus OR "Pen shell*" OR snail* OR abalone* OR Nassarius OR "whelk*" OR Bolinus OR "murex" OR ormer OR Haliotis OR "true limpet*"OR Patella OR Cellana OR Buccinum OR Concholepas OR conch* OR winkle* OR periwinkle* OR octopus OR squid* OR cuttlefish OR nautilus* OR Todarodes OR Loligo OR Sepia OR Paracentrotus OR Strongylocentrotus OR "Echinus esculentus" OR "sea cucumber*" OR "cukes" OR piure* OR pyura OR "sea violet*" OR "sea tulip*" OR "sea peach*" OR "sea pineapple*" OR "ice floe" OR "sea squirt*" OR gravad OR graved OR "gravad lax" OR gravlax OR sushi OR sashimi OR surimi OR ceviche OR caviar OR albacore OR amberjack OR anchovy OR anchovies OR angler OR anglerfish* OR anguilla OR argentine OR Argyrosomus OR bacha OR barbel OR barracuda OR basa OR bass OR beluga OR bib OR bigeye OR blackfish OR bleak OR blenny OR bluefish OR "blue runner" OR "blue shark" OR bonito

OR branzino OR bream OR brill OR burbot OR butterfish OR Capellin OR carp OR catfish OR catshark OR "Chelon auratus"OR chub OR "clupea harengus" OR cod OR comber OR conger OR corb OR cutlassfish OR cyclopterus OR Cyprinus OR cyprinidae OR dab OR "danubian wels" OR dentex OR dicentrarchus OR dogfish OR eel OR emperor OR engraulis OR flathead OR flounder OR "flying fish" OR forkbeard OR gadus OR garfish OR garrick OR goby OR goldline OR grouper OR guitarfish OR gunard OR haddock OR hake OR halibut OR hammerhead OR herring OR hippoglossus OR hoki OR huss OR icefish OR "John dory" OR "Katsuwonus pelamis"OR labrus OR lamprey OR lanternfish OR leerfish OR ling OR "little tunny" OR "Liza aurata"OR lophius OR lumpfish OR lythe OR mackerel OR "mahi mahi" OR "mallotus villosus" OR marlin OR meagre OR megrim OR melva OR merluccius OR Micromesistius OR monkfish OR moonfish OR mugil OR mullet OR "mullus barbatus" OR needlefish OR Oncorhynchus OR oreo OR osmeridae OR pacu OR pandoras OR panga OR pangasius OR parrotfish OR "parrot fish" OR perch OR picarel OR pike OR pikeperch OR pilchard OR pilotfish OR "pilot fish" OR platichthys OR plaice OR pleuronectes OR pollan OR Pollack OR Pollock OR ponyfish OR porbeagle OR pout OR pouting OR ray OR ribbonfish OR rigg OR rockfish OR rosefish OR sablefish OR sailfish OR salmon OR salmo OR sandeel OR sardine OR sardina OR sardinella OR scabbardfish OR scomber OR scophthalmus OR scorpionfish OR "sea bass"OR seabass OR seabream OR "sea bream" OR seriola OR sheatfish OR "shi drum" OR sild OR sillago OR skipjack OR smelt OR smooth hound OR "smooth-hound" OR snapper OR snook OR sole OR solea OR sparidae OR sparus OR sparling OR spearfish OR sprat OR sprattus OR "St Peter's fish" OR stargazer OR stingray OR stizostedion OR sturgeon OR "surgeon fish" OR trachurus OR swordfish OR tailor OR tench OR theragra OR thunnus OR tilapia OR tinca OR threadfin OR triggerfish OR trisopterus OR trout OR tubefish OR tuna OR turbot OR tusk OR walleye OR weever OR whitebait OR whiting OR wrasse OR yellowtail

Seafood Composite: meal OR meals OR food OR foods OR "buffet meal*" OR "complex food" OR "frozen meal*" OR multi-ingredient OR "multi ingredient" OR ready-to-eat OR RTE OR "ready meal" OR "ready prepared" OR "ready to eat" OR "under vacuum" OR composite* OR convenience OR cured OR dip OR dips OR dish OR dishes OR dressing* OR dumpling* OR fermented OR filling OR gravy OR macerated OR marinad* OR marinate* OR mayonnaise OR pasta OR pizza OR pickled OR preserved OR pudding* OR puree* OR salsa OR salsas OR salted OR sandwich* OR sashimi OR ceviche OR sauce* OR smoked OR snack OR snacks OR soup OR soups OR stew* OR surimi OR sushi OR topping* OR chowder

AND NOT Terms (Title-Keywords-Abstract):

"in vitro" OR "in-vitro" OR "challenge study" OR "essential oil*" OR attribution OR biofilm* OR "plant extract" OR "extracts" OR feed OR livestock OR sanitiser OR sanitizer OR spiked OR "feed supplement*"