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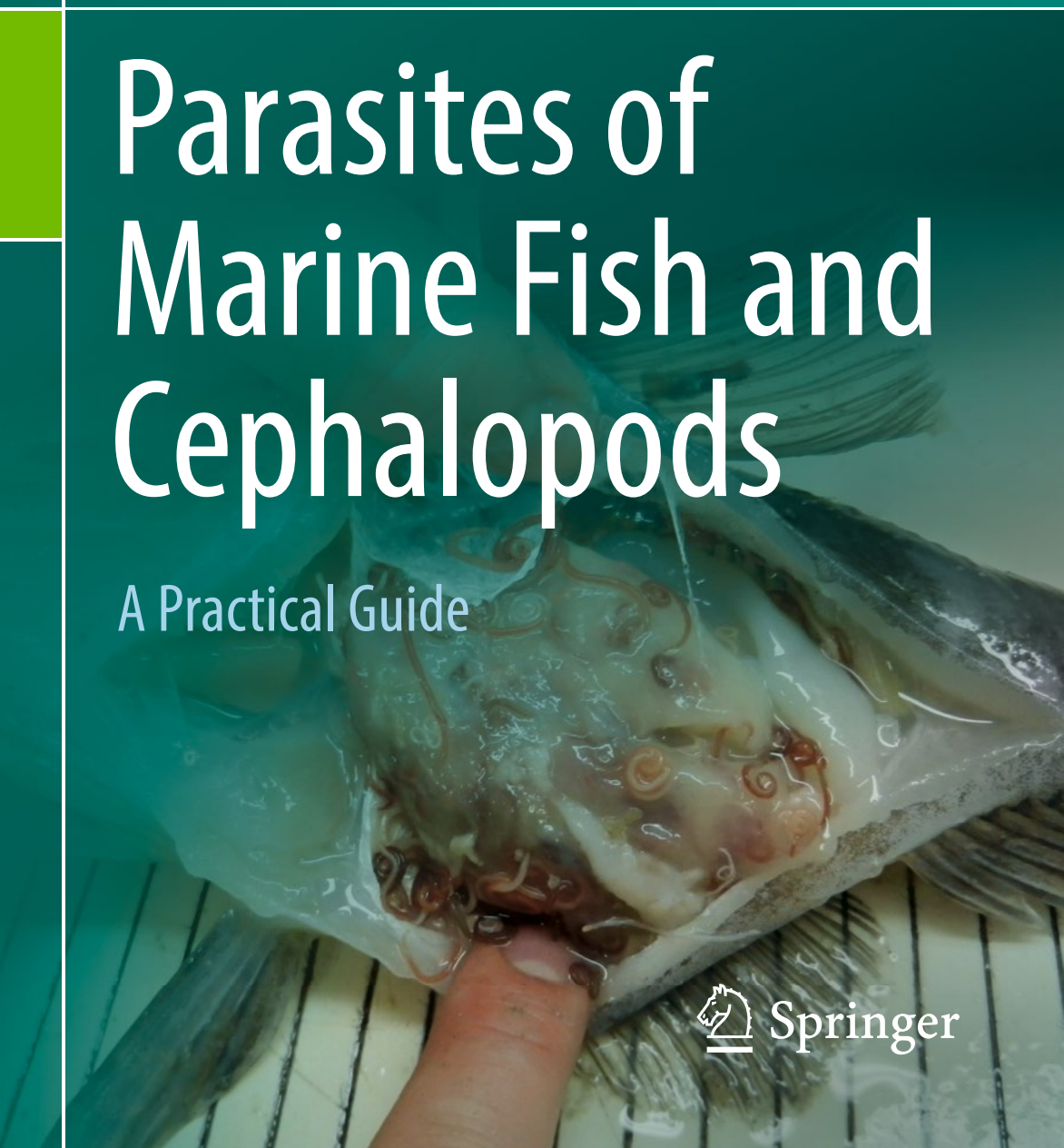
Parasites of Marine Fish and Cephalopods

A Practical Guide



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ISBN 978-3-030-16218-4 ISBN 978-3-030-16220-7 (eBook)
https://doi.org/10.1007/978-3-030-16220-7

This Springer imprint is published by the registered company Springer Nature Switzerland AG.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface and Acknowledgements

Parasites are an integral but often neglected part of any ecosystem. It is very likely that nearly every organism gets parasitised, at least temporarily, during its lifespan. According to today's knowledge, it is assumed that more parasitic than non-parasitic organisms exist on earth. The number of marine fish parasites alone is estimated at 20,000–100,000 species. Parasites live inside (endoparasites) or on (ectoparasites) host organisms, using them as a source of nourishment. The damage parasites can cause to the host can be either direct (e.g. mechanical destruction of cells, tissues and organs) or indirect (e.g. withdrawal of nutrients and intoxication). Parasites are also often the cause of secondary bacterial infections. In the field of marine fish parasitology, so-called host–parasite and parasite–host lists exist for certain geographical regions, which provide evidence of the regional differences in parasite diversity in fish and are related to abiotic and biotic factors, e.g. water temperature, salinity, water depth, food and the availability of intermediate and final hosts. Numerous studies have shown that these factors have direct and indirect effects on the marine food web. However, abiotic and biotic factors also affect the parasites as well as their developmental stages directly. This applies to parasites with a direct life cycle, but also to those with an indirect life cycle. The latter require one or more intermediate hosts (e.g. Cephalopoda, Crustacea, Elasmobranchii and Teleostei) for their development to reach sexual maturity in the final hosts. Knowledge of the life cycles of marine parasite species is important to understand and interpret the parasite load of fish. At the same time, parasites can be used as bioindicators, i.e. they can provide detailed information about their hosts and their habitats. Several studies from various areas and regions exist in which the benefits of parasites have been investigated or in which parasites have been successfully used as bioindicators. In fisheries biology, parasites are used to e.g. separate fish stocks or even subspecies of fish species, show migration habits of fish or solve questions concerning the diet of individual fish species and their position in the food web. Parasites are also used to monitor the pollution and eutrophication of water bodies.

Facing a world population of more than 7.5 billion people and an annual increase of about 40 million, the world's adequate food supply presents a major challenge for

the future. In 2005, approximately 80% of the world's population lived in so-called developing countries, mainly concentrated in coastal areas. Fishing has served as a food source for these people for centuries and led, among others, to the current settlement structure. Still today, there are nomadic peoples who build their livelihoods exclusively on fishing. In industrialised countries, the demand for fish and fishery products is also steadily increasing. As the population continues to grow, the pressure on natural food resources such as the sea also increases. Despite highly modern and technologically advanced fishing fleets and fishing gear, it is no longer possible to guarantee an increase in yield which at the same time accounts for a sustainable use of natural resources. The need to develop alternative methods for fish production is therefore becoming more and more apparent. In this context, aquaculture as a growing global industry plays a crucial role.

Due to their central position in aquatic ecosystems and their economic importance, fish are crucial research objects for parasitological studies. Fish is considered of high quality for human nutrition, and the question about the risk potential of fish parasites as pathogens for the fish host as well as for humans is of high scientific relevance. Parasitological work on fish has been intensified worldwide in recent decades, and various research vessels and fishing gear are used for diverse scientific research. Although all host organs can be infected by parasites, the infection of the fish muscles is of particular interest for the fish industry and human consumption. Muscle infection can lead to loss of muscle, which reduces the swimming speed of free-living fish (Richards and Arme 1981; Sprengel and Lüchtenberg 1991; Rohlwing et al. 1998). The consequence is a greater selectivity, for example in trawl fishing, and leads to greater landings of infected fish, which is then offered for consumption on the markets. Furthermore, parasitic diseases have a negative impact on fish farming, resulting in loss of production and a negative impact on the sustainable development of (marine) fish farming.

The consumption of parasitised fish can lead to serious diseases in humans. Diphyllobothriasis and anisakidosis are particularly common diseases in regions where traditionally raw or semi-cooked fishery products are consumed. They are caused by the ingestion of living larval stages of cestode or nematode parasites, which leads to the infection of the human gastrointestinal tract. One of the fastest developing disciplines in this field of research is studying the cause and spread of anisakidosis caused by species of the genera *Anisakis, Contracaecum* and *Pseudoterranova,* which use whales and seals as their final hosts. This disease can occur after the consumption of raw or insufficiently cooked fish (sushi, sashimi). Anisakidosis is currently a serious problem with more than 20,000–25,000 disease cases in humans per year; however, the true infection rate could be much higher due to symptoms that are similar to those of other gastrointestinal diseases. A continuous increase of this zoonotic disease is to be expected worldwide.

The idea for this book emerged from teaching regular courses in parasitology and infection biology at different institutions and universities (especially at the Helmholtz Centre for Ocean Research Kiel, Heinrich-Heine-University Düsseldorf and Goethe University Frankfurt/Main). The need of undergraduate and postgraduate students for a comprehensive presentation of practices and methods in aquatic parasitology was

equally a crucial factor. The content of this book therefore differs from but does not replace textbooks on parasitology or specific literature for the identification of protozoan and metazoan parasites. It is primarily intended for students, doctoral students, applied parasitologists and fish ecologists, employees of the fishing industry with a basic biological understanding, anglers and 'interested laymen'.

The structure of the book is very concise. In the introduction, more general information is given. This includes the presentation of fish and cephalopod morphology and anatomy as well as the most important taxonomic groups of parasites. The main part contains the techniques of dissections and analyses, both accompanied by graphic representations and photographs. At the end of each chapter, the most important information is summarised in boxes and a list of important original papers, review articles and monographs is given as well. Textbooks are only mentioned if they are particularly useful for the respective chapter.

I am grateful to a number of colleagues who have provided information, assistance and other material for this edition. Among them is Dr. Ken MacKenzie (University of Aberdeen, School of Biological Sciences), who has edited this text with a lot of patience, time and expertise. I had invaluable support from creative staff and colleagues during the organisation and textual implementation of the book, and they deserve my sincere appreciation: Birgit Nagel, Gabriele Elter, Dr. Sarah Cunze, Katharina Alt, Sina Zotzmann, Fanny Eberhard (all from Goethe University Frankfurt/Main, employed at the Department of Integrative Parasitology and Zoophysiology), Prof. Dr. Jörg Oehlmann (Goethe University Frankfurt/Main, Department of Aquatic Ecotoxicology), Dr. Horst Karl (Max Rubner-Institut—Federal Research Institute of Nutrition and Food, Department of Safety and Quality of Milk and Fish Products, Hamburg), Dr. Heino Fock (Johann Heinrich von Thünen Institute—Federal Research Institute for Rural Areas, Forestry and Fisheries, Institute of Sea Fisheries, Bremerhaven), Dr. Uwe Piatkowski (GEOMAR—Helmholtz Centre for Ocean Research Kiel) and Dr. Arne Levsen (University of Bergen, National Institute of Nutrition and Seafood Research).

I am especially grateful to Prof. Dr. Heinz Mehlhorn (University of Düsseldorf, Institute of Zoomorphology, Cell Biology and Parasitology) and Prof. Dr. Dr. h.c. Volker Mosbrugger (General Director of the Senckenberg Gesellschaft für Naturforschung, Frankfurt/Main), who always supported our work in the field of parasitology and infection biology.

My heartfelt thanks goes to my wife Svenja and my children Lina and Jarne, who have been incredibly patient over the last few years. They have always been supportive, although I have spent a lot of time with 'parasitology and infection biology'. Finally, I would like to thank Springer Verlag for being responsive to all my ideas and wishes, which made it possible to publish 'Fish parasitological field guide' in its present form and scope.

Frankfurt/Main, Germany Sven Klimpel

Contents

Introduction

Seas and oceans represent the largest continuous ecosystem on our planet, comprising a total area of about 360 million km^2 and an estimated volume of 1375 billion km^3 (Hempel et al. 2006; Sommer 2005; Tardent 2005). They absorb and store considerable amounts of carbon dioxide and heat, thereby playing a key role in regulating the earth's climate (Hoegh-Guldberg and Bruno 2010). Furthermore, they provide habitats for a large number of organisms and thus represent the most important biogenic resource for humans. For example, various micro- and macrophyta are used as food. However, first and foremost, fish and fishery products offer a valuable and inexpensive source of high-quality animal protein. The livelihood of many people depends on fish, especially in regions of the world designated as 'developing countries'. Over the last decades, the overall demand for marine products has increased dramatically. About 65% of the world's population live within 150 km of a coastline, and an estimated 54.8 million people earn their living from fishery products (Hewitt and Campbell 2007; FAO 2011). In 2009, fish accounted for 20% of the total animal protein intake for 3 billion people (FAO 2011). In 2010, 79,500,000 tonnes of marine fish were landed and processed for humans. According to current calculations by the Food and Agriculture Organization (FAO) of the United Nations, the average demand for fishery products has risen from around 9.9 kg (live weight equivalent) per capita per year to 18.6 kg (average over the period 1961–2009) (FAO 2012). With an average growth rate of approx. 3.2% per year, the demand for fish is growing faster than the global human population with 1.7%. Fish and fishery products are not only the main resource for protein and micronutrients but are also of enormous economic importance, especially for the leading fish-exporting countries, with a total estimated value of US$ 217.5 billion (FAO 2011, 2012; Woo 2006). With the importance of fishery products for the daily human diet increasing, research into harmful organisms in fish products has also become more important. Besides a number of different viruses (e.g. Birnavirus, Paramyxovirus), bacteria (e.g. *Vibrio* spp., *Flexibacter* spp.) and fungi (e.g. *Ichthyophonus hoferi*, *Exophiala* spp.), unicellular (protozoic) and multicellular (metazoic) parasites have become of great public interest (Möller and Anders 1983;

S. Klimpel et al., *Parasites of Marine Fish and Cephalopods*,
https://doi.org/10.1007/978-3-030-16220-7_1

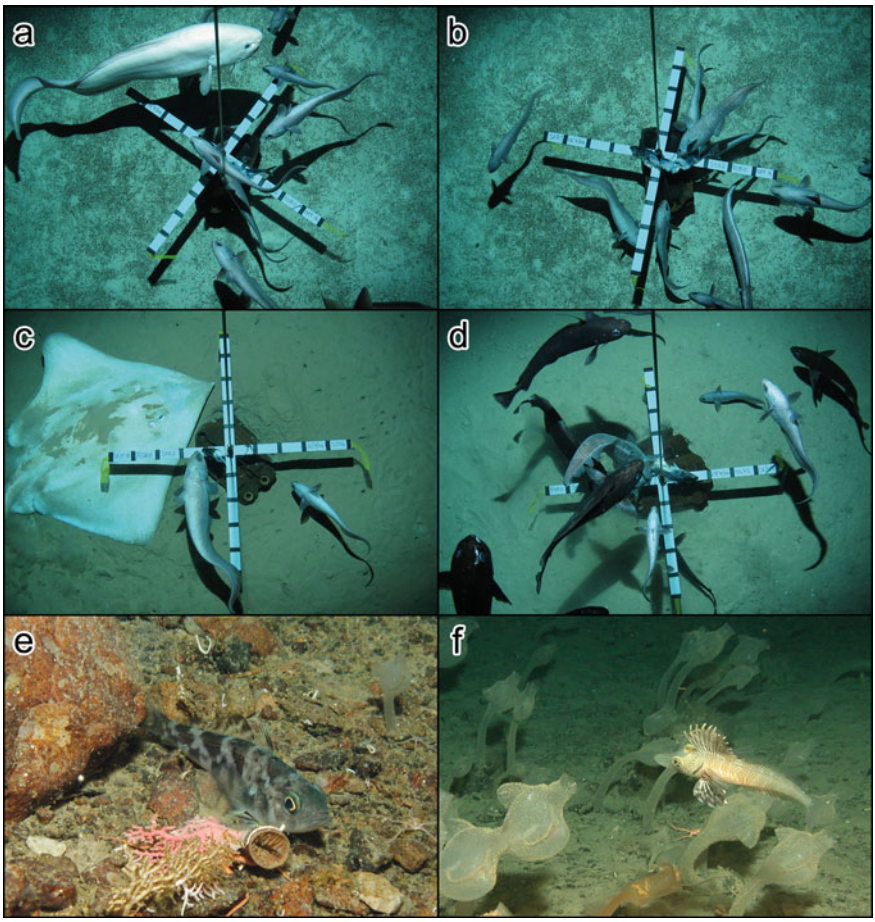

Fig. 1 Fish and ray intermediate and final hosts for metazoan parasite species. (**a**–**d**) Baited camera observations of deep-sea demersal fishes of the Mid-Atlantic Ridge Ecosystem. The camera was programmed to take digital photographs at 60 s intervals from a height of 2 m above the seafloor, with the ballast, bait (500 g mackerel *Scomber scombrus*) and reference cross (1 m × 1 m marked at 10 cm intervals) in the centre of the field of view. Ribbons were attached to the end of each arm of the scale cross to visually corroborate current direction. (**a**) Pudgy cusk eel (*Spectrunculus grandis*) and several species of Abyssal grenadier (*Coryphaenoides armatus*). (**b**) Arrowtooth eel (*Histiobranchus bathybius*) and nine Abyssal grenadiers. (**c**) Pale ray (*Bathyraja pallida*) and two Abyssal grenadiers. (**d**) Blue antimora (*Antimora rostrate*, black) and three Abyssal grenadiers. (**e**) Blunt scaly head (*Trematomus eulepidotus*). (**f**) *Pagetopsis macropterus* is a species of the Fam. Channichthyidae (Crocodile icefishes)

Muroga 2001; Woo 2006). In the aquatic sector, the level of knowledge about the global diversity of medically important species (pathogens, parasites) varies greatly. Fish are the most diverse vertebrate class in the sea, functioning as intermediate and final hosts for many parasites (Figs. 1 and 2). The high diversity of host species is paralleled by a high diversity of their parasites and has been illustrated in numerous

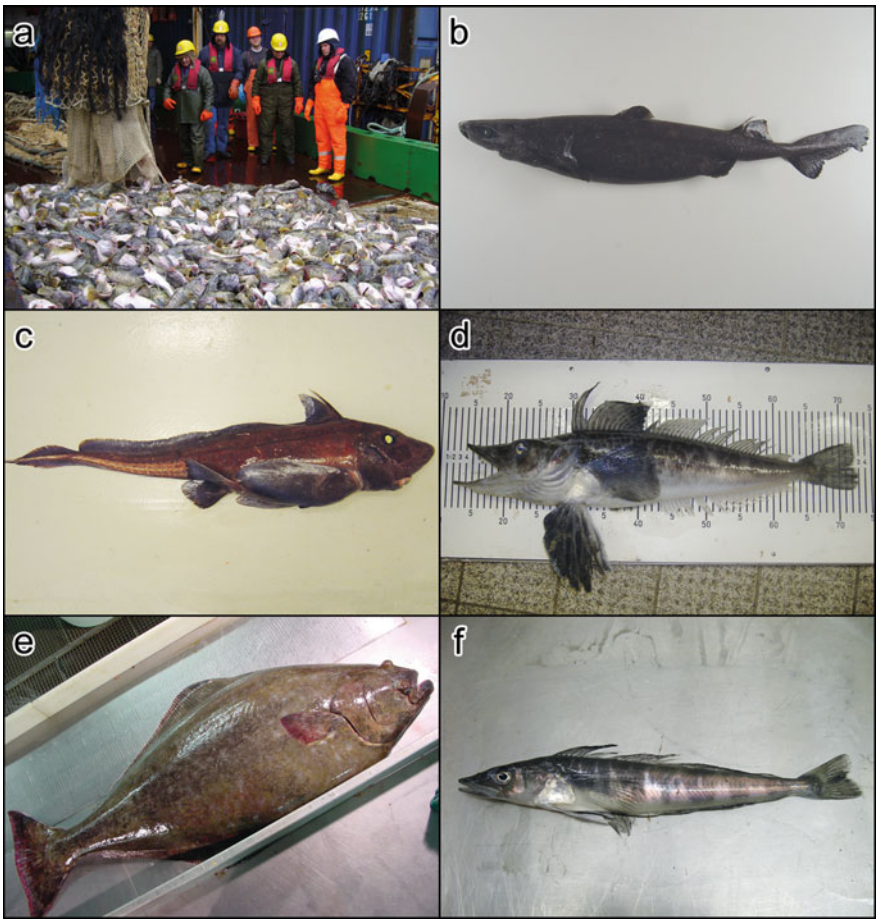

Fig. 2 Fish, Elasmobranchii and Holocephali intermediate and final hosts for metazoan parasite species. (**a**) Complete haul of fish emptied from the net. (**b**) Great lanternshark (*Etmopterus princeps*). (**c**) Small-eyed rabbitfish (*Hydrolagus affinis*). (**d**) Jonah's icefish (*Neopagetopsis ionah*). (**e**) Atlantic halibut (*Hippoglossus hippoglossus*). (**f**) Mackerel icefish (*Champsocephalus gunnari*)

parasite-host checklists for different aquatic ecosystems, in which the respective infection parameters are compared between fish species. Today, fish parasites make up a significant part of marine biodiversity. Based on current lists, three to four parasite species can be found in a single fish specimen (Klimpel et al. 2009). However, these values are determined by factors like habitat, geographical region, fish species, abiotic and biotic factors and their interactions. Thus, water movements, hydrostatic pressure, salinity, temperature and light conditions cannot only affect the composition of aquatic parasites in fish hosts but also food web structures, migration behaviour and predator-prey relationships. Parasites can also be used as biological indicators or markers, which can help evaluate a wide variety of ecological issues

and anthropogenic influences on water bodies (Lafferty 1997; MacKenzie et al. 1995; Sasal et al. 2007). For example, they can be used to differentiate local fish stocks (Baldwin et al. 2012; Kijewska et al. 2009; Klapper et al. 2017; MacKenzie 2002; Mattiucci et al. 2007, 2008) or to describe the population dynamics of their hosts (e.g. Arthur and Albert 1993; Mattiucci 2006; Williams et al. 1992). In addition, they are valuable for monitoring environmental stressors such as eutrophication (e.g. Reimer 1995; Valtonen et al. 1997), heavy metal input (Sures et al. 1999; Sures 2003; Sures and Siddall 1999) or industrial pollutants (Madanire-Moyo et al. 2012). Due to their longevity and the fact that they are present in almost all marine food webs at all trophic levels, they can also be used to determine the role of their hosts in the food web (Klimpel et al. 2003a, b; Marcogliese 2002, 2005). A long retention time of their intermediate stages in the hosts enables important ecological information to be collected through a combination of stomach content and parasitological analyses. These include, for example, the type and origin of the individual food organisms or short-term ontogenetic changes in the diet of the host (e.g. Klimpel et al. 2003b; Klimpel and Rückert 2005; Münster et al. 2015). Using parasites as biological indicators is essential for the study of host organisms that do not allow in vivo analysis due to their habitats inaccessibility (e.g. Klimpel and Palm 2011; Lafferty et al. 2008). The co-evolution of marine fish parasites and their hosts benefits the study of important ecological questions using various methods. Depending on the type and the number of hosts required to complete the life cycle of a parasite, a distinction is made between monoxenous (one-host) and heteroxenous (multiple-hosts) parasites (Mehlhorn and Piekarski 2002, Table 1). The host specificity of a parasite, i.e. the degree of its specialisation with regard to the host, can be strict (stenoxenous) or loose (euryxenous). Thus, if the host spectrum of a certain parasite is known, the presence of a parasite can be taken as

Table 1 Life cycle stages of different parasitic groups

	Digenea	**Monogenea**	Cestoda	**Nematoda**	Acanthocephala
water	egg	egg	egg	egg	egg
water	**miracidium**	oncomiracidium	coracidium	1./2. larva in egg	acanthor in egg
1. intermediate host	redia or sporocyst		procercoid	2./3. larva	acanthella or cystacanth
water	cercaria				
2. intermediate host	**metacercaria**		plerocercoid	3. larva	
final host	adult	adult	adult	4. larva adult	adult

evidence for the presence of the respective intermediate and final hosts in a specific area, since these hosts are required for the completion of the parasite's life cycle. Consumption of parasitised fishery products can lead to serious diseases in humans (e.g. Audicana and Kennedy 2008; Klimpel and Palm 2011; Mattiucci and Nascetti 2008). In a globalised world, with networks of trade, increased travel and cultural and demographic changes, the need for studies on the potential risk of fish parasites as pathogens for the host or for humans becomes more pressing.

Parasitism/Parasites

Parasitism is the most successful form of life on earth, with more parasitic than free-living species having been described (Price 1980; Windsor 1998). Parasitism is defined as the relationship between two unrelated organisms with the parasite being temporarily or permanently present at or in its host due to physiological and/or morphological characteristics (Lucius and Loos-Frank 2008; Zander 1998). The host provides the necessary nutrients for the parasite, offers protection and enables the survival of various developmental stages of the parasite or its reproduction (Rohde 2005). This process happens mainly at the expense of the host organism which is affected by its parasite in many different ways. Parasites can cause mechanical damage through their histophagous or haematophagous diet or by removal of important nutrients from the host (food competitors). Metabolic products produced by the parasite ('excretions') can lead to intoxication of the host, and thus directly to the disintegration of its vital organ functions. Secondary infections often occur with parasite infection as parasites aid pathogenic microorganisms (e.g. viruses, bacteria, fungi) to also enter the host (Mehlhorn and Piekarski 2002). Furthermore, the behaviour of a host can change once infected with parasites, which might lead to, e.g. being exposed to higher risk of predation (e.g. Bakker et al. 1997; Baldauf et al. 2007; Milinski 1985). Thus, parasites can be characterised as a driving force in the evolution of their host organisms as, like the abiotic environment itself, they generate selection and adaptation pressure (Lucius and Loos-Frank 2008; Palm and Klimpel 2007).

In the course of evolution, parasitism has independently developed within the most diverse groups of organisms. Studies show that every organism has been parasitised, at least temporarily, within its life span (Marcogliese and Price 1997). Parasites are therefore considered an integral part of all ecosystems and account for a significant proportion of biodiversity (Palm and Klimpel 2007). For example, if we compare the number of recent Elasmobranchii (cartilaginous fish) with the number of their associated parasite species, we can see that the number of parasite species clearly exceeds the number of host species. This finding becomes even more significant knowing that the Elasmobranchii themselves are much better studied than their parasite fauna. It can be assumed that the currently known number of parasite species is significantly underestimated (Palm 2004). The same applies to other parasite taxa in various ecosystems, where research either has not, or has

insufficiently, considered the biodiversity of parasitic life forms. Parasitism as a general way of life is an ecological concept that can only be studied in combination with the host organisms and the specific ecosystems due to its polyphyletic origin (Cruickshank and Paterson 2006; Wenk and Renz 2003).

Parasites always receive attention when they use humans as hosts. Many human pathogenic parasites have been known for centuries, such as the nematode species *Dracunculus medinensis*, which was initially mistaken as a 'nerve concrement' (Adams 1844, in Foster 1965). At the end of the twentieth century, scientists were able to detect *Schistosoma haematobium* eggs in Egyptian mummies dated back to 1250–1000 AC (Cox 2002). The earliest evidence of nematode eggs of the species *Ascaris lumbricoides*, isolated from coprolites from Peru and Brazil, dates back to 2277–1420 AC (Cox 2002). The first knowledge about 'intestinal worms' during the early Middle Ages in Europe was derived from Greek and Roman writings, especially from Hippocrates (460–370 AC) and Aristotle (384–322 AC). Other studies did not exist at that time.

The beginnings of 'scientific' parasitology lie in the first half of the eighteenth century. From then on, German physicians and natural scientists were particularly active in the field of parasitic helminthology, concentrating on the description and systematic classification of parasites, which was unchartered scientific territory at the time (Enigk 1986). Many anatomical and morphological studies of parasites and their stages of development were carried out at the end of the nineteenth century, when technology was progressing and new techniques, such as histology and light microscopy, were being developed. While Karl Asmund Rudolphi (1808–1810) still classified and separated different life stages (larva, adult) into different tapeworm genera in his systematics, it now became possible to study the heteroxenous life cycles. Thus, the life cycles of trematodes, cestodes and nematodes, e.g. *Clonorchis sinensis*, *Taenia saginata* and *D. medinensis*, were studied and published between 1830 and 1918 (Cox 2002; Grove 1990). Until the twentieth century, self-experiments by scientists often played a major role in the clarification of life cycles of human pathogenic parasite species (Enigk 1986). In comparison to the development of terrestrial parasitology, the study of aquatic zoonotic diseases was only advanced during the last century. Today, the Digenea genera *Clonorchis* and *Opisthorchis*, the Cestoda genera *Diphyllobothrium* (Diphyllobothriasis), *Ligula* and the Nematoda genera *Anisakis*, *Contracaecum* and *Pseudoterranova* (Anisakiasis) are known worldwide as human pathogenic aquatic parasite species (Audicana et al. 2002).

The necessity to detect non-visible pathogens, such as viruses, led to the development of the electron microscope in the early twentieth century. The first electron microscope was developed in 1931 by the two electrical engineers Ernst Ruska (1906–1988) and Max Knoll (1897–1969). It laid the foundation for a transmission electron microscope, which was completed in 1938. In the 1960s, high-performance types of electron microscopes were developed. These microscopic techniques have been used in parasitology to describe surfaces and ultrastructures of various types of parasites. With the more recent development of molecular biological techniques, more fields of application continued to open up, such as molecular species

Fig. 3 Scientists in action. (**a**) Sorting of fish catch during the Antarctic research cruise ANT XXIII/8 with the German research icebreaker Polarstern. (**b**) Sven Klimpel isolates monogenean parasites from the gills of the Patagonian toothfish (*Dissostichus eleginoides*). (**c**) Julian Münster sorting fish in the wet lab of the research vessel Walther Herwig III. (**d**) Dorian D. Dörge during parasitological investigations in the laboratory of the Goethe University. (**e**) Thomas Kuhn during genetic processing of nematode samples. (**f**) Sven Klimpel shortly before helicopter takeoff to whale watching

diagnostics, population genetics and molecular phylogeny. Today, parasitology is an interdisciplinary field of research that uses a wide range of different methods, techniques and devices (Fig. 3). There are a multitude of interdisciplinary topics within parasitology, for example, phylogeny, host specificity, virulence, host change biology, behavioural manipulation by parasites, evolution of specific immunological recognition and the relevance of sexual reproduction for hosts and parasites. Generally, parasitology can be divided into two major disciplines, aquatic (freshwater, marine) and terrestrial parasitology. While marine parasitology mainly focuses on

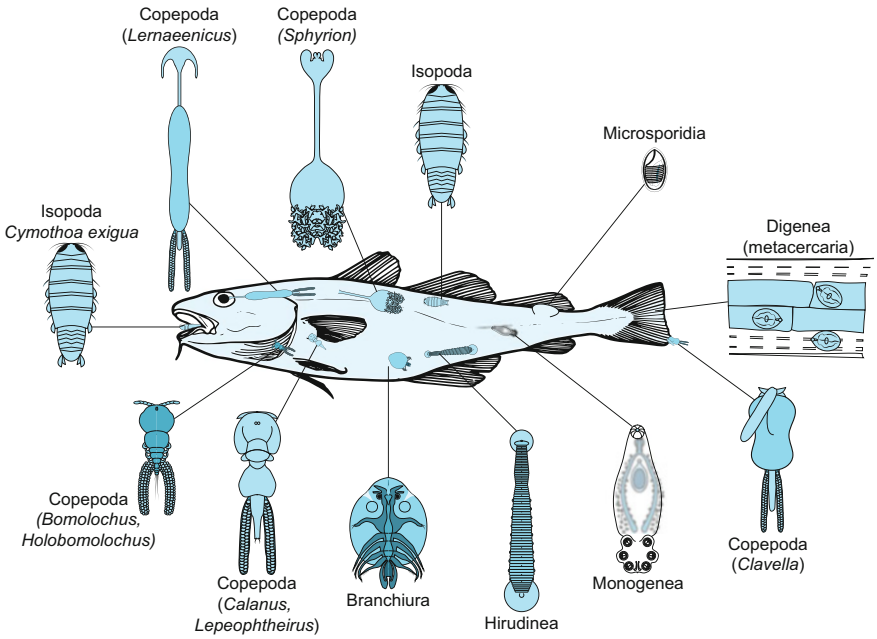

Fig. 4 Diagrammatic representation of different parasites and their location on fish hosts

fish parasites (ectoparasites, endoparasites; Fig. 4) and their transmission mechanisms, terrestrial parasitology primarily studies pathogens of farm animals and humans (e.g. Palm 2004; Wenk and Renz 2003).

From a scientific point of view, the two systems are closely related, since the largest parasite taxa originate either from the terrestrial (Nematoda) or the aquatic (Cestoda) environment. The phylogenetic relationships within the different parasite groups thus enable comparative studies of parasites in aquatic and terrestrial systems. For example, they can help elucidate the origin of certain life cycles and stages or taxa. Most of the highly developed terrestrial ascarid animal and human nematodes, such as the genera *Ascaris* and *Toxocara*, have a specialised direct life cycle that no longer requires an invertebrate intermediate host (Anderson 2000; Mehlhorn 2016). Some families of the order Strongylida, however, require a mollusc for their transmission to the vertebrate final host, whereas in a few species of the vertebrate parasitic orders Ascaridida and Spirurida, annelids and insects are interposed as invertebrate hosts (Anderson 2000; Mehlhorn 2016). In the aquatic environment, the life cycle of nematodes always requires crustaceans as invertebrate intermediate hosts (Anderson 2000; Moravec 1994). Here, the highly developed Ascaridida have not specialised as much as in the terrestrial environment, but have acquired a highly generalistic way of life, which allows them to colonise almost all aquatic habitats, from the Antarctic to the deep sea. In conclusion, the colonisation of habitats and the importance of specialisation for the biology and distribution of ascaridoid nematodes

as well as other parasitic helminths appear to underlie different conditions and processes in terrestrial and aquatic areas.

Biology and Ecology of Marine Fish Parasites

The diversification of aquatic organisms manifests itself in their many adaptations to the abiotic and biotic environment, such as light conditions, hydrostatic pressure, water movements, salinity, temperature, food spectra, migrations, predator-prey relationships and population dynamics. Among extant marine organisms, Mollusca is the most diverse group, followed by Crustacea, Teleostei, Cnidaria, Porifera and Polychaeta (Froese and Pauly 2018; Ott 1996). Among the vertebrates, fishes represent the most diverse class and are distinguished into Chondrichthyes and Osteichthyes. More than 34,000 different fish species have been described globally, with approx. one half occurring in freshwater and the other half in marine habitats (Froese and Pauly 2018). Within the marine environment, 3100–4600 fish species inhabit the deep sea and more than 374 can be found in the Antarctic Ocean (Duhamel et al. 2014; Klimpel et al. 2009; Kock 2005). This high biodiversity of potential hosts and the great age of some marine habitats (e.g. coral reefs, deep sea) have allowed parasite species to thrive. For example, 54 different parasite species have so far been detected globally in the deep-sea grenadier or rattail (*Macrourus berglax*), 37 species in the Antarctic black rock cod (*Notothenia coriiceps*), 125 in the commercially heavily exploited North Atlantic fish species cod (*Gadus morhua*) and 82 in the herring (*Clupea harengus*) (Arthur and Arai 1984; Hemmingsen and MacKenzie 1993; Klimpel et al. 2006, 2009; Münster et al. 2015, 2016; Palm and Klimpel 2008; Palm et al. 1998).

Margolis and Arthur (1979) and McDonald and Margolis (1995) found an average of 3.2 species of parasites per fish species in Canadian waters (marine/freshwater). Similarly, values of 3.1 were found in German coastal waters of the North and Baltic Seas (Klimpel et al. 2009; Palm et al. 1999). These rather high values of parasite species most likely reflect the increased research effort in these waters due to the fish economic importance for the fishing industry. Estimates for tropical waters (e.g. Philippines, Bangladesh) are significantly lower, with between 1.2 and 1.7 species of parasites per fish species (Arthur and Lumanlan-Mayo 1997; Arthur and Ahmed 2002; Klimpel et al. 2009). Based on the number of fish species from marine and brackish water described to date, Klimpel et al. (2001) estimated a total between 20,000 and 43,200 different species of marine metazoan parasites. Based on a fish fauna of 30,000 species (freshwater and marine), Rohde (2002) estimated about 100,000 parasite species, which corresponds to an average of about 3.3 parasites per fish species. The above-mentioned lists of hosts and parasites show how inadequately and unevenly the various aquatic habitats have been studied. However, the results clearly suggest that both biotic and abiotic factors play a major role in the different occurrences of parasite species, although abiotic and biotic factors have been mainly considered independently in the interpretation of

parasitological data. There are numerous case studies on the influence of abiotic and biotic factors, such as temperature (Rohde 1993; Poulin and Rohde 1997), geomorphological barriers (Rohde and Hayward 2000; Rohde 2002), salinity (Valtonen et al. 2001; Zander and Reimer 2002), water depth (Klimpel et al. 2006; Palm et al. 2007; Walter et al. 2002), food and availability of intermediate and final hosts (Lile 1998; Klimpel et al. 2004, 2006, 2007) and migrations of hosts (Walter et al. 2002), which regulate the interactions between parasites and fish species and their food organisms. Interpreting the results factor by factor only, no clear conclusions can be drawn as the effect of an examined parameter is often masked by or correlated to another factor. Therefore, simultaneous recordings of different biotic and abiotic factors are a basic requirement for the study of marine parasites.

Fish as Food

Due to their central position within aquatic ecosystems and their economic importance, fish are crucial objects of study for parasitological research. As a high-quality food, fish is indispensable for human nutrition. A significant proportion today comes from aqua- or mariculture, which increasingly complements catches from wild stocks. The infection of fish with parasites has been known as a problem for a long time (Jütte 1987). The first evidence of regular human consumption of fish dates back to the Neolithic inhabitants of modern Denmark and to the time of 800 AC on Canada's Pacific coast (Hoursten and Haegele 1980; Schnack 1996). Especially during the formation of the Hanseatic League, catches and marketing of herring (*Clupea harengus*) from the North and Baltic Seas played a significant role. The first economic losses due to parasites occurred in Cologne as early as 1582, when nematodes were found in salted herring (Jütte 1987). Due to these findings, the purchase of marine fish as food for the military was banned in Bavaria in 1912 (Kahl 1936). The discovery of living nematodes in edible fish products reported and screened on German television [tv-programme: Monitor-Reportage (ARD = broadcaster)] in 1987 caused an extreme loss of turnover in the German and European fishing industry. It led to the realisation that there was a lack of knowledge about the occurrence and spread of potentially human pathogenic parasites in economically exploited fish. Furthermore, it was proof that aquatic parasitology had been a neglected field of research for a long time.

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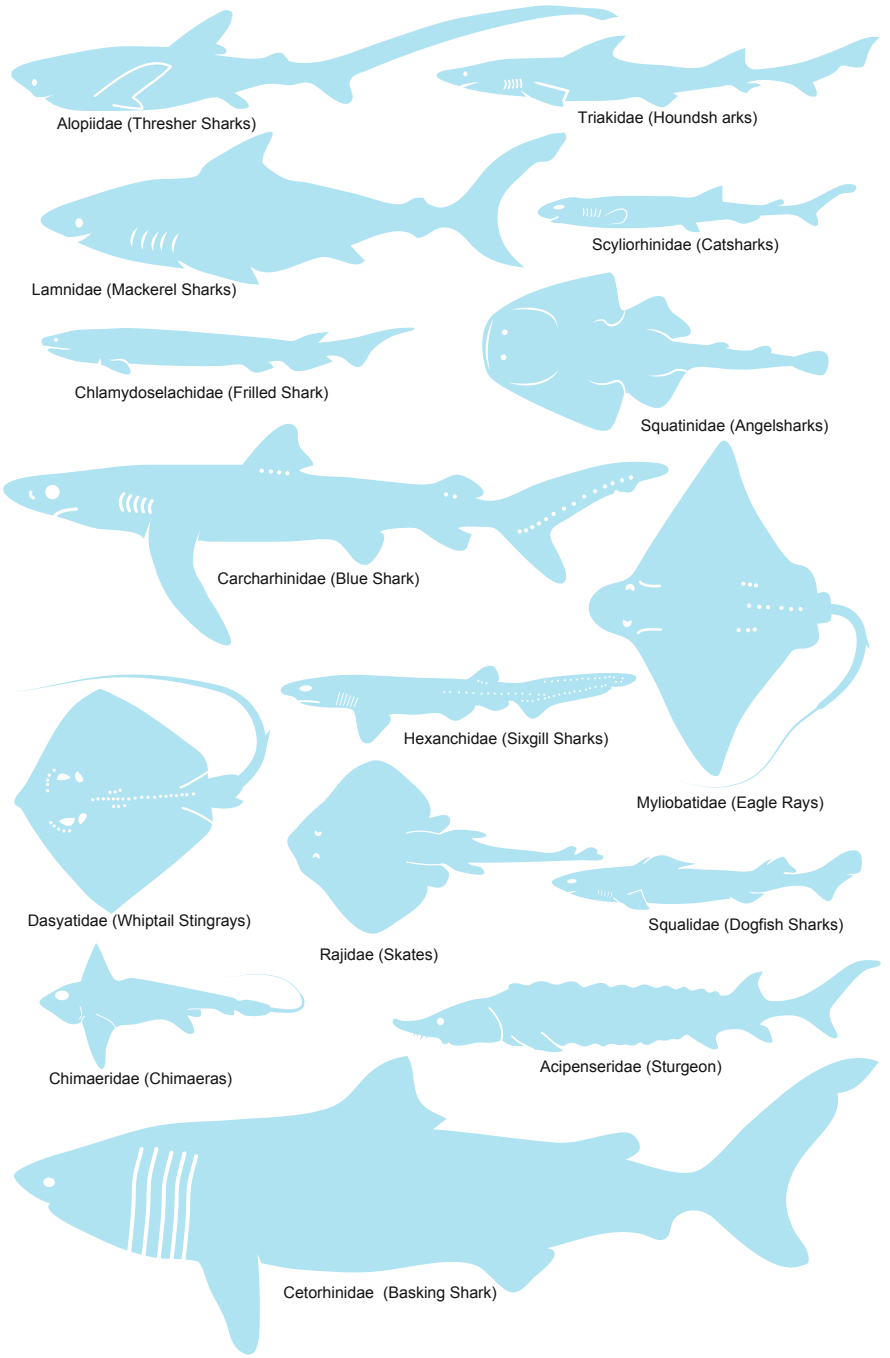
Anatomy and Morphology of Fish and Cephalopods

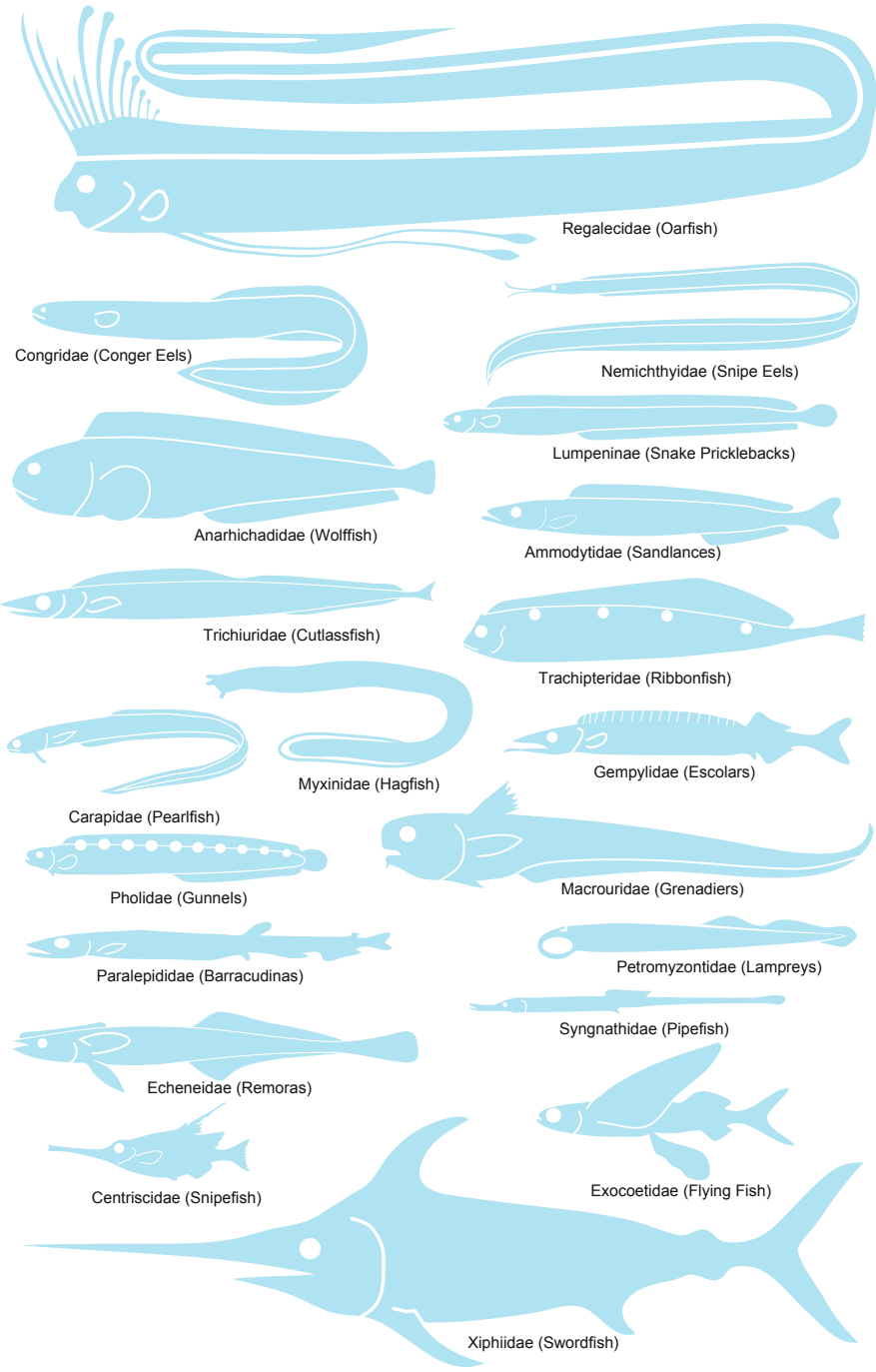
Fish and Cephalopod Illustrations

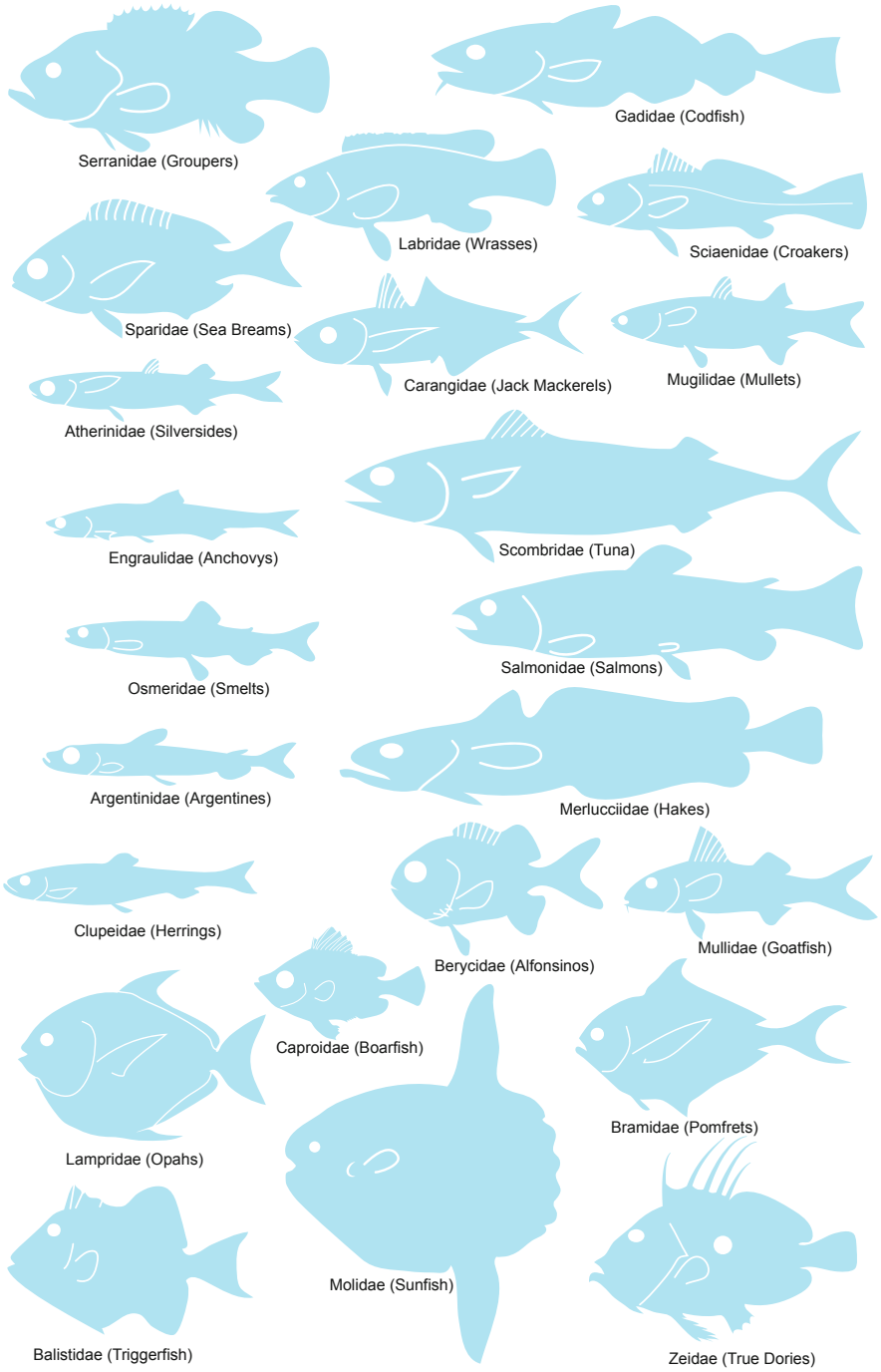
Fish do not form a uniform group of animals such as birds or mammals. Fish are rather all vertebrates that can be characterised by tooth-bearing jaws, living in the water and having paired and unpaired fins. They form the most species-rich vertebrate group in aquatic ecosystems. Their shape, size and internal structures vary considerably. Currently, more than 34,000 different fish species are known, and new species are being described every year. The majority are bony fish, mainly Teleostei, followed by cartilaginous fish species and jawless fish. Half of the fish species known today can be found in freshwater; the other half inhabit marine ecosystems. Fifty percent of the fish species occurring in marine waters live in warm and nearshore water zones (e.g. coral reefs). However, fish can be found in all marine habitats, with some remarkable adaptations to prevailing biotic and abiotic conditions. These adaptations enable them to live in the deep sea, polar regions and close to the coast.

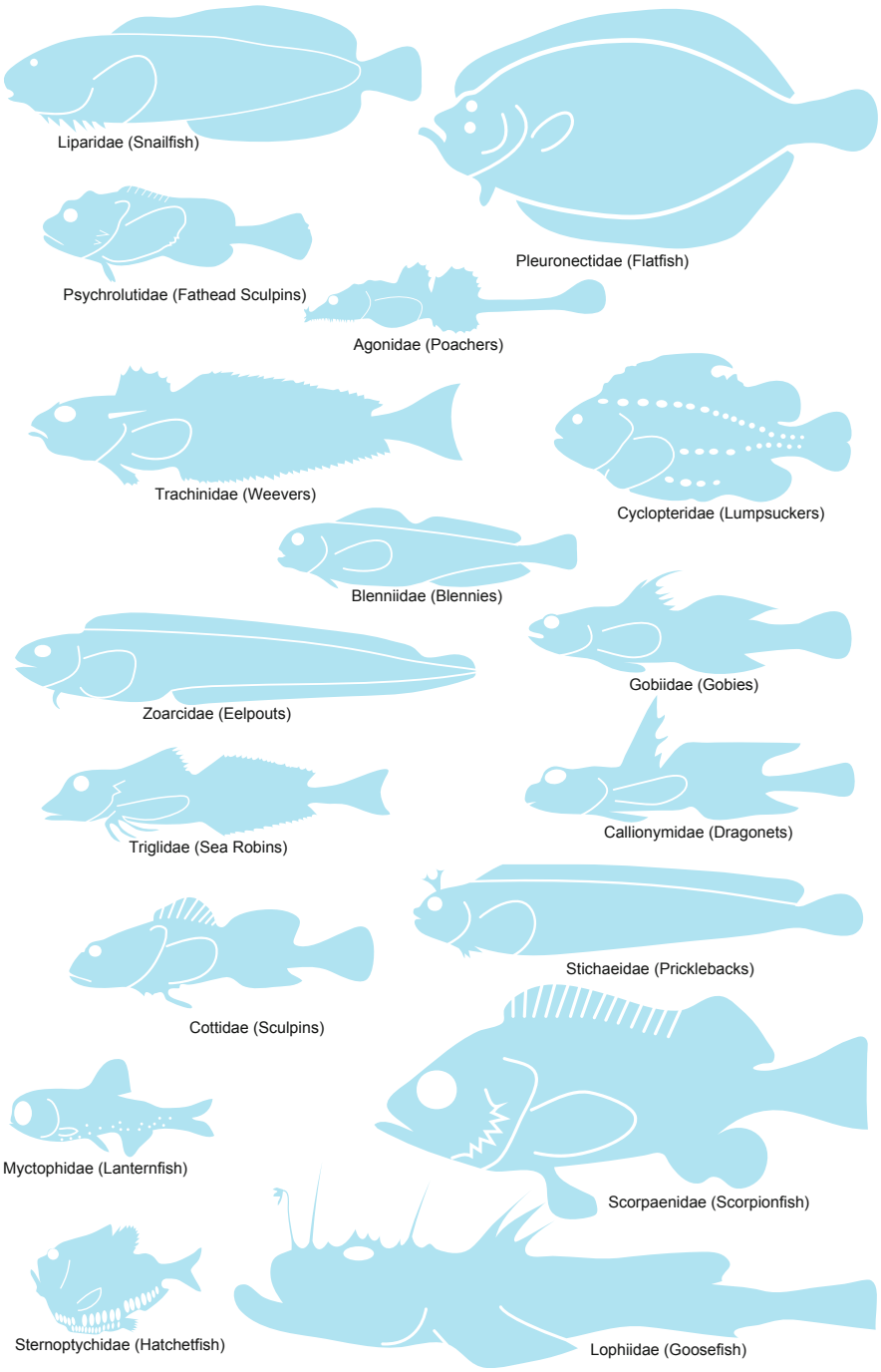
The following compilation should allow the pre-classification of fish specimens into groups based on their shape. Detailed information can then be found in more specific technical literature or in the 'Fishbase' database (www.fishbase.org). The presentation in this book does not include any systematic classification. The anatomy and morphology of common fish groups and of cephalopods are exemplarily presented in the following sections.

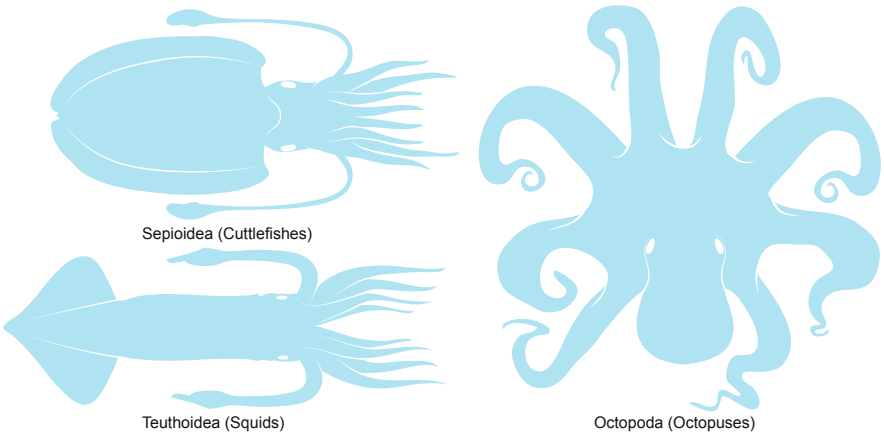
S. Klimpel et al., *Parasites of Marine Fish and Cephalopods*,
https://doi.org/10.1007/978-3-030-16220-7_2


Alopiidae (Thresher Sharks)
Triakidae (Houndsh arks)
Lamnidae (Mackerel Sharks)
Scyliorhinidae (Catsharks)
Chlamydoselachidae (Frilled Shark)
Squatinidae (Angelsharks)
Carcharhinidae (Blue Shark)
Hexanchidae (Sixgill Sharks)
Myliobatidae (Eagle Rays)
Dasyatidae (Whiptail Stingrays)
Rajidae (Skates)
Squalidae (Dogfish Sharks)
Chimaeridae (Chimaeras)
Acipenseridae (Sturgeon)
Cetorhinidae (Basking Shark)


Regalecidae (Oarfish)
Congridae (Conger Eels)
Nemichthyidae (Snipe Eels)
Lumpeninae (Snake Pricklebacks)
Anarhichadidae (Wolffish)
Ammodytidae (Sandlances)
Trichiuridae (Cutlassfish)
Trachipteridae (Ribbonfish)
Myxinidae (Hagfish)
Gempylidae (Escolars)
Carapidae (Pearlfish)
Macrouridae (Grenadiers)
Pholidae (Gunnels)
Petromyzontidae (Lampreys)
Paralepididae (Barracudinas)
Syngnathidae (Pipefish)
Echeneidae (Remoras)
Exocoetidae (Flying Fish)
Centriscidae (Snipefish)
Xiphiidae (Swordfish)


Serranidae (Groupers)
Gadidae (Codfish)
Labridae (Wrasses)
Sciaenidae (Croakers)
Sparidae (Sea Breams)
Carangidae (Jack Mackerels)
Mugilidae (Mullets)
Atherinidae (Silversides)
Scombridae (Tuna)
Engraulidae (Anchovys)
Salmonidae (Salmons)
Osmeridae (Smelts)
Merlucciidae (Hakes)
Argentinidae (Argentines)
Clupeidae (Herrings)
Berycidae (Alfonsinos)
Mullidae (Goatfish)
Caproidae (Boarfish)
Bramidae (Pomfrets)
Lampridae (Opahs)
Molidae (Sunfish)
Balistidae (Triggerfish)
Zeidae (True Dories)


Liparidae (Snailfish)
Pleuronectidae (Flatfish)
Psychrolutidae (Fathead Sculpins)
Agonidae (Poachers)
Trachinidae (Weevers)
Cyclopteridae (Lumpsuckers)
Blenniidae (Blennies)
Gobiidae (Gobies)
Zoarcidae (Eelpouts)
Callionymidae (Dragonets)
Triglidae (Sea Robins)
Stichaeidae (Pricklebacks)
Cottidae (Sculpins)
Myctophidae (Lanternfish)
Scorpaenidae (Scorpionfish)
Sternoptychidae (Hatchetfish)
Lophiidae (Goosefish)



Atlantic Cod (*Gadus morhua*, Gadidae)

The Atlantic cod (*Gadus morhua*) is considered one of the most abundant fish species in the North Atlantic and adjacent seas. Its distribution reaches from boreal to polar zones, from Cape Hatteras to the southern coast of Svalbard. In these waters, cod is one of the most important high trophic level predators of small-sized fish and invertebrates in inshore and offshore ecosystems and is among the most important species of all commercial fishes. Due to its high economic value, Atlantic cod has been given the name 'beef of the sea'. It was one of the first fish specifically targeted in fisheries and has been one of the most important food fish since the sixteenth century. The economic importance of cod escalated in the so-called Cod Wars between Great Britain and Iceland in 1958 and 1975. The economic peak with a catch of 3.9 million tons was reached in 1968, followed by a collapse of large parts of the stocks. Whether the collapse was caused by overfishing or climatic changes or a combination of both is uncertain. Since 1996, because of these collapses, *G. morhua* has been listed as vulnerable on the 'Red List of Threatened Species' of the IUCN (International Union for Conservation of Nature and Natural Resources, status 2017) although the population in the North East Arctic is currently thriving.

Ecology

Gadus morhua is a generalist species in many ways. Its temperature spectrum ranges from 0 °C up to 20 °C, while salinity is only of minor importance as cod occurs in nearly fresh as well as full oceanic water. The distribution reaches from shorelines to continental shelf edges. While juveniles prefer shallow, sublittoral water layers, adults can be found down to 1000 m but usually live in 150 to 200 m depth above the continental shelf. The migratory behaviour is very variable. Inshore stocks of Greenland's fjord systems are known to be stationary, while offshore stocks tend to migrate between feeding and spawning grounds. Timings of maturity and spawning seem to be related to the different regions where cod occur. Reproduction of cod requires oxygen-rich water (min. 2 mg/L O_2) with a high salinity (min. 11 psu). Factors such as salinity and temperature influence the density of the water and thus also the drift behaviour and the water depth at which the eggs float. This is problematic in the Baltic Sea, for example, where the required conditions only prevail in ocean basins, partially due to natural and partially to anthropogenic influences, such as industry and agriculture, making conditions hypoxic and therefore lethal for eggs.

Mature specimens usually reach an average total length of 100 cm, although exceptional specimens of 200 cm have been reported. The generalist, broad diet of cod depends on the size of the fish. Cod larvae feed on plankton, juvenile specimens prey on small invertebrates (e.g. Decapoda) and larger fish feed on pelagic invertebrates, while fish as prey becomes generally more important with increasing size. Stomach content and parasitological analyses revealed an ontogenetic shift in diet from primarily Crustacea to a more piscivorous diet when reaching a total length of 30 cm.

Due to its abundance and economic importance, cod is one of the best studied species today. More than 120 metazoan parasites are known from this fish species. As for many fish species of north Atlantic waters, the known parasite fauna of Atlantic cod is diverse. In relation to the number of species, the trematode group Digenea is the dominant group, with at least 35 different species. A little less diverse, but often much more abundant, are the Nematoda with 26 species. The other represented major metazoan parasite taxa are Monogenea (9 species), Cestoda (19), Acanthocephala (10), Hirudinea (4) and Crustacea (19). The important role of cod as a host for parasites can be seen in the large number of generalist parasite species infecting this fish (83 out of 122 species). Of the remaining species, 15.9% are specific for Gadidae and 6.5% strictly host-specific for *G. morhua*. This diverse parasite fauna can be explained by its broad food range as well as extensive distribution in North Atlantic waters.

Anatomy and Morphology

Scientific name: *Gadus morhua*
Common name: Atlantic cod (English), Atlantisk Torsk (Danish), Kabeljau (German), Bacalao (Spanish)
Distribution: Shorelines and continental shelf of the North Atlantic
Size: max. 200 cm
Weight: max. 96 kg
Age: 25 years

The Atlantic cod has an elongated body, roughly round in cross-section. It grows to an average length of approx. 63 cm. However, the size depends on the region and the sex of the fish, with females being slightly larger than males. The dorsal body is normally spotted and appears brown to greenish in colour, which turns to silver on the ventral side. The lateral line is whitish and clearly visible. Located under a distinct upper and shorter lower jaw, Atlantic cod has a well-developed chin barbel, which is a typical characteristic of Gadidae. The three dorsal fins and the two anal fins are clearly separated. The pectoral and pelvic fins are relatively slender, the latter ending in an elongated filament.

Related Literature

Hemmingsen W, MacKenzie K (2001) The parasite fauna of the Atlantic cod, *Gadus morhua* L. Adv Mar Biol 40:2–60

Münster J, Klimpel S, Fock HO, MacKenzie K, Kuhn T (2015) Parasites as biological tags to track an ontogenetic shift in the feeding behavior of *Gadus morhua* off West and East Greenland. Parasitol Res 114:2723–2733

Atlantic Herring (*Clupea harengus*, Clupeidae)

As one of the most important food fish species, the Atlantic herring (*Clupea harengus*) has been targeted for thousands of years. Due to its importance, herring even influenced early settlements, as towns were often found near coastal spawning grounds of herring, which had an influence on the political power structures in medieval Europe. Its economic importance remained also during World War II and reached its peak in 1965 with catch volumes of over 1 million tons, followed by a collapse of stocks thereafter due to over-exploitation. After a fishing ban lasting several years, stocks are slowly recovering again. Today, stock sizes are increasing,

and herring can be found among the top five of the most exploited fish species worldwide. In Europe, it is an essential ingredient in many traditional dishes. In Germany, it is typically consumed in fried, marinated form as 'Brathering' or marinated with water and vinegar as 'Rollmops'. It is served smoked as 'Kipper' in the UK or fermented as 'Surströmming' in Sweden.

Ecology

The Atlantic herring is spread across the North Atlantic and can be found from Spitsbergen to the US Coast off South Carolina and to the Bay of Biscay of France in Europe. The relatively small (45 cm) and slender, silver-coloured fish is usually found in the pelagic zone and near the bottom of coastal waters between 2 and 400 m depth. Spawning takes place once a year, while spawning season varies between different local populations. Spawning grounds are typically found in shallow waters along coast lines or offshore banks, with gravel as substrate. At an age of 3–7 years, Atlantic herring reaches maturity with a typical spawn size of 20,000–50,000 eggs. Larvae hatch after a development period of 10–14 days. The huge spawning schools are an important food source for many larger predators, e.g. tuna, seals and whales.

Herring form large schools and show a migratory behaviour between coastal grounds for spawning and overwintering, and offshore grounds for feeding. The migration routes are acquired from older generations. Apart from the horizontal migration, herring is known to move slowly through the water column upwards at night to follow planktonic prey organisms. Adults feed mostly on small crustaceans, especially copepods, whereas larvae are known to prey on diatoms as well as larvae of molluscs and crustaceans.

Due to its high abundance and high nutritional value, herring is one of the most important food fish today, which at the same time has made it the focus of many parasitological studies. More than 90 different parasite species use the herring as an intermediate or final host. Although *C. harengus* has a diverse parasite fauna, it only shows low infection rates (e.g. prevalence and intensities), which is typical for planktivorous fish species. The origin of the diverse parasite fauna can be found in its wide distribution range, the diverse habitats it occurs in and in its great osmotic tolerance ranging from oceanic to nearly fresh waters.

Anatomy and Morphology

The body shape of *C. harengus* is slender and elongated and is covered with thin, silvery scales. The ventral side of the fish is silver and turns into greenish blue dorsally. Its ventral and anal fins are transparent, while the other fins are dark coloured. In contrast to many other fish species, *C. harengus* has no lateral line. The average length is around 20 cm, with individual specimens growing up to 45 cm.

Scientific name: *Clupea harengus*
Common name: Atlantic herring (English), Atlantisk sild (Danish), Hering (German), Arenque (Spanish)
Distribution: Shorelines and continental shelf of the North Atlantic
Size: max. 45 cm
Weight: max. 1.1 kg
Age: 25 years

Related Literature

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ICES (2017) ICES-FishMap *Clupea harengus*

European Plaice (*Pleuronectes platessa*, Pleuronectidae)

The European plaice (*Pleuronectes platessa*) is a widespread, medium-sized flatfish found in waters of the North Atlantic in Europe, the Baltic Sea, the southern Barents Sea and the southeast coast of Greenland. In terms of fisheries, it is the most important flatfish of European coastal fisheries. Since the nineteenth century, it has been a commercial target species. Landings peaked in 1989 with a maximum of 169,000 tons, followed by a decline during subsequent years. After the reduction of the spawning stock biomass down to a critical level, catch volumes were reduced. Today, stocks have recovered from the over-exploitation, and biomass of *P. platessa* is increasing again. Due to its economic relevance, the parasite fauna of *P. platessa* has been well studied. The parasite fauna consists mainly of Digenea and Nematoda. Most recorded species are generalists with a wide host range (e.g. *Derogenes varicus, Hemiurus communis*, *Echinorhynchus gadi*).

Ecology

As a coastal species, plaice can be found in estuaries but rarely enters freshwater habitats. They occur at depths between 0 and 200 m, but mainly between 10 and 50 m, with smaller specimens inhabiting shallow and larger specimens deeper regions. Small individuals are regularly found in the intertidal zone on substrates

like mud or sand, where they prey on benthic species, e.g. molluscs and various polychaetes. Large specimens additionally feed on sand eels.

European plaice reaches maturity at an age of 2–5 years depending on sex. Spawning takes place in offshore waters at temperatures around 6 °C, with eggs and larvae being part of the pelagic zooplankton until metamorphosis. At this point, the larvae return to the bottom and move back to their shallow nursing grounds. Typically, small fish prefer shallow waters, while larger specimens migrate to deeper areas.

Anatomy and Morphology

The European plaice shows an oval, compressed body which is typical for flatfish. The ventral side of the fish is brown with red or orange spots, while the dorsal side is white. The scales of plaice are small. The average length is approx. 40 cm with an average weight of 1 kg. Maximum sizes can reach 100 cm with a weight of up to 7 kg.

Scientific name: *Pleuronectis platessa*
Common name: European plaice (English), Scholle (German), Plie (French), Palaia anglesa (Spanish), Rødspætte (Danish)
Distribution: Shorelines and continental shelf of the North Atlantic
Size: 40 cm TL, max. 100 cm SL
Weight: 1 kg, max. 7 kg
Age: max. 50 years

Related Literature

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European Squid (*Loligo vulgaris*, Loliginidae)

The common squid or European squid (*Loligo vulgaris*) is an abundant squid species found in coastal, temperate waters off the East Atlantic coast and adjacent marginal seas, such as the English Channel, the North Sea, the Baltic Sea as well as the Mediterranean Sea. Due to its high abundance in these waters, it is the main target species for the European all year-round squid fisheries. It is also often caught as by-catch in bottom and pelagic trawling fisheries.

Ecology

The depth range of *Loligo vulgaris* stretches from the water surface to 500 m depth. It commonly occurs between 20 and 250 m and rarely appears in inshore waters shallower than 100 m. During winter months, *L. vulgaris* usually migrates to deeper water layers. These actively hunting predators feed mainly on fish, crustaceans and other cephalopods. However, seasonal variations in food composition, e.g. different fish species, do occur. Stomach content studies on cephalopods are rather difficult as they use their beaks to bite the prey into small pieces, which are then quickly digested. Parasites can therefore be used as biological indicators to gain a better long-term insight into the feeding ecology of *L. vulgaris*. European squid has only a short life span, which varies from 9 months to 1.5 years depending on the area of distribution. This short life span is typical for squid (Order: Teuthida) and is caused by a high metabolism, the highest known from marine invertebrates so far. Spawning takes place throughout the year, with fecundity generally increasing with female body size, sometimes exceeding 70,000 eggs. The eggs are placed inshore on solid substrate (e.g. stones) on top of sandy or muddy sediment.

Anatomy and Morphology

Loligo vulgaris can be described as a slender and elongated squid with rhomboid-shaped fins that cover two thirds of the mantle. Their colour can be greyish or reddish. Individuals can reach a maximum mantle length of 48.5 cm in females and 64 cm in males, with an average mantle length of approx. 20 cm. The maximum total weight for males is 2.3 kg and 1.3 kg for females.

Scientific name: *Loligo vulgaris*
Common name: European squid (English), Gemeiner Kalmar (German), Encornet européenne (French), Calamar europeo (Spanish)
Distribution: East Atlantic, from southwestern African coast to the British Isles and the Baltic Sea
Size: 20 cm, max. 64 cm
Weight: max. 1.3 kg for females, 2.3 kg for males

Related Literature

Storch V, Welsch U (2014) Kükenthal-Zoologisches Praktikum. Springer, 554 pages
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Parasitic Groups

Protozoa

The term protozoa describes heterotrophic, single-celled eukaryotes of different origin and comprises a variety of species, most of which are free-living. The term is not a category of systematics but is currently used in parasitology to distinguish single-celled parasites from multicellular parasites. One third of about 40,000 different species of protozoa have a parasitic lifestyle.

Classification

Phylogenetic analyses of the different groups of protozoa continuously generate new insights about their relatedness and often lead to changes in the systematics. A clear systematic presentation is therefore not possible here. Overall, the following groups of protozoa are important in fish: Microsporea (spore-forming), Kinetoplastea (flagellated), Ciliophora (ciliated) and Apicomplexa (spore-forming).

Development

Some protozoan groups multiply asexually by binary or multiple fission; others alternate between asexual and sexual reproduction. Protozoa usually go through complex life cycles in one or multiple hosts.

S. Klimpel et al., *Parasites of Marine Fish and Cephalopods*,
https://doi.org/10.1007/978-3-030-16220-7_3

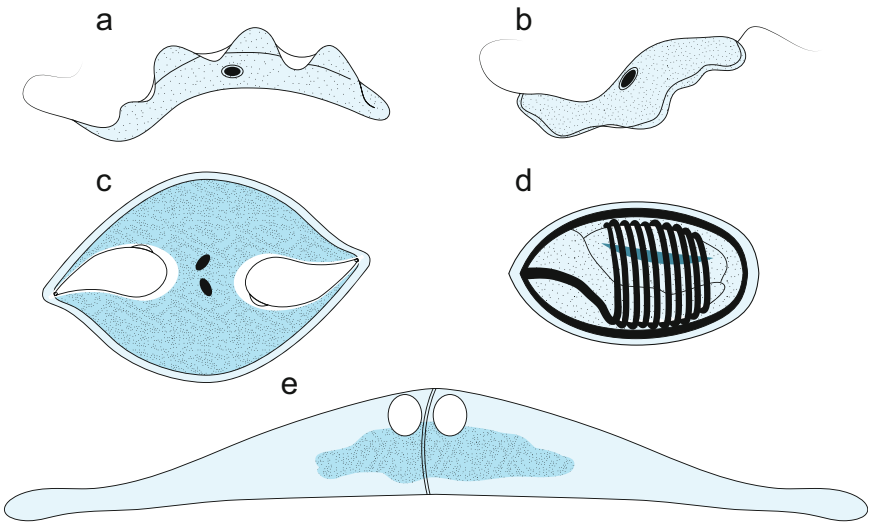

Fig. 1 Diagrammatic representation of different protozoan and metazoan parasites. (**a**, **b**) Typical *Trypanosoma* and *Trypanoplasma*. (**c**) *Myxidium gadi* in gall bladder of gadids. (**d**) Microspora (**e**) *Ceratomyxa auerbachi* in the gall bladder of herring (*Clupea harengus*)

Morphology and Marine Species (Figs. 1 and 2)

In marine fish, ectoparasitic protozoa occur with a relatively small number of species. More specifically, Ciliophora of the genus *Trichodina*, which comprises numerous species, can be found on the gills and on the body surface of almost all marine fish. Endoparasitic Microsporea include the important genera *Glugea*, *Loma* and *Pleistophora*. Numerous species of these endoparasitic protozoa, in particular those affecting the gall bladder, urinary bladder and musculature of fish, were already described at the turn of the twentieth century. With more and more fish species being studied, new forms and species of blood parasites of the class Kinetoplastea (genera *Trypanosoma* and *Trypanoplasma*) are discovered. However, the detection of intracellular parasitising Amoebae and Coccidia is significantly more difficult. Apart from some very characteristic forms, the identification of protozoan species is quite problematic since the inventory of their diversity is still in the early stages and some fundamental problems of classification have not been solved yet. Current species identification is based on the shape and size of the parasites and with molecular methods.

Microsporea: to date, more than 1300 species of the class Microsporea have been identified, and approx. 156 of these protozoan parasite species live in fish. Microsporea are considered to be a very original group of eukaryotes as they do not have any organelles of endosymbiotic origin. A relationship to fungi seems plausible and is currently supported by sequence analyses. Microsporea are relatively small parasites measuring only 1–7 μm. One of their characteristics are

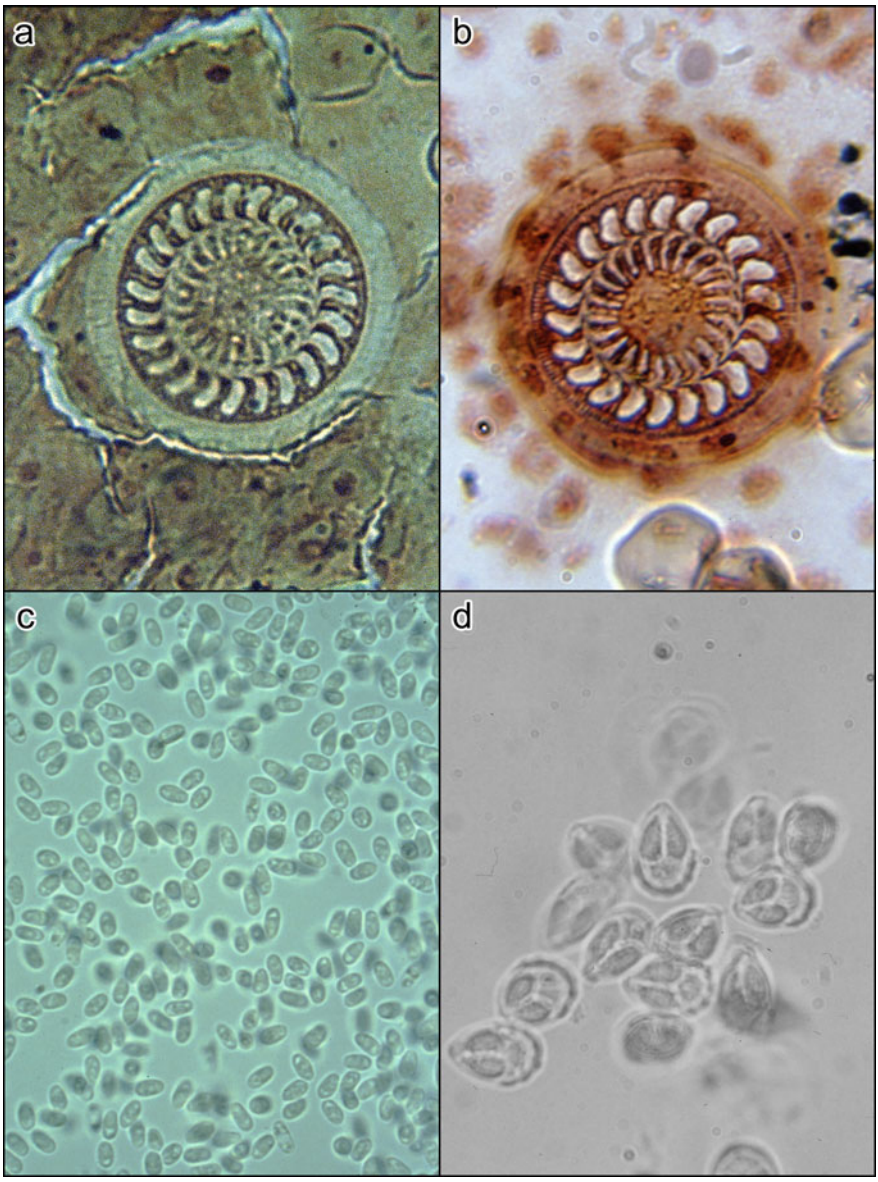

Fig. 2 Pictures of different protozoan and metazoan parasites. (**a**, **b**) Trichodinids with silver impregnated adhesive discs (*Trichodina* sp.). (**c**, **d**) Frontal view of spores of *Glugea stephani* (**c**) and *Myxobolus* sp. (**d**) isolated from the intestine or the gall bladder, respectively

infectious spores, each with a coiled polar filament. Spores are released into the environment with the faeces, with urine or during decay of the host but can also infect cells of the infected individual. Most species parasitize intracellularly and

thereby cause hypertrophy of the infected host cells, which results in the formation of mostly white pseudocysts. Some species cause mass mortality among fish stocks or destroy the musculature of fish. Infection with *Glugea* species leads to gigantism of the infected host cell, which later surrounds the spore mass as a thin layer. Species of the genus *Pleistophora* decompose the contents of the host cell without causing hypertrophy, but their mass replication in the fish musculature can lead to the formation of swellings. Most of the life cycles that have been described for Microsporea so far are monoxenous life cycles. However, there are also some species with obligatory intermediate hosts.

Kinetoplastea (older term: flagellates): As most flagellates, Kinetoplastea are free-living aquatic organisms with only few species infecting marine organisms. More than 4000 species have been described, of which about 140 species have a parasitic life style. The characteristic feature of this group is the kinetoplast, a highly organised accumulation of mitochondrial DNA. The parasites are equipped with one (*Trypanosoma*) or two (*Trypanoplasma*) flagella. The original taxon of Kinetoplastea includes ecto- and endoparasites of fish that either have a monoxenous life cycle or are transmitted by leeches constituting an intermediate host (heteroxenous life cycle).

Ciliophora (older term: Ciliata): Ciliophora are characterised by the presence of two nuclei ('vegetative macronucleus', 'generative micronucleus') and a variety of cilia, which either surround the entire outer body or are arranged annularly. The most important group is the genus *Trichodina*, which parasitises the gills or the skin and fin surfaces of most marine fish. Trichodines are circular and hat-shaped. The cilia are in a constant, whirling motion. They can anchor themselves in the mucous layer and in the epithelium of the fish by using ventral hooks (identification feature). In the case of mass infections, these hooks might damage outer layers to such an extent that secondary infections of the host occur.

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Myxozoa

Myxozoa are metazoan parasites with a worldwide distribution. Their life cycle alters between vertebrate and invertebrate hosts. At the time of their discovery in 1838, the class was mostly assumed to be protozoan and placed accordingly into the same taxa as diatoms or, as Sporozoa, in the order Microspora. Shortly after, it was suggested that they might be multicellular; however, it took more than 140 years before they were finally recognised as Metazoa at the end of the 1990s. After being classified as Bilateria, they were finally identified as Cnidaria. The dominant vertebrate hosts for Myxozoa are bony fish (Teleostei), while Amphibia, Reptilia or cartilaginous fish (Chondrichthyes) are rarely used as hosts by Myxozoa. Single host records were made from ducks and hedgehogs. It is assumed that invertebrates were the first hosts and vertebrates were only later integrated into their life cycle.

Classification

Kingdom: Animalia

Phylum: Cnidaria

Class: Myxozoa

Subclass: Malacosporea
Subclass: Myxosporea

Myxozoa are divided into the two subclasses Myxosporea and Malacosporea, depending on their invertebrate host. Myxosporea typically use Annelida as invertebrate hosts, while Malacosporea rely on Bryozoa. Malacosporea (one famous representative is *Tetracapsuloides bryosalmonae*) are to the current state of knowledge only distributed in freshwater, whereas Myxosporea can be found in fresh, brackish and salt water. Today, more than 2180 species from 62 different genera are known. The majority belong to the Myxosporea, while only a small proportion (four species) are considered Malacosporea. A non-taxonomic classification distinguishes the species depending on their infection site. Species parasitising the gall bladder and urinary ducts are called coelozoic species, whereas species predominantly infecting soft tissue are called histozoic species. The latter include economically important species, e.g. of genus *Kudoa*, the causative agents of the soft-flesh syndrome.

Morphology (Fig. 2)

Morphological characteristics of Myxozoa are one to several polar capsules, which resemble those of other Cnidaria and are surrounded by one to multiple spore valves. Each of those capsules contains a filament, which is used for attachment to the host's skin or the surface of the mouth or stomach. After the spore is anchored to the host tissue, a sporoplasm is injected behind the polar capsules. The forms of the spores are very diverse, depending on the developmental stage. While spores of the Myxosporea mostly have a droplet or spherical shape with varying numbers of polar capsules depending on the species, the spores of Actinosporea are more diverse in their form. Most have an anchor-like shape, and often long filaments to enable the spore to remain in the water column for a longer time, which increases the probability of their encountering and infecting a host organism.

Development (Fig. 3)

Despite being a species-rich and economically important group, the life cycle of the microscopic metazoans was discovered only 40 years ago. One of the first species with a full description of its life cycle was *Myxobolus cerebralis*, a parasite that causes whirling disease in salmonid fish. The life cycle of Myxozoa is rather unusual, as the transition between their hosts is realised by sporocysts. The life cycle alternates between invertebrates and vertebrates. Vertebrates, mostly fish, get infected when the actinosporean stage contacts the host's skin or mouth tissue. The spores anchor themselves with filaments stored in the polar capsules, after which a

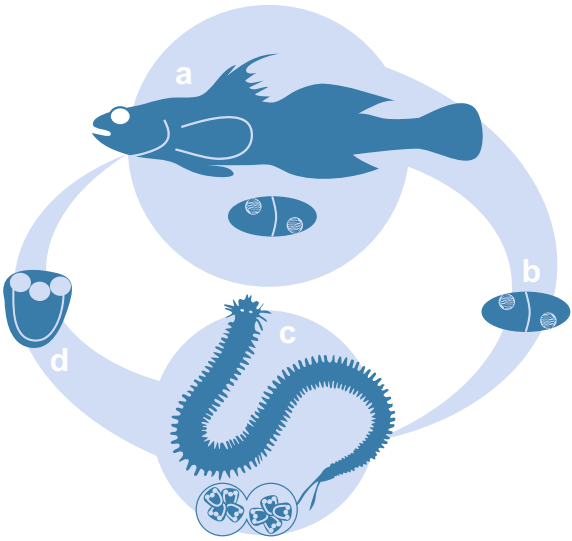

Fig. 3 General myxozoan life cycle. The life cycle showing alternation between (**a**) fish and (**c**) Annelida (e.g. Polychaeta, Oligochaeta) worm hosts, infected by (**b**) myxospore or (**d**) actinospore, respectively. Myxospores have two polar capsules, and actinospores have three

motile sporoplasma, also referred to as amoebula, is injected. The sporoplasma then migrates to the infection site within the host and develops into a syncytium (multinucleated cell), in which the infectious spores arise. After formation of the spores, the surrounding sporoplasma falls apart, and the spores are released. Depending on the infection site within the host, spores of Myxosporea are either excreted by the host itself, by a predator of the host after ingestion, or while the host fish is decaying. Once in the water column, the spores sink to the bottom where they are ingested by their invertebrate host (e.g. *Tubifex tubifex*). As during the myxosporean stage, the spore anchors with its polar filaments in the intestinal wall and injects the infectious germ cells, which start replicating and evolving into the actinosporean stage. Similarly to the process in the vertebrate host, spores are released with the faeces of the host into the water column. Actinosporean stages usually develop long filaments which help them get dispersed and remain in the currents. As soon as the spores come into contact with fish tissue, they attach themselves with the help of the polar filaments, and the life cycle is completed. For some marine Myxozoa, several studies have indicated a direct transmission from fish to fish.

Marine Species

Today, 14 different myxozoan families and 41 genera are known to infect marine fish species. Of those, the most diverse family is the Sinuolineidae, with nine genera occurring in all oceans. The number of known marine myxozoan species is only small in comparison to the still unknown existing species of this group. True distribution patterns cannot really be recognised today as sampling effort is concentrated in the northern hemisphere with only some hot spots off Australia and South America.

While most families exhibit a variety of genera, some with single genera are also known, e.g. the genus *Kudoa* for the family Kudoidae. Despite the low generic diversity, *Kudoa* harbours more than 70 species. While most species within this genus are host-specific for one particular species (e.g. *Kudoa musculoliquefaciens* in *Xiphias gladius, K. clupeidae* in *Clupea harengus*), more than 37 host species from 18 different families are known for *K. thyrsites*. *Kudoa* is, together with the less well-known genera *Unicapsula* and *Hexacapsula*, the causative agent of the so-called soft-flesh syndrome which affects different economically important species (e.g. *Clupea harengus*, *Salmo salar, Scomber scombrus*). Studies found no changes of the host behaviour or life span despite an infection with *Kudoa*. The soft-flesh syndrome is caused by the release of proteolytic enzymes. These enzymes are released to create space for the developing plasmodia. However, studies suggest that surrounding tissue is not harmed. Enzymes start to liquefy the tissue only after the death of the host. The enzymes are Cathepsin L proteases, which are responsible for the myoliquefication, and probably cause the release of the spores into the water column.

At present, the invertebrate hosts for most of these marine myxozoans remain unknown. Today, six complete life cycles of marine species are discovered (e.g. *Sigmomyxa sphaerica*). Apart from economic losses in aquaculture and fisheries, myxozoans are important as causative agents of food-borne disease. With over 100 cases per year, *K. septempunctata* causes severe diarrhoea and vomiting in Japan.

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Digenea

Together with the Aspidogastrea, the Digenea form the class Trematoda and represent by far the most diverse taxon within the group of internal metazoan parasites. Digenean trematodes are endoparasites with obligatory hosts, a generational change,

and complex life cycles that may vary considerably among the taxa. Many species from terrestrial and freshwater habitats are of significant medical importance (e.g. *Schistosoma* spp.) and pose a major threat to the health of both animals and humans. In marine habitats, adults commonly occur in the alimentary tract of all classes of vertebrates. In fish, they might also occur under the scales, on/in the eyes, gills, in the swim/urinary/gall bladder, the body cavity, the musculature, ovaries and circulatory system. Most digeneans have an oral sucker, opening into a blind gastrointestinal tract, and a ventral sucker which is used only for attachment. However, the latter is also absent in many species.

Classification

Kingdom: Animalia

Phylum: Platyhelminthes

Class: Trematoda

Subclass: Digenea

Due to the highly variable classification parameters and the large number of species and genera, the taxonomy of the Digenea is complicated. Following the biological systematics of the Trematoda, provided in three volumes by Gibson et al. (2002), Jones et al. (2005) as well as Bray et al. (2008), the most recent, fully revised classification is based on molecular ssrRNA and lsrRNA analyses of nearly 170 taxa. In broad terms, the Digenea form a dichotomy with two orders: Diplostomida and Plagiorchiida. The first order includes among others Sanguinicolidae, Spirorchidae and Schistosomatidae, which inhabit the bloodstream. The second group consists of all other members of the Digenea separated into 13 superfamilies.

Morphology (Fig. 4)

Despite some extreme exceptions reaching several centimetres or even metres (*Nematobibothrioides histoidii*), most digeneans are comparatively small and not more than 0.5–5 mm in length. Although a great variety of forms exists among the digenean species, the most typical digenean trematode is dorsoventrally flattened, leaf- or tongue-shaped and commonly bears two suckers, one at the anterior end and one around mid-body. Based on the structure and arrangement of these suckers, adult specimens can be divided into several groups: gasterostome, monostome, distome, amphistome, echinostome, holostome and schistosome. The structure of the body's surface, the intestine, the excretory system and the organs of the genital apparatus are

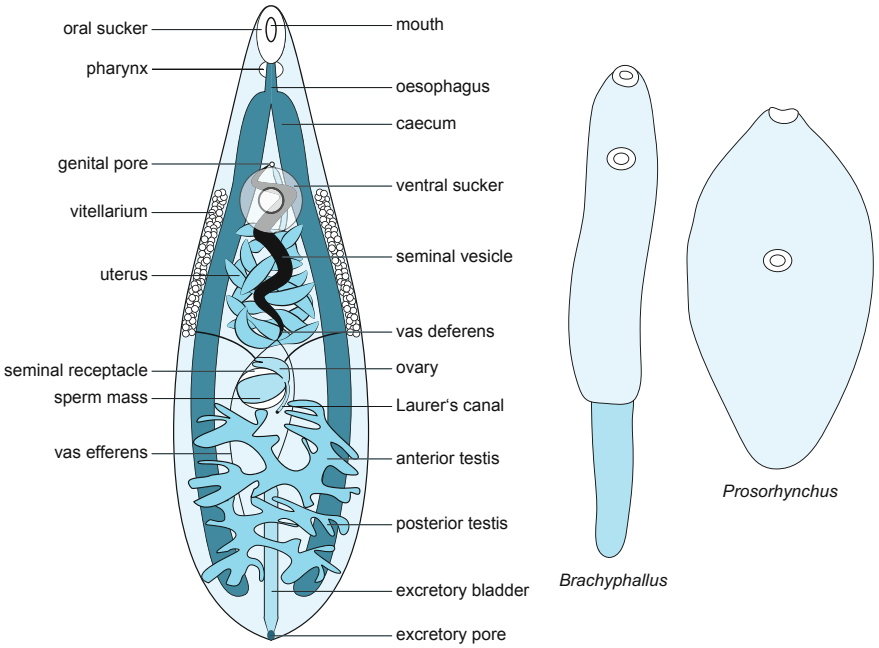

Fig. 4 Diagrammatic representation of the inner structure of digenean trematodes and form variations

clearly visible, even at low microscopic magnification. The alimentary tract is usually blind-ending and comprises a short tubular prepharynx, oesophagus and two (sometimes single) caeca.

Except for a very few species (e.g. *Schistosoma*), digeneans are hermaphrodites, and the reproductive system fills most of the body. The most prominent structures in the male system are typically the two testes, which are usually clearly visible even in fresh, uncleared samples and are considered an important taxonomic feature. The female system is more complex, with vitelline follicles, the uterus (filled with eggs) and the ovary as the most prominent and most clearly visible structures. The excretory system comprises flame cells, ducts and an excretory pore at the posterior end of the body.

Development (Fig. 5)

Even with a terrestrial definitive host, the life cycle is associated with water (e.g. wetlands, marshes). It is usually heteroxenous and involves both free-living and parasitic stages as well as asexual and sexual reproduction. Most of the Digenea alternate between a mollusc as the first obligate intermediate host and a vertebrate as definitive host. A very typical life cycle includes the motile and ciliated miracidium

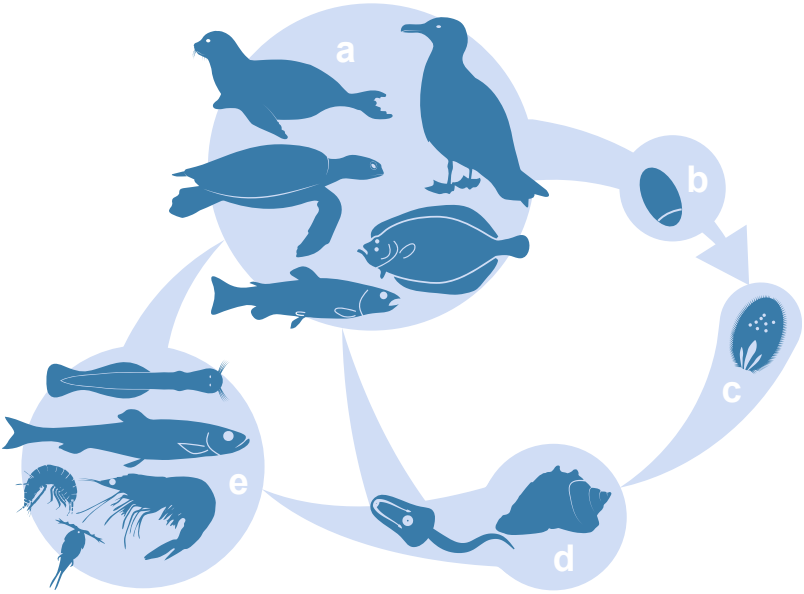

Fig. 5 General Digenea life cycle. (**a**) The hermaphroditic adults live within the gastrointestinal tract of the final hosts (e.g. planktivore and piscivore fishes, piscivore birds). (**b**) Eggs are excreted by the final hosts through faeces and contain a miracidium. Egg size and shape vary with species. (**c**) The egg is ingested by the first intermediate host (aquatic snails), and the miracidium hatches inside its intestine or the miracidium hatches in the water column and actively penetrates the molluscs. (**d**) Within the snail, the miracidium penetrates the hepatopancreas (digestive gland) and transforms into a redia or sporocyst (depending on the species). These form cercaria, which are released into the water and can directly infect the final host (ingestion or penetration of the skin) or (**e**) infect another intermediate host (e.g. Pisces, Chaetognatha, Crustacea: Cyclopoida, Amphipoda, Euphausiacea) where they transform into metacercariae, which mature over time until the intermediate host is ingested by the final host

stage, which hatches from an egg and is released into the environment by sexual adults. Molluscs (e.g. gastropods, bivalves, scaphopods) are the first obligate intermediate hosts in which the miracidium develops into an asexually reproducing sporocyst that may either produce further generations of sporocysts (daughter sporocysts) or, alternatively, one or multiple generations of rediae, in turn releasing infective stages known as cercariae. Both daughter sporocysts and rediae reproduce asexually in the same way as the initial mother sporocyst. Cercariae leave the mollusc and will be either encysted (as metacercariae) in or on a second intermediate host (e.g. crustaceans, plants, fish) or directly infect the definitive host and grow into the mature stage. Additional intermediate hosts may be incorporated into the life cycle.

Marine Species (Figs. 6 and 7)

About 70 families of digeneans occur in teleost fishes. More than 5000 species known from marine and freshwater fishes can be assigned to 10 families: Acanthocolpidae, Bucephalidae, Cryptogonimidae, Derogenidae, Didymozoidae, Fellodistomidae, Hemiuridae, Lecithasteridae, Lepocreadiidae and Opecoelidae. These account for about two-thirds of records of digeneans in fishes. In addition, many groups of birds have a strong association with aquatic habitats and may act as definitive hosts for digeneans which include fishes as intermediate hosts in their life cycles. The most important digenean parasites of marine fishes are briefly introduced below.

Derogenes varicus is probably the most widespread digenean species. It is found in over 100 teleost species and occurs in temperate waters from the subarctic regions in the north to subantarctic regions in the south. It is known to include a variety of intermediate hosts in its life cycle. While the first intermediate hosts are presumably various species of moon snails like *Natica alderi* or *N. clausa,* the second intermediate hosts are more diverse, ranging from copepods (e.g. *Calanus finmarchicus*) to Paguridae. Metacercariae were also detected in the chaetognath *Sagitta* spp., which were probably infected by preying on infected copepods. *Derogenes varicus* was also recorded from the parasitic crustacean *Lernaeocera lusci,* which is a case of hyperparasitism.

Another widely distributed digenean species is *Hemiurus communis*. It can be found in the boreal zone of the North Atlantic. Like *D. varicus*, *H. communis* is considered a generalist parasite with a broad host spectrum. Its first intermediate hosts are snails, e.g. *Retusa truncatula*. These snails occur in the intertidal zone down to depths of 50 m in the North Sea, the Baltic Sea as well as coastal waters of the North Atlantic. Second intermediate hosts are copepods and chaetognaths, which are preyed upon by small fish. Final hosts include a variety of non-clupeid, mostly piscivorous fish like *Gadus morhua* or *Pollachius virens*. *Gonocerca phycidis* is a generalist representative of the Digenea. It shows a wide host spectrum including teleosts, but also the cartilaginous Holocephali. This parasite has a circumpolar or even cosmopolitan distribution and occurs typically at depths of 130–1400 m. Despite its wide distribution, the life cycle of *G. phycidis* is not entirely known. The intermediate hosts are probably Mollusca and small Crustacea, e.g. copepods. Its final hosts are fish like *G. morhua* or *Macrourus berglax*.

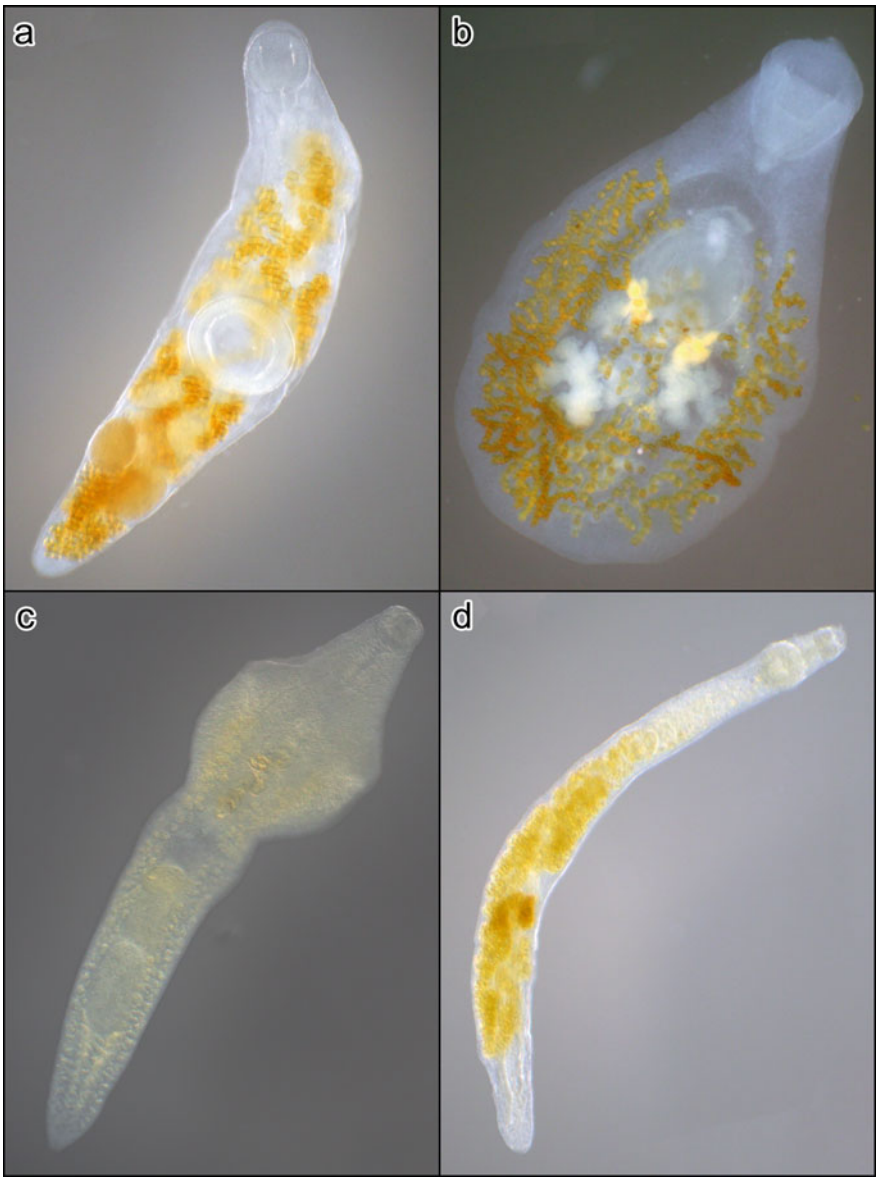

Fig. 6 Different digenean parasites. (**a**) *Derogenes varicus* from the stomach of Atlantic cod (*Gadus morhua*). (**b**) *Degeneria halosauri* from the ureter of Abyssal halosaur (*Halosauropsis macrochir*). (**c**) *Gibsonia borealis* from the digestive tract of Roughhead grenadier (*Macrourus berglax*). (**d**) *Hemiurus luehei* from the stomach of herring (*Clupea harengus*)

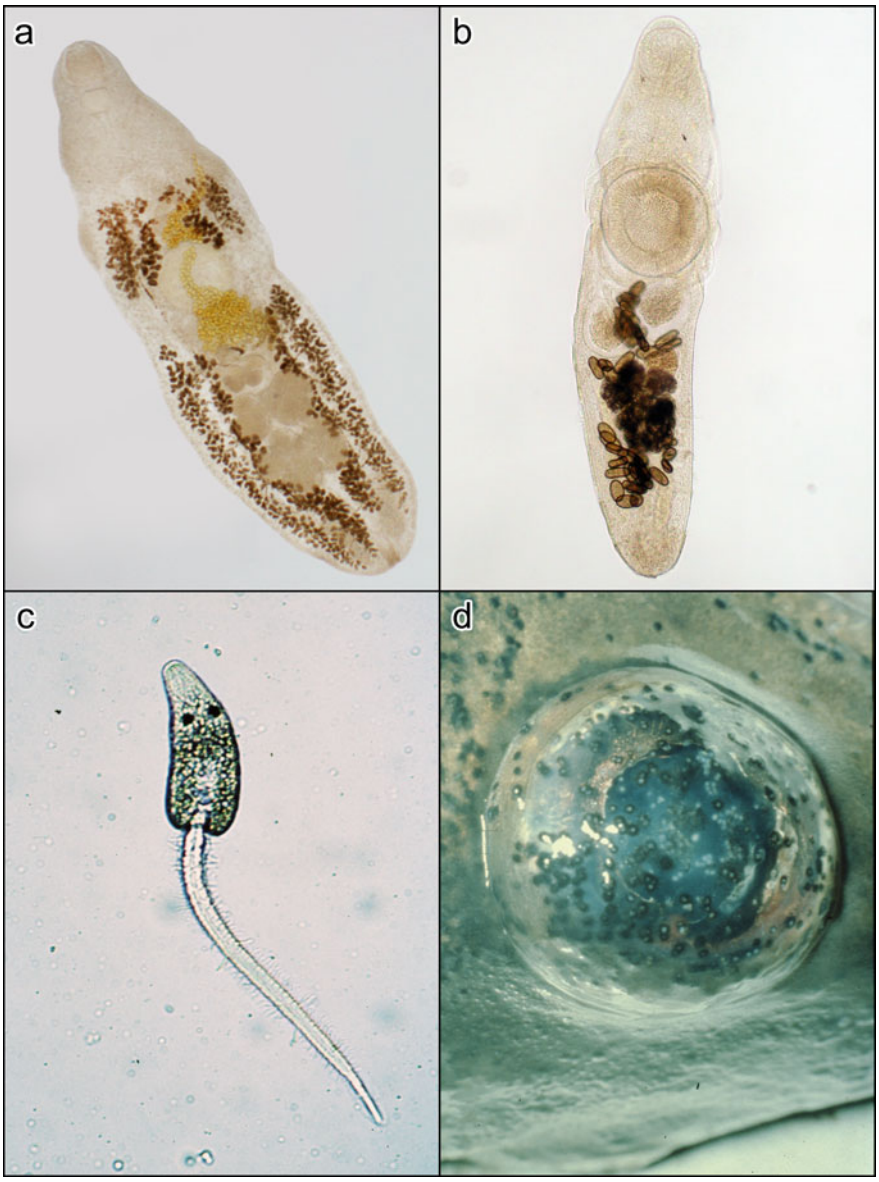

Fig. 7 Different digenean parasites. (**a**) *Discoverytrema gibsoni* from the intestine of Smalleye moray cod (*Muraenolepis microps*). (**b**) *Lecithophyllum botryophoron* from the stomach and intestine of Goiter blacksmelt (*Bathylagus euryops*). (**c**) Cercaria of the trematode genus *Cryptocotyle*. (**d**) Fish (Atlantic cod) with multiple metacercaria infection sites (eye, skin)

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Monogenea

The monogenean flatworms are a group consisting mainly of ectoparasites parasitising on freshwater as well as marine fish species. However, a few species have switched from an ectoparasitic to an endoparasitic mode of life. Monogeneans are a diverse group, with 25,000 estimated species known both from teleosts and elasmobranchs. Occasionally, they can also be present in amphibians and reptiles, and a single species has been recorded from a hippopotamus. Despite this large number of species, only 3000–4000 species have been described so far. These are divided into the Monopisthocotylea and Polyopisthcotylea, which are morphologically separated by the form of their opisthaptor, a clamp or hook-bearing attachment organ at the posterior end of the parasite. Due to these morphological adaptations to specific host organs, most Monogenea are highly host-specific. However, species

with a broad host range, such as *Neobenedenia melleni* with more than 100 different fish hosts, are also known. While Monopisthocotylea parasitise on gills, skin and fins, Polyopisthocotylea are only found on gills. While Monopisthocotylea feed on the epidermis, Polyopisthocotylea feed solely on host blood.

Classification

Kingdom: Animalia

Phylum: Plathyhelminthes

Class: Monogenea

Subclass: Monopisthocotylea
Subclass: Polyopisthocotylea

The class Monogenea belongs to the phylum Platyhelminthes and is separated into the subclasses Monopisthocotylea and Polyopisthocotylea.

Morphology (Fig. 8)

The haptor of the Monopisthocotylea has a symmetrical, platelike shape. It consists of one, two or rarely three pairs of large hooks, known as hamuli, and 14 or 16 smaller hooklets (<15 μm). The haptor is used for attachment to the host's surface. The haptor can be present as a large single sucker or may be divided into smaller suckers called loculi. Polyophistocotylea have several small clamps that can be equipped with small hooks or act as suckers. Clamps are stabilised internally by several sclerites, which can vary in number and orientation. The number, form and arrangement of the hooks and hamuli are species-specific. In contrast to Polyopisthocotylea, Monopisthocotylea have no haptoral clamps.

Anteriorly, Monogenea commonly have another organ of attachment. Its shape varies and can, for example, consist of suction cups or glands. An oral sucker may also be present. The intestine is usually divided into two blind caeca, although a sac-like, ring-shaped or highly branched intestine can occur. All monogeneans are hermaphroditic, with separate male and female reproductive organs.

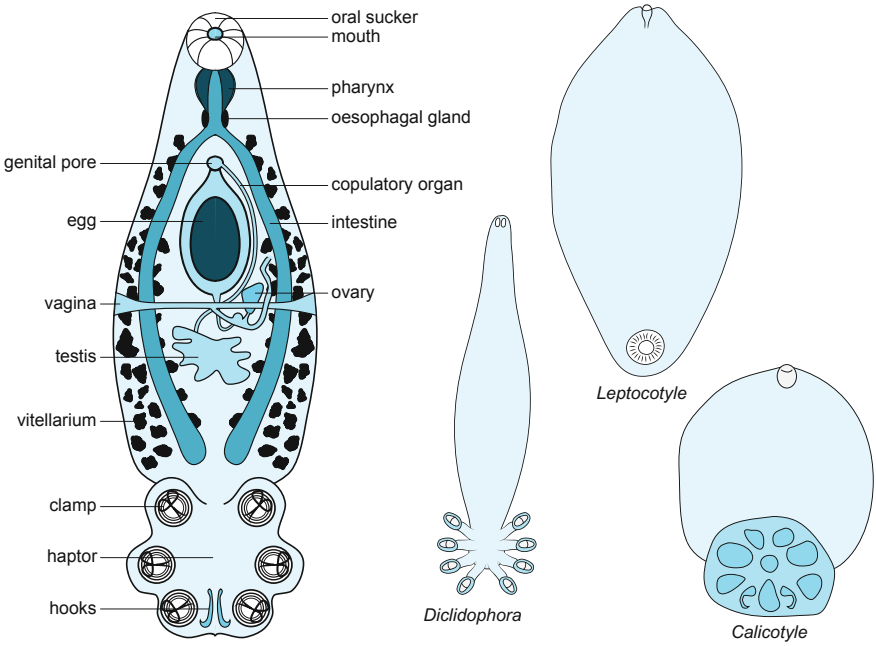

Fig. 8 Diagrammatic representation of the inner structure of monogenean trematodes and form variations

Development (Fig. 9)

The hermaphroditic monogeneans have a direct life cycle with a single host. For at least two species of polyopisthocotyleans (*Gotocotyla acanthura, Pricea multae*), small fish are known to serve as intermediate hosts. Most marine species use fish as hosts and are ovoviviparous and lay unembryonated eggs; however, most Gyrodactylidae are viviparous. Inside the egg, the oncomiracidium, a ciliated larva, matures. After hatching, the larva is infective for its host. Gyrodactylidae larvae locate their hosts using sensory receptors (e.g. eyespots). When coming into contact with a potential host, the ciliated layer is ejected, and the larvae attach themselves to the skin, fins or gills of the host. Buccal, branchial and nasal cavities are less common infection sides. Knowledge of the generation times and longevity of Monogenea is very fragmented.

Marine Species (Fig. 10)

Today 4000 to 5000 species of monogeneans are known, but the actual number is expected to be much higher. The distribution of marine monogeneans ranges from

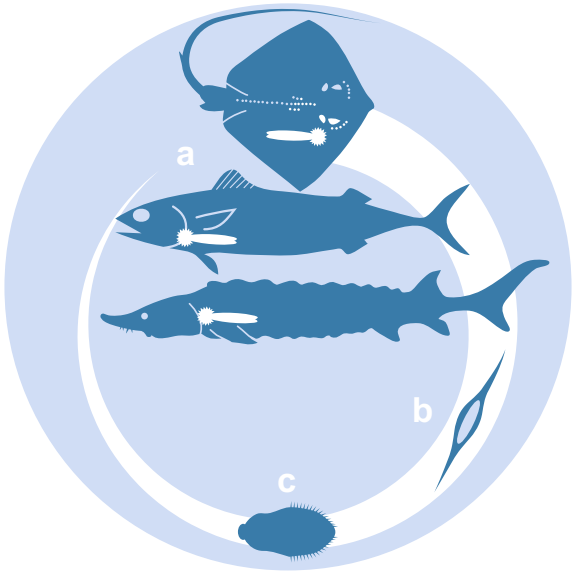

Fig. 9 General Monogenea life cycle. (**a**) The hermaphroditic adults live ectoparasitic on the host (e.g. planktivore and piscivore fishes), attached to the skin, eyes or gills. (**b**) Eggs are released and get caught on the same host or are swept away, possibly attaching to another host. The eggs of most monogenean species have long appendages, serving for attachment to gill filaments. (**c**) From the egg, the ciliated and with multiple hooks equipped oncomiracidium hatches. After hatching, the oncomiracidium larva attaches to the surface of the same or new host and creeps to its final site, where the parasite usually stays for the rest of its life

polar to tropical waters and varies from cosmopolitan to a very restricted range, usually depending on the endemic distribution of their hosts. The diversity of marine monogeneans, at least for coastal regions, is higher at low latitudes than at higher latitudes. The polyopisthocotylean *Entobdella soleae* parasitises mainly on the gills of the flatfish *Solea solea* in North Atlantic shelf waters off the European coast. This species has relatively large eggs and free-swimming larvae of 250 μm length, which can be easily spotted. *Diclidophora merlangi* typically parasitises the first gill arch of the whiting *Merlangius merlangus*. This species belongs to the family Diclidophoridae, the predominant family of deep-sea Monogenea. It usually parasitises fish species of the order Gadiformes, mainly Gadidae, Macrouridae and Moridae. The distribution of *D. merlangi* ranges along the Northeastern Atlantic and corresponds to the distribution of its host.

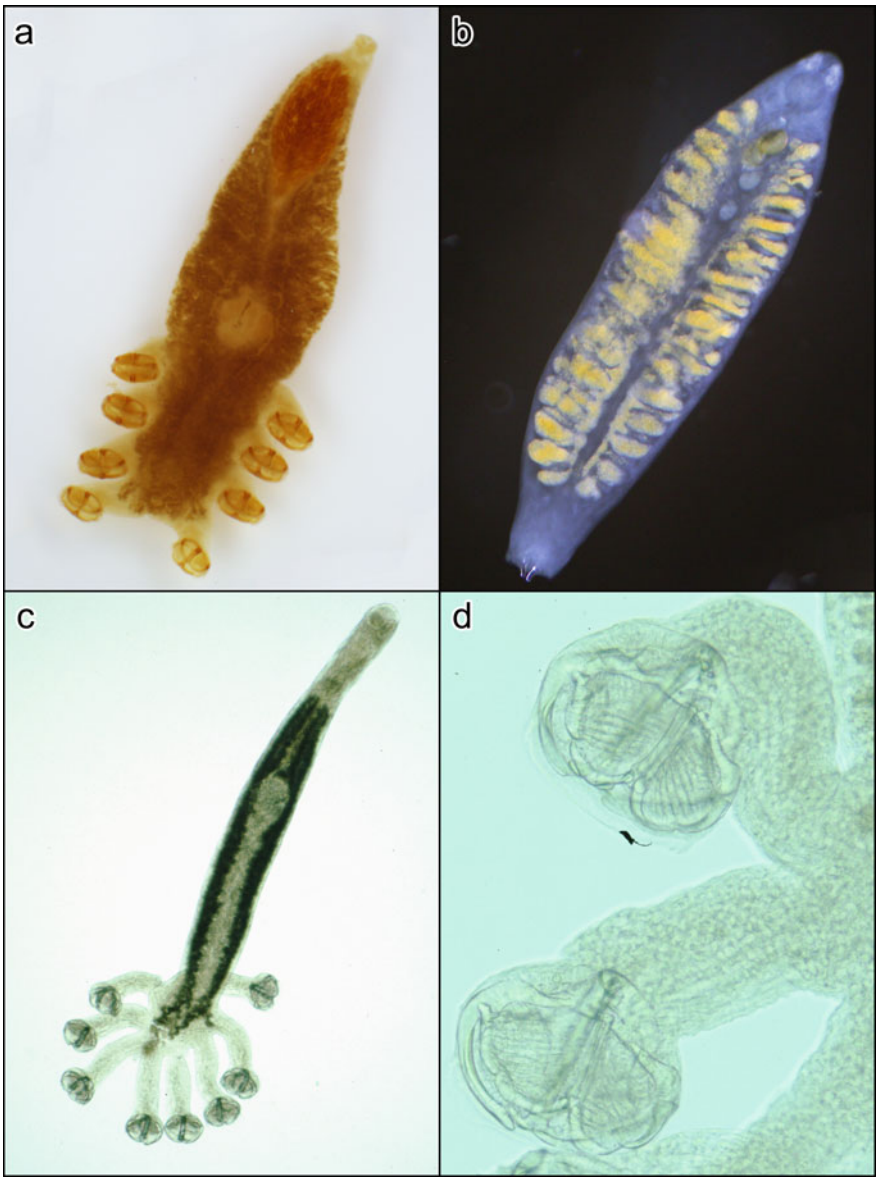

Fig. 10 Different monogenean parasites. (**a**) *Diclidophora pollachii* form the gills of the Saithe (*Pollachius virens*). (**b**) *Paradiplectanotrema lepidopi* from the oesophagus of Silver scabbardfish (*Lepidopus caudatus*). (**c**) *Cyclocotyloides bergstadi* from the gills of the grenadier *Coryphaenoides brevibarbis*. (**d**) Detail of haptoral peduncles of *C. bergstadi*

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Cestoda

Cestodes (or tapeworms) are exclusively endoparasitic. They infect many teleost and bony fish species but usually do not appear to cause much harm to their vertebrate definitive hosts. Adults inhabit the digestive tract or occasionally its associated organs. Once anchored to the host's intestinal wall, cestodes absorb nutrients and remain stationary for the rest of their life span. They may reach a considerable length of several metres (e.g. marine *Tetragonoporus calyptocephalus*, freshwater *Diphyllobothrium latum*) and continuously produce proglottids. These proglottids increase in size towards the end of the body where they also reach a more developed stage of maturity and are finally shed within the faeces of the host. Fish may serve as second intermediate hosts and definitive hosts or both.

Classification

Kingdom: Animalia

Phylum: Platyhelminthes

Class: Cestoda

Subclass: Cestodaria

Order: Amphilinidea
Order: Gyrocotylidea

Subclass: Eucestoda

The class Cestoda includes three groups of Platyhelminthes, the Gyrocotylidea and Amphilinidea, which both belong to the subclass of Cestodaria, and the Eucestoda. Only Eucestoda, which are commonly referred to as Cestoda in the literature, are considered to be 'true tapeworms' (elongated and dorsolaterally flattened). Since Amphilinidea are of low economic importance and Gyrocotylidea are a very small group of parasites infecting only Chimaeriformes, the focus in this book will be on the true tapeworms (hereafter referred to as Cestoda). For a very recent update on the deep phylogenies of the Cestoda found in vertebrates, the publication by Caira et al. (2017) is highly recommended.

Morphology (Fig. 11)

Cestodes are hermaphroditic polyzoic flatworms. They have three distinct body regions, the scolex, the neck and an elongated segmented strobila consisting of individual proglottids. The scolex is a distinct anterior holdfast organ, which is often armed with hooks and/or equipped with suckers and bothria that function like suction cups. Following a thin neck, the flattened body is composed of successive segments, the proglottids. Together, they form the tapeworm chain (strobila). The proglottids are continuously produced in the neck region posterior to the scolex, and each comprises one or more sets of reproductive organs. As the cestode matures, the segments are shifted towards the posterior end of the individual and are separated (e.g. apolysis) by the time they have reached the end. They lack a digestive system and absorb nutrients through their neodermis. Like other flatworms, cestodes use protonephridia (flame cells), located in the proglottids, for excretion. The reproductive system includes a lobed or unlobed ovary, oviduct and uterus (female organs) as well as testes, cirri, vas deferens and seminal vesicles (male organs).

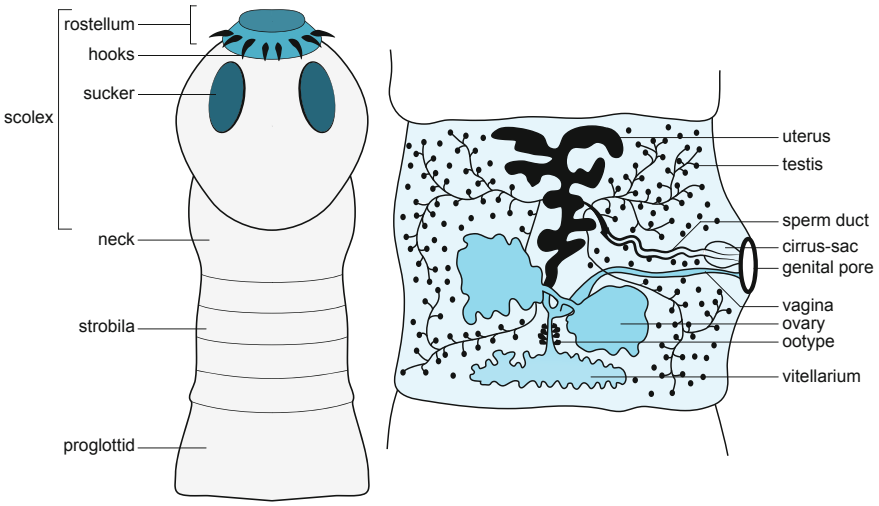

Fig. 11 Diagrammatic representation of cestodan anatomy

Development (Fig. 12)

Life cycles of marine cestodes lack free-living stages, with the exception of those involving the free-swimming stage known as coracidium. Thus, they rely on the natural dynamics of the food web to achieve transfer between hosts. Unfortunately, life cycles of marine cestodes are still poorly known, and only a few have been studied in detail. In marine habitats, vertebrates function as definitive hosts. At least one, but usually several intermediate hosts, may be included in the life cycle. Paratenesis is common. Excreted eggs develop into a stage known as hexacanth embryo which remains inside the eggshell or into a coracidium which is surrounded by a ciliated membrane and hatches from its egg. Hexacanth embryos do not hatch and remain passively within the egg until ingested by a suitable host. Following the stage of the hexacanth embryo, the presence and sequence of larval stages may vary between cestode orders. Diphyllobothriidea and Bothriocephalidea, for example, possess two parasitic larval stages, the procercoid and plerocercoid. Procercoids have been reported from several crustacean groups (copepods, amphipods). Plerocercoids, on the other hand, may infect a wide range of teleosts; small, planktivorous teleosts serve as intermediate hosts, whereas larger piscivorous fish serve as paratenic hosts. Plerocercoids emerge from the procercoid and might already possess a scolex with suction pits as well as some degree of strobilisation. Plerocercoids develop into adults upon predation by the definitive host.

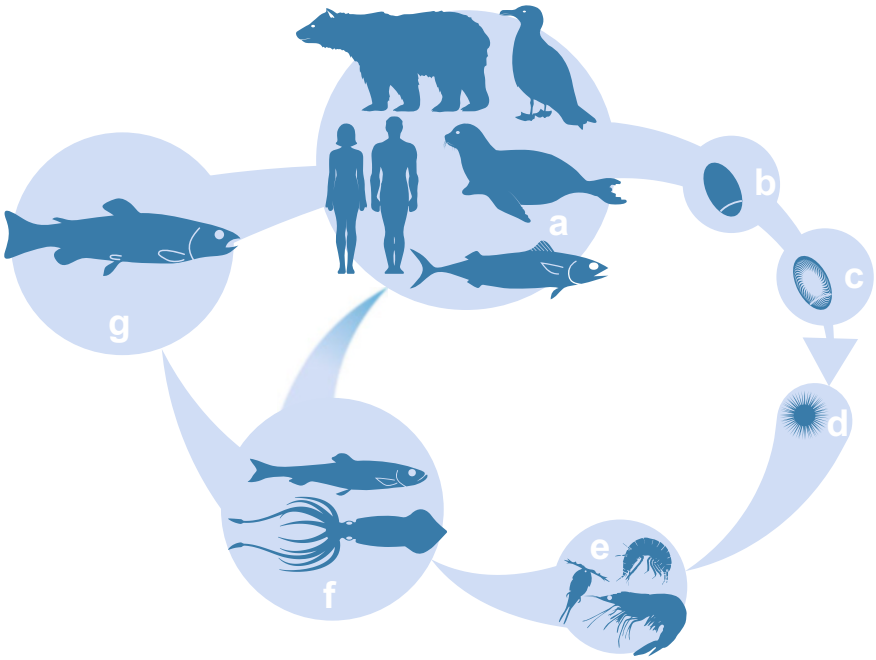

Fig. 12 General Cestoda life cycle. (**a**) The hermaphroditic adults live, attached with the scolex, within the intestine of their final hosts (e.g. Ursidae, Pinnipedia, piscivore birds, planktivore and piscivore fishes, humans). (**b**) Operculated eggs are directly excreted unembryonated or from detached proglottids and transported into the water with the hosts faeces. (**c**) Within the water column, they develop over a time span dependent on the water temperature. From this egg, the coracidium larva containing the oncosphere, which is endowed with six hooks, hatches. (**d**) The coracidium is ingested by the first intermediate host-small crustaceans (e.g. Cyclopoida, Amphipoda, Euphausiacea). (**e**) Within the crustaceans, it develops into the procercoid larva. (**f**) The infected crustaceans are ingested by the next paratenic host (small fish, Cephalopoda), where they penetrate the intestine wall and bore into the muscle and body tissue where they develop into plerocercoid larvae. The infected second intermediate hosts are then either eaten directly by the final host or (**g**) another paratenic host, where the plerocercoid larvae can accumulate within the tissue. The last paratenic host is then eaten by the final host, where the plerocercoid larvae enter the digestive system and rapidly grow into adults. Multiple cestoda species are able to carry out their life cycle within several final hosts, while in accidental hosts, the plerocercoid larvae accumulate and migrate through the body

Marine Species (Fig. 13)

More than 1400 cestode species are known to occur in marine habitats, and 465 species have been described from bony fishes alone. Among these, Diphyllobothriidea, Bothriocephalidea (together formerly known as Pseudophyllidea), Trypanorhyncha and Tetraphyllidea are the predominant orders. In addition, some species of the Spathebothriidea (6) and several hundred species from the Cyclophyllidea are also known from euryhaline and marine environments.

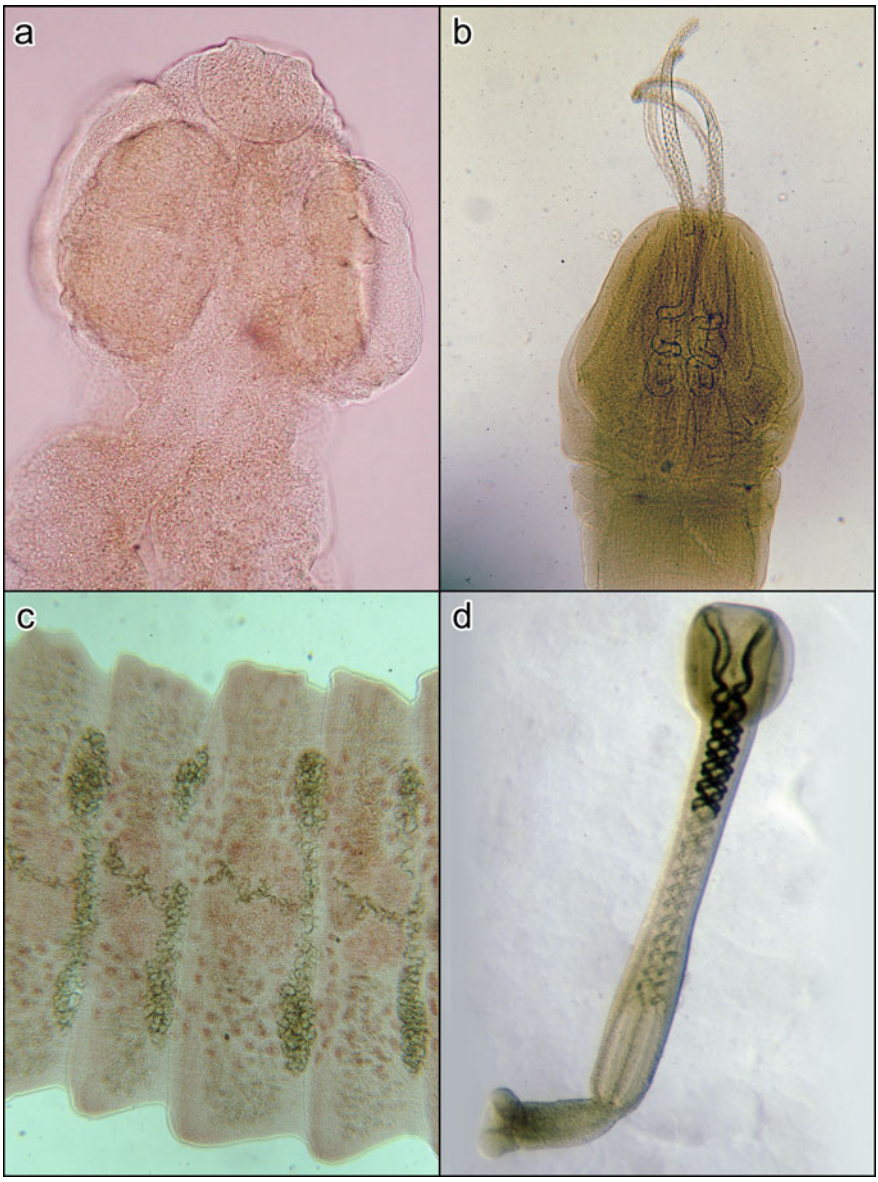

Fig. 13 Different cestode parasites. (**a**) *Scolex pleuronectis* refers to larval stages of species of cestodes in the order Tetraphyllidea. (**b**) *Mixonybelinia southwelli* (trypanorhynch cestode) from the Venus tuskfish (*Choerodon venustus*). (**c**) Mature cestode proglottids isolated from fish intestine. (**d**) The trypanorhynch cestode species *Lacistorhynchus tenuis* is widely distributed in different hosts within the North Atlantic

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Nematoda

With more than 250 known families and an estimated 40,000 species, nematodes (roundworms) are one of the most species-rich and diverse taxa within the Metazoa. They have colonised every habitat and ecosystem on earth with most of them being free-living and feeding on decomposing organic material. Although only a small number of all known genera are parasitic in freshwater and marine fishes (approx. 4%), nematodes are known to be frequent endoparasites of a wide range of aquatic organisms. The use of paratenic hosts, a low host specificity, free-living stages that actively search for susceptible hosts, as well as the infection of short-lived organisms (e.g. small invertebrates) that occur in vast numbers, are only a few examples of how nematodes achieve their temporal and spatial dispersal. These characteristics also explain how they increase the likelihood of encountering a successive host in a diluted environment, where contact between successive hosts may be periodic or even rare. The occurrence of certain nematodes (e.g. Anisakidae: *Pseudoterranova*, *Anisakis*) in the musculature or internal organs of their intermediate or paratenic hosts is well documented and has been extensively investigated in a variety of fish, especially those with high commercial value. This is not only related to their significance as pathogens and potential triggers of zoonotic diseases but also to the economic aspects resulting from the spoilage of infected fish products.

Classification

Kingdom: Animalia

Phylum: Nematoda

Class: Chromadorea
Class: Enoplea

The original classification of the phylum Nematoda into the classes Phasmidea/Aphasmidea was based mainly on the presence of paired sensory organs (phasmids) at the posterior end of individuals. However, the designation was revised and replaced with Adenophorea (= Aphasmidea = Enoplea) and Secernentea (= Phasmida = Rhabditea). The first comprehensive molecular analyses divided the Phylum Nematoda into five classes, contradicting the classification into Adenophorea/Secernentea. They also provided evidence of a paraphyletic origin of animal and plant parasitism. Currently, there is strong support for the monophylogeny of the classes Chromadorea and Enoplea. However, phylogenetic relationships that go beyond the systematic level of the class are currently the focus of ongoing molecular analyses and are intensively discussed in the literature. Thus, a very detailed description of the very recent working hypotheses would not be appropriate here and probably soon outdated.

Morphology (Fig. 14)

Nematodes (roundworms or threadworms) are bilaterally symmetrical, unsegmented and cylindrical to filamentous in shape. They are dioecious with males typically being smaller than females and possessing needle-like mating structures (spicules), which serve to open the vulva of females. The body wall consists of a firm, multilayered noncellular cuticle, a thin cellular hypodermis and longitudinal club-like muscle cells. The rigid cuticle serves to counter the internal pressure of the liquid-filled pseudocoel and the longitudinal muscle cells. Nematodes have a relatively distinct head with a lipped mouth, which often bears a series of teeth. In contrast to most other endoparasites, nematodes have a complete digestive tract with mouth (buccal cavity), pharynx, oesophagus, intestine and anus. The excretory system consists of either an excretory gland and a pore or a more complex structure which is usually H-shaped. Arrangements and morphometrics of the internal structures, alimentary tract and the orientation of the excretory pore are often taxonomically important features.

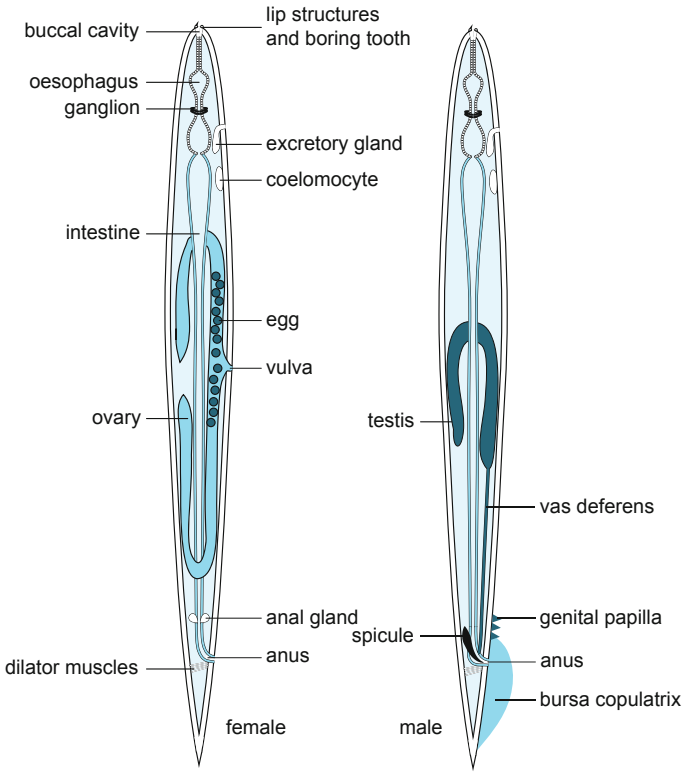

Fig. 14 Diagrammatic representation of ascarid anatomy

Development (Fig. 15)

Heteroxeny and paratenesis are very common phenomena in the transmission pathways of aquatic parasitic species and include intermediate and definitive hosts at almost every trophic level. Aquatic parasitic nematodes have evolved numerous elaborate means of maintaining themselves in an environment where contact between successive hosts within a life cycle may be periodic or even rare. The typical life cycle of a nematode is characterised by four moultings and larval stages. Eggs are usually shed within the faeces of the definitive host, and the development depends on abiotic factors such as salinity and temperature. In many species, moulting takes place within the egg, and L2 stages are found within the sheath of their L1 larval stage. Upon predation by an intermediate host, the L2 larva hatches and develops into the L3 larva, a stage which is infective to the definitive host.

The microhabitat within the intermediate host strongly depends on the nematode species. Haemocoel (for crustacean hosts), body cavity (and mesenteries), surfaces of internal organs and muscles are common sites of infection. If hosts are preyed

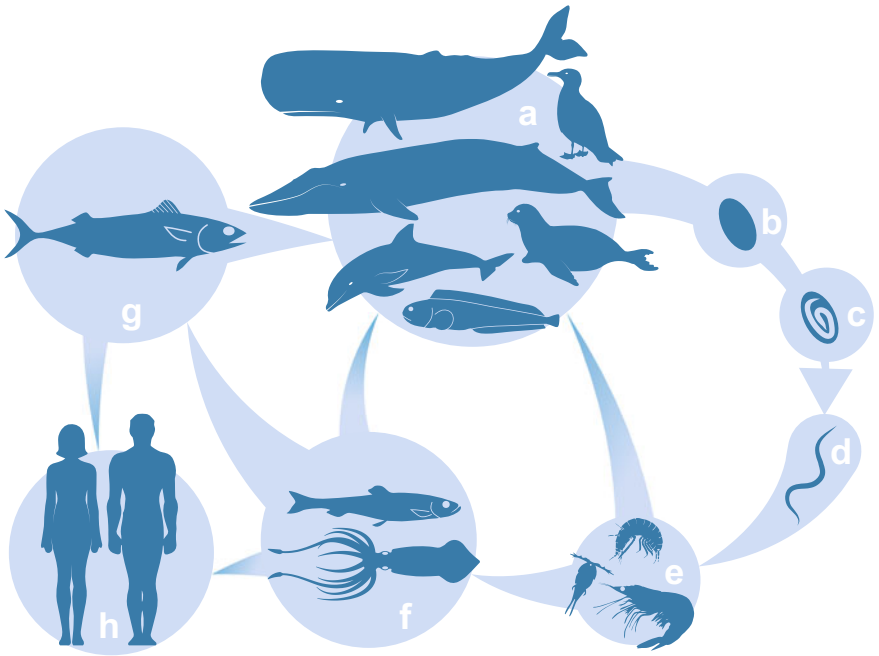

Fig. 15 General Nematoda life cycle. (**a**) Adults live within the intestine of their definitive hosts (Cetacea, Pinnipedia, piscivore birds, piscivore fish). After copulation, females produce eggs which are excreted by the host. (**b**) Eggs mature in the water column from L1 to L2 larvae. (**c**) L2 larvae hatch in the water column. (**d**) L2 larvae become ingested by small crustaceans (Cyclopoida, Amphipoda, Euphausiacea). (**e**) Within the crustaceans, they develop into infective L3 larvae. The infected crustaceans are rarely eaten directly by the final host and (**f**) mostly ingested by a paratenic host (small fish, Cephalopoda), where they penetrate the intestine wall and encapsulate on organs. These paratenic hosts are either eaten by (**g**) further paratenic hosts (predatory fish), where they encapsulate on organs again or by (**h**) accidental hosts like humans or the final hosts

upon by larger predators, the larvae are capable of reinfecting the latter without further moulting.

Consequently, predatory hosts act as paratenic hosts and may accumulate enormous numbers of larvae over time. When entering the digestive tract of a suitable definitive host, most commonly a vertebrate, the L3 stage moults into the pre-adult L4 stage and soon after into the female or male adult, which remain in the intestinal tract to reproduce. Vertebrates such as cetaceans, pinnipeds, teleosts, elasmobranchs and piscivorous birds are the most common definitive host classes (Fig. 16). It is generally accepted that invertebrates such as crustaceans and chaetognaths play a crucial role as intermediate hosts for most species. Cephalopods and teleost fish species are important paratenic intermediate hosts on several trophic levels.

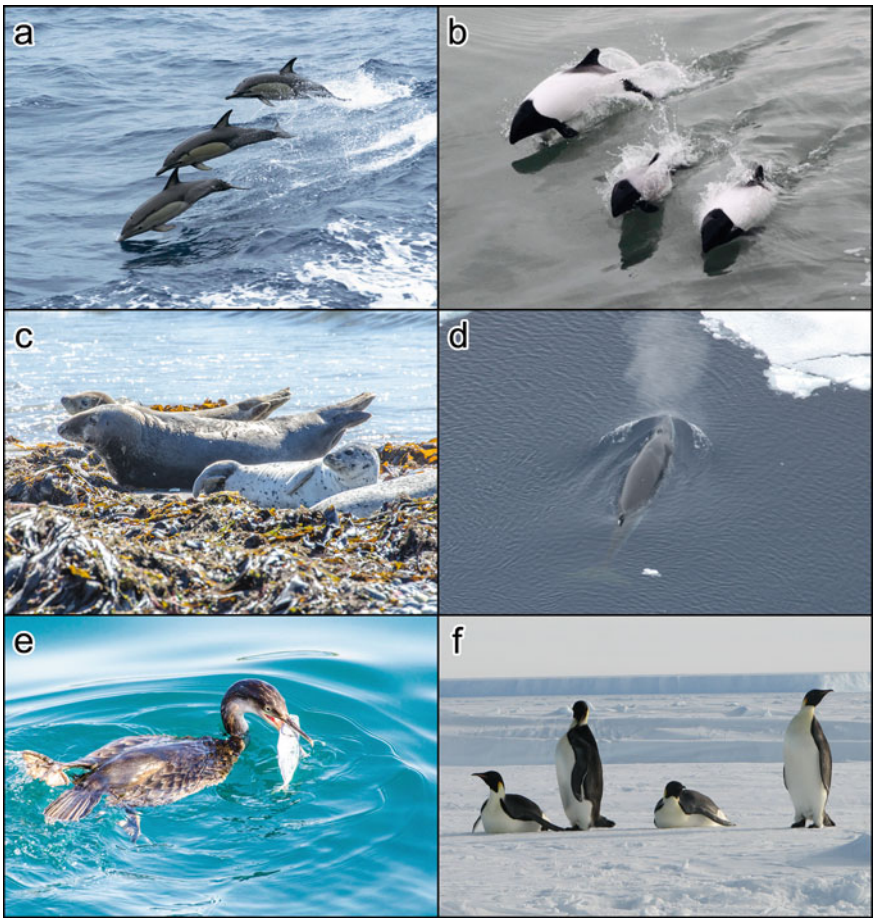

Fig. 16 Possible Cetacea, Pinnipedia and bird final hosts of metazoan parasites species, especially for marine nematodes. (**a**) Short-necked Common dolphin (*Delphinus delphis*). (**b**) Commerson's dolphin (*Cephalorhynchus commersonii*). (**c**) Harbour seal (*Phoca vitulina*) and grey seal (*Halichoerus grypus*). (**d**) Antarctic Minke whale (*Balaenoptera bonaerensis*). (**e**) Common or European shag (*Phalacrocorax aristotelis*). (**f**) Emperor penguin (*Aptenodytes forsteri*)

Marine Species (Fig. 17)

Nematodes represent a highly diverse and abundant group of marine parasitic organisms, often occurring at high infection levels in their invertebrate and vertebrate hosts. Marine parasitic nematodes are found in several orders, i.e. Ascaridida, Benthimermithida, Dioctophymatida, Marimermithida, Mermithida, Oxyurida, Spirurida, Strongylata and Trichurida. Some found in marine fish and squid are not primarily parasitic, and only a very few of them are of major importance for humans, due to their abundance and/or significance as pathogens. In this context,

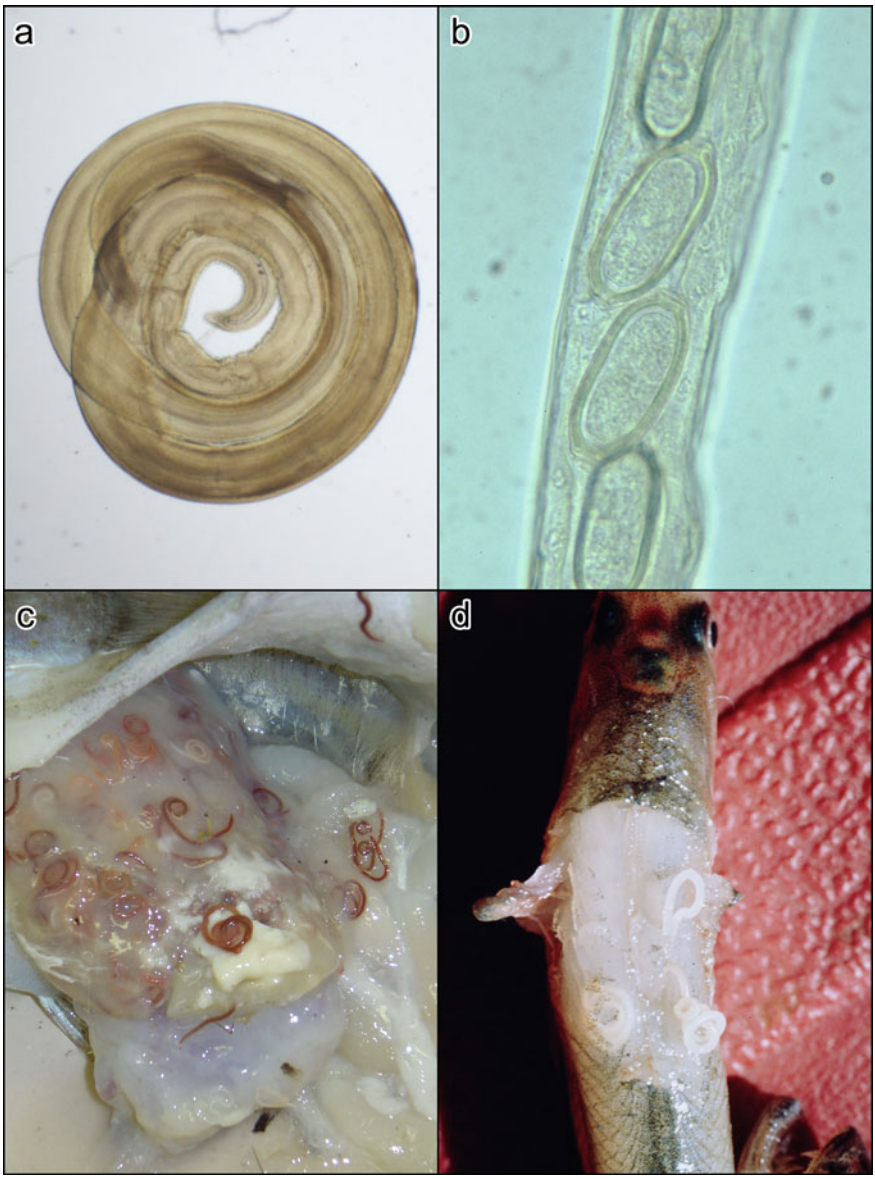

Fig. 17 Different nematode parasites. (**a**) *Anisakis* sp. third-stage larva (L3). (**b**) Female *Capillaria gracilis* with typical unembryonated eggs with a thick shell and polar plugs. (**c**) Numerous anisakid nematode larvae (L3) of the genus *Pseudoterranova* (red) and *Contracaecum* (white) in the viscera of the Mackerel icefish (*Champsocephalus gunnari*). (**d**) *Pseudoterranova decipiens* larvae (L3) in the musculature of the Smelt (*Osmerus eperlanus*)

members of the order Ascaridida are probably the most important representatives. In combination with the Spirurida, they are responsible for the vast majority of nematode infections in marine fish. The order Ascaridida includes parasites of the gastrointestinal tract of all vertebrate classes. They are characterised by the presence of three prominent lips, external labia and numerous caudal papillae.

Acanthocheilidae, Anisakidae, Ascaridae and Heterocheilidae are important families, with anisakids being the most numerous and diversified members of the group. The family Anisakidae can be divided into the subfamilies Anisakinae (e.g. *Anisakis*, *Contracaecum, Pseudoterranova, Phocascaris*), *Goeziinae* and *Raphidascaridinae* (e.g. *Hysterothylacium*). They have a worldwide distribution, and some are known as causative agents of the human anisakidosis, a painful inflammation of the gastrointestinal tract caused by the ingestion of the third-stage larvae (L3) in insufficiently cooked fish products. Species of the genus *Anisakis* have been most frequently associated with this zoonosis, together with the genera *Pseudoterranova* and *Contracaecum*. A wide range of marine mammals (Cetacea, Pinnipedia) and piscivorous birds are utilised as final hosts, whereas invertebrates (e.g. Crustacea, Chaetognatha) and sometimes Cephalopoda as well as a variety of fish are involved as intermediate and/or paratenic hosts. Due to the morphological homogeneity among the species and even genera, an ad hoc identification can be difficult, and a genetic identification using DNA sequence data is recommended.

Spirurida are characterised by the presence of six lips, a usually well-developed buccal capsule and an oesophagus divided into anterior (muscular) and posterior (glandular) portions. They occur subcutaneously, in deeper tissues, and also in the organs of all classes of vertebrates. The family Cystidicolidae is usually found in the intestine, stomach and pyloric caeca of marine and freshwater fishes, while some have adapted to the swim bladder of physostomous fishes. The life cycle includes aquatic insects and crustaceans that serve as intermediate hosts, while marine fish are used as final hosts. The cosmopolitan *Ascarophis* is the largest genus within this family and is found in the digestive tracts of marine and estuarine fishes. The Philometridae contain a large number of species that are exclusively parasitic in various ray-finned freshwater, brackish water and marine fishes. Very similar to anisakid nematodes, parasites of the genus *Philometra* are pathogenic and very common in commercially available fish species. They utilise crustaceans as intermediate hosts, and the predatory fish hosts get infected by feeding either on infected copepods or by paratenesis.

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Acanthocephala

Acanthocephala, or ‘spiny headed worms’, are exclusively intestinal parasites of vertebrates and comprise approx. 1150 species. About half of these are parasites of fish. Human pathogenic acanthocephalan species seem to be restricted to terrestrial habitats. However, it cannot be excluded that the consumption of marine Acanthocephala in fish food might cause allergic reactions, which so far have been only known from anisakid nematodes. In fish, acanthocephalans are known to cause serious pathological damage, including irreversible lesions of intestinal tissues leading to digestive and absorptive malfunctions.

Classification

Kingdom: Animalia

Phylum: Acanthocephala

Class: Archiacanthocephala
Class: Eoacanthocephala
Class: Palaeacanthocephala
Class: Polyacanthocephala (unverified)

The classification of the Acanthocephala is largely based on the concepts of Meyer and Van Cleave and has been summarised by Amin (1985) in a new system that became a widely accepted standard for this group. The recent classification presented above distinguishes three (possibly four) classes within the phylum Acanthocephala, with a majority of the species primarily infecting aquatic hosts. Around 57% of the acanthocephalan species belong to one of the two orders Echinorhynchida and Polymorphida within the Palaeacanthocephala, representing the most diverse and common acanthocephalan groups in marine teleost fish. However, as in the Nematoda, detailed phylogenetic relationships are currently the focus of ongoing molecular analyses and are intensively discussed in the literature. They will therefore not be dealt with in this context.

Morphology (Fig. 18)

Acanthocephalans are bilaterally symmetrical, slender worms that may reach lengths of a few centimetres in marine fish and may have pseudo-segmentation. They consist of a metasoma (trunk) and an anterior prosoma, comprising a neck and the characteristic protrusible holdfast organ, called a 'proboscis', which is usually covered with spiny, recurved hooks arranged in horizontal rows. It is usually kept semi-invaginated and is used to pierce the intestinal wall of hosts and hold the parasite in position. Number, form, size and position of the hooks are important taxonomic criteria. Acanthocephala have neither a gut nor a mouth. Similar to the cestodes, adult stages in the intestines of hosts take up nutrients directly through their body surface. Excretion organs (protonephridia) only occur in species parasitising terrestrial vertebrates. The tegument is a syncytium, and the sub-tegumental musculature consists of longitudinal as well as circular muscles. Acanthocephala are dioecious with females typically being larger than males.

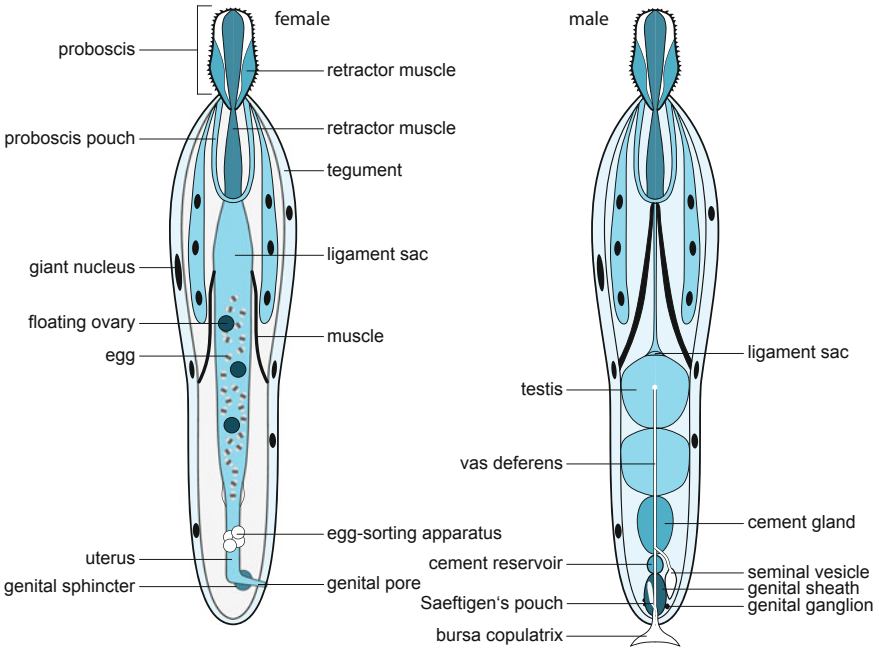

Fig. 18 Diagrammatic representation of acanthocephalan anatomy

Development (Fig. 19)

Adult Acanthocephala live attached to the intestinal wall of their vertebrate hosts. Life cycles of marine species usually involve one or two intermediate hosts, typically with a benthic association. Eggs are shed with the faeces of the final host. The eggs, which contain the 'acanthor' (larva), are released into the water column. First intermediate hosts are often detritus-feeding zooplankton organisms (e.g. Amphipoda), which acquire the larvae by oral ingestion. After penetrating the intestinal wall of their host, the acanthor will be surrounded by a cyst shell of host origin in which it further develops into the 'acanthella' stage with a protrusible proboscis. At the end of this development, the stage is called 'cystacanthus'. Paratenic hosts (e.g. fish) and final hosts (e.g. fish, birds, seals, whales) become infected by preying upon the intermediate hosts. Generally, specificity for intermediate hosts is low for marine acanthocephalan species, and crustaceans belonging to several families are known as transmitters. For most of the Echinorhynchida, amphipods usually serve as intermediate hosts and fish as definitive hosts. Polymorphida use mammals/birds as definitive hosts and fish as common paratenic hosts.

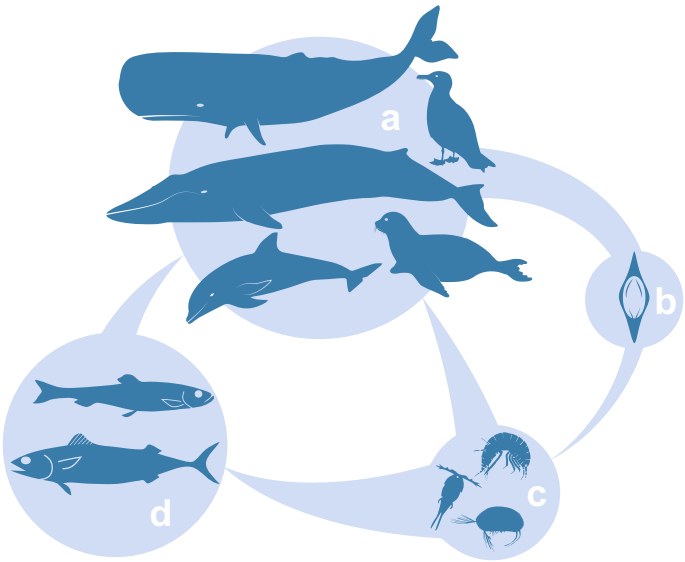

Fig. 19 General Acanthocephala life cycle. (**a**) Adults live, attached with their proboscis, within the intestine of their final hosts (Cetacea, Pinnipedia, piscivore birds). After copulation, females produce eggs for up to several months (patent period), which are excreted by the host with faeces. (**b**) The fully embryonated eggs are ingested by the intermediate hosts (Ostracoda, Cyclopoida, Amphipoda). (**c**) Within the intestine of these hosts, the egg hatches and releases the acanthor, which travels into the body and transforms into the acanthella. The intermediate host with this infective stage is then either ingested by the final host or (**d**) a paratenic host (planktivore or piscivore fishes)

Marine Species (Figs. 20 and 21)

Compared to, e.g. nematodes, acanthocephalan parasites are much less diverse in the marine realm, and some vertebrate groups, such as elasmobranchs, do not seem to be suitable definitive hosts at all. However, acanthocephalans are very abundant in certain marine habitats (e.g. deep sea, Antarctica) and may have wide distributional ranges. Abundant and well-known marine species are mainly represented in the classes Palaeacanthocephala and Eoacanthocephala. Within the Palaeacanthocephala, the echinorhynchid species *Echinorhynchus gadi* as well as the polymorphid genera *Corynosoma* and *Profilicollis* are the most important representatives. *Echinorhynchus gadi*, frequently occurring in North Atlantic marine hosts, includes benthic Crustacea of the families Gammaridae (e.g. *Gammarus* spp.) and Caprellidae as obligatory first intermediate hosts and follows a benthic life cycle strategy. Teleost fish are final hosts for *E. gadi*. Species of the genus *Corynosoma* are abundant parasites of marine cetaceans, pinnipeds and sea otters while infecting amphipods and fish as intermediate and paratenic hosts. *Profilicollis* species are associated with ducks and shorebirds that become infected by preying upon infected decapods. *Neoechinorhynchus agilis* is the most common representative of the

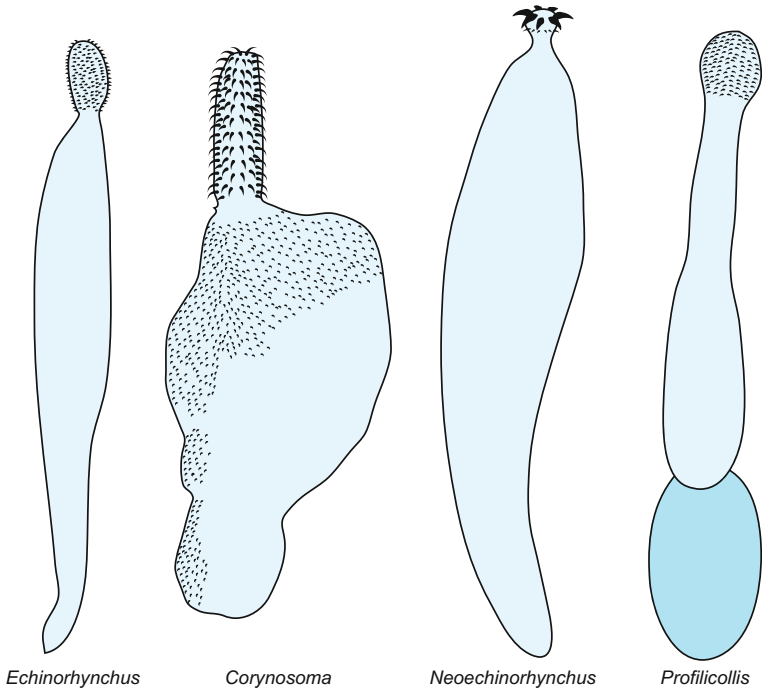

Fig. 20 Diagrammatic representation of acanthocephalan diversity of shapes

Eoacanthocephala. This species is specific for mugilid fish which are distributed in temperate waters worldwide. Ostracods are thought to be intermediate hosts.

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Fig. 21 Different acanthocephalan parasites. (**a**) *Echinorhynchus gadi* with inverted proboscis. (**b**) *Aspersentis megarhynchus* isolated from the intestine of Black rockcod (*Notothenia coriiceps*). (**c**) The acanthocephalan species *Corynosoma bullosum* is a parasite of the jejunum, ileum and colon of pinnipeds. (**d**) *Pomphorhynchus laevis* is a typical parasite in the intestine of many marine fish hosts from coastal and brackish environments

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Hirudinea

Hirudinea (leeches) are a class within the phylum Annelida and are distributed worldwide in aquatic (marine/freshwater) and terrestrial ecosystems. Parasitic species are typically not very host-specific and can act as haematophagous ectoparasites on fish, amphibians, reptiles, birds and mammals. They can also be predatory, feeding on a range of invertebrates. About 15% of the approx. 680 described species are associated with marine habitats, while the vast majority live in freshwater ecosystems. Adult leeches are often found attached within or near gill chambers or at fin bases and have been known to reduce the value of fish catches, particularly when they have reached high densities on economically important fish species. They may cause local skin injuries, although serious pathogenic manifestations have not been described.

Classification

Kingdom: Animalia

Phylum: Annelida

Class: Clitellata

Subclass: Hirudinea

Infraclass: Acanthobdellidea
Infraclass: Euhirudinea

The taxonomy and systematics of leeches are currently being reviewed. Among other things, discussions are ongoing on as to whether leeches represent a class on their own or should be positioned within the Clitellata as a subclass. There is also some dispute as to whether the primitive Acanthobdellidea are a separate clitellate group. Furthermore, the phylogenetic relationships within and/or among the taxon Oligochaeta are still unclear. Molecular analyses suggest that the class Clitellata is synonymous with the more commonly used class Oligochaeta and that leeches, branchiobdellidans (crayfish worms) and acanthobdellids should be regarded as

orders. The infraclass Acanthobdellidea consists of only two species of salmonid ectoparasites within the genus *Acanthobdella*. The 'true leeches' (Euhirudinea) are divided into two major groups associated with freshwater and terrestrial habitats—the Arhynchobdellida and the Rhynchobdellida, which contain both freshwater and marine species. They can be distinguished based on the presence/absence of the protrusible proboscis, a muscular pharynx and their vascular/haemocoelomic system.

Morphology (Fig. 22)

The hermaphrodite leeches are most prominently characterised by two suckers, one at either end of the body. They are segmented, although the external segmentation does not correspond to the internal segmentation of their organs. The first 4 of the 32 post-oral somites (metameres) are designated head segments, which include the anterior brain and sucker. The mid-body segments (21) include ganglia, reproductive organs (testisacs and ovisacs) and the last seven segments form the posterior sucker and the posterior brain.

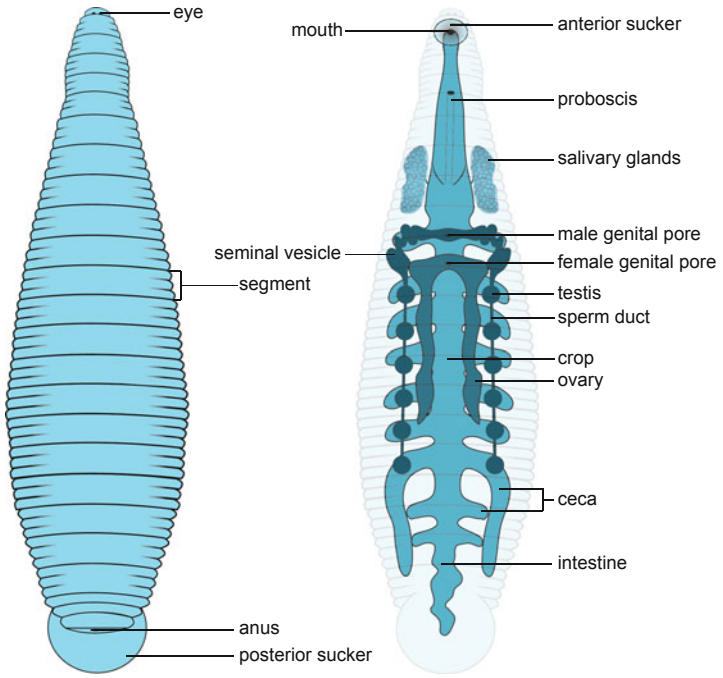

Fig. 22 Diagrammatic representation of hirudinean anatomy

Several sensory structures, chemo-/mechanoreceptors (eyes/eyespots, sensillae, papillae) might be present on the head structure and along the rest of the body. The digestive system consists of a jawed mouth at the anterior end, pharynx, oesophagus, crop (functions as blood storage after feeding), gizzard and the intestine which ends at the posterior sucker. Leeches produce an anticoagulant that prevents the ingested blood from clotting. This characteristic finds application in medicine and is of economic importance.

Development

The ontogeny of Hirudinea is direct, without any larval stages. They are typically simultaneous hermaphrodites with internal fertilisation. Self-fertilisation is rather rare. Upon maturity, leeches mate and exchange their gametes. They successively produce several fluid-filled chambers (cocoons) containing the eggs and nutrients. These will usually be abandoned after they have been attached to substrates or the host. Some species (glossiphoniid) show extended care, even after hatching. Within 3–4 months after hatching, the juveniles reach maturity and mate again.

Marine Species (Figs. 23 and 24)

Marine Hirudinea are represented by members of two groups within the Rhynchobdellida. Species of the Ozobranchidae are marine parasites of sea and freshwater turtles represented by the genus *Ozobranchus.* Parasites of fish can be found within the Piscicolidae. This family is comparatively large and contains species of marine, brackish and freshwater habitats separated into more than 40 genera. Leeches preferably attack demersal fish species although prevalence and intensity of infections are usually fairly low. Unusually large numbers of *Trulliobdella capitis*, however, have been frequently observed on the Antarctic *Champsocephalus gunnari*. Species of the genus *Calliobdella* reach a length of up to 50 mm and have been reported from several fish species (e.g. *Lophius piscatorius*) in North Atlantic waters. A remarkable intensity of infection by *Calliobdella carolinensis* was observed on the Atlantic Menhaden, *Brevoortia tyrannus*. Members of the genus *Oceanobdella* are among the most common and most diverse marine fish leeches in boreal waters, infecting several teleost fish species.

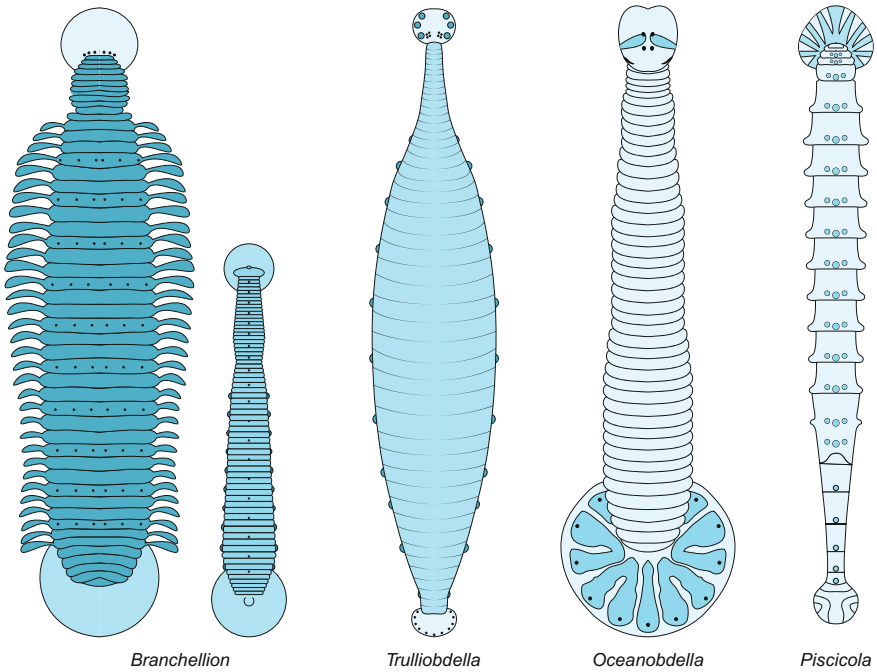

Fig. 23 Diagrammatic representation of marine hirudinean diversity of shapes

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Fig. 24 Different hirudinean parasites. (**a**) *Piscicola geometra* on the pectoral fin of a smelt (*Osmerus eperlanus*). (**b**, **c**) Blackfin icefish (*Chaenocephalus aceratus*) and Mackerel icefish (*Champsocephalus gunnari*) with parasitic leeches

Crustacea

Crustaceans are the most diverse parasitic group in the marine environment. Moreover, as the main component of the zooplankton, crustaceans form the most individual-rich animal group on the planet. The lifestyle of crustacean parasites varies from temporarily to fully parasitic and can either be endo- or ectoparasitic. Their development from the egg stage to the adult comprises a large variety of larval stages, starting with the nauplius and metanauplius, and several other group-specific stages.

The largest group of crustaceans is the Copepoda. This group consists of more than 11,500 species, and most of them are probably parasitic, e.g. *Sphyrion lumpi*. Most parasitic copepods show monoxeny, i.e. they use only one host, but a few species include fish as intermediate hosts in their life cycle, e.g. *Lernaeocera branchialis.* Another parasitic group of crustaceans is the Isopoda. Their morphology varies from typical amphipod-like (but dorsally flattened) to a sac-like body shape. The main parasitic suborder within the Isopoda are Cymothoida, which parasitise the skin, mouth or gills of their fish hosts, including the famous tongue-biting species *Cymothoa exigua.* Branchiura are solely parasitic Crustacea, with approx. 130 described species, including the genus of fish lice *Argulus*. Cirripedia, the so-called barnacles, are sessile filter-feeders but also include parasitic species like *Anelasma squalicola*, which parasitises different shark species. Another group of crustacean fish parasites is the order Amphipoda. Among the amphipods, the whale louse family (Cymidae) is the best-known group. Compared to other crustaceans, Amphipoda contain only few species that are parasitic, e.g. *Cyamus boopis*. Although the morphologies and life cycles of Crustacea are very diverse, only a few examples of very common parasitic crustaceans among fish hosts are mentioned here. For information that is more detailed, please consider additional literature.

Classification

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

The currently 67,000 described species of Crustacea belong to the phylum Arthropoda. The first attempts on crustacean phylogenetic relationships were made by Haeckel in 1866 and Claus in 1876. Today, approx. 52,000 species are known, separated into six classes (Branchiopoda, Remipedia, Cephalocarida, Maxillopoda, Ostracoda, Malacostraca) containing more than 850 families.

Morphology (Fig. 25)

Typically, the crustacean body is divided into three segments: the cephalon (head), pereon (thorax) and pleon (abdomen). However, as expected with such a high number of species, the morphology of Crustacea is very diverse. The most uniform part is the head; but this is often not easily recognisable in parasitic Crustacea. In addition to the various morphologies, crustaceans have a large size spectrum, ranging from a few mm (*Clavella adunca,* 4.3 mm cephalothorax length) to about 3.7 m (leg span of *Macrocheira kaempferi,* nonparasitic). Larger parasitic crustaceans, like *Sphyrion lumpi,* reach a length of several centimetres.

Development

The development of Crustacea usually starts with a fertilised egg. The earliest larval form is the nauplius (in copepods), whereas a zoea occurs in larger Crustacea (e.g. decapods). The different larval moults are characterised by fully functional appendages. However, depending on the group of crustaceans, development and developmental stages vary and are therefore not explained here in detail.

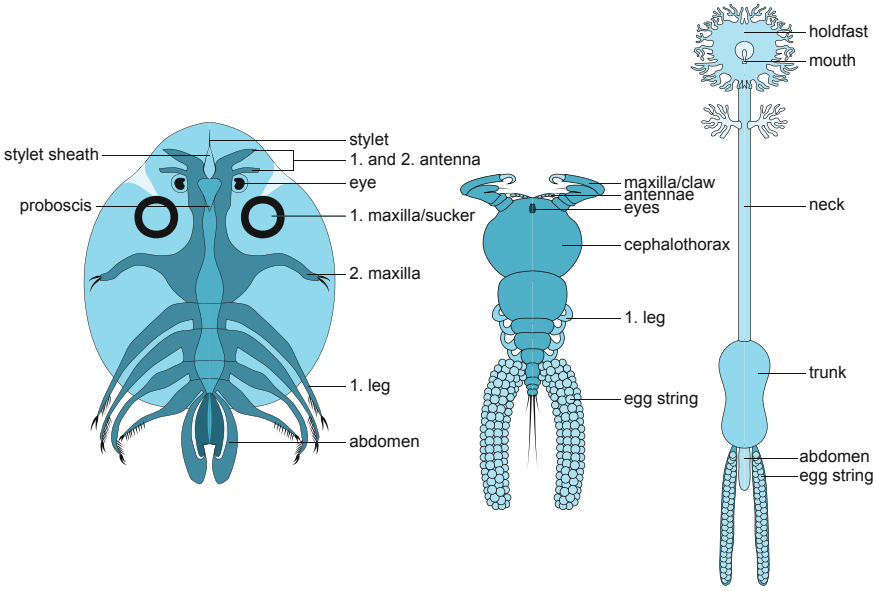

Fig. 25 Diagrammatic representation of crustacean parasite anatomy

Marine Species (Figs. 26 and 27)

Sphyrion lumpi is a mesoparasitic copepod with several bony and cartilaginous fish species as hosts. The main hosts are redfish of the genus *Sebastes*. *Sphyrion lumpi* shows a monoxenous development and a distinct sexual dimorphism, with females reaching several centimetres in length, while males only grow to 1.12 mm. The females anchor themselves in the muscle tissue of their hosts. The distribution of the copepod *Clavella adunca* (Lernaeopodidae) ranges over North Pacific, Atlantic and Antarctic waters. The stationary ectoparasite usually infects fins, anus, gills or the mouth cavity. Specimens from the gills or mouth seem to be smaller than those found on the body surface of a host. *Clavella adunca* shows a broad host spectrum, but species of Gadiformes are the main hosts, showing seasonal variations in infection intensities. The developmental stages of *C. adunca* are reduced to one nauplius stage. This free-swimming stage is restricted to a short life span; therefore, new infections often take place on the same host.

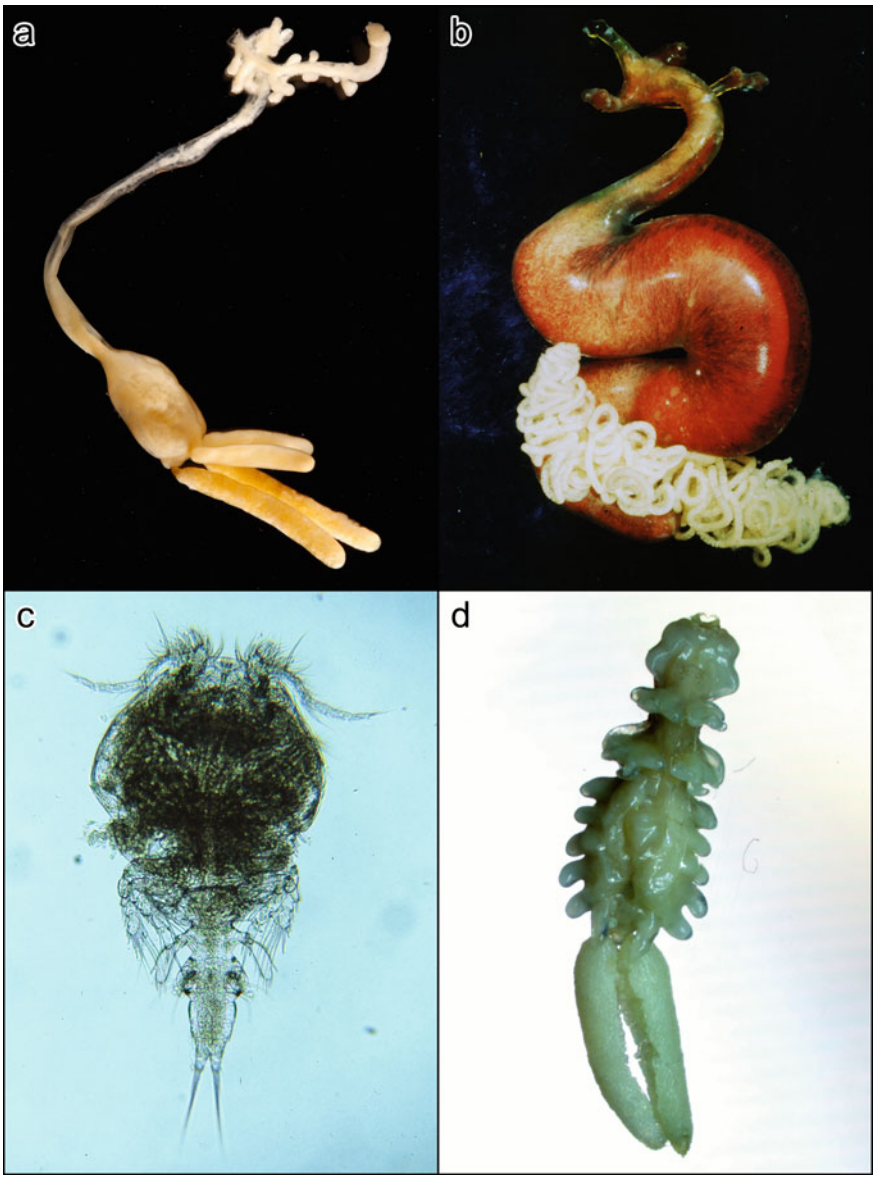

Fig. 26 Different crustacean parasites. (**a**) *Paeonocanthus antarcticensis* isolated from the musculature of the Goiter blacksmelt (*Bathylagus euryops*). (**b**) *Lernaeocera branchialis*, a copepod crustacean parasite of gadoids. (**c**) *Acanthocolax exilipes* from the gills of Solenette (*Buglossidium luteum*) caught at the Dogger Bank (North Sea). (**d**) Female *Chondracanthus nodosus*; a large variety of chondracanthiform copepods occur exclusively on marine fish species. In some species, the body of the female carries several wing-like processes. Males of this group live as pygmy forms attached to the females

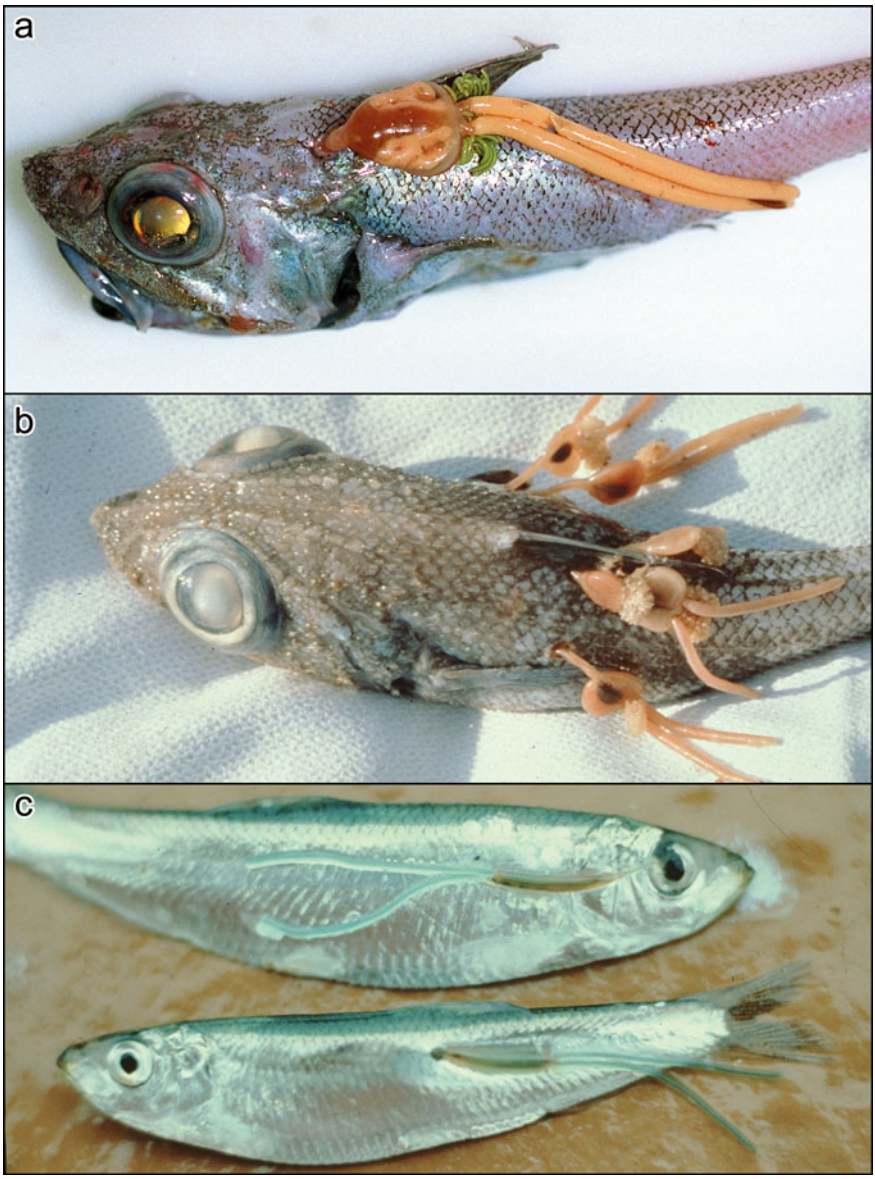

Fig. 27 Different crustacean parasites on their hosts. (**a**) The sphyriid copepod *Lophoura szidati* isolated from the Whitson's grenadier (*Macrourus whitsoni*) from the Southern Ocean. (**b**) *Sphyrion lumpi* from the Roughhead grenadier (*Macrourus berglax*) Greenland Sea. The mesoparasitic copepod is known to parasitise the musculature of several cartilaginous and bony fish. (**c**) *Lernaeenicus sprattae* (top) and *L. encrasicholi* on Sprats (*Sprattus sprattus*). *Lernaeenicus sprattae* anchors its head into the eye of the fish, while *L. encrasicholi* anchors it somewhere in the fish body musculature

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Techniques

Depending on the aim and the purpose of the study, qualitative and quantitative parasitology uses a broad range of techniques and methods that are usually feasible in any regular laboratory. With the exception of the UV-press method after Karl and Leinemann (see below), no special tools are necessary. The described techniques and methods presented in this chapter have been proven practical and effective for routine use in a variety of laboratory settings and partially also in the field. They can be easily acquired and require only a minimum of apparatus/equipment.

Before You Start

In every scientific study, a well-developed study design, with adequate preparation and care on the part of the researcher, is needed in order to achieve the best and most reproducible results. It is thus advisable to give beginners and students an intensive training on test fish which will not be included in the study to reduce errors later on. Due to the small size and the sometimes unusual shape and appearance of adult and larval stages, some parasites are very difficult to spot and require a more trained eye when sorting between parasite specimens and pieces of organ tissue of the hosts. The training should be supervised by an experienced researcher and may take, depending on the skills of the student and the researcher, at least a few days. A training period of about 2 weeks, including 1–2 fish per day, has been proven most effective.

Basic Equipment and Instruments

The basic equipment of every researcher includes a set of dissection instruments which can be obtained at most retailers for medical and laboratory supplies at a

S. Klimpel et al., *Parasites of Marine Fish and Cephalopods*,
https://doi.org/10.1007/978-3-030-16220-7_4

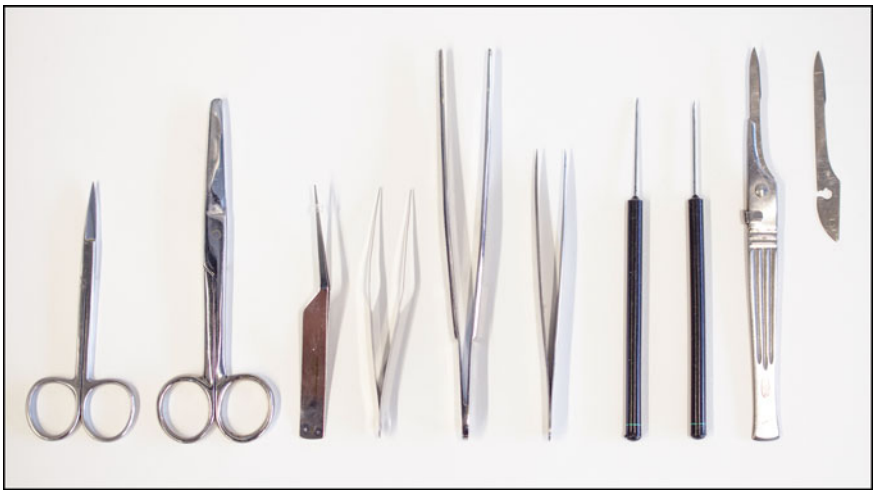

Fig. 1 Tools for fish dissection

reasonable price. Unfortunately, many of those precompiled sets only contain instruments that are rather unwieldy and not delicate and precise enough for the dissection of fish and the handling of small parasites. The right choice is therefore to compile a customised set of instruments that fit the personal needs of the researcher.

Basic Set of Dissection Instruments (Fig. 1)

- A pair of small, straight microscopy scissors with pointed ends
- A large robust pair of straight scissors with blunted ends
- A set of forceps including two delicate, very fine ones (e.g. Dumont 5); one blunt-nosed thumb forceps with serrated tips for increased grip; and one pointed light metal forceps
- Two preparation needles, straight (140 mm)
- A scalpel with interchangeable blades

The work space should be well organised and equipped with a sink and a desk, the covering of which should be washable and easy to disinfect. For examinations lasting for a longer period of time, the use of a ventilated room is strongly recommended. A mandatory prerequisite for a proper investigation of the entire parasite fauna is good optical equipment. The bright-field stereomicroscope is the main working tool when working with parasites and should always be equipped with a transmitted light function; otherwise small endoparasites can easily be overlooked, and inner structures of parasites will not be visible. Practical classes should be held in situations that are equipped with a sufficient number of transmitted light microscopes, and at least one instrument should be available for purposes of demonstration.

Fig. 2 Workspace arrangement for parasitological examinations

Workspace Organisation (Fig. 2)

- A bright-field stereomicroscope with transmitted and reflected light option (magnification: 6–45×)
- A bright-field stereo light microscope (1000×)
- An external gooseneck lamp, dimmable (optional)
- A laboratory scale with a measurement range sufficient for the weight of the host specimen and a fine scale for measurements of small amount of tissue and organs
- A wax-filled dissection dish
- A sufficient amount of petri dishes suitable for the size of the dissected host
- A sieve to prevent organic material from dropping into the sink and potentially causing odour nuisance
- A squeeze bottle filled with 0.9% NaCl solution
- A squeeze bottle filled with 70% EtOH (denatured)
- Phosphate-buffered formaldehyde (e.g. Histofix®)
- A small bottle of EtOH (absolute)
- Reaction tubes
- Small block glass dishes (at least one for every organ), labelled or alternatively placed on a labelled sheet of paper

Sampling, Sampling Size and Sample Storage

Origin, transport and storage can have a great impact on the quality of the study results. For statistically valid results, the sample size for host species should not be less than 35 individuals, with the more the better. Regarding sample collection,

various options are available. The advantages and disadvantages of some of the most common sources of material are discussed in the following section.

Fish from the Local Fish Market or from Fishermen

Fishes from the local fish market are usually very fresh (or even still alive) and in a good condition. Thus, fish markets are a reliable source for samples, especially when the parasites need to be studied alive. People also usually gain a good overview of the local fish fauna and the habits of fish consumption of the local residents when visiting fish markets. There, the fish is often cheaper, and fishermen may provide information about the fishing location. However, depending on the region, the catch can be quite small and too irregular to provide sufficient material for a whole study. In addition, ectoparasites might have been lost from the host during the handling process, which can lead to biased infection parameters.

Fish from Fishery Research Vessels

Using fishery research or industrial trawling cruises is probably the most cost- and time-intensive way to obtain fish samples (Fig. 3). At the same time, it might be the most practical one as catches are usually bigger and might contain fish species that are not available from local fishermen operating on a small scale (e.g. deep sea fish/cephalopods). The researcher gets very detailed information on the exact coordinates of the sampling location and, even more important, information on oceanographic features which are essential for ecological studies, e.g. water temperature, salinity, primary production, water depth and ocean currents (Fig. 4).

Samples that cannot be immediately examined on board during the expedition can be frozen separately and kept in plastic bags for subsequent analyses. Freezing should be done as quickly as possible to avoid degradation of the internal organs. Depending on the fishing technique (e.g. longline fishing, bottom trawl, pelagic net fishing, Fig. 5), ectoparasites may be lost due to the mechanical stress in the net. Stomach content analyses might become biased when living fish have been feeding on the by-catch during the haul.

Fish Caught by Hand

If the aim of the study is simply to examine live fish and their parasites (especially protozoan parasites), small-scale sampling with small nets or a fishing rod might be the best option. This is also a common technique when studying ectoparasites or catching freshwater fish. Basic abiotic factors can be measured with handheld devices. However, this kind of sampling method requires appropriate equipment and know-how on the part of the researcher or fisherman. Although some fish species can be targeted using specific bait, the process seems rather arbitrary and can be very time-consuming.

Fig. 3 Research vessels (RV). (**a**) Polarstern is a German research icebreaker (length, 118 m; beam, 25 m). (**b**) G.O. Sars is a Norwegian research vessel (length, 78 m; beam, 16 m). (**c**) Maria S. Merian is Germany's second most modern research vessel (length, 95 m; beam, 19 m). (**d**) Walther Herwig III is a German research vessel which cruises in sea areas with deep offshore fishing (Baltic Sea, North Sea, North Atlantic Ocean) (length, 65 m; beam, 15 m). (**e**) Alkor is a German medium-sized research vessel; it operates mainly in the North Sea and the Baltic Sea (length, 55 m; beam, 12 m, Photo: Daniela Krellenberg, GEOMAR). (**f**) Senckenberg is a German research vessel operating in coastal areas of the North Sea (length, 30 m; beam, 7 m)

Sample Defrosting

If it is not possible to examine the samples right after the haul, the sampled fish need to be stored in a fridge at 0–2 °C or, if processed much later, at temperatures of at least −20 °C (keep in mind that some analyses, e.g. protozoan screening on flagellates, will be impossible once the fish are frozen). The best method to defrost a fish is to place it in a fridge at 4 °C the night before the dissection is planned. If the

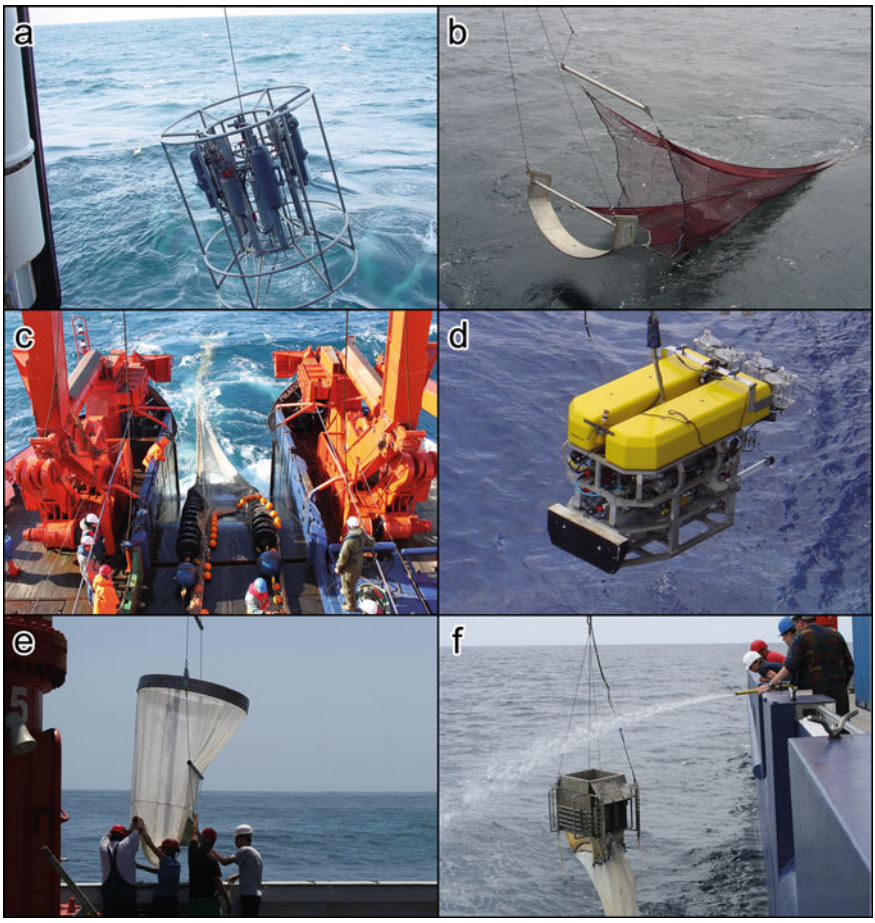

Fig. 4 Scientific equipment. (**a**) Multi Water Sampler with CTD (is an oceanography instrument used to measure the conductivity, temperature and pressure of Seawater = depth). (**b**) The Isaacs-Kidd Midwater Trawl Net (IKMT) is the standard net for collection of larger quantities of macrozooplankton and micronekton. (**c**) Fish trawl (RV Polarstern) from the waters around Elephant Island (Southern Ocean), (**d**) remotely operated vehicle (ROV). (**e**) Bongo net, oceanographic ring net for horizontal or oblique tows. (**f**) Multiple Plankton Sampler (MultiNet) for collection macrozooplankton and micronekton

fish is still frozen the next morning, place it in a dissection bowl at room temperature, and wait until the intestines can be pressed but are still slightly frozen. Previously defrosted specimens can be kept on ice (but still remaining in their plastic bags) until the dissection begins.

Small fish specimens of only a few centimetres in length can be defrosted within minutes to hours before dissection. The fastest and easiest method is to put the fish in a watertight plastic bag under cold water until it is defrosted. However, this method can only be recommended for fish that are not relevant for the study (e.g. for

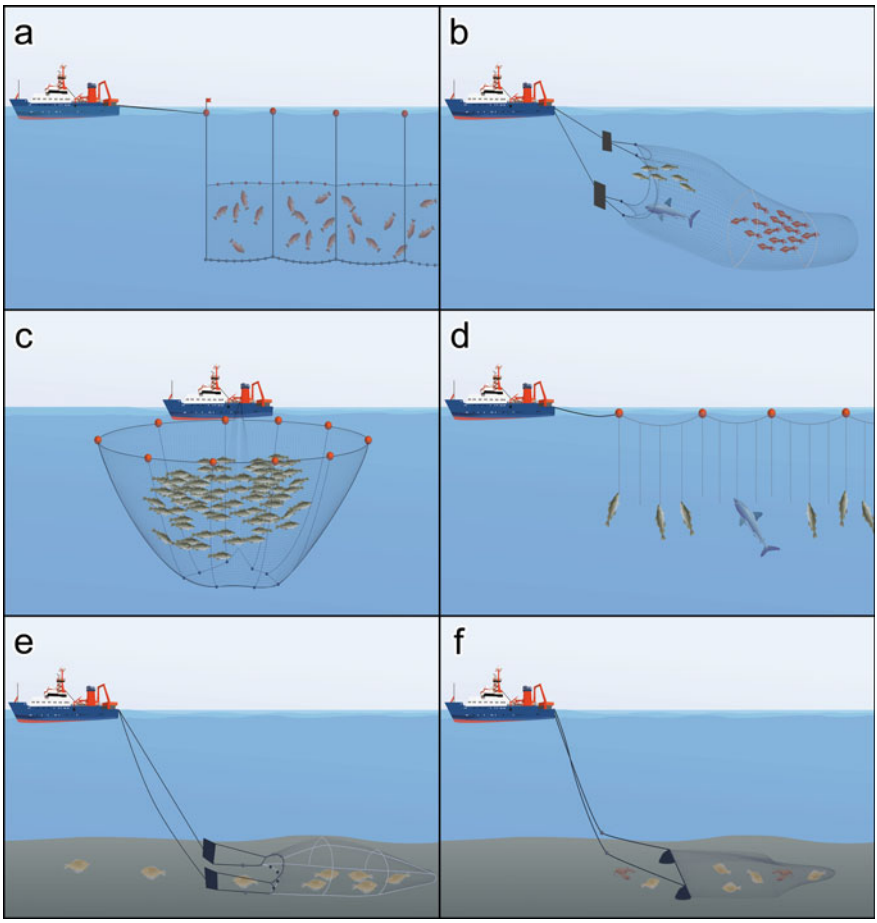

Fig. 5 Different types of nets and fishing methods used in scientific and commercial fishing. (**a**) Gillnet: gillnets are drawn out in the open water and can be as long as 2500 m; fish are caught by entanglement in the net. (**b**) Pelagic trawl: size can be up to 200 m long and 150 m wide; nets are towed by one or two ships and have trawl doors to keep the net spread. (**c**) Purse seine net: sizes range from 2000 m length and 200 m depth in offshore fishing to 200 m length and 20 m depth in inland fishing. The net is towed to surround a swarm of fish and is then closed from the bottom. (**d**) Longline: consists of a main line, which can be up to 130 km in length, and baited hooks (quantity can reach more than 20,000) attached at intervals. (**e**) Bottom trawl: similar to the pelagic trawl, but the net is built to withstand ground contact and has a special shape which is more broad than high. (**d**) Beam trawl: a ground fishing method mainly used for catching prawns and flatfish; the metal beam and two shoes at the side disturb the ground which causes bottom dwelling organisms to swim up a bit and get caught in the net

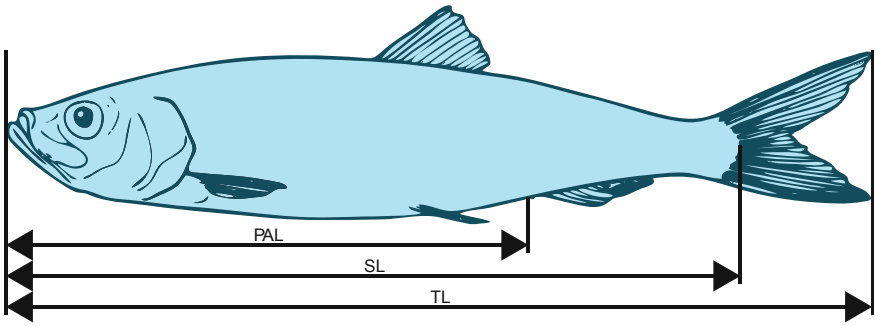

Fig. 6 Diagrammatic representation of roundfish measurements for catch statistics and parasitological examinations

demonstration purposes) and should only be applied in cases without other alternatives. Longer periods of storing defrosted fish without any processing should be avoided to prevent the internal organs, in particular the liver, from decomposing. The plastic bag should also be checked for parasites that might have become detached from the fish during the freezing/defrosting processes.

Host Dissection

Roundfish

Host Biometric Data

The dissection starts by taking some basic morphometric data, i.e. the total length [TL] and standard length [SL] as well as the pre-anal length [PAL] and the total weight [TW].

Important Host Morphometric Parameters (Fig. 6)

Total length [TL]: Length [cm] between the most forward point of the head (usually the closed mouth) and the tip of the caudal fin while the fish is lying on its side.

Standard length [SL]: Length between the most forward point of the head (closed mouth of the fish) to the base of the tail fin while the fish is lying on its side.

Pre-anal length [PAL]: Length between the most forward point of the head and the anus while the fish is lying on its side. This parameter is used on fish

(continued)

samples with elongated tails and fins (e.g. Macrouridae) which might have broken off during the freezing and/or defrosting process.

Total weight [TW]: Weight [g] of the defrosted fish before dissection.

Carcass weight [CW]: Weight [g] of the fish without its intestines and isolated parasites.

Blood Sample and Skin Smear Examination

Accurate analyses of the protozoan parasite fauna can only be applied on fish that have been expertly killed immediately before the dissection. The parasites have then to be collected before the blood starts clotting. An appropriate method to kill a fish is by stunning it through a blow on the head followed by cervical dislocation and a cardiac stab (Alternatively, you might follow the respective Animal Protection Law applied in your country).

Blood Sample Preparation

– Collect a drop of blood immediately after the cardiac stab using a Pasteur or capillary pipette by:

1. Cardiac puncture through the ventral body wall between the pectoral fins puncture of the caudal artery using a (Vacutainer®) syringe along the midline of the fish body, or
2. Severing the caudal peduncle posterior to the anal fin with a scalpel or sharp knife, or
3. Cutting the gill vessel

It may also be advisable to use tools that contain anticoagulants (e.g. heparin) in order to avoid blood clots. A more detailed description of how to take blood samples of fish are provided by Ostrander (2000, "The laboratory fish").

Fresh mounts of blood can be directly examined for live blood parasites such as *Trypanoplasma* or *Trypanosoma*. Place a droplet of blood on a microscope slide, use a cover slip on top and examine the slide under a light microscope at 100× magnification. Living flagellates are very easily spotted due to their vigorous movements. Mobile myxosporeans can be found using 200–400× magnification.

Dry blood smears should also be prepared (Fig. 7). Place one drop of blood (approx. 4 mm in diameter) near the end of a defatted microscope slide with frosted end and spread the drop out using another slide. Place the spreading slide at a 45° angle and back it into the drop of blood until it catches the drop and spreads along the edge. Push this slide across the sample slide in a smooth action. The length of the smear can be adjusted by different angles at which the spreading slide is pushed

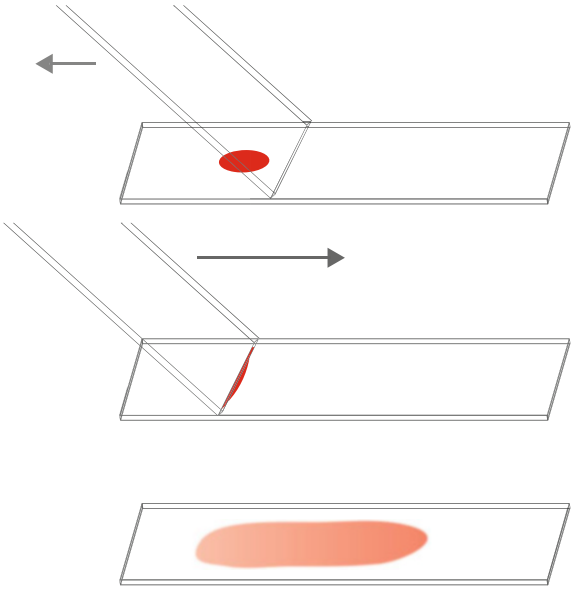

Fig. 7 Diagrammatic representation of blood smear fabrication

across the sample slide. The steeper the angle, the shorter (and thicker) the smear. It is important to prepare smears that are not thicker than one layer of erythrocytes in order to achieve good results. Smears should be air-dried and then immersed in 100% methanol or ethanol for about 10 min. Do not dip the frosted end into the jar. The smear can be stained in GIEMSA solution for some minutes, rinsed in distilled water, and dried and studied under the microscope without a cover slip. Immersion oil should be placed directly on the blood smear.

Mucus samples from the skin, fins, nasal pits and gills and the inner side of the opercula should be scraped very gently using a knife, scalpel or the back end of the forceps. The mucus is then placed and spread on a microscope slide and examined at 100× magnification for the presence of parasites such as ciliates (e.g. *Trichodina*). Particular emphasis should be placed on the caudal area of the gill openings where parasites may accumulate. Small white spots, raw ulcers or loose scales on the body are typical indicators of infections.

Macroscopic Examination

As a next step, the body surface and openings (eyes, skin, fins, gills, nostrils, anus, mouth cavity) of the fish should be macroscopically checked for ectoparasites. True ectoparasites that are visible without a microscope are Crustacea (Copepoda, Isopoda, Branchiura), Monogenea, some digenean metacercariae and cyst-producing protozoans (Myxosporea). Isolated parasites should be placed in labelled block glass dishes filled with 0.9% saline solution (see work place organisation). The exact position of each parasite on the host body should be noted separately (Fig. 8).

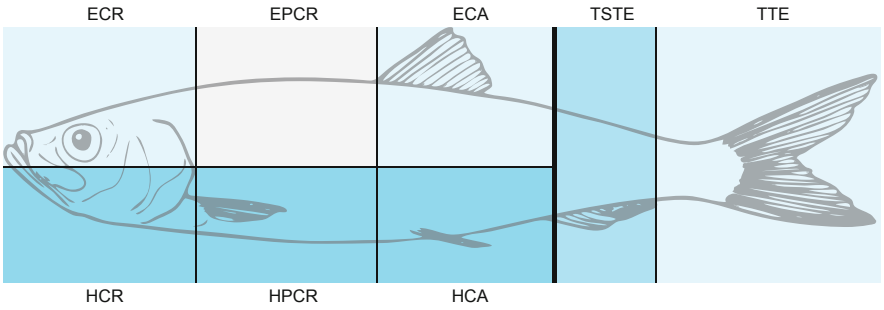

Fig. 8 Diagrammatic representation of fish sectors for parasite location determination. *ECR* epaxial cranial, *HCR* hypaxial cranial, *EPCR* epaxial postcranial, *HPCR* hypaxial postcranial, *ECA* epaxial caudal, *HCA* hypaxial caudal, *TSTE* tail subterminal, *TTE* tail terminal

Fish Dissection (Figs. 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20)

Once the external examination has been finished and the parasites are isolated and separated by origin, the fish can be dissected.

Operculi

Lift each operculum from the gill arches underneath with strong forceps and cut as close to the cranium as possible. Rinse the inner surface with saline solution into a petri dish, but check both again for parasites using a microscope. Cover the petri dish with a lid and set aside. The dissected tissue parts will be needed later to assess the "carcass weight (CW)".

Gill Arches

The gills can either be isolated as a whole with the arches separated afterwards and placed into a petri dish, or the individual arches can be cut out separately directly from the fish. Use a pointed and sharp pair of scissors to cut through the cartilaginous ends at the upper and lower extremities. Put them in petri dishes filled with saline solution to avoid dehydration. Now rinse the gill cavity with saline solution into another dish through the mouth and nasal openings and check for parasites. If not done already, carefully check the filaments of the gills for parasites. Crustaceans, although not always recognisable as such, are usually easy to spot due to their size and unusual appearance. *Lernaeocera branchialis*, for example, a common crustacean parasite from fish of the North Atlantic, are deeply red in colour. They can grow up to 50 mm in size and can be spotted without any magnification. For smaller parasites, use the back of your forceps to gently scrape out residuals from the filaments. It is not uncommon that endoparasites (e.g. Digenea, Nematoda), possibly regurgitated with the food pulp from the fish stomach during the hauls, can be found on gill filaments. Of course, the origin of these specimens should be labelled as "stomach". The gill filaments and operculi are part of the carcass weight as well, so keep the remains.

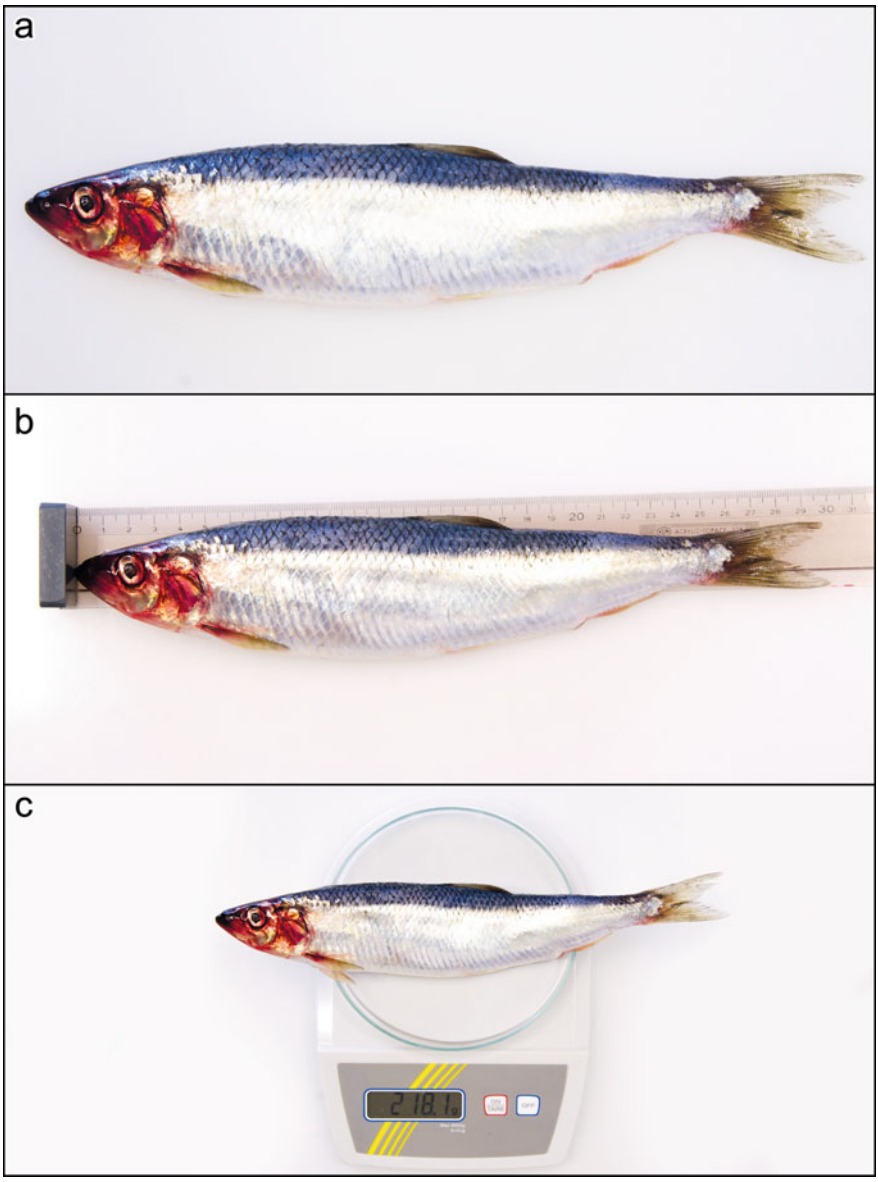

Fig. 9 Roundfish dissection. (**a**) Lateral view of the habitus of *Clupea harengus.* (**b**) Measuring the body length. (**c**) Weighing of the body

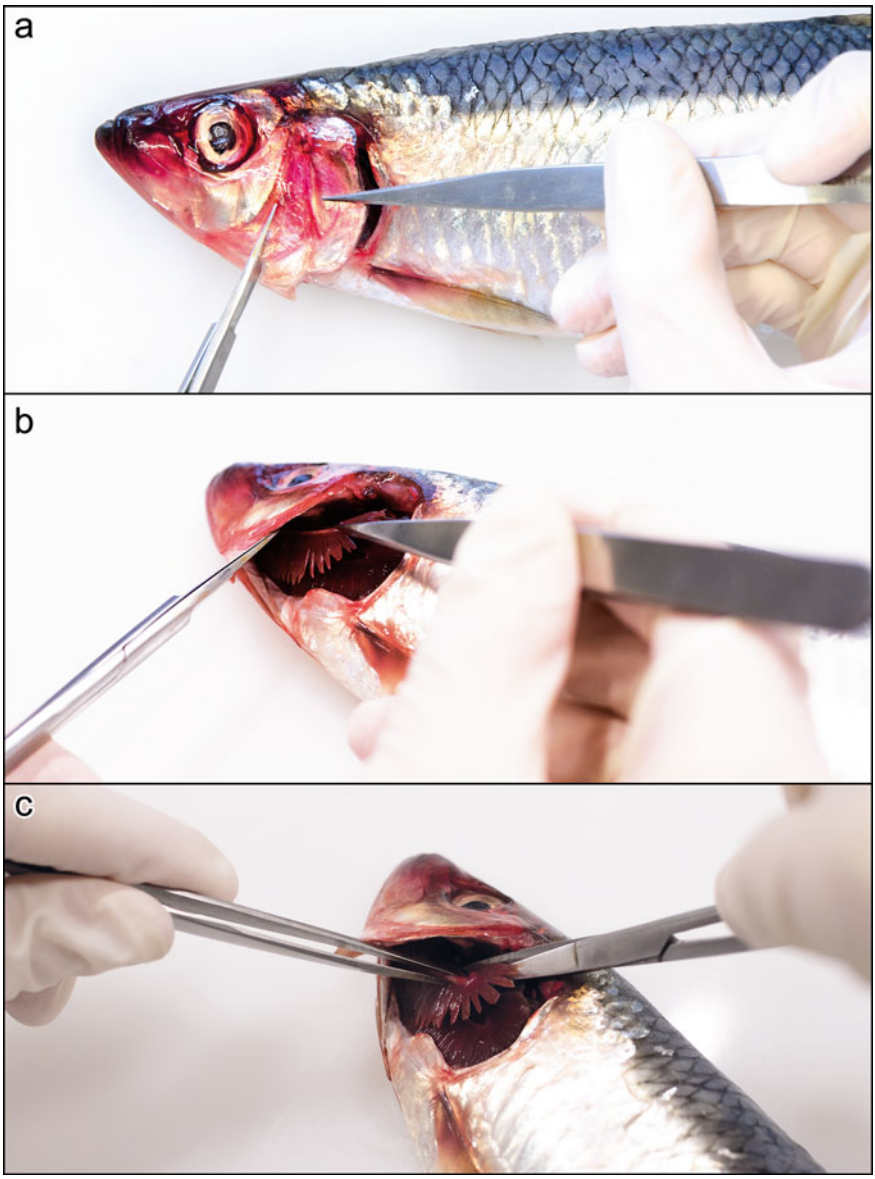

Fig. 10 (**a**) Removal of the gill cover. (**b**) Examination of the gills and gill cavity. (**c**) Removal of the gills

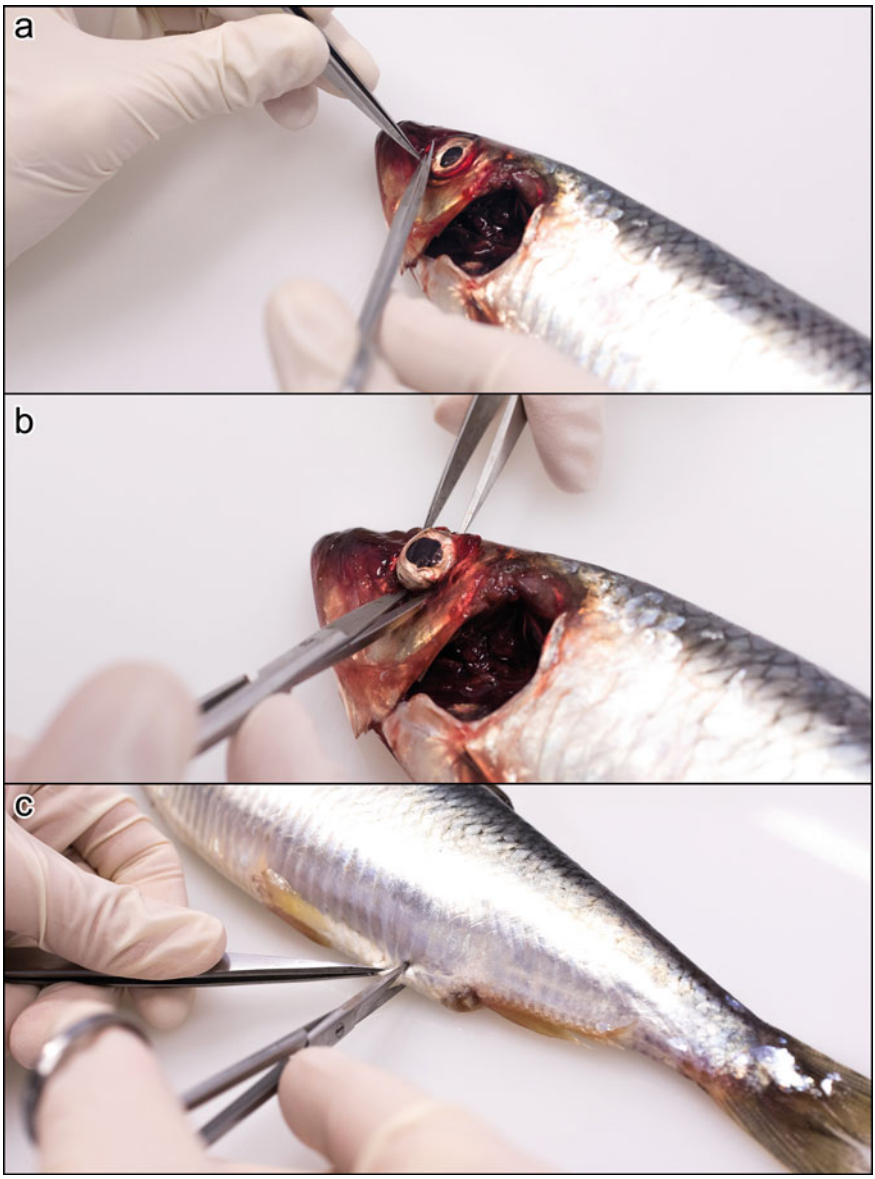

Fig. 11 (**a**) Preparation for eye removal, cut through the eye capsule around the eye. (**b**) Removal of the eye. (**c**) Pre-anal cut for opening of the abdominal cavity

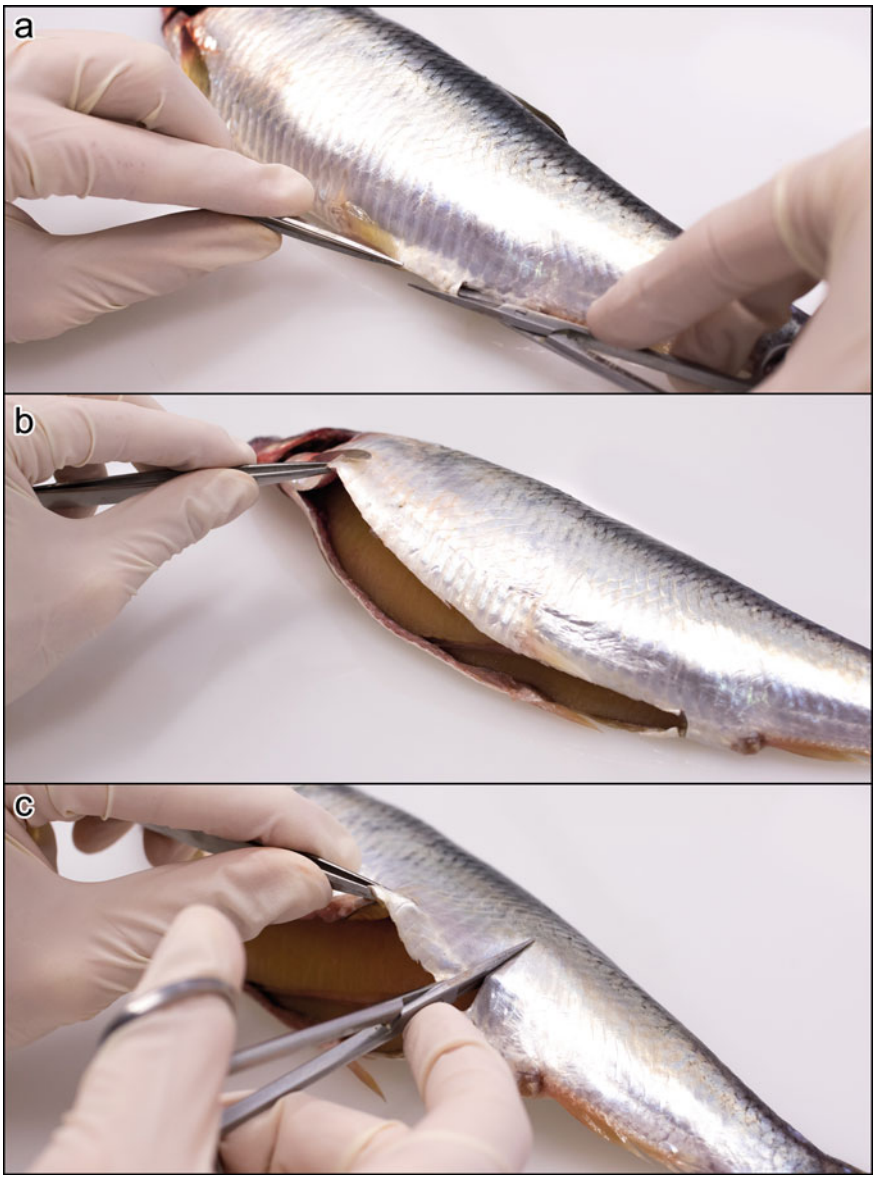

Fig. 12 (**a**) Ventral cut in cranial direction. (**b**) Finished ventral cut, opening of abdominal cavity. (**c**) Cut from starting point (pre-anal cut) in ventral direction

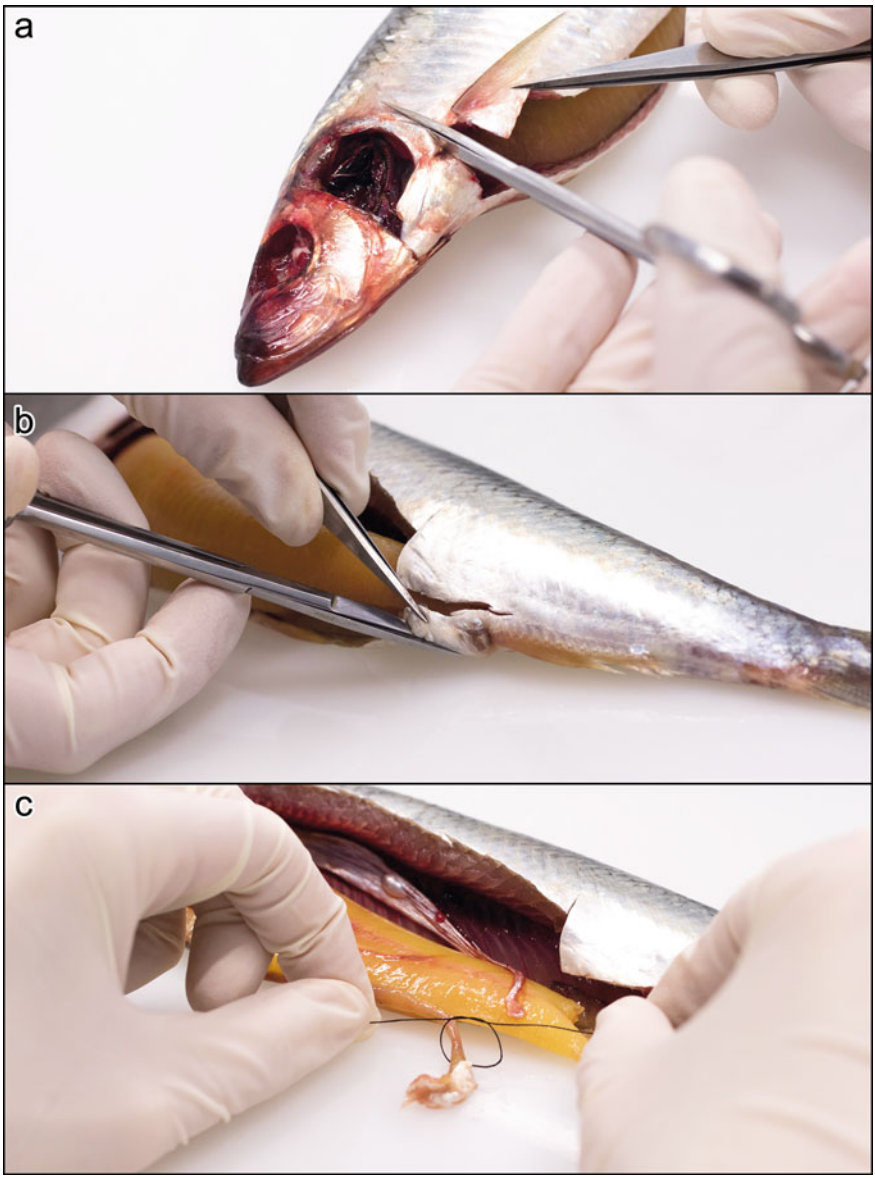

Fig. 13 (**a**) Cut in ventral direction behind the branchial arch. (**b**) Cut around the anus for extraction of the intestine. (**c**) Tie off of posterior end of the intestine

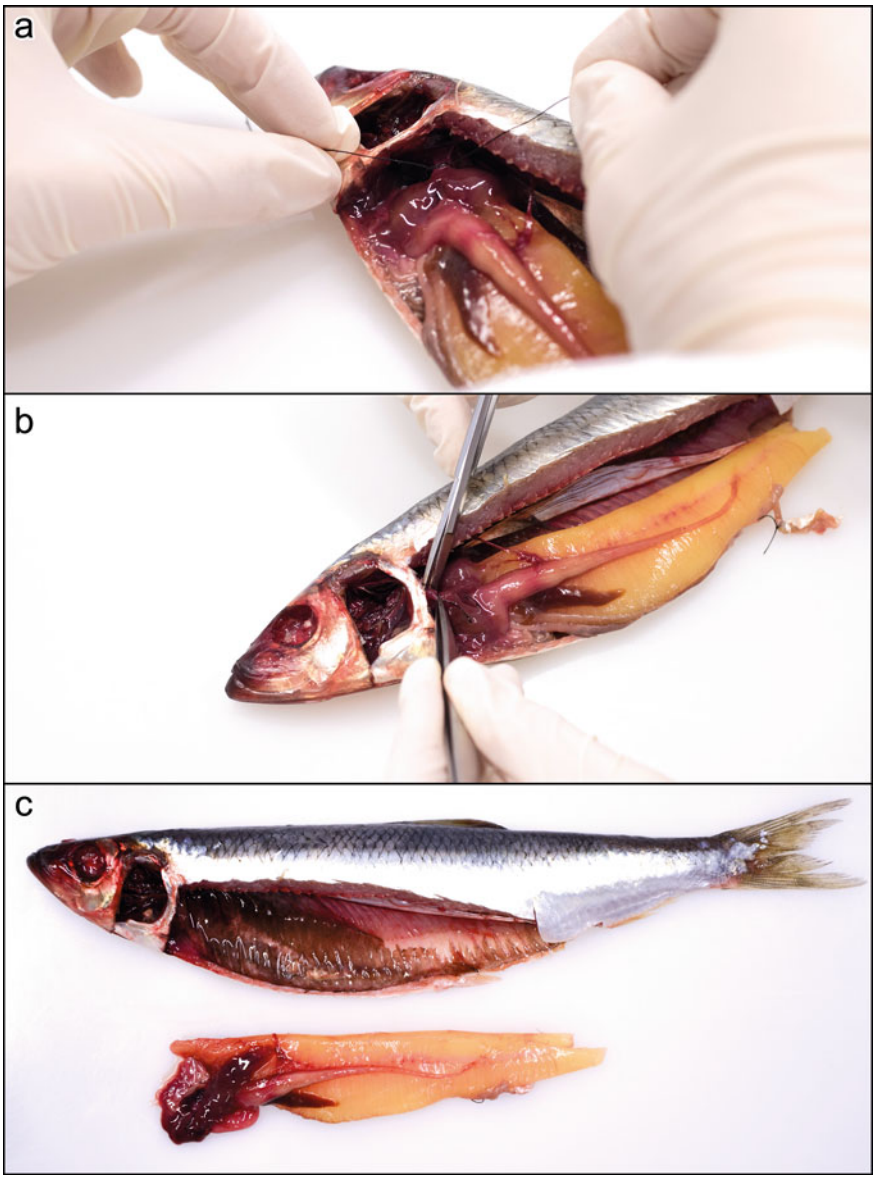

Fig. 14 (**a**) Tie off of anterior end of the oesophagus. (**b**) Cut through the oesophagus. (**c**) Removal of internal organs

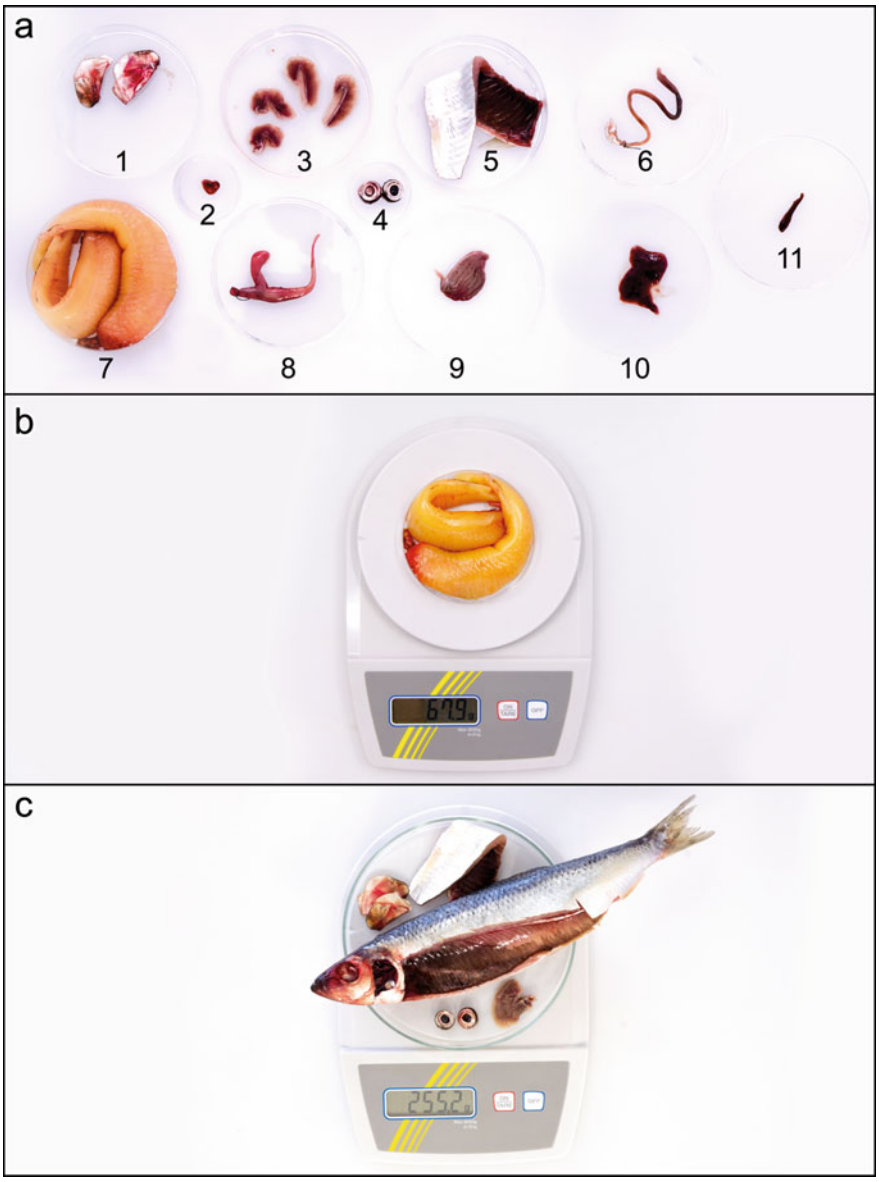

Fig. 15 (**a**) Overview of the (1) operculi, (2) heart, (3) gills, (4) eyes, (5) belly flap, (6) intestine, (7) gonads, (8) stomach, (9) pylorus, (10) liver and (11) spleen. (**b**) Weighing of internal organs (e.g. gonads). (**c**) Determining of carcass weight

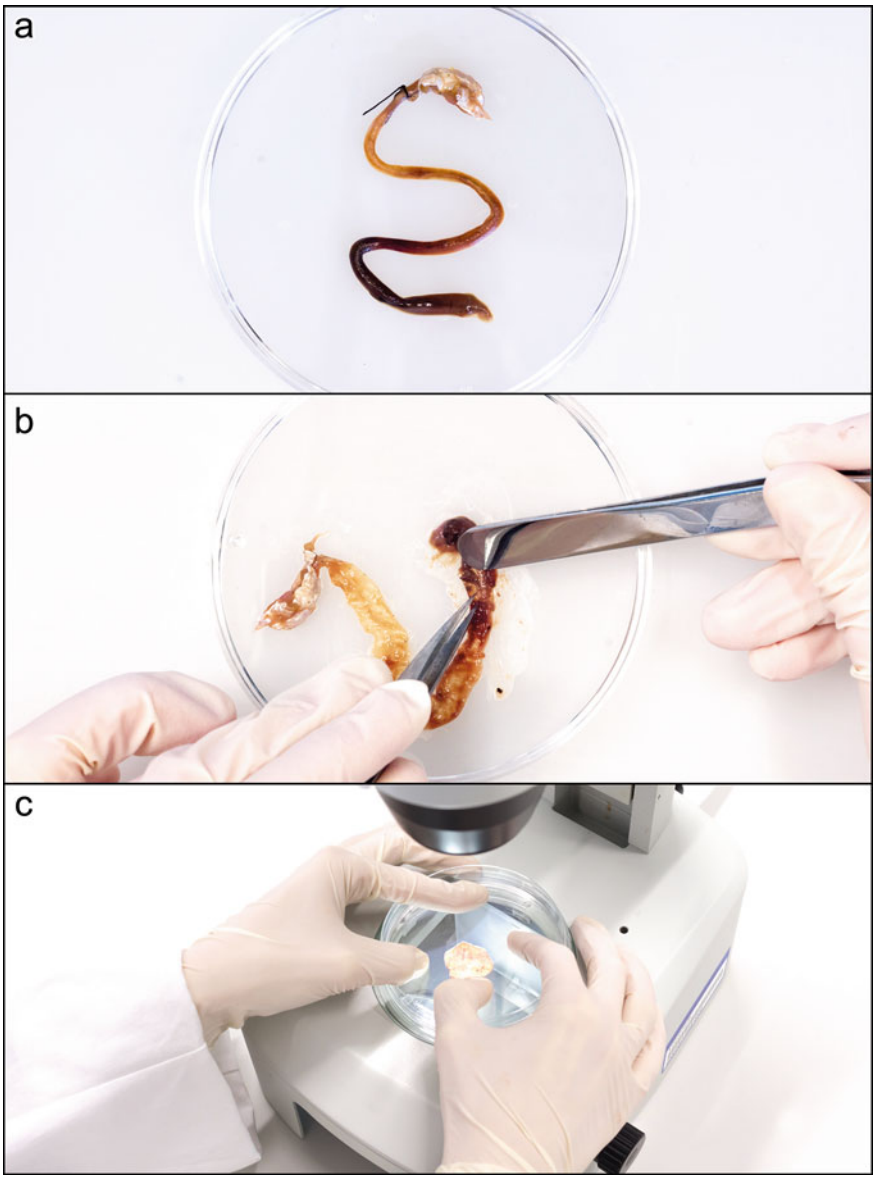

Fig. 16 (**a**) Preparation of intestine for content analysis. (**b**) Scraping of intestinal wall for microscopic examination. (**c**) Tissue compression and screening of internal organs for parasites

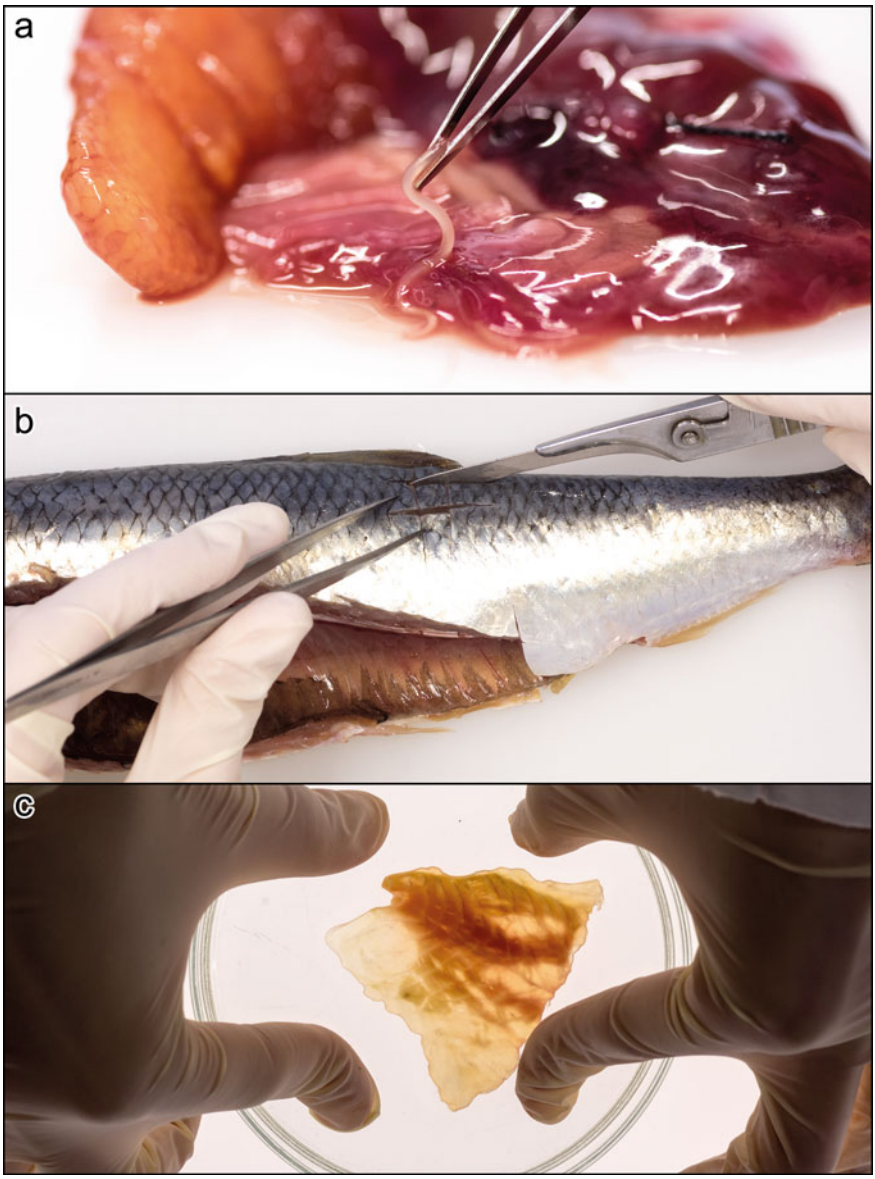

Fig. 17 (**a**) Examination of internal organs for parasites. (**b**) Cutting of tissue sample for analysis. (**c**) Tissue compression of belly flap for embedded parasites

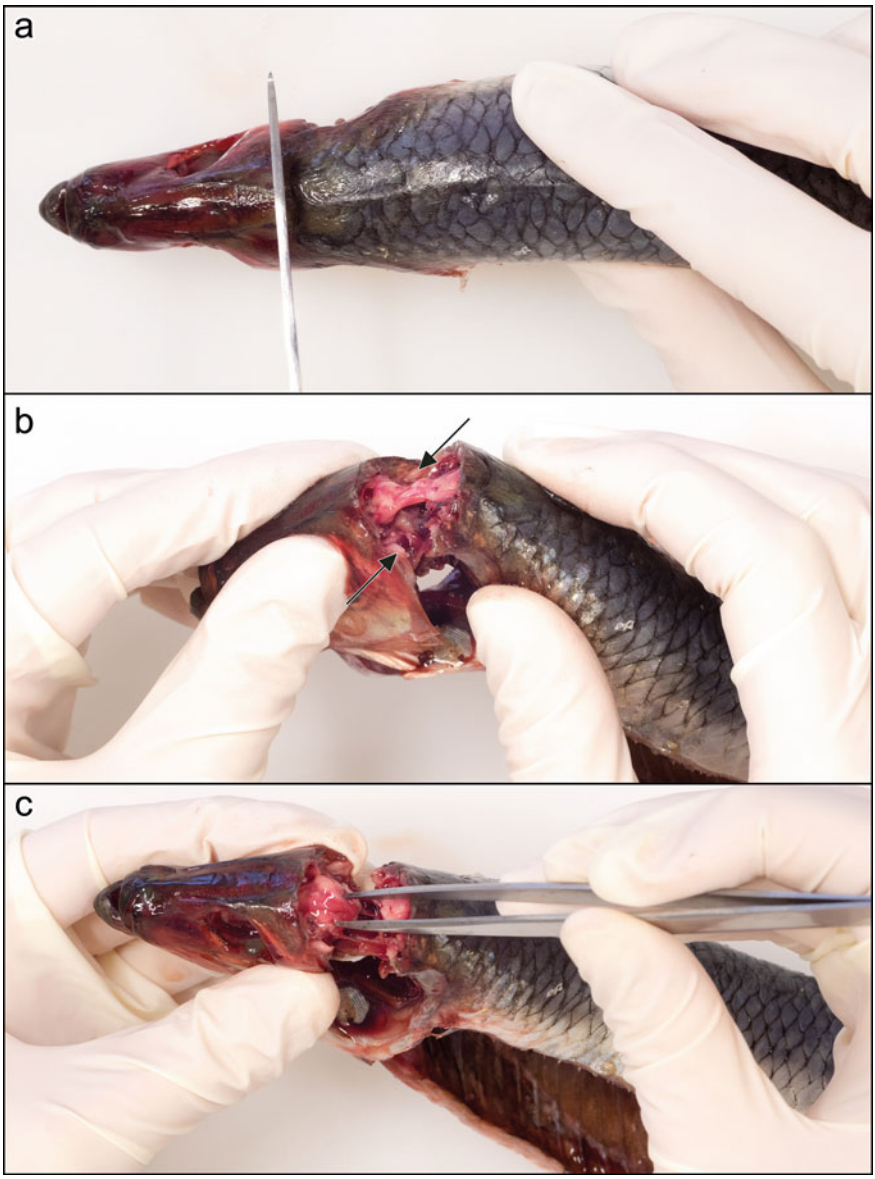

Fig. 18 (**a**) Precise cut on the ventral side of the head for brain samples and otoliths. (**b**) Cut open the brain with otoliths. (**c**) Sampling of brain tissue

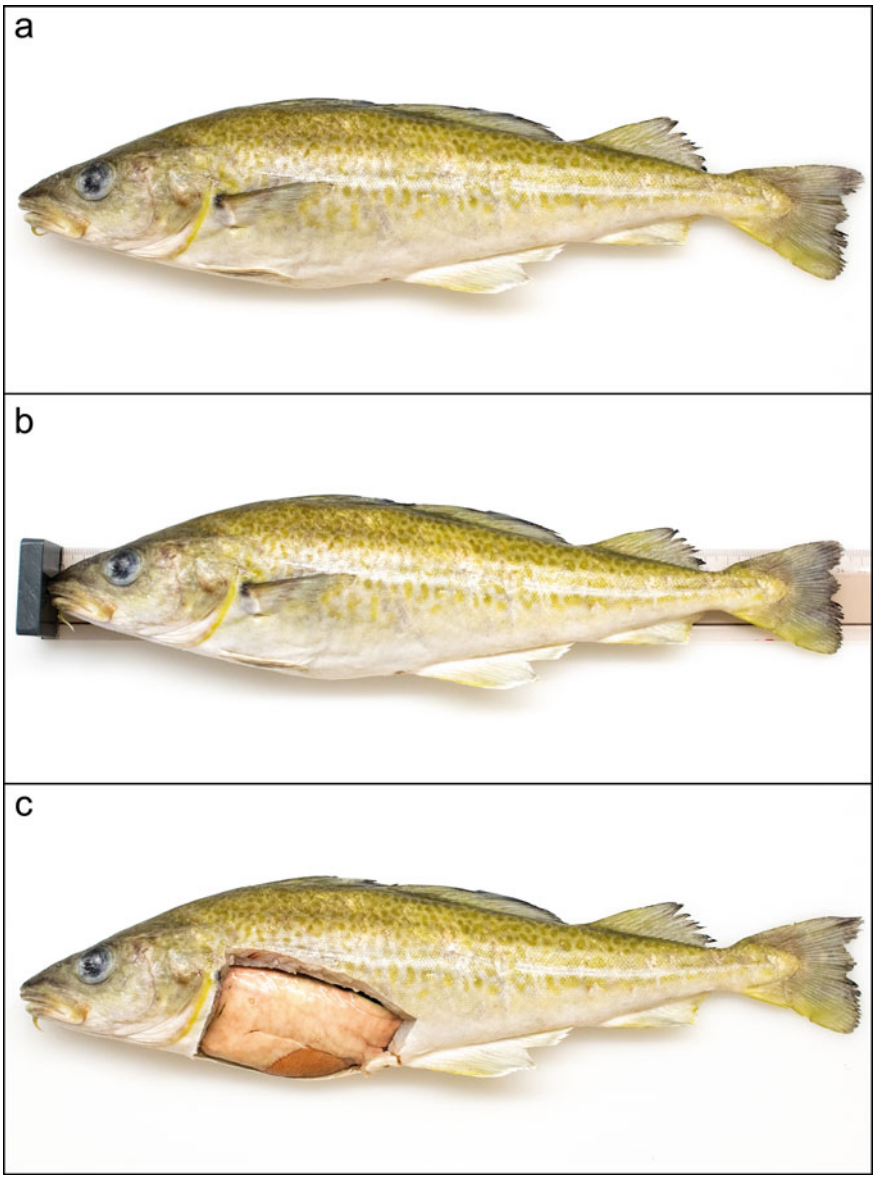

Fig. 19 Roundfish dissection. (**a**) Lateral view of the habitus of *Gadus morhua*. (**b**) Measuring the body length. (**c**) Lateral view of the habitus with opened body cavity

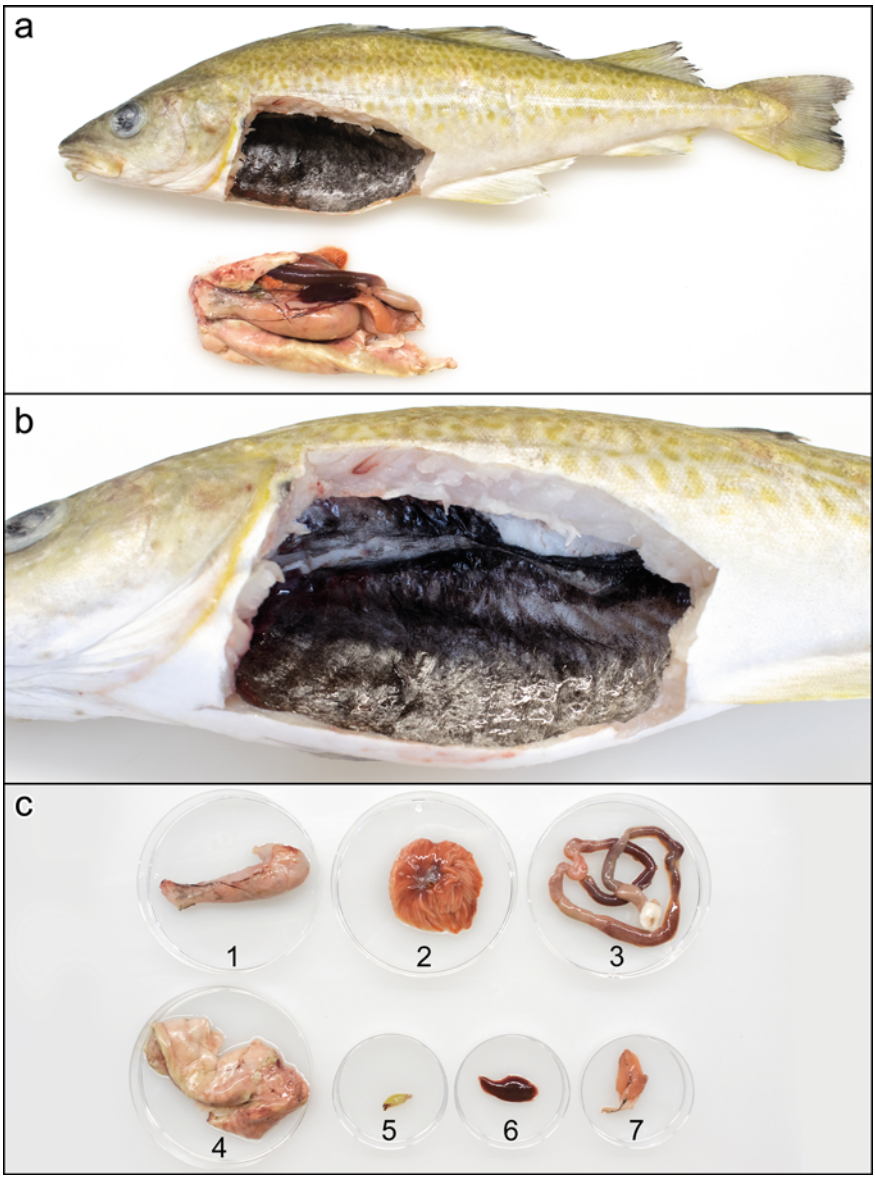

Fig. 20 (**a**) Lateral view of the habitus with removed organs. (**b**) Lateral view of the opened abdominal cavity with kidneys. (**c**) Overview of the extracted organs: (1) stomach, (2) pylorus, (3) intestine, (4) liver, (5) gall bladder, (6) spleen, (7) gonads

Enucleation

In most fish species, the eyeballs are tightly enclosed by the bony orbital cavity. The eyeballs (bulbi oculi) can be easily removed using forceps and a pointed and rounded pair of scissors. Carefully grasp the conjunctiva palpebrae in the corners of the eyes with the forceps, cut a small hole in it and carefully cut the connective and fatty tissue as well as the optic nerve and the muscles on the backside of the bulbi. Place the isolated eyeballs in a clean petri dish and punctuate the bulbi using a syringe or straight needle. Check the eye fluid for the presence of digenean metacercariae (e.g. of the genus *Diplostomum*) using the stereomicroscope.

Body Cavity

The dissection begins with the incision of the thin skin and the muscle layer on the ventral side of the fish just a few millimetres anterior to the anal opening (vent). Do not cut through the anus itself as this would damage the rectum and would result in intestinal contents (and possibly parasitic stages) leaking from the incision. The incision should be large enough to insert the rounded tip of a sharp pair of scissors. Cut along the ventral midline towards the isthmus between the operculi. While cutting, the rounded tip of the scissors should point slightly towards the ventral skin and not the body cavity in order to avoid damage of the internal organs. After this, turn the fish on its side and perform a second cut towards the dorsal end of the body cavity from the two ends of the first incision. The resulting flap of the skin and muscle can be lifted, cut off and rinsed on the inside with saline solution. Rinsed off contents should be kept in a petri dish and checked for parasites under the microscope.

The next step is to locate and identify the respective organs of the fish. Examine the ventral part of the body cavity and mesenteries for macroscopically visible parasites (e.g. nematode larvae that might appear as coiled-up structures between the mesenteries) and isolate them using a fine pair of forceps. Place them, separated by origin (e.g. body cavity, mesenteries), in labelled block glass dishes.

Extraction of Internal Organs

Before the internal organs can be separated from the carcass, the posterior extremity of the gut has to be sealed with sewing thread in order to avoid the contents leaking from the vent. Separate the vent from the carcass first, by cutting the tissue around the vent with a fine pair of scissors. Carefully pull and loosen the adipose tissue and organ-convolute from the dorsal part of the body cavity using your hands (the gloves have to be carefully rinsed and checked for parasites afterwards) or a pair of forceps, depending on the size of the fish.

Cut the oesophagus at the most anterior position possible and place the organs in a larger petri dish before separating and placing them individually in labelled dishes. Be careful not to puncture the gall bladder. Weigh the liver (LW), gonad (GW) and the stomach (SW). Cover each dish with a lid to avoid drying out. Examine the

empty body cavity for parasites and rinse the buccal cavity and body cavity with saline solution, which should also be checked for parasites. Pat dry the carcass and place it together with the gills, operculi and skin flaps on a scale to assess the carcass weight (CW).

Liver and Gall Bladder

The liver with its very fatty and energy-rich tissues is a common settlement site for several different parasitic stages (e.g. nematodes) and should be examined thoroughly. Nematode stages, for example, L3 larvae of anisakid nematodes (e.g. *Anisakis* spp., *Pseudoterranova* spp.), are usually visible without any magnification and appear as tightly coiled (*Anisakis*) or more extended forms on the liver surface. Try not to tear them apart by using two forceps and maybe a preparation needle to isolate them. It can be difficult to separate the parasites from their capsules and any remaining host tissue. However, try to remove any excess tissue since this is a mandatory prerequisite for any successful morphological and especially subsequent genetic analysis.

Once all macroscopically and microscopically visible parasitic stages have been removed, the remaining liver tissue can be further analysed using the "crush preparation" technique (see grey box). This is probably the most efficient method to examine even large quantities of liver tissue. The gall bladder should be isolated beforehand, without damaging the thin mantle. Transfer the gall bladder into a separate glass, puncture it with a fine needle, mount a drop of gall liquid on a microscope slide and check for parasites (Myxozoa).

Crush Preparation

- Dissect liver, spleen and pylorus into subsamples of approx. 1 cm^3 portions.
- Place individual subsamples into the lid of a petri dish.
- Cover with the bottom half of the petri dish.
- Crush the sample into a thin layer using the thumbs and fingers.
- Examine via transillumination using a stereomicroscope, applying pressure throughout the examination.

Pylorus

Depending on the fish species examined, the pylorus can be quite voluminous. Very similar to liver tissue, the crush preparation is recommended to efficiently examine large fish specimens. Make sure to dissect the pylorus into small pieces before crushing and opening up each caecum with a very fine pair of scissors. Keep the tip of the scissors flat to prevent damage of the potentially present parasitic stages. Watch out for Digenea, Cestoda, Nematoda and Acanthocephala larvae.

Stomach

Measure the weight of the full stomach [Stomach $Weight_{filled}$] before dissecting it. Afterwards, the stomach can be cut open by using a fine pair of scissors, with the tip pointing upwards. Spread the tissue open and transfer its content into a new dish. Transfer the stomach into a clean, separate petri dish and scrape out the mucus using the rounded back end of a pair of forceps or the edge of a microscope slide. Measure the mucus weight. Pat dry the inner membrane and weigh the empty stomach [Stomach $Weight_{empty}$]. Check the contents for digeneans, cestodes and nematodes (see chapter stomach contents).

Intestine

The intestines of an organism are a very common habitat for a plethora of different parasitic groups and thus require special attention during dissection and preparation. In many cases, and usually depending on the size of the study fish, the intestines are too long to be dissected and examined as one piece. Small portions of up to 2 cm are sufficient to avoid an excessive pollution of the saline solution by the intestinal contents. The gut should be cut along a median line starting from the small intestines using a fine pair of scissors. Keep the tip pointed upwards and try not to damage the contents. It helps to squeeze out the contents on a length of a few centimetres before cutting to avoid damaging larger parasites such as nematodes or cestodes that might be coiled up within the lumen. If necessary, the subsamples can be further divided until examination, using transmitted light if possible. If cutting of parasite individuals cannot be fully avoided (e.g. large cestodes), unique body parts such as the scolex of a cestode or the anterior parts of nematodes are reliable indicators for the number of individuals present.

Muscular Tissue

Once the dissection of the inner organs is finished, the muscular tissue should be inspected for parasitic stages such as metacercariae of Digenea (e.g. *Cryptocotyle*) cestode plerocercoids (Diphyllobothriidae) and Nematoda (e.g *Anisakis*, *Hysterothylacium*, *Pseudoterranova*). Based on the type of study, i.e. whether it is a practical course or large-scale study with different numbers of hosts and sample sizes (see grey box), different methods can be applied. The first step should always be to cut out a small piece of tissue (0.5 cm^3) that should be stored as a sample for potential genetic studies of the hosts (e.g. genetic species identification, population genetics).

Muscle Tissue Preparation

Crush preparation

Crush preparation can be used as a "fast and easy" method and is suited best for dissections as part of a practical course and when the host individuals are small enough.

- Place a small portion of tissue in the lid of a heavy glass petri dish.
- Press carefully until a thin tissue layer allows transillumination against daylight or examination using reflected light under a dissecting microscope.

Digestion method after the European Reference Laboratory for Parasites

The digestion method allows the examination of several individuals' tissue simultaneously without too much effort. It is also effective for digesting the cyst wall that might be secreted by some metacercariae. However, due to the implementation of hydrochloric acid (and the enzyme pepsin), this method is only partly suited for practical courses and should only be applied after careful introduction. The basic principle is to digest the tissue in a solution that resembles the gastric acid (and body temperature) of the host. In an additional step, a solution containing trypsin allows the parasites to exit from their cyst wall.

Solution for nematode extraction (after Buchmann)

- Pepsin: 10 g (30 ml, 660 EP)
- HCl (25%, molar concentration 7.8–7.9): 16 ml
- Distilled water: 2000 ml
- Adjust to pH 2
- Fillet: 100 g

Solution for metacercariae (after Buchmann)

- Pepsin: 10 g (30 ml, 660EP)
- HCl (25%, molar concentration 7.8–7.9): 10 ml
- Distilled water: 2000 ml
- Adjust to pH 2
- Fillet: 100 g

Trypsin solution (after Buchmann)

- Trypsin: 2 g
- PBS (pH 7.5): 100 ml
- Bile (optional): 0.5 g

Procedure

- Dissect the tissue into defined parts (e.g. belly flap left/right, anterior dorsal/ventral, posterior dorsal/ventral, tail).

(continued)

- In a ratio of 1/10 (tissue/medium), place the respective tissue in a glass beaker with digestion solution.
- Incubate one to several hours by using a magnetic stirrer under constant and slight stirring at 37–45 °C.
- Extend incubation if needed until the host tissue is dissolved.
- Filter the remainder through a sieve and recover the macroscopically visible parasitic stages.
- Recover the solution in a centrifuge tube and leave to sediment for at least 5 min.
- Aspirate supernatant up to 10 ml.
- Pour 10 ml pellet in a petri dish and analyse under stereomicroscope to detect metacercariae.
- Put parasites in a glass well with dd trypsin solution.
- Incubate until excystation is completed (a few seconds up to 30 min).
- Remove excysted parasites from the well and place in a block glass dish with saline solution.

UV-press method for nematode detection

This method is based on the crush preparation and can also be applied for internal organs. It allows, depending on the degree of automation and size of the equipment, the analyses of large tissue samples. Thick glass plates and a set of bolts and nuts can be used to build a handheld tool for small samples, whereas a large hydraulic press and tissue samples pressed in large plastic bags can be used to perform large-scale monitoring in a fast and easy way. The detection of the nematodes is based on the fact that nematodes, once they have been deep frozen, emit light when exposed to UV light.

Brain and Otolith Extraction

As the last step of the dissection, the cranium and brain will be examined, and the sagittal otoliths should be extracted. Otoliths are small mineral structures found in the head of all fishes other than sharks, rays and lampreys. Otoliths allow fish to hear and provide a sense of balance, but to a fisheries biologist, the otolith is one of the most important tools for understanding the life history of the individual fish or the fish population. Although not directly relevant for the parasitological examination, counting the annual growth rings on these otoliths, also called “ear bones”, is a common technique for estimating the age of fish. In addition, studying the trace elemental composition or isotopic signatures of trace elements within a fish otolith can be used to study the environment, including diet and temperatures, in which the fish lived throughout its lifetime, as well as their natal origin. Even if none of the above reasons apply to the specific aim of the study, following the recommendations of good scientific practice, an extraction is strongly recommended to meet potential requests from interested scientists.

The extraction of otoliths is neither very time-consuming, nor does it require special dissection skills. However, it can be a bit tricky to not lose the small white otoliths in the vestibular system once the fish skull has been cut open. There are different techniques for extracting otoliths from fish. The "scalp" method is one of the quickest and easiest methods for retrieving otoliths. The top of the head is carefully cut transversely to the dorsal midline using a sharp, serrated knife to expose the brain case. In order to avoid cutting through the otolith, a common practice is to cut the head bone and then carefully break the head apart using the bare hands. By keeping the brain aside using forceps, the two largest otoliths, the Sagittae, can be extracted from near the bottom of the brain case. The otolith is often still inside the fluid-filled sac (sacculus) that surrounds it, but the sac is easily removed. The otoliths are then cleaned and stored or prepared for analysis. The brain should be transferred to a petri dish lid and crushed using the bottom of a glass well.

Flatfish

The dissection of flatfish closely resembles the preparation protocol for round fish. In the following, the focus is set on the relevant differences that arise from the specific morphology of flatfish.

The dissection starts by measuring the basic morphometry, i.e. the total length [TL] and standard length [SL] and the pre-anal length [PAL] as well as the total weight [TW] (Fig. 21).

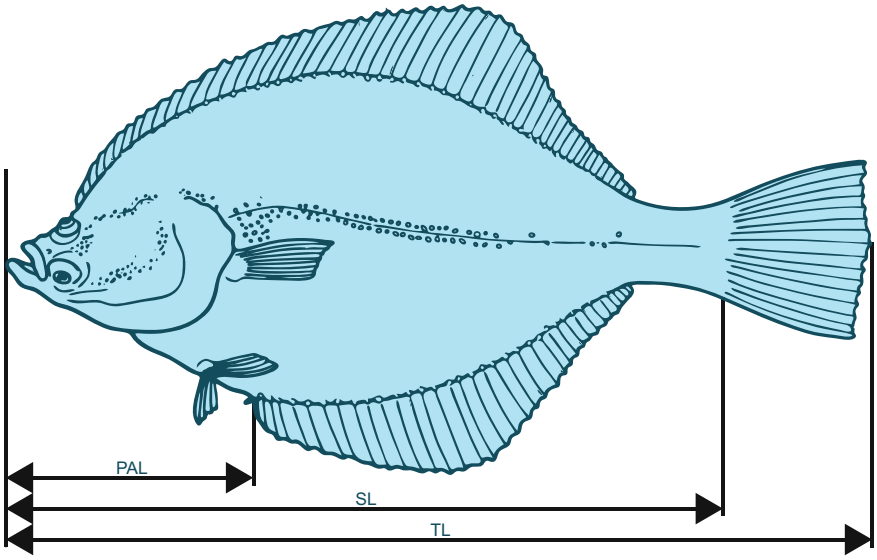

Fig. 21 Diagrammatic representation of flatfish measurements for catch statistics and parasitological examinations

Blood Sample and Skin Smear Examination

The easiest method to collect a blood sample of a flatfish is by puncturing the caudal artery along the midline of the fish's body using a syringe. Skin smears should be prepared from both the eyed and blind sides of the fish.

Macroscopic Examination

Begin by inspecting the overall condition of the body surface and openings, check for ectoparasites and record any anomaly. Note that flatfish possess pairs of pectoral and pelvic fins, a single elongate dorsal and ventral fin as well as the typical caudal fins.

Fish Dissection (Figs. 22, 23, 24 and 25)

Operculi, Gill Arches, and Enucleation

Using the same method as described for roundfish, examine the gill filaments, gill arches and gill rakers after removing the opercula with strong forceps as close as possible to the cranium. Remove the eyeballs and check the fluid for the presence of metacercariae.

Body Cavity

To expose the organs in the peritoneal cavity, place the flatfish with the blind side facing upward. Use a pair of fine scissors to make an incision just caudal to the anus and be careful not to cut through the anus itself or the underlying ovary or testis. Cut posteriorly and dorsally along the caudal margin of the peritoneal cavity following a roughly semicircular shape. Make another incision near the anus and cut along the ventral midline between and past the pelvic fins. Finally trim the resulting flap of skin by making a dorsal cut along the anterior margin of the peritoneal cavity. Examine the body cavity for parasites.

Extraction of Internal Organs

Seal the end of the gut with sewing thread and separate the vent from the carcass as described above. Carefully loosen the organ-convolute from the peritoneal cavity and cut the oesophagus as close as possible to its opening. Place the organs in a petri dish and separate them into single and labelled dishes. Proceed by examining the organs as described in the previous chapter.

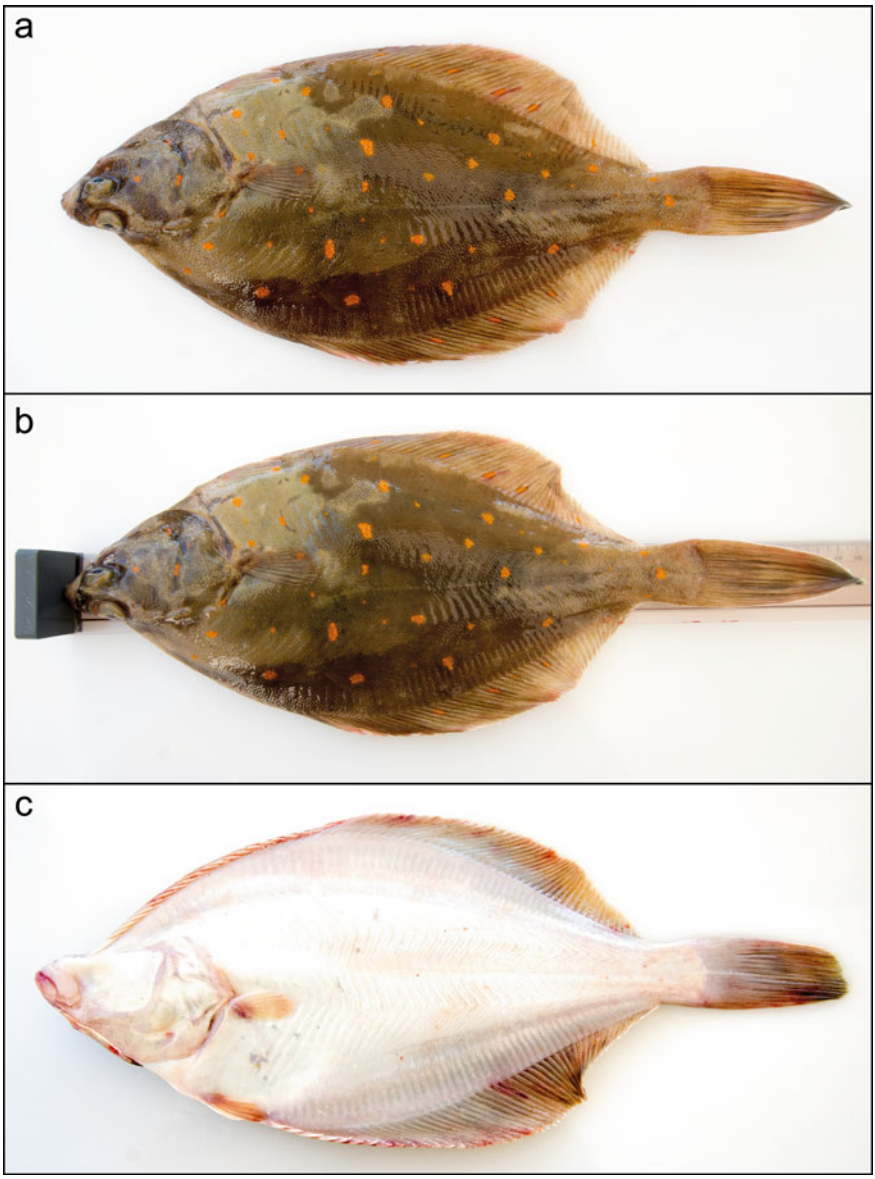

Fig. 22 Flatfish dissection. (**a**) Lateral (dorsal) view of the habitus of *Pleuronectes platessa*. (**b**) Measuring the body length. (**c**) Lateral (ventral) view of the habitus

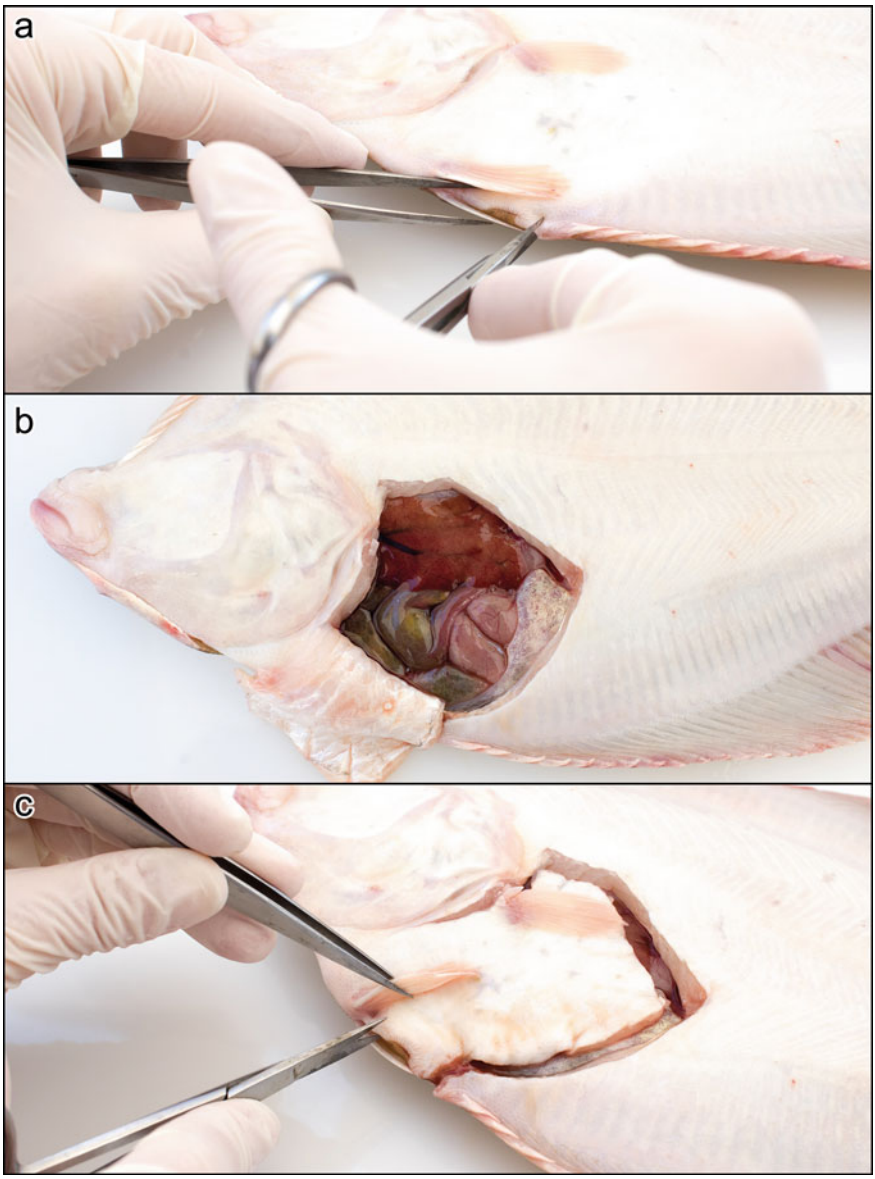

Fig. 23 (**a**) Postanal cut for opening of the abdominal cavity. (**b**) Opened abdominal cavity with belly flap. (**c**) Cut around the anus for extraction of the intestine

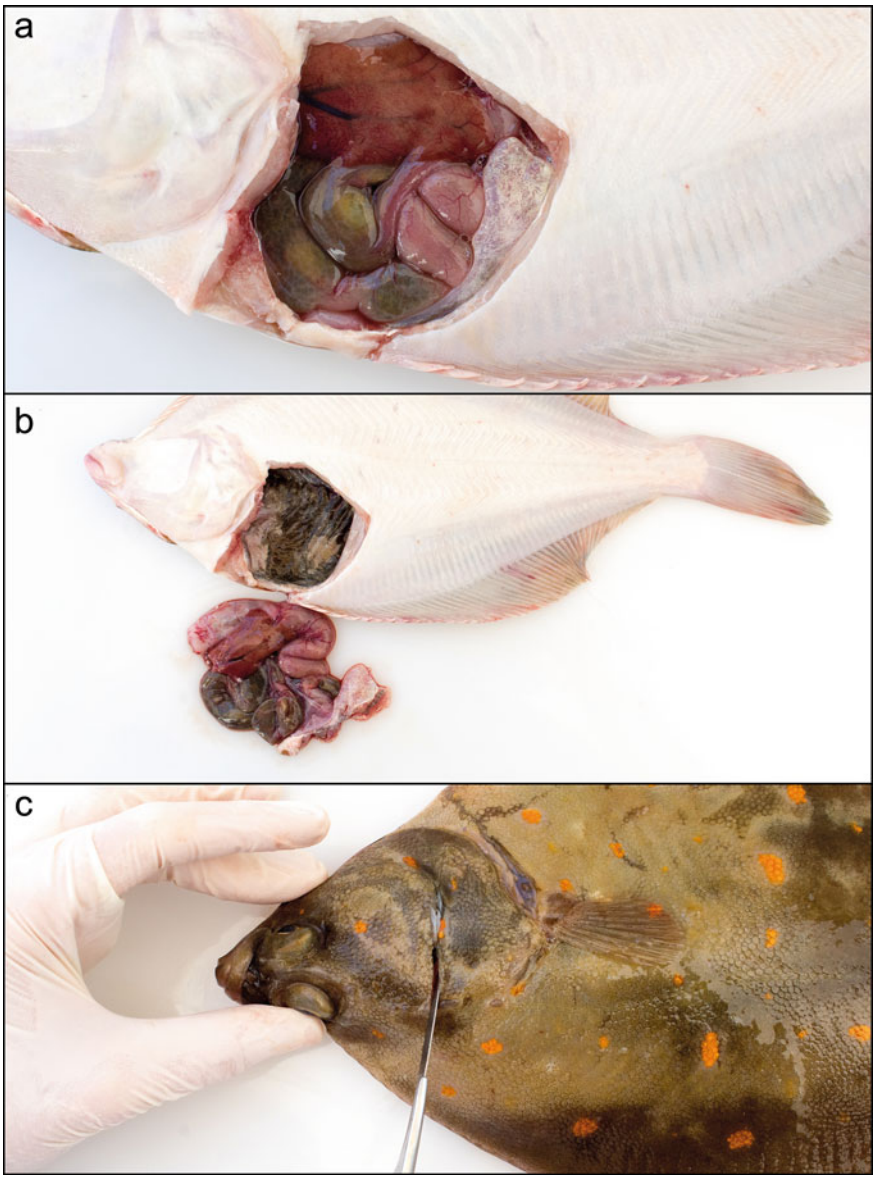

Fig. 24 (**a**) Opened abdominal cavity with organs. (**b**) Opened abdominal cavity with removed organs. (**c**) Precise transverse section at the beginning of the branchial arch for brain samples and otoliths

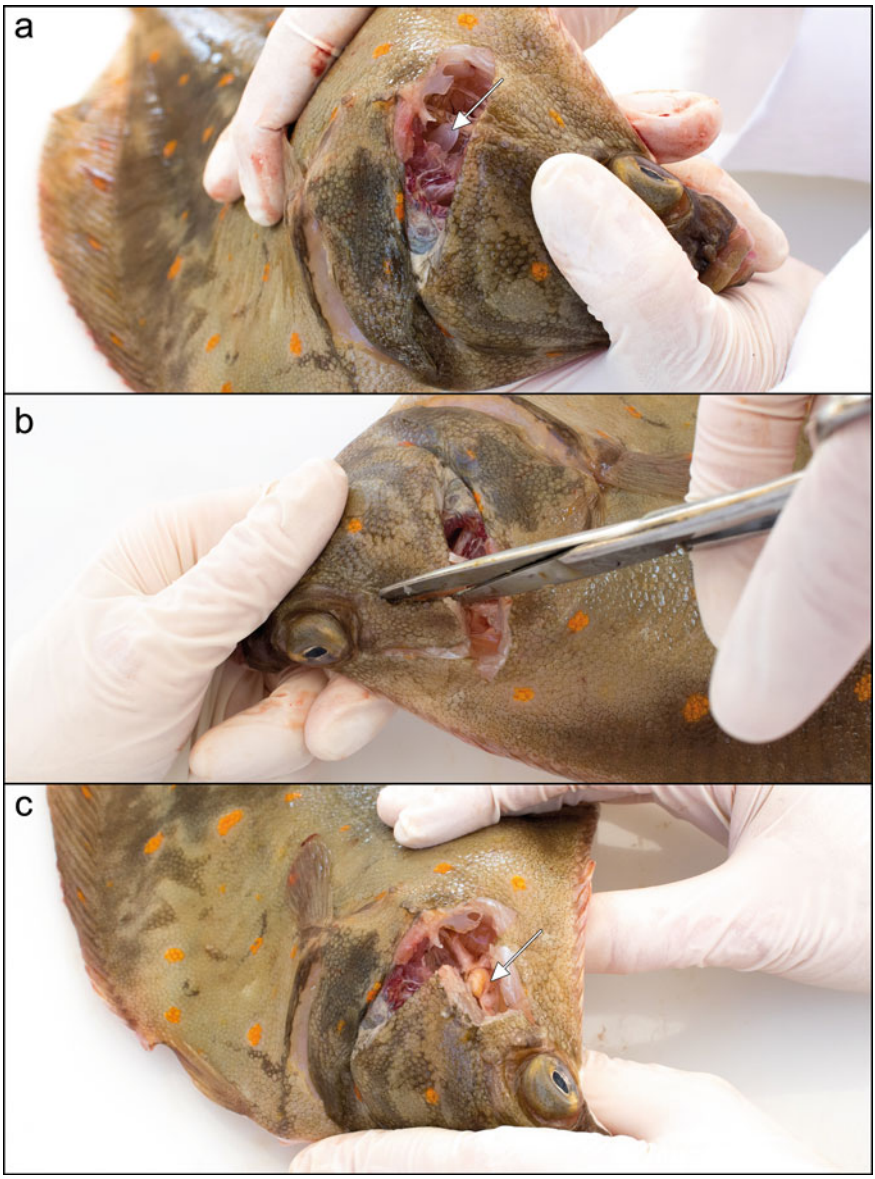

Fig. 25 (**a**) Widening of the cut to reveal otoliths (arrow). (**b**) Longitudinal section in cranial direction from the centre of the previous cut. (**c**) Revealed brain (arrow)

Brain and Otolith Extraction

Very similar to the otolith extraction of round fish, it is almost impossible to extract more than two of the otoliths of flat fish. Place the fish on its dorsal side (the eyes facing upwards) and cut the cranium at a level approx. midway between the posterior edge of the preoperculum and the posterior edge of the operculum on the dorsal side of the head. After collecting the otoliths using pointed forceps, make another cut along the dorsal margin of the brain cavity. Be careful not to damage the fragile brain tissue. Make another parallel cut along the ventral margin of the cranial cavity and lift the flap with a strong set of forceps. Extract and crush the brain, check for parasites.

Cephalopoda

Dissecting a cephalopod can be quite messy, so make sure you have a few additional cloths at hand. Cephalopoda are comparatively fragile and susceptible to maceration by digestion enzymes that are active even during the freezing process. If the specimens cannot be deep-frozen using a very fast freezing apparatus working at very low temperatures, it is highly recommended to examine them immediately after the catch. The following protocol is based on the dissection protocol for a squid but can be applied to any other cephalopod species as well. In many aspects, cephalopods resemble fish in their patterns of parasitic infection, although prevalences and intensities are rather low.

Host Biometric Data

Due to the fact that cephalopod limbs can be easily stretched beyond their natural length, the tentacles are very fragile and might easily be torn off during freezing and storage. The only two robust and reliable measures of the cephalopod biometry are the mantle length [ML] as well as the total body weight [BW]. When examining eight-limbed cephalopods (Octopodiformes), the mantle length is taken from the midpoint between the eyes to the posterior end of the mantle. Place the specimen with the ventral side (funnel side) facing upwards onto a measuring board.

Important Host Biometric Parameters (Fig. 26)

Mantle length [ML]: Measured dorsally above the midline of the mantle and defined as the length in [cm] between the anterior edge of the mantle (near the head) and the posterior end of the mantle or the apex of the joint fins.

Body weight [BW]: Defined as the weight in [g] of the defrosted cephalopod before dissection.

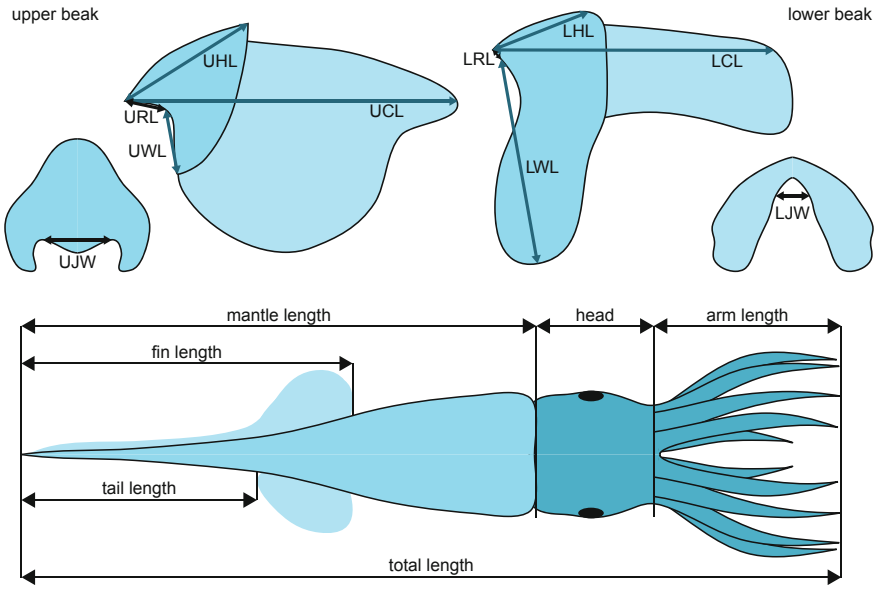

Fig. 26 Diagrammatic representation of cephalopod measurements for catch statistics and parasitological examinations. *UHL* upper hood length, *URL* upper rostral length, *UCL* upper crest length, *UWL* upper wing length, *UJW* upper jaw width, *LHL* lower hood length, *LRL* lower rostral length, *LCL* lower crest length, *LWL* lower wing length, *LJW* lower jaw width

Blood Sample and Skin Smear Examination

Blood samples can be obtained by either inserting a needle into the cephalic vein lying dorsal to the funnel or placing it directly into the branchial heart. Whereas the first method can be applied after taking the biometric data, the latter method can only be applied once the mantle has been opened. Prepare skin smears.

Macroscopic Examination

Begin by inspecting the overall condition of the body surface and openings and record any anomaly. In contrast to fish, infections with ectoparasites are rather uncommon among cephalopods. However, some monogenean or crustacean parasites have been documented in the past and might be present on the skin, eyes or in body openings.

Cephalopod Dissection (Figs. 27, 28, 29, 30, 31, 32 and 33)

Enucleation

The eyes of a cephalopod are rather large for an invertebrate and are located on both sides of the head. Remove eyeballs and check the fluid for the presence of metacercariae.

Body Cavity

To expose the organs in the body cavity, place the cephalopod with the ventral side (dark in colour) facing upward. Lay down the tentacles and determine the sex of the specimen if possible. Mature males have developed a hectocotylus (metamorphosed reproductive arm) to store and transfer spermatophores to the female. They may be shaped in many distinctive ways and vary considerably between species.

Use a pair of fine scissors to make an incision approx. 1 cm next to the ventral mantle line starting from the edge of the mantle right above the funnel. Cut an incision next to the mantle line and continue cutting up to the tip of the visceral sac. Then spread the resulting flaps to the sides. Examine the body cavity for parasites and determine the sex and state of maturity according to the key below.

Sex and Maturity State According to Lipinski (1979) (Fig. 34)

Males (spermatophore sac present)		
Juvenile	0	Not identifiable
Juvenile	I	Spermatophoric complex visible using magnification
Immature	II	Spermatophoric complex visible without magnification
Preparatory	III	Spermatophoric complex whitish
Maturing	IV	Needham sac long, spermatophores not yet formed
Mature	V	Spermatophores present in Needham sac
Spent	VI	No or degenerated spermatophores in Needham sac. Condition poor

Females (nidamental glands present)		
Juvenile	0	Not identifiable
Juvenile	I	Nidamental glands visible using magnification
Immature	II	Nidamental glands visible without magnification
Preparatory	III	Immature ova visible
Maturing	IV	Eggs pressed together at least in the proximal part of the oviduct
Mature	V	Stage IV and eggs are translucent
Spent	VI	No or degenerated eggs. Nidamental glands small. Animal condition poor

Gills and Funnel

Once the mantle cavity has been opened, the gills can be identified as feathery structures that lie on either side of the body cavity with the gill-heart at the base of

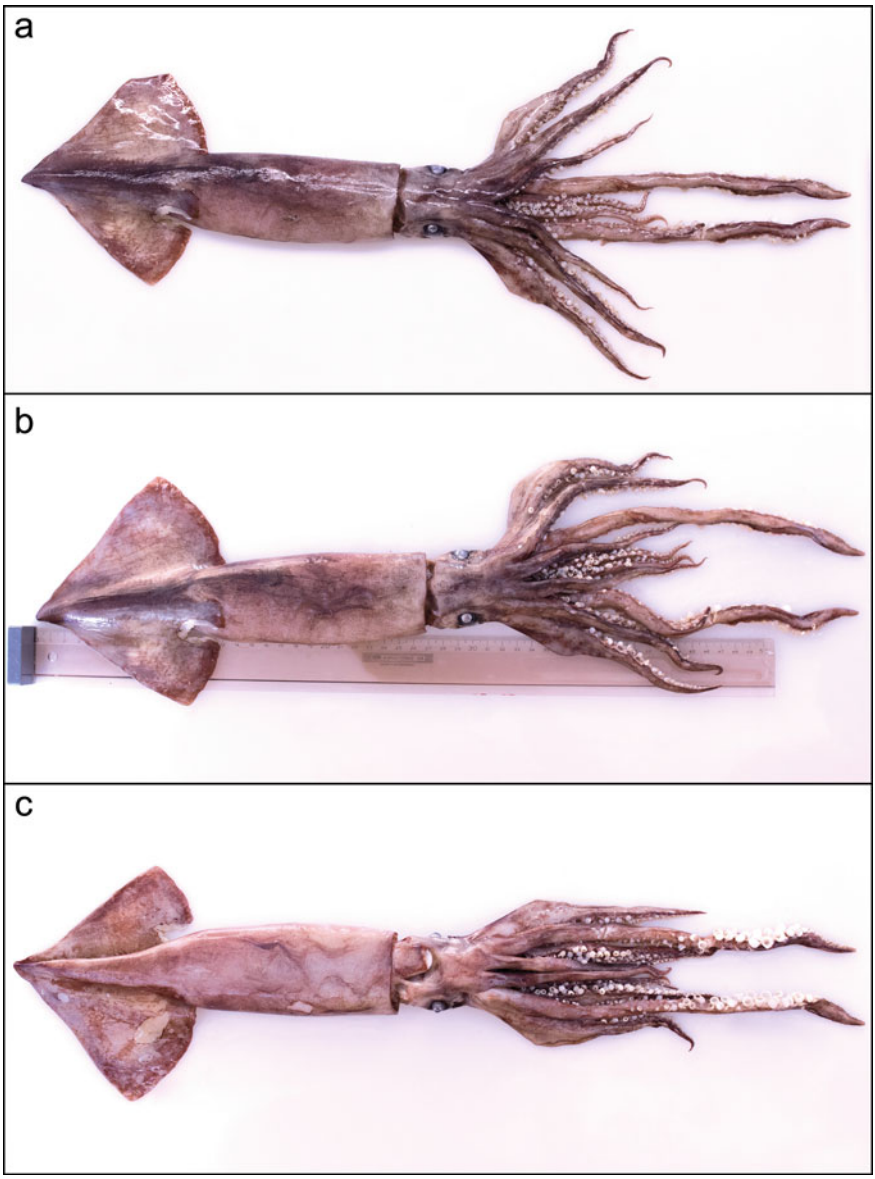

Fig. 27 Cephalopod dissection. (**a**) Dorsal view of the habitus of *Loligo vulgaris*. (**b**) Measuring the body length. (**c**) Ventral view of the habitus of *L. vulgaris*

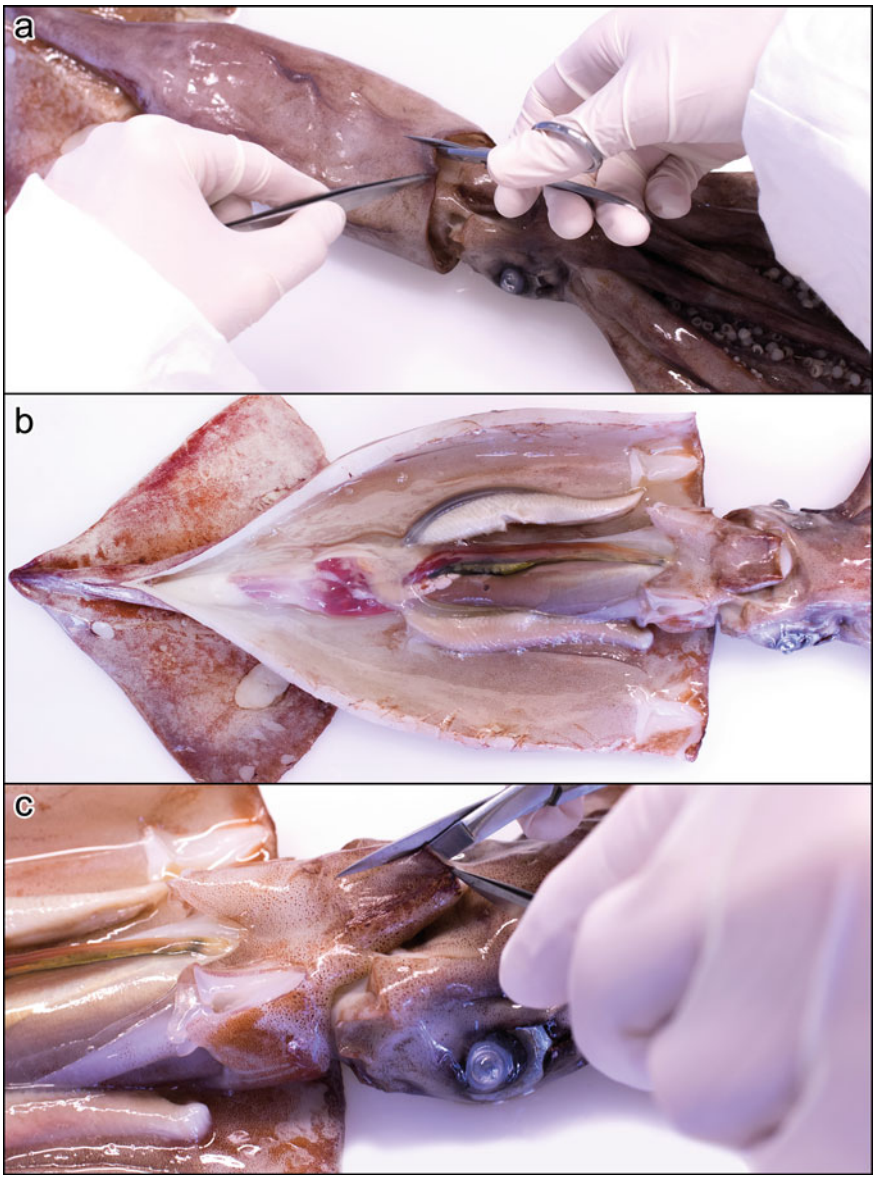

Fig. 28 (**a**) Central cut on the ventral side to open the mantle. (**b**) Overview of the mantle cavity with gills and the organ sac. (**c**) Central cut to open the funnel

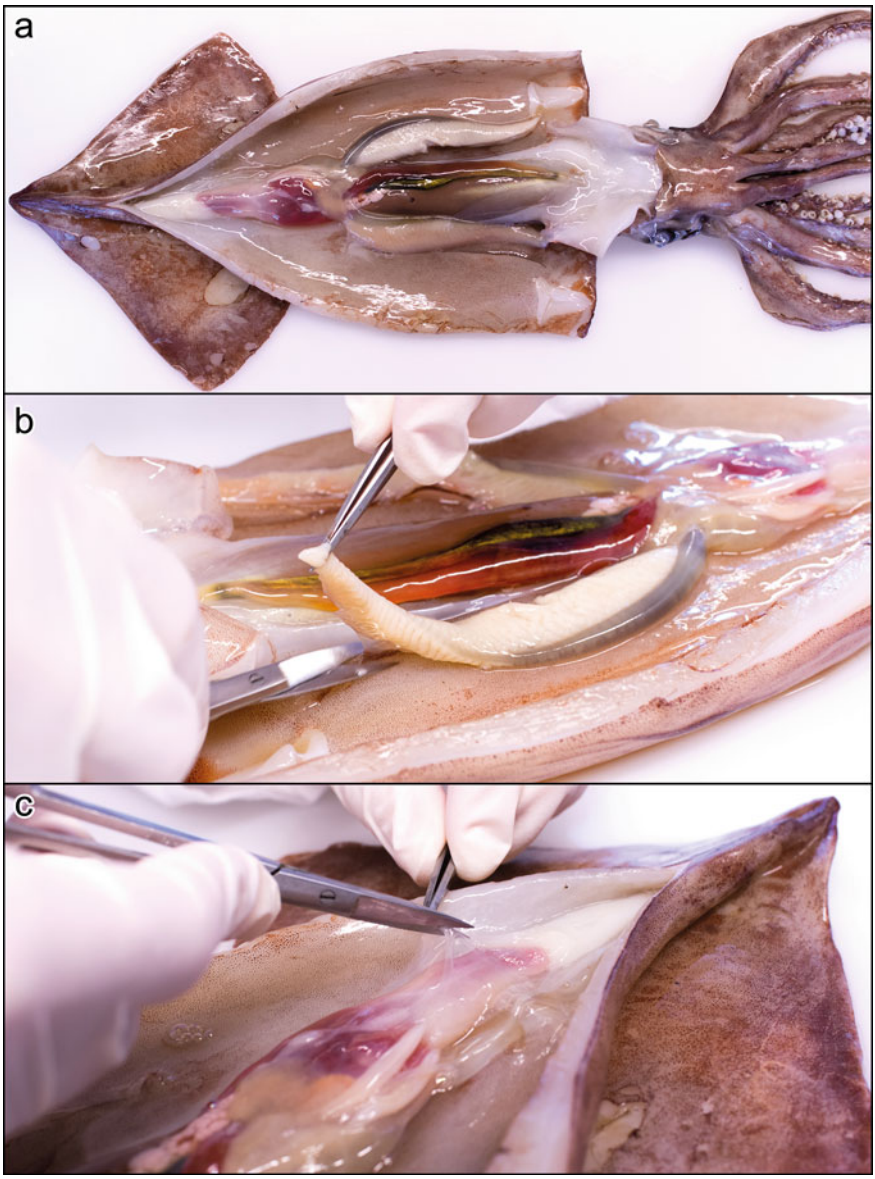

Fig. 29 (**a**) Overview of the mantle cavity with gills and the organ sac with opened funnel. (**b**) Cutting of the ligament holding the gills and removal of gills. (**c**) Opening of the organ sac

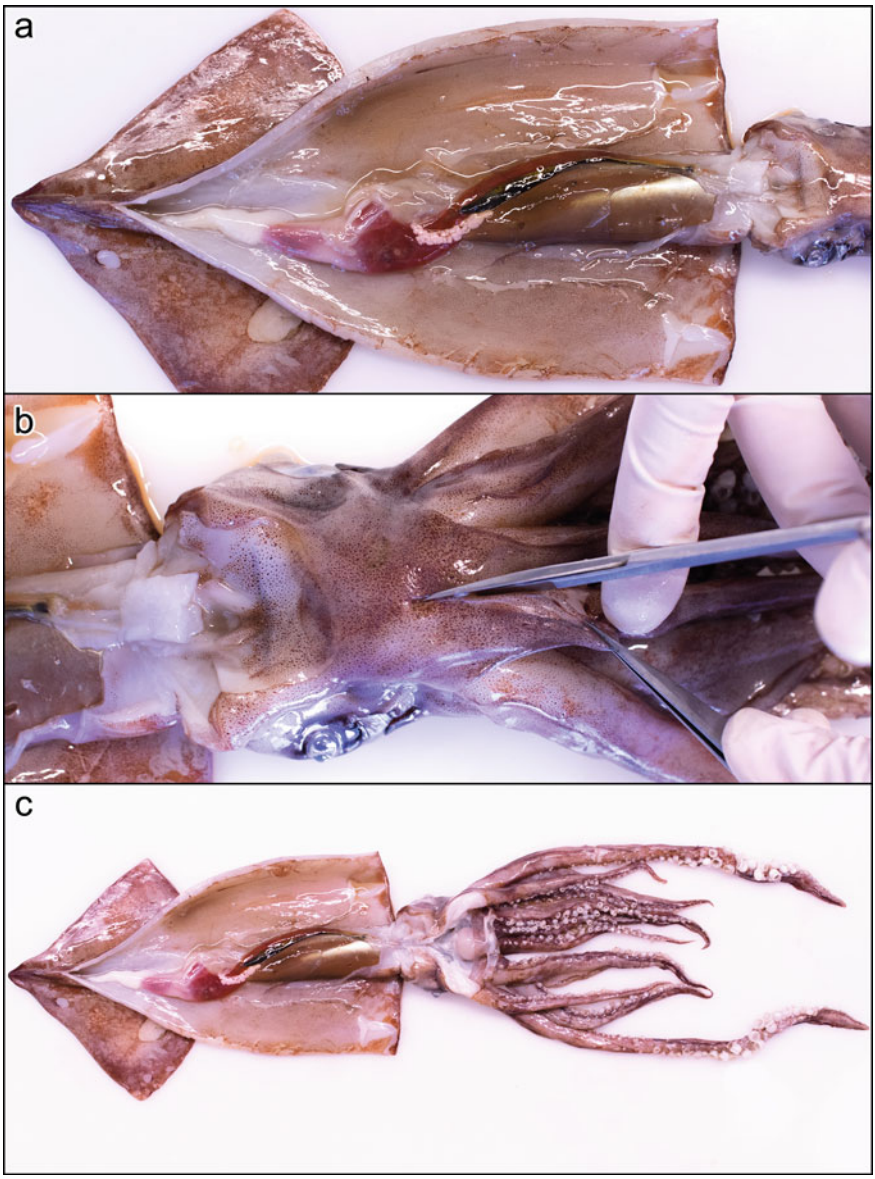

Fig. 30 (**a**) Overview of the mantle cavity with removed gills. (**b**) Opening of the head. (**c**) Overview of the habitus with exposed mouth capsule

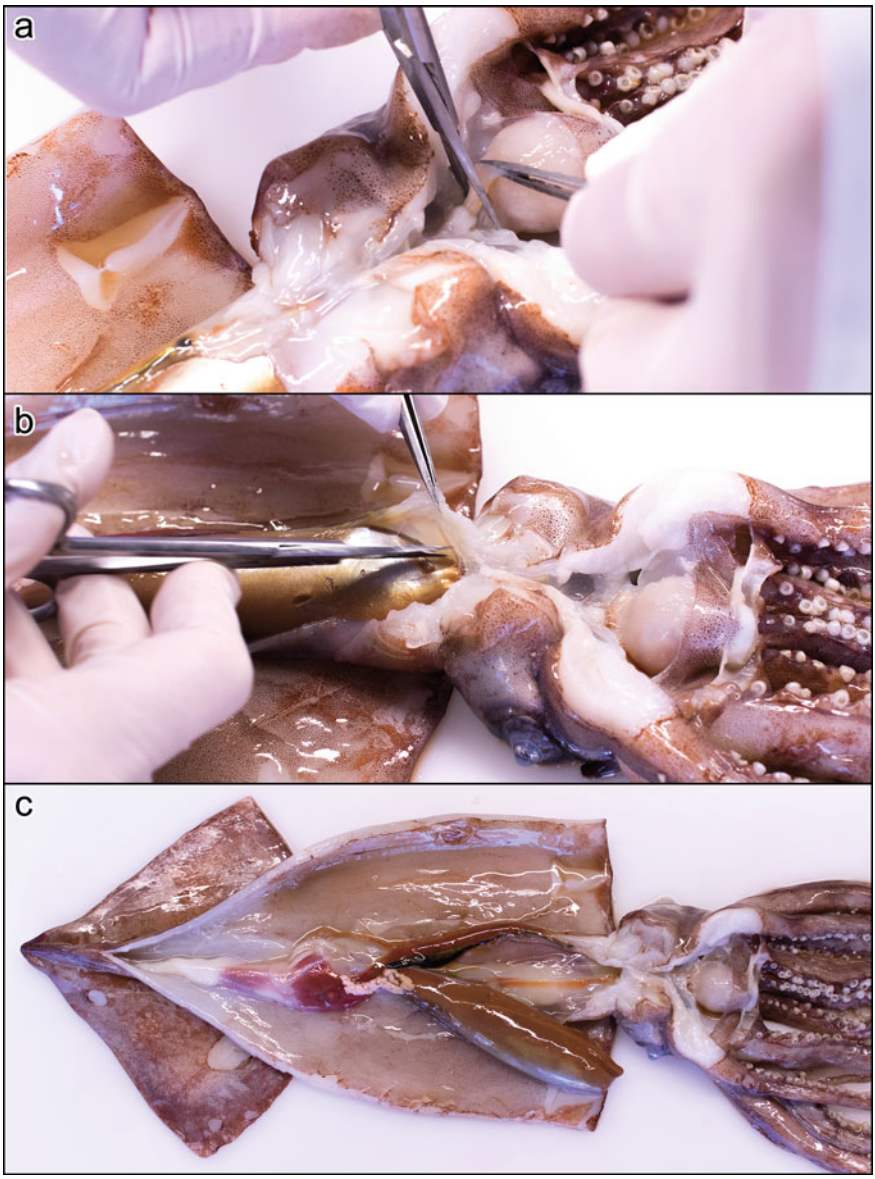

Fig. 31 (**a**) Cut through the oesophagus at the mouth capsule. (**b**) Excision of the liver. (**c**) Overview of the mantle cavity with excised liver

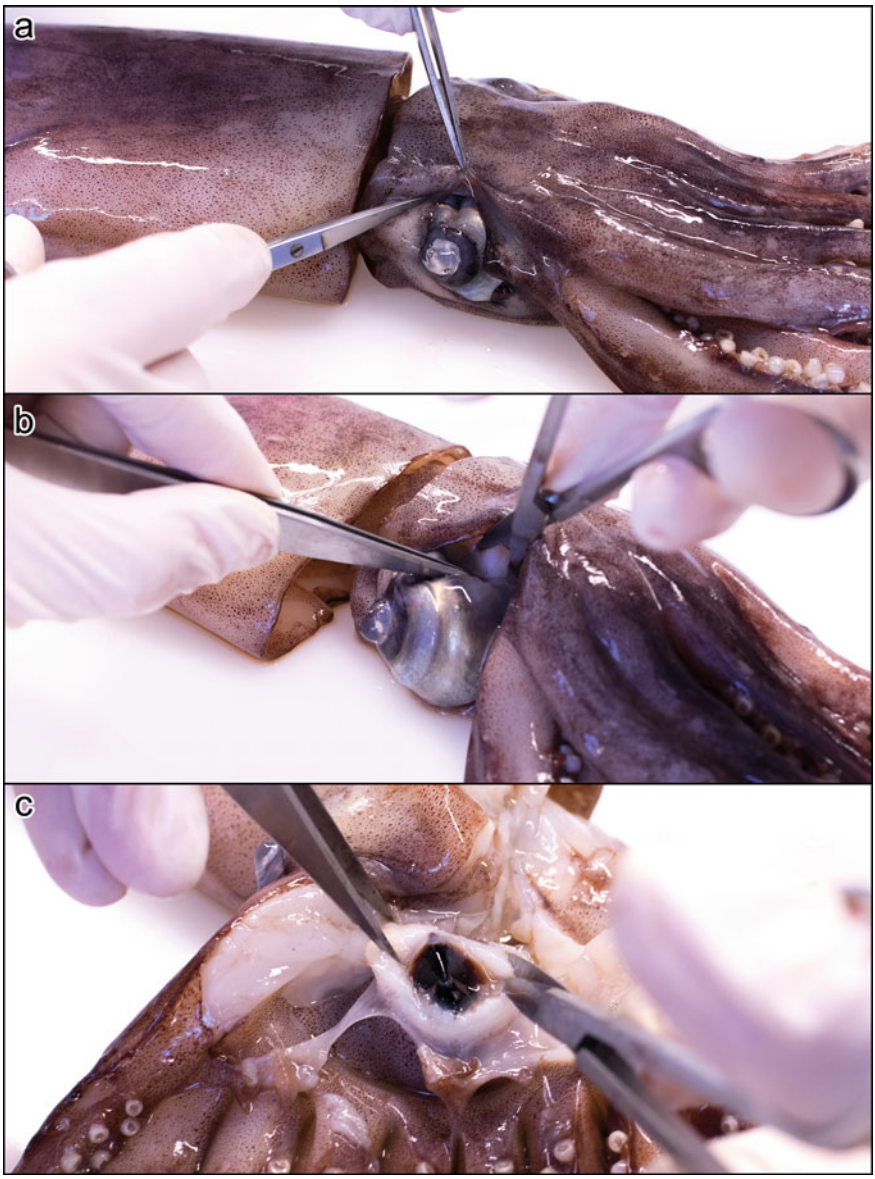

Fig. 32 (**a**) Opening of the eye cavity. (**b**) Cut through the eye stem and removal of the eye. (**c**) Opening of mouth capsule and removal of the two-part beak

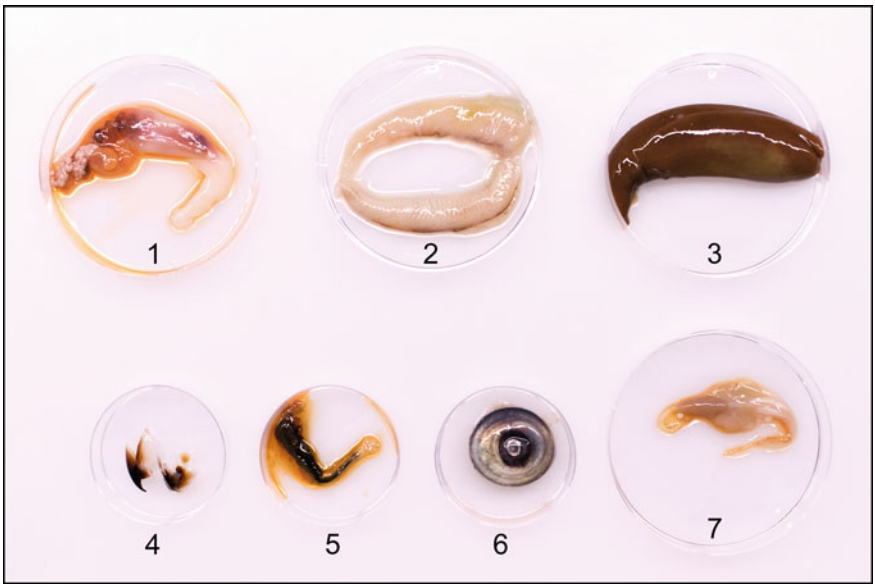

Fig. 33 Overview of the extracted cephalopod organs: (1) stomach and salivary glands, (2) gills, (3) liver, (4) beak, (5) intestine, (6) eye, (7) gonads

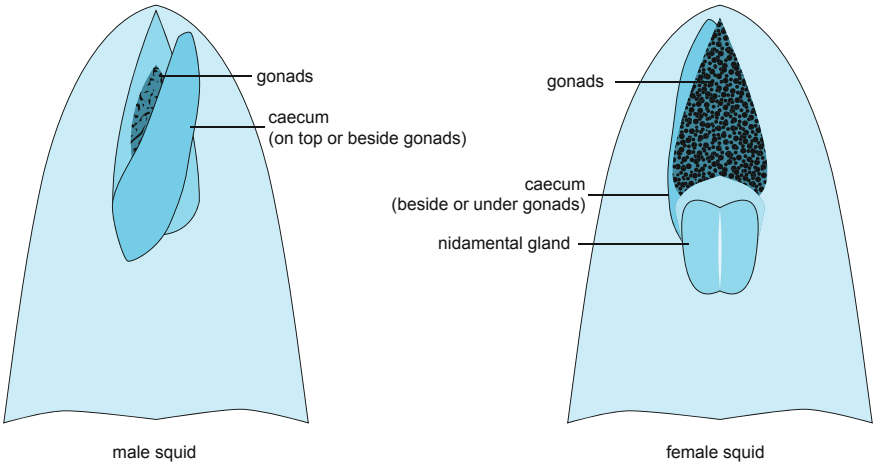

Fig. 34 Diagrammatic representation of isolated cephalopod sexual organs for sex and age determination

each cavity. Cut the gills at their base; transfer them into a petri dish filled with saline solution and check for ectoparasites. Open the funnel by a median cut and check it for parasites.

Extraction and Examination of Internal Organs

Carefully open the thin visceral sac with rounded-tip scissors. Take care not to damage the ink sac. Then loosen the organ-convolute and cut the oesophagus as close as possible to the cranium. Place the organs in a petri dish and separate them into single and labelled dishes. Proceed by examining the organs.

Parasite Preparation and Preservation

A large number of methods and techniques are commonly used to preserve and prepare parasitological material for subsequent analyses. Specific procedures may vary depending on the starting material (e.g. protozoan/ metazoan parasites, Cestoda, Nematoda, blood smears) and subsequent evaluation method (imaging, molecular genetics), but they can generally be divided into two major workflows: the preparation for structural/ morphological analyses and the preparation for genomic, proteomic and other molecular methods. The latter requires samples to be completely free from any chemicals or compounds that would inhibit enzymes or interfere with other compounds from the molecular tool kit. In most samples, the starting point would be a single or a bulk sample of extracted parasites, either in a block glass dish filled with saline solution (e.g. Digenea, Monogenea, Cestoda, Nematoda, Acanthocephala) or as part of a liquid sample (e.g. cephalic liquor, blood, gall, eye fluid, etc.) (Fig. 35).

Fixation

Fixation conserves the parasitic tissue and stops any physiological process, prevents denaturation and decay and ideally results in a preparation that provides a snapshot of the current physiological/morphological state of an organism. Tegument, intestines, gonads, hooks, foregut and other important distinctive characteristics will be preserved and prepared for subsequent (time-independent) microscopic analyses.

Small parasites are particularly suited for so-called immersive fixation during which, preferably recently, inactivated samples are transferred into a chemical fixative. In principle, all chemicals that conserve cell and tissue structures in a natural way are suitable. Ethanol, ether and acetone are fast-reacting; they cause precipitation of proteins and have a dehydrating effect. Protein coagulants such as formalin and glutaraldehyde are very commonly used and lead to a coagulation but not precipitation of proteins.

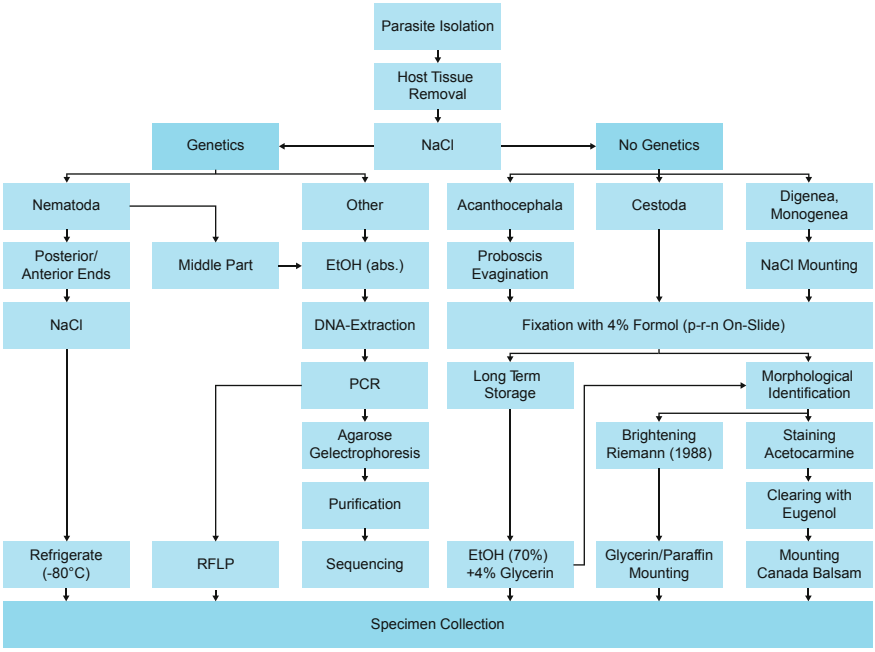

Fig. 35 Diagrammatic representation of the workflow for genetic and morphological determination of parasites

Host Tissue/Cyst Removal (Fig. 36)

The removal of any excess material (e.g. cysts, host tissue, food/gut residues) from the extracted parasites is an essential and crucial first step that has to be conducted very diligently. Any contamination could negatively affect the quality of the microscope slide and complicate morphological identification. Even the smallest amount of (host-) DNA can adversely impair genetic analyses that include broad-range genetic or barcoding markers, such as, e.g. cytochrome c oxidase I (cox1) or internal transcribed spacer (ITS).

In order to recover parasites from any tissue residues or cysts, the digestion method or the manual dissection method are commonly applied. Nematode larvae (e.g. *Anisakis*, *Pseudoterranova*, *Hysterothylacium*) and other large and easy-to-handle parasites can be well prepared using a pair of fine light metal forceps, two preparation needles and a binocular microscope (7–40× magnification). Use the first needle to fixate the cyst on the surface of a petri dish and use the second to carefully tear the cyst wall and/or host cell capsule. Transfer the parasites into a new glass dish filled with saline solution using fine forceps.

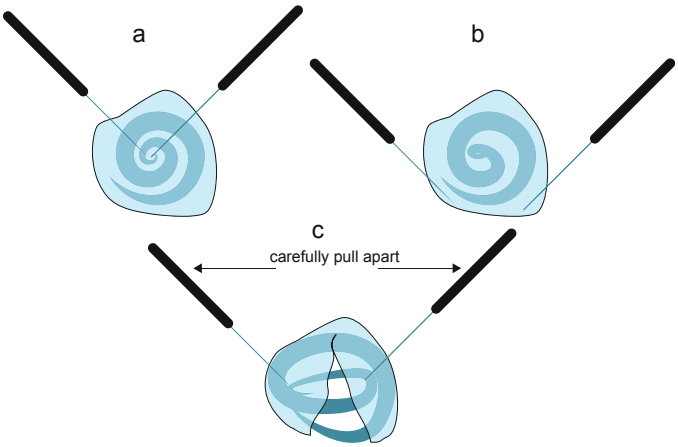

Fig. 36 Diagrammatic representation of nematode extraction from a cyst. (**a**) is recommended if the centre of the nematode is reachable with needles, (**b**) when it is not

Bulk Fixation

The most common and easiest method used for fixation is to simply transfer the isolated parasites in a block glass dish filled with the fixative. Individuals that might be still alive after isolation should be immediately killed in a boiling NaCl solution (0.9%) beforehand. They can then be fixed directly in the dish by adding a few drops of 4% (phosphate buffered) formalin. The fixation time depends on the size of the specimen and the concentration of the fixative. After fixation the specimens can be transferred into a 70% EtOH solution. Adding a few drops of glycerine to the EtOH prevents the parasites from drying out in case of accidental evaporation of the alcohol.

Fixation on the Slide (Fig. 37)

For certain parasitic groups (e.g. Digenea, Monogenea), it may be advisable to align and fix the specimens directly on the slide in a position that exposes all the relevant morphological characteristics essential for identification. For this, position the parasite in a drop of NaCl solution on a microscope slide. Place a small bead of plasticine (modelling clay, e.g. “Fimo”) or vaseline on each corner of a glass coverslip and cover the parasite on the microscope slide with the coverslip. Put a droplet of the fixative directly at one side and a piece of tissue paper at the opposite side of the coverslip until the NaCl solution is completely substituted by the fixative. Approximately 5 min after, depending on the size of the individual, the coverslip can be removed, and the parasite can be stored in 70% EtOH.

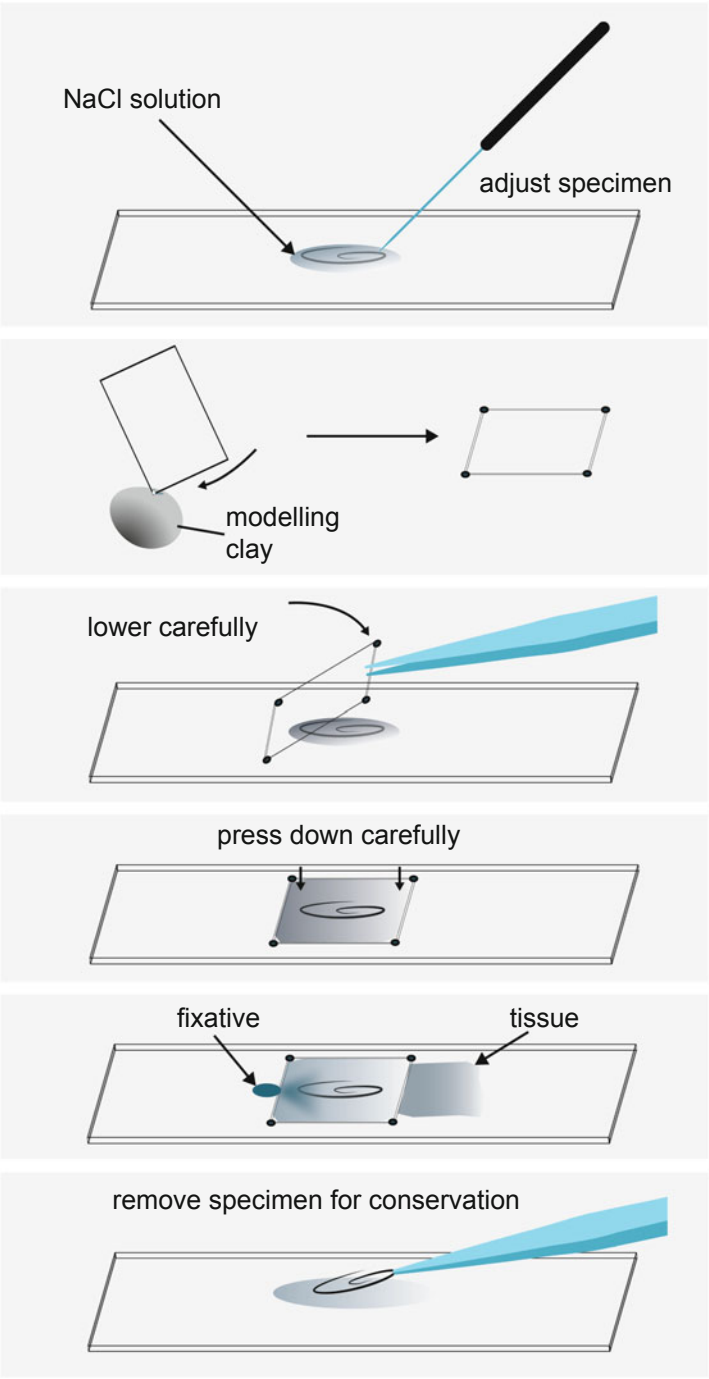

Fig. 37 Diagrammatic representation of the parasite staining/fixation method

Ciliate Fixation

The easiest method for fixing ciliates such as *Trichodina* from skin smears is by air-drying. Smear-slides that have been produced from gills, opercula and body surface should be stored in a dry and dust-free place until completely dried.

Fixation for Genetic Analyses

If the isolated specimens are to be analysed using modern molecular techniques (e.g. barcoding of mtDNA, rDNA), fixation in absolute ethanol is strongly recommended. Any other fixative, such as formalin, might interfere with subsequent molecular reactions and hinder a successful amplification. Unfortunately, even if properly washed in EtOH or TE-buffer, molecular analyses are very rarely successful if a specimen has been fixed in formalin previously. On the other hand, if the parasite has been fixed in ethanol, proper morphological analyses are complicated due to the tissue-shrinking effects of the alcohol. It is therefore very important to think about the fate of the sample before fixing it. In some cases, e.g. for nematodes whose middle body part does not contain essential morphological diagnostic features, it might be worth cutting off and conserving a piece of (morphologically irrelevant) tissue for genetic analyses later while preparing the rest (nematodes: anterior/posterior extremities) for morphological/ histological analysis.

Fixatives

Ethanol

Absolute, undenatured ethanol is the preferred fixative and storage agent for genetic samples as it usually does not interfere with the enzymes in molecular reactions. The duration of the fixation can be regulated by using different concentrations of ethanol. Ethanol (70%) is sufficient for long-term storage and available as a ready-to-use solution at most chemical suppliers. However, keeping a large container of absolute EtOH (denatured/undenatured) in the laboratory is of course the most flexible way to allow the production of dilutions that fit specific requirements.

Formalin

Formalin is another standard fixative and usually available as saturated solutions of approx. 37% formalin gas in water. Dilution ratios of 1:10 produce a solution of 3.7%. It fixes tissue in an instant, and when used in a phosphate-buffered form (e.g. Histofix®), it is perfectly suitable for most applications.

Phosphate-buffered formalin (1000 ml):
Formalin stock solution (37%) 100 ml
PBS 900 ml

Glutaraldehyde

For the fixation of samples intended for electron microscopy, 2–6% dilutions of buffered glutaraldehyde solutions (pH 7.2) with a subsequent additional fixation in buffered osmium acid are commonly used. Glutaraldehyde is usually available in the form of a 25% solution and has to be diluted with either phosphate buffer or cacodylate buffer.

0.1 M phosphate buffer, pH 7.4 (1000 ml):
$NaH_2PO_4 * H_2O$ 3.1 g
Na_2HPO_4 (anhydrous) 10.9 g

Fill up with distilled water to make a total volume of 1000 ml. Dilute glutaraldehyde stock solution with the phosphate buffer in a 1:10 ratio for a 2.5% solution.

0.2 M sodium cacodylate buffer, pH 7.4 (100 ml):
$Na(CH_3)_2\ AsO_2 * 3H_2O$ 4.28 g
Distilled water 100 ml

Add 5.6 ml of 0.2 N hydrochloric acid to 100 ml sodium cacodylate buffer for a solution with pH 7.4. Prepare in a fume cupboard (arsenic gas!). Store at 4 °C and use within 2 weeks.

2.5% glutaraldehyde in 0.1 M buffer
Glutaraldehyde (25%) 10 ml
0.2 M sodium cacodylate buffer 50 ml
Distilled water 40 ml

Store at 4 °C and use within 2 weeks.

Berland's Fluid

This solution is used to uncoil and stretch out nematodes. It also makes them more transparent and can kill them. It can generally be used as a long-term storage medium, although storage in 70% EtOH is preferred.

Glacial acetic acid 1 Part
Formalin (37%) 19 Parts

Bouin's Fluid

The Bouin fixative contains aqueous picric acid, formalin and glacial acetic acid and achieves better nuclear staining than buffered formalin. With some restrictions, samples treated with Bouin's fluid can still be used for DNA extraction and subsequent PCR.

Glacial acetic acid	5 ml
Formalin (37%)	25 ml
Picric acid (saturated solution)	75 ml

AFA (Alcohol-Formalin-Acetic-Acid)

AFA can be used for both killing and fixing of parasites and is the preferred fixative for tapeworms.

Ethanol (95%)	50 ml
Distilled water	40 ml
Formalin (37%)	6 ml
Glacial acetic acid	4 ml

FAA (Formalin-Acetic-Acid-Ethanol)

FAA is a good "general-purpose" fixative for tissues.

Ethanol (95%)	100 ml
Distilled water	70 ml
Formalin (37%)	20 ml
Glacial acetic acid	10 ml

Staining

Parasites that are in a good state of condition and properly cleared and/or stained should be permanently mounted using one of the following methods.

Mayer-Schuberg's Acetocarmine Staining (After Reichenow et al. 1969)

This method is specifically suited for staining the morphologically relevant characteristics of Digenea, Monogenea and Cestoda. Fixed specimens are first washed in 70% EtOH and then transferred into the alcoholic Mayer-Schuberg staining solution

for a few minutes. Small specimens can also be stained directly on the slide with diluted MS solution, depending on the thickness of the specimen. The aim is to yield specimens that are light red in colour. If the product is either dark red or purple, it can be unstained using acid ethanol. After staining and dehydration (ethanol series 70%, 80%, 90%, 99%), the parasites have to be cleared using, e.g. eugenol and methyl salicylate until a clear appearance is obtained. If eugenol is used for clearing, an additional step 1:1 eugenol:ethanol should be integrated before the specimens are transferred into 100% eugenol. Stained individuals can be embedded using the methods described below.

Mayer-Schuberg's Acetocarmine Staining

- Transfer fixed specimens into a dish with 70% EtOH and incubate for 15–30 min.
- Transfer specimens into a dish with Mayer-Schuberg's staining solution, and leave for 30 s up to 5 min until light red.
- Transfer into a dish with 70% EtOH and wash away any excess staining solution.
- If the colour is dark red or even purple, transfer into a dish with acid-EtOH (see below) until the specimen is light red in colour.
- Prepare dishes with an increasing alcohol concentration.
- Dehydrate the parasites by incubation in concentrations of 70%, 80%, 90% and 99% EtOH for 15–30 min each, depending on the size of the individuals.
- Transfer into a second dish of 100% EtOH (overnight).
- During incubation, prepare a mixture of EtOH and eugenol (1:1).
- Clear specimens in eugenol. This can take up to 1 h.
- Mount as described below.

Silver-Nitrate Staining (After Klein 1926, 1958)

Staining of marine ciliates can be performed using a slightly modified version of the silver impregnation method as described by Klein (1926, 1958). Dried slides are washed in distilled water in order to resolve any remaining chloride. After air-drying them again, the slides are coated with 5% silver-nitrate solution ($AgNO_3$) and incubated for 30 min in the complete dark. Wash the slides in distilled water, irradiate them for 40–50 min with UV light, and air-dry them once more. Check each slide for ciliates and seal the positive slides using resin embedding (e.g. Eukitt).

Silver-Nitrate Staining

- Air-dry the slides according to Lom (1958).
- Prepare one staining dish filled with distilled water and one dish filled with 5% $AgNO_3$ solution.
- Wash air-dried slides with distilled water using a Pasteur pipette to remove any excess chloride.
- Allow slides to air-dry again.
- Transfer the washed slides into the dish with silver nitrate, and incubate for 30 min in the dark (e.g. incubator, closet with a sealed door).
- Wash again in distilled water.
- Expose to UV light for 30–40 min.
- Air-dry again and embed if needed.

Staining Solutions

Mayer-Schuberg's Acetocarmine Solution

Ethanol (95%)	85 ml
Hydrochloric acid (conc.)	1.5 ml
Distilled water	15 ml
Carmine	4 g

Add the carmine and the acid to the distilled water and heat for approx. 30 min with back-flow cooling. Add the EtOH and filter.

Silver-Nitrate Aqueous Solution (5%)

This staining solution is very easy to prepare but also available as a ready-to-use solution at several suppliers specialising in histology or chemistry.

For 100 ml of 5% silver nitrate, mix until completely dissolved:

$AgNO_3$ (s)	5 g
Distilled water	100 ml

Acid Ethanol

Easy solution for unstaining dark red/purple carmine-stained specimens.

Ethanol (70%)	100 ml
Hydrochloric acid (conc.)	2.0 ml

Add HCl to ethanol and mix gently.

Clearing

Studying morphological features is a necessary process to describe and identify a species. For visibility of important structures, the use of a clearing agent is required. Clearing agents have a refractive index very similar to the tissue/proteins of the parasite which makes the tissue appear transparent under the microscope. Hydrophilic or lipophilic agents can be used, depending on the qualities of the mounting medium, i.e. lipophilic in the case of, e.g. resin-based embedding media (DePeX).

Glycerine Clearing After Riemann (1988)

This method is commonly used in combination with the paraffin-embedding procedure described below and easy to apply without the need of expensive or harmful chemicals. Glycerine has a refractive index of 1.45 which is not ideal, but a good compromise in relation to the required effort. Based on the original work of Berland (1984), Riemann (1988) described this technique. The individuals are first dehydrated in EtOH and then transferred into a mixture of EtOH, distilled water and glycerine. Samples are incubated at a temperature of 60 °C until the EtOH and the water have completely evaporated. A fast and easy method omits the step of increasing alcohol concentrations, and parasites are directly transferred from the fixative into the clearing mixture.

Glycerine Clearing

- Prepare dishes for increasing alcohol concentrations.
- Dehydrate the parasites by incubation in concentrations of 70%, 80%, 90% and 99% EtOH for 5–10 min each, depending on the size of the individuals.
- During incubation, prepare a mixture of EtOH-glycerine (70% absolute EtOH, 5% glycerine, 25% distilled water).
- Transfer the dehydrated parasites in the clearing mixture, and incubate at 60 °C overnight or until the EtOH and water are evaporated and the individuals remain in pure glycerine.
- Omit the dehydration steps for quick and easy mounts.

Mounting/Embedding

Mounting/embedding is the very last step of the preparation and enables the isolated parasitic individuals to be studied under a light microscope.

Glycerine/Paraffin Embedding After Riemann (1988)

This technique is a "quick and easy" technique and probably the most common method to prepare parasites for long-term storage without too much effort. It can be applied directly to freshly fixed individuals or after an initial clearing step. Individual specimens are carefully placed in a small amount of glycerine on a clean and greaseless microscope slide. Try to arrange the parasite in such a way that all morphologically important characters are visible (using binocular microscopy). Ideally, the parasites have been already fixed in a correct position using the method described before. The drop of glycerine should be small enough to leave a few millimetres of air between the glycerine and the edges of the coverslip that will be used on top subsequently. Use a needle to remove any air bubbles that might be present. Apply a small bead of plasticine to each corner of the coverslip by scraping them carefully off a well-kneaded piece of synthetic modelling clay (e.g. Fimo™). Using a very fine pair of forceps, place one edge of the coverslip on the slide next to the parasites and lower the opposite edge. With the rounded end of the forceps, gently press each corner with plasticine down on the slide. Do not apply too much pressure on the centre of the coverslip as it might break easily. If the space between coverslip and slide is completely filled, you have used too much glycerine and need to start over again. If there is some space/air remaining around the parasite and the edges, you may proceed by placing one or two small paraffin pellets just next to the left and right edges of the coverslip. Place the slide on a hotplate preheated to 60 °C. Once the paraffin starts melting, it will run between the coverslip and slide and fill out the remaining space. Take care to apply enough paraffin and to not leave air underneath the coverslip (otherwise it might break easily and ruin your mounting). When the paraffin has completely melted, carefully lift the slide from the table and place it on a cold surface without moving it too much. The paraffin will then immediately solidify. Remove any excess paraffin from the coverslip edges and seal them with ready-to-use coverslip sealing lacquer or acrylic nail varnish.

Glycerine/Paraffin Mounting (Fig. 38)

- Preheat a hotplate to 60 °C.
- Clean the microscope slide with EtOH to remove excess fat.
- Put a small drop of glycerine in the middle of the slide.
- Place the fixed (and cleared) sample in the glycerine and position the specimen well.
- Remove any air bubbles using a fine needle.
- Scrape small beads of plasticine off a piece of well-kneaded synthetic modelling clay, and use them on the corners of a coverslip.
- Carefully lower the coverslip on the sample by applying one edge of the slide first.
- Gently press down the corners to keep the coverslip in position.

(continued)

- Leave enough air for the paraffin to fill the gaps around the sample.
- Place a few paraffin pellets next to the coverslip.
- Heat the slide on the hotplate until the paraffin melts and has completely enclosed the glycerine.
- Place the slide on a cold surface and allow the paraffin to solidify.
- Remove any excess paraffin by scraping it off with a razor blade or sharp knife.
- Seal the edges of the slide using coverslip sealing lacquer or acrylic nail varnish.

Resin Embedding

Samples that have been embedded using natural (Canada balsam) or synthetic (e.g. Eukitt, DePeX) resin-based embedding media are nearly everlasting. There are several ready-to-use media available, each with specific pros and cons. Canada Balsam (*Balsamum canadese*) is the most natural embedding resin still used frequently despite its proven disadvantages, e.g. the very long curing time or its tendency to show striation in samples "contaminated" with water. With time, this embedding medium became widely substituted by artificial alternatives with improved qualities and characteristics. Two commonly used artificial resin-based media are Entellan and Eukitt. They have a refractive index of 1.49 and a comparatively short drying time of less than 1 h. Users sometimes criticise the opacity or the insufficiently bright preparations previously stained with borax carmine and a rather mediocre fluidity. However, both media are quite tolerant to traces of water and alcohol. Another alternative is DePeX, which has a slightly longer drying time but appears to have a higher refractive index and does not tend to cause too much shrinkage. The refractive index of Euparal, a medium soluble in alcohol, is 1.53–1.54 and, therefore, almost ideal. Drying time ranges from 6 to 12 h. The medium is especially recommended for mounts that have been stained with borax carmine solution. Malinol is similarly versatile as DePeX but is a resin-based medium. The refractive index is 1.52 with an ideal fluidity and minor shrinkage. It has a rather long drying time (3–6 days), but the slides can be carefully handled after 6–12 h of initial drying.

Resin Embedding (Fig. 39)

- Clean the microscope slide with EtOH to remove excess fat.
- Place a small drop of embedding medium in the middle of the slide.
- Place the fixated (and cleared) stained or unstained sample in the medium and arrange correctly.
- Carefully lower the slide on the sample by placing one edge of the slide first.
- Place the slide on a straight surface (in a fume hood), and keep it in a horizontal position until the medium has completely dried.

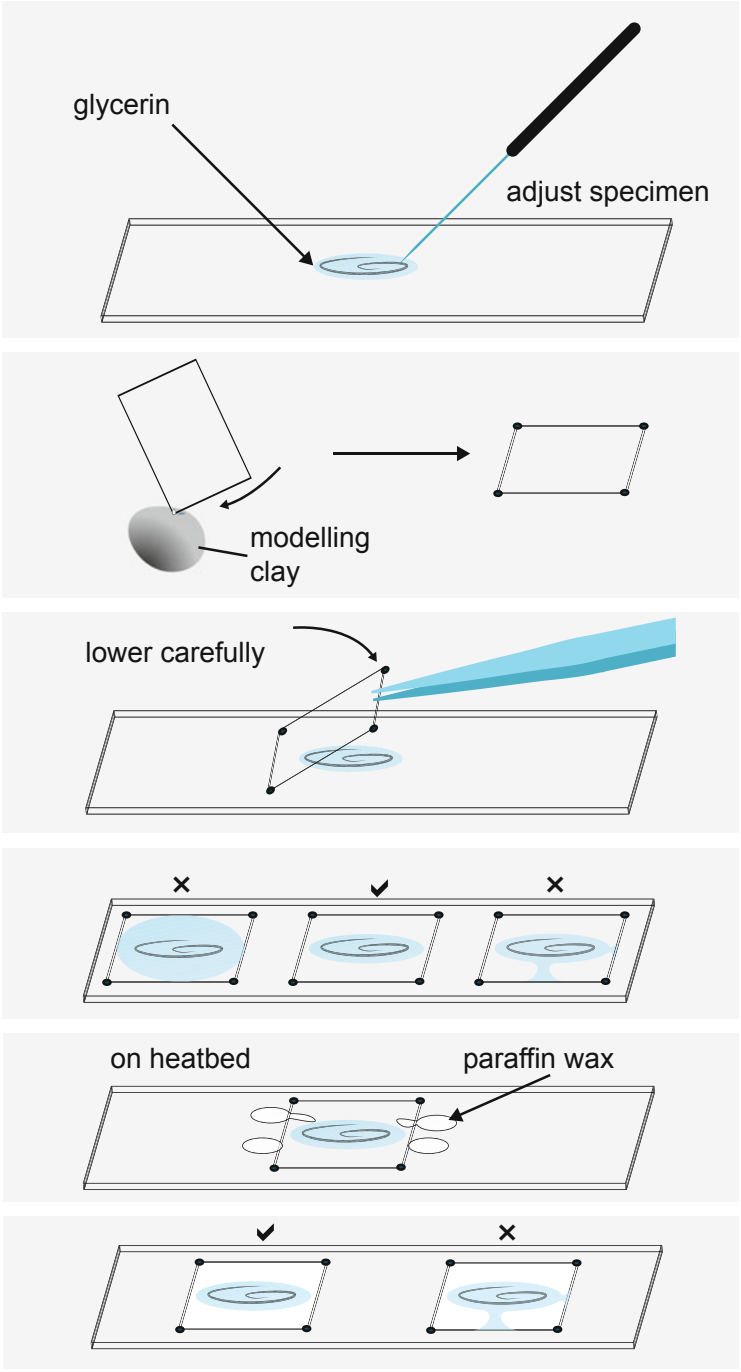

Fig. 38 Diagrammatic representation of the production of a permanent microscope slide specimen using paraffin wax

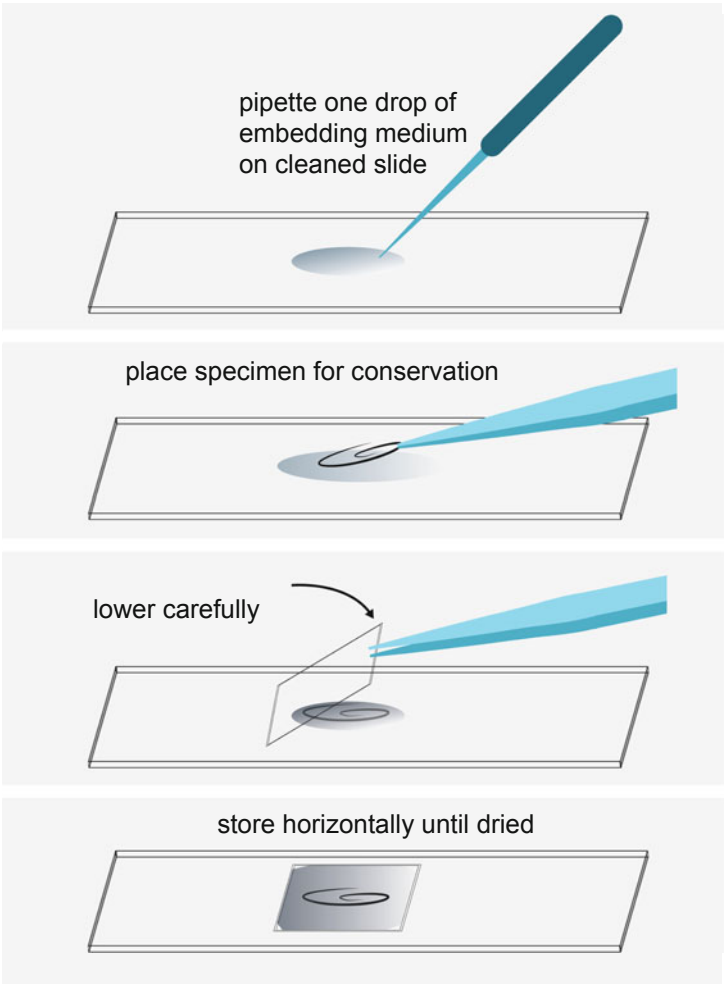

Fig. 39 Diagrammatic representation of the production of a permanent microscope slide specimen using resin

Genetic Analyses

Modern methods of molecular biology are currently being developed at a rapid pace; specific methods will therefore not be presented and explained in great detail here. A particularly widespread method is direct sequencing (Sanger sequencing, NGS) of previously amplified (and annotated) markers or whole genomes. Species are identified by direct comparison of a nucleotide sequence and homologous sequences stored in international and online-available gene banks (e.g. NCBI). This approach allows the total amount of nucleotides being used as individual characteristics of a species and is fundamental for the analysis of phylogenetic relationships. The choice

of the respective method should be based on the current state of the art and more particular on the availability of suitable references.

Recommended Application of Parasite Preservation

Ciliates (Skin Smears)

- Clean the microscope slide with EtOH to remove excess fat.
- Prepare skin smears of, e.g. the skin or gills.
- Air-dry the slides according to Lom (1958).
- Stain the slides with 5% silver nitrate.
- Prepare permanent mounts with, e.g. Eukitt, Malinol and Entellan.
- Store horizontally until completely dried.

Myxozoa/Microsporea

- Clean the microscope slide with EtOH to remove excess fat.
- Clean spores from host tissue to avoid bacterial growth.
- Mount and embed parasite spores on microscope slide, and seal with paraffin after Riemann (1988).
- Can be stored for short time in refrigerator.

Alternative

- Spores can be transferred to distilled water and preserved at 4 °C for approx. 1 year.

Capillary tube method

- Puncture cysts with a capillary tube (diameter, 1 mm; length, 10 cm) (see Lom & Dyková 1992).
- Fill half a capillary tube by capillary suction with spores.
- Openings of the capillary tube must be sealed and stored vertically.

Digenea/Monogenea

- Clean the microscope slide with ethanol to remove excess fat.
- Kill live individuals with hot NaCl solution.
- Arrange and align the specimens and fix them on the slide.

(continued)

– Transfer to 70% EtOH if needed.

Fast and easy (without staining)

– Clear the specimens after Riemann (1988).
– Mount and embed parasites in glycerine and paraffin after Riemann (1988).

Staining and permanent mount

– Stain with Mayer-Schuberg's acid carmine solution.
– Mount and embed with resin-based medium.
– Store horizontally until completely dried.

Cestoda

– Clean the microscope slide with ethanol to remove excess fat.
– Fix cestodes using hot or almost boiling 4% formalin (under a fume hood!).
– Leave specimens in the fixative solution for 1 week.
– Transfer to 70% EtOH or stain directly.
– Stain with Mayer-Schuberg's acid carmine solution.
– Prepare permanent mounts with, e.g. Eukitt, Malinol and Entellan.
– Store horizontally until completely dried.

Nematoda

– Prepare nematodes for either morphological or genetic analyses.

Genetic analyses

– Clean the microscope slide with EtOH to remove excess fat.
– If possible, cut the nematode into three pieces (anterior section, middle section, posterior section).
– Fix the middle section in absolute EtOH for genetic analyses and store in a reaction tube.
– Fix the anterior and posterior section using formalin or FAA, store in 70% ethanol if needed, or directly prepare for morphological analyses.

Morphological analyses (whole nematode)

– Clean the microscope slide with EtOH to remove excess fat.
– Fix the whole nematode using formalin or FAA.
– Transfer and store the specimens in 70% EtOH if needed.
– Use clearing/mounting/staining as described for Digenea/Monogenea.

Acanthocephala

- Clean the microscope slide with EtOH to remove excess fat.
- After isolation, transfer specimens to distilled water until the proboscis has emerged (approx. after 1 h).
- Proceed as described for Digenea/Monogenea.

Crustacea

- Clean the microscope slide with EtOH to remove excess fat.
- Wash isolated specimens in saline solution.
- Fix in 4% formalin.
- Store in 70% EtOH or (for small individuals) embed in paraffin.

Stomach Content Analyses (Fig. 40)

Analysing the contents of fish stomachs is standard practice in parasitological studies of fish and provides important information on the hosts' feeding ecology. It allows for indirect conclusions on the life cycle biology and possible transmission pathways of their parasites. Variation in diet and/ or dietary composition between different subsamples (e.g. year classes, habitats, seasonal variation of catch) might also have an effect on the specific infection parameters. Although rather time-consuming, stomach content analyses, generally representing snapshots of the recent diet and food composition of the host, can be used to infer predator-prey interactions when combined with parasitological analyses. This can be seen as an advantage especially in inaccessible environments difficult to observe in vivo.

Isolation/Identification/Preservation

After isolating the stomach, the stomach content should be separated into the different components. If applicable, each group should be identified to the lowest possible taxonomic level using keys and descriptions that correspond to the area and host group of the study. Depending on the level of digestion, an identification might not be feasible in some cases. If the stomach is empty, or only the remains of strongly digested food items are present, pat dry its remains and determine its weight as "stomach content indet". Every individual of each group has to be counted; however, some difficulties might occur with *Crustacea* (e.g. *Euphausiacea*). To get an approximate idea, the eyes (appearing as small black spherules in the dish) are

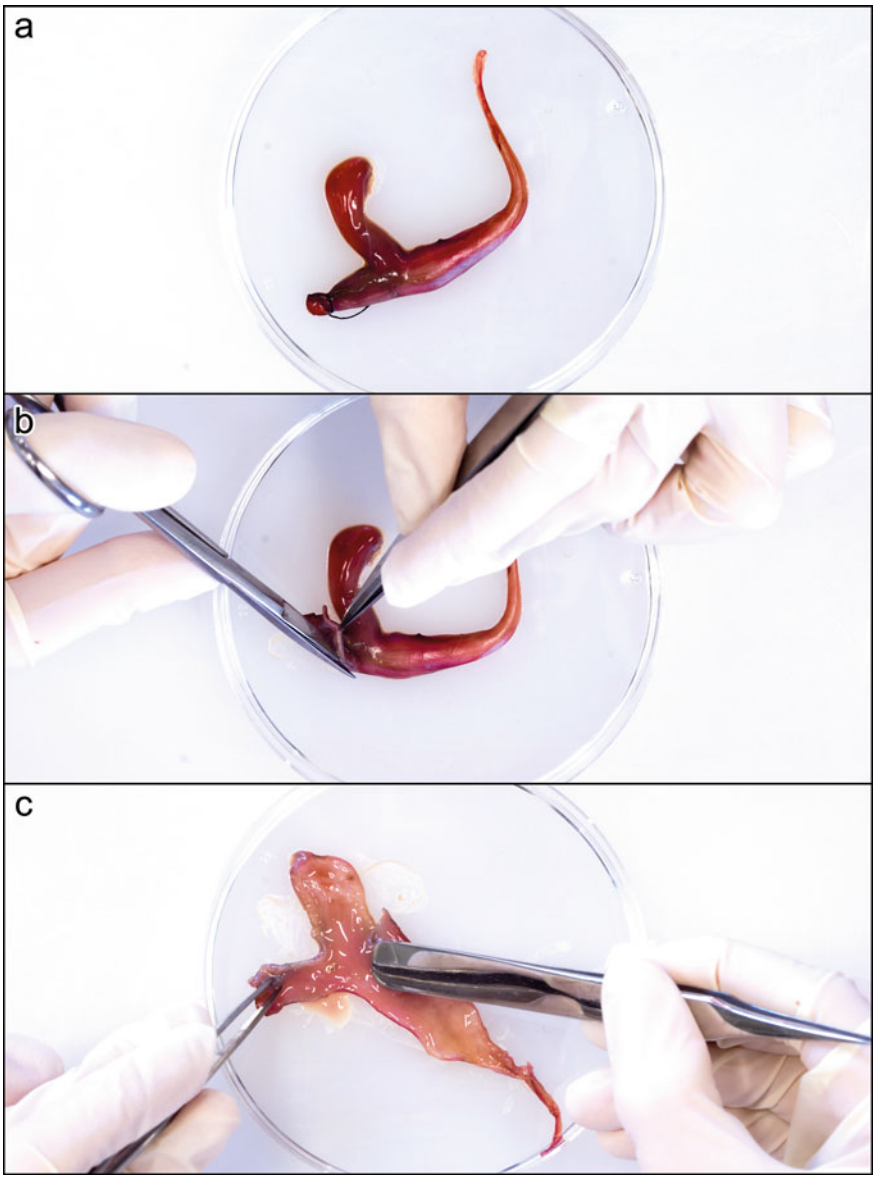

Fig. 40 Stomach content analyses. (**a**) Preparation of stomach for stomach content analysis. (**b**) Cutting open the stomach. (**c**) Scraping of stomach wall for microscopic examination

usually counted and divided by two to obtain the number of individuals. Keep in mind that this might bias your data. Pat dry each component (taxonomic group) carefully with an absorbent paper and measure its weight. If possible, measure the mucus weight as well. Each component can be preserved in glass vials or another

form of airtight container filled with diluted ethanol. If the aim is to perform any subsequent genetic analysis, the use of non-diluted, absolute EtOH is recommended. In any other cases, 70% EtOH should be sufficient. For caloric analyses of prey, the content should not be preserved in liquids, but rather deep-frozen untreated in a suitable lab container.

Quantitative Calculations

Frequency of Occurrence [*F%*]

A very basic way of documenting data from stomach contents is to record the number of stomachs containing one or more individuals of each food/diet category. Calculating the frequency of occurrence [*F%*] is quick but gives only little indication of the relative amount of each food category and provides a rather crude qualitative picture of the food spectrum.

Frequency of Occurrence [*F%*]
Describes the percentage of fish that consumed a specific food component. Defined as the number of fish stomachs that contained a specific prey item divided by the overall number of stomachs that contained food items. Expressed as a percentage.

$$F[\%] = S_i / S_{\sum}{}^{*}100$$

S_i Number of stomachs with a specific prey item i
$S_{\sum}$ Number of stomachs that contained food

Weight Percentage of Prey [*W%*]

To calculate the weight percentage of prey [*W%*], the weight of each food category is determined either in a wet (pat-dried) or dry (evaporating water, freeze drying) condition. The total weight of a category can then be expressed as a percentage of the overall weight of the stomach contents. Gravimetric measurements tend to overemphasize the contribution of single heavy food items such as ingested fish but are relatively easy to apply otherwise (volume or weight).

Weight Percentage of Prey [*W%*]
Compares the different food components by weight and is defined as the total weight of a specific prey item (or group) divided by the weight of all food components. Expressed as a percentage.

$$W[\%] = W_i / W_{\sum}{}^{*}100$$

W_i Total weight of a specific prey category i
$W_{\sum}$ Summed weight of all food categories

Numerical Percentage of Prey [*N%*]

The numerical method is relatively quick and easy and might be most applicable for samples with prey items of the same size range. This method takes into account the number of individuals in one specific food category in relation to all individuals in all food categories (expressed as a percentage). This descriptor gives an indication of the amount of effort exerted towards selecting and capturing certain organisms but overemphasises the importance of small prey items present in large numbers. In addition, it is often very difficult to estimate numbers due to an advanced state of digestion in most samples. If an advanced state of digestion occurs, calculating the percentage occurrence or volume composition instead is suggested. Another drawback of this method is that it is not applicable to food items that do not appear in discrete units (e.g. detritus).

Numerical Percentage of Prey [*N%*]
Compares the food components in a numeric way and is defined as the sum of the individuals of a specific prey group divided by the sum of all individuals of all prey groups. Expressed as a percentage.

$$N[\%] = N_i / N_{\sum}{}^{*}100$$

N_i Number of individuals of specific prey category i
$N_{\sum}$ Sum of all individuals from each food category

Index of Relative Importance [IRI]

For studies that only take a small number of samples but have a great variation in food categories, a separate application of the above-mentioned descriptors may produce different results and biased estimations of the importance of specific food categories. If the aim is to describe or compare diets, the consideration of both the weight and numerical importance produces a more realistic and representative

picture of the food ecology of a host. The index of relative importance [IRI] incorporates the number, volume and frequency of occurrence and is a very common descriptor in studies of food ecology.

Index of Relative Importance [IRI]
Provides a measure of the importance of each food component and is defined as the sum of the number and weight of prey multiplied by the frequency of occurrence. The food item with the highest IRI is considered most important

$$\mathrm{IRI} = F^{*}(N^{*}W)$$

N Number of individuals of a specific prey category
W Total weight of a specific prey category
F Frequency of occurrence of a specific prey category

Short Protocol "Stomach Content Analyses"

- Measure the weight of the filled stomach [Stomach $\mathrm{Weight}_{\mathrm{filled}}$].
- Cut the stomach open using a fine pair of scissors (tip pointing upwards).
- Spread the stomach evenly.
- Transfer contents into a clean petri dish.
- Scrape out the mucus with the rounded end of a pair of forceps and measure its weight.
- Pat dry the inner stomach membrane and measure it [Stomach $\mathrm{Weight}_{\mathrm{empty}}$].
- Sort the components according to the lowest possible taxonomic level.
- Count the individuals of each category ($=N$).
- Pat dry and measure the weight of each food category ($=W$).
- Preserve items according to the successive analyses.
- Determine ecological descriptors ($F\%$, $N\%$, $W\%$, IRI).

Quantitative Parasitology

A variety of descriptors is frequently used to quantify parasites in a sample of hosts or comparing the infection rates among several samples. Since aggregated (right-skewed) distributions among the host individuals is a common feature in parasitological studies, the confidence interval should be provided in order to indicate the accuracy of the estimation. The frequency distribution of parasites should be reported, i.e. a histogram of the intensity, or alternatively a box-whisker plot including the percentiles or quartiles of distribution and a measure for the skewness. The most common descriptors used in fish parasitology are introduced below.

Common Descriptors

Prevalence [*P%*]

Prevalence is the most widely used descriptor for presence/ absence data on parasites in a sample of hosts and requires only detection of the presence of the parasite, regardless of the number of individuals present. It should be complemented with a confidence interval for the prevalence based on the binominal distribution. If the aim is to compare the prevalence between two or more samples, Fisher's exact test or the Chi-squared test can be applied.

Prevalence [*P%*]
Describes the number of hosts infected with one or more individuals of a particular species divided by the number of hosts examined. Expressed as a percentage.

$$[P\%] = n_{\mathrm{i}}/n_{\sum}{}^{*}100$$

n_{i} Number of hosts with a specific parasite i
$n_{\sum}$ Total number of hosts examined

Intensity [*I*]

Intensity (of infection) is a very basic descriptor providing a general overview on the range (min/ max number) of a specific parasite. It does not consider the actual sample size of the study and should be provided in combination with the mean intensity of infection [mI].

Intensity [*I*]
Described as the number of individuals of a particular parasite species found in a single infected host, i.e. the number of individuals in an infra-population. Expressed as a range.

$$I = I_{\min} - I_{\max}$$

$I_{\min}$ Lowest number of a specific parasite species found in a single host
$I_{\max}$ Highest number of a specific parasite species found in a single host

Mean Intensity [mI]

The mean intensity (of infection) is the average number of parasites found in the infected hosts. Unlike mean abundance [*A*], non-infected hosts are excluded from the calculation. Providing the standard deviation of the mean intensity is not recommended for aggregated distributions. Instead, the bias-corrected and

accelerated bootstrap (BCa Bootstrap) for the confidence interval is recommended (see Software Recommendations). Mean intensities between two samples can be compared using a Bootstrap *t*-test (see Quantitative Parasitology 3.0).

Mean Intensity [mI]
Described as the average intensity of a particular parasite species and defined as the total number of parasites of a particular species divided by the number of hosts infected with this species in a sample.

$$\mathrm{mI} = I_{\sum \mathrm{i}} / n_{\sum \mathrm{i}}$$

$I_{\sum \mathrm{i}}$ Total number of a specific parasite species i
$n_{\sum(\mathrm{infected})}$ Number of hosts infected with the specific parasite i

Median Intensity [medI]

Very similar to the mean intensity, the median intensity is the median number of parasites found in the infected hosts. It may be used instead of or in addition to mean intensity. Provide the confidence interval to give an estimation of accuracy. Mood's median test is recommended for comparison between samples.

Median Intensity [medI]
Described as the median intensity of a particular species and defined as the intensity value separating the upper half of infected hosts from the lower half. For a data set, it may be thought of as the centre of intensity values, in order of smallest to largest values.

Mean Abundance [mA]

The mean abundance [mA] is the average number of parasites found in the examined hosts, regardless of whether or not the hosts are infected. It can yield an indication of the dispersion of parasites among hosts. A Bootstrap *t*-test will show whether parasite abundance differs significantly among samples (Quantitative Parasitology 3.0)

Mean Abundance [mA]
Defined as the total number of parasites of a particular species divided by the number of hosts examined in a sample.

(continued)

$$\mathrm{mA} = I_{\sum \mathrm{i}} / n_{\sum}$$

$I_{\sum \mathrm{i}}$ Total number of a specific parasite species i
$n_{\sum}$ Total number of examined hosts

Derived Calculations

Shannon's Diversity Index [H']

Shannon's diversity index [H′], also known as the Shannon–Wiener index, the Shannon–Weaver index and the Shannon entropy, was originally proposed to quantify the entropy in strings of text. In ecological studies, it is commonly used to measure the diversity of species in a specific habitat, e.g. the parasites in a fish host. This indicator can take on values between 0 (only one parasite species in the host) and H_{max} (high diversity of species in a host, each with equal amounts).

Shannon's Diversity Index [H']
Described as the degree of uncertainty to find a specific species in a random sample. The more species occur and the more even they are distributed in a habitat, the higher is the uncertainty and therefore the diversity.

$$H' = \sum_{i=1}^{S} p_{\mathrm{i}} \ln p_{\mathrm{i}}$$

$$p_{\mathrm{i}} = \frac{n_{\mathrm{i}}}{N}$$

$$H_{\max} = \sum_{i=1}^{S} \frac{1}{S} \ln \frac{1}{S} = \ln S$$

N Number of specimens
n_{i} Number of specimens of species i
S Number of species
p_{i} Proportional abundance of species
$H_{\max}$ Maximum possible value of H'

Dominance Index [J']

The dominance index, Pielou's evenness index or simply evenness, is also a measure of biodiversity and quantifies how numerically equal a community of species is. It sets the Shannon's diversity index H' in relation to the highest possible diversity in a host (H_{max}). J' is restricted between 0 and 1.

Dominance Index [J']
Described as the Shannon's diversity index in relation to the maximum diversity in a host (H_{max}).

$$J' = H'/H_{max}$$

H' Shannon's diversity index
H_{max} Maximum possible value of H'

Fulton's Condition Factor [K]

Fulton's condition factor [K] or Fulton's K provides a measure of the length-weight relationship, using the carcass weight (CW) and total length (TL). It is a morphometric condition index which provides a useful tool to examine the overall growth of a fish. If the fish is growing isometrically, weight increases as the cube of length, and the exponent takes the value 3.0. Values significantly larger or smaller indicate allometric growth, i.e. the fish are relatively heavy in relation to their length. It might change, e.g. during the spawning season.

Fulton's K [K]
Defined as the carcass weight multiplied by 100 divided by the cubed total length.

$$K = (\mathrm{CW}^{*}100)/\mathrm{TL}^{3}$$

CW Carcass weight
TL Total length

Hepatosomatic Index [HSI]

Similar to Fulton's condition factor [K], the hepatosomatic index [HSI] provides a rough measure of the physical state and energy status of the fish host. It puts the liver weight (LW) in relation to the total weight (TW). When fish acquire more energy than necessary to meet basic metabolic and growth requirements, excess energy is stored in the liver (as glycogen). The size of the liver relative to the body is large

when a considerable amount of energy is stored in the liver and indicates a well-fed condition of the fish.

Hepatosomatic Index [HSI]
Defined as the liver weight multiplied by 100 divided by the total weight.

$$\mathrm{HSI} = (\mathrm{LW}^{*}100)/\mathrm{TW}$$

LW Liver weight
TW Total weight

Software Recommendations

Whereas the very basic descriptive parasitological parameters described above can easily be calculated with or without the use of a pocket calculator, advanced statistical methods may require the use of specific computer software. Given the rapid pace at which new commercially or freely available software bundles are being introduced and updated, an extensive presentation of specific tools and programmes might not be helpful here. However, generally speaking, it will certainly be a good idea to invest in a tool or programme that covers most commonly applied statistical methods used in natural sciences.

GraphPad Prism

Distributed by GraphPad Software, Inc., California, USA
Available at: www.graphpad.com

GraphPad Prism is a very well-structured statistical software available for both Windows and Mac computers. It offers a broad range of features to analyse, graph and present scientific data in a fast and intuitive way. It offers *t*-tests, non-parametric comparisons, ANOVA and more. It lets you perform non-linear and linear regressions of (multiple) data sets in an easy way. The programme is especially suited for undergraduate and graduate students as it offers extensive explanations and descriptions of the statistical methods included in a comprehensive help section. GraphPad Prism is available at a reasonable price with a discount for students.

Quantitative Parasitology 3.0

Distributed by Reiczigel J. & Rózsa L. (2005), Budapest
Available at: www.zoologia.hu

Quantitative Parasitology is a non-commercial software tool especially designed to analyse data from parasitological studies. According to the developers, the tool

provides statistically valid ways to analyse the highly aggregated frequency distributions exhibited by parasites and offers methods to describe parasitic infections within a sample of hosts and across different samples. It is distributed freely, used in education and science, quick and easy to install and a very handy software for the commonly used methods (prevalence, mean/median intensity, variance/mean, Bootstrap *t*-test, Mood's Median test, etc.).

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Food Safety Considerations

While there is a broad knowledge on parasitic diseases related to the consumption of meat in industrial countries, such as trichinellosis and cysticercosis, food-borne zoonoses related to fish are less studied. Today, increased trade and transportation, demographic changes due to globalisation and changing food trends (e.g. the consumption of sushi and sashimi and low-temperature cooking in countries where raw or undercooked fish is not traditional) are facilitating the spread and increase of these parasitic diseases.

Fish-Borne Diseases Related to Digenea, Cestoda and Nematoda

Among the most common diseases associated with fish consumption are opisthorchiasis, intestinal trematodiasis, diphyllobothriasis and anisakidosis. The main parasite groups involved are digenean trematodes (families Heterophyidae, Opisthorchiidae and Nanophyetidae), cestodes (genus *Diphyllobothrium*) and anisakid nematodes (*Anisakis simplex* (s.s.), *A. pegreffii*, *Pseudoterranova decipiens*).

Most of these parasites can be ingested through the consumption of freshwater fish, while for fishery products originating from the marine habitat, anisakid nematodes are a major risk for fish-borne zoonoses. Despite the fact that digenean trematodes are the most common parasites causing fish-borne diseases worldwide, zoonotic species are mainly restricted to freshwater fish as intermediate hosts and play a minor role in diseases associated with the consumption of marine fishery products. Cestodes are distributed in both marine and freshwater habitats worldwide. Most species of *Diphyllobothrium* are found in freshwater fish, but some marine examples are reported, e.g. *Diphyllobothrium cameroni*, *D. cordatum* and *D. hians*. Diphyllobothriasis is often asymptomatic but can cause abdominal and digestive

S. Klimpel et al., *Parasites of Marine Fish and Cephalopods*,
https://doi.org/10.1007/978-3-030-16220-7_5

discomforts. In rare cases, vitamin B12 intake by the tapeworm can lead to megaloblastic anaemia as in the case of *Dibothriocephalus latus* infections.

Due to their wide-ranging distribution and high abundance in nearly all marine fish species in the North Atlantic, anisakid nematode larvae constitute a major threat to food safety of fishery products in Europe. More specifically, two aspects are of immediate concern: direct infections and the triggering of allergies. Humans can get infected with anisakid nematodes after consumption of flesh or viscera of fish or cephalopods infected with larvae and may thus become accidental hosts in the life cycle of anisakid nematodes. Once ingested, the larvae of the families Anisakidae and possibly Raphidascaridae may penetrate the alimentary tract and associated organs. This may cause mild to severe symptoms and is commonly diagnosed as anisakiasis or anisakidosis. The most common agents for anisakidosis are *Anisakis* spp. (*A. simplex* (s.s.)*, A. pegreffii*), but humans may also get infected by sealworms *P. decipiens* and rarely by *Contracaecum* spp. Third-stage larvae of *A. simplex* are the most important agents for the disease, constituting over 90% of the 25,000 reported cases of anisakidosis, while the remaining cases are related to *P. decipiens* or *A. physeteris*.

In its acute phase, human anisakidosis is associated with mild to severe gastrointestinal symptoms such as epigastric pain, nausea, vomiting and diarrhoea. The most common form of anisakidosis is the penetration of the gastric or intestinal mucosa. Larvae that enter these regions may cause an abscess, and some worms may even invade the peritoneal cavity or other organs. Diagnosis of anisakidosis is either made through gastroscopy and involves physical removal of the larvae. Alternatively, patients are treated with antihelminthics, anti-inflammatories or analgesics. Allergic reactions to anisakid antigens can occur after consumption of infected fish products and are primarily triggered by *A. simplex* (s.s.). Sensitisation occurs during ingestion of viable parasites, whereas hypersensitivity reactions can also occur after ingestion of non-viable parasites, including immunological cross-reactions to proteins of related nematodes and other invertebrates such as crustaceans and house dust mites. Allergic patients suffer from acute urticaria and anaphylaxis; however, diagnosis relies on the detection of serum lgE antibodies to allergenic proteins. These allergic reactions to anisakid nematodes are frequently observed in Spain, possibly due to the frequent consumption of traditionally raw or only lightly marinated seafood.

Food Hazard Reduction Measures

Preventive measures are laid down by the seafood industry and official authorities in order to mitigate the risk of food hazards. These measures include a variety of regulative steps: the identification of food safety hazard, development of suitable control mechanisms and implementation of actual control measures, e.g. monitoring for parasites during the fish processing steps. Several regulations and criteria are currently set up by the European Union. The EC Regulation 853/2004 sets out rules

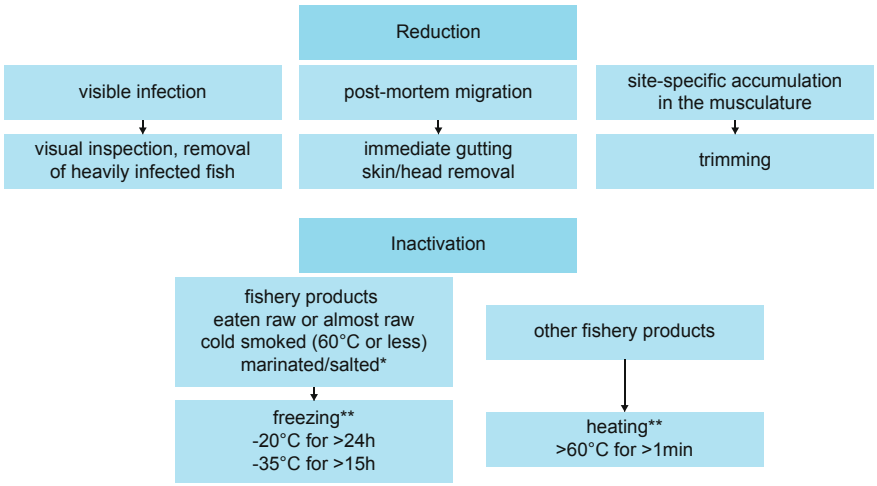

Fig. 1 Measures to minimise a potential risk of viable parasites in fishery products. *Core temperature in all parts of the fish. **Depending on the treatment

for foodstuff that may constitute a hazard to human health and therefore requires specific hygienic rules. Regulation 853/2003 ensures that food business operators must not place fishery products obviously infected with parasites on the market intended for human consumption. Closely related to this, the European Food Safety Authority (EFSA) has been appointed to (a) assess the food safety concerns associated to parasites that may be present in fishery products, (b) evaluate alternative treatments for killing viable parasites in fishery products and evaluate their effectiveness compared to the freezing method described in the hygiene regulations and (c) set criteria for when products are eaten raw, almost raw or cold-smoked from fishing grounds and from aquaculture that do not present a health hazard due to the presence of parasites. In general, measures on the reduction of parasites in fish and fishery products can either be taken during harvesting or during processing and include special handling practices, e.g. reducing the number of parasites and inactivating viable parasites potentially zoonotic (Fig. 1).

Besides the health risk due to the zoonotic potential of some parasite species, the presence of visible parasites alone poses an aesthetical problem and thus affects the marketability of commercially produced fish. In order to avoid heavily infected fish on the market, measures are taken to either reduce the infection level or sort out visibly infected fish. Several handling steps may be performed by processors and include heading, gutting, filleting, skinning and trimming.

Regulation EC (2005) 2074/2005 lays down rules for visual inspection, i.e. the non-destructive examination of fish and fishery products under good light conditions, to check for parasites during handlings of fishery products on land or on board of the vessels. The inspection of eviscerated fish must be carried out by an experienced person with a sufficiently high number of samples and be focused on the abdominal cavity, liver and roe intended for human consumption. For fish fillets or

slices, visual inspection must be performed during trimming and after filleting/slicing, either by individual examination or according to a sampling plan. The commonly used method for the detection of parasites in the fish flesh is candling, i.e. placing fish on a light table in a darkened room. This method should be used by the fish processing industry and be included in the sampling plan if necessary from a technical viewpoint. However, the candling method has some weaknesses, e.g. a successful detection of nematode larvae varies depending on the thickness of the fillet, the presence of skin on the fillet, oil content, pigmentation and the level of experience of the operator. Experiments have shown that only 7–10% of anisakid nematodes actually present as larvae in the fillets of pelagic fish from the Northeast Atlantic are detected through candling.

Anisakid nematodes are usually not distributed evenly in the fish musculature but are mostly located in the hypaxial part of the fillets in the majority of fish species. This might be explained by the short distance for larvae dwelling from the viscera into the musculature. Therefore, the most successful method for reducing the number of anisakid nematodes in fish products is the trimming of belly flaps, which is a method used by many fish operators.

Measures to Inactivate Parasites in Fish

Regulation 853/2004 specifies that food business operators must ensure that fishery products intended for raw or almost raw consumption undergo a freezing treatment that ensures killing viable parasites. This regulation was extended with some specifications based on the scientific advice of the EFSA in EC Regulation 1276/2011.

Freezing or heating are effective measures to inactivate viable parasites (Fig. 1). The duration and temperature of the freezing treatment must consist of at least −20 °C for 24 h or −35 °C for 15 h in all parts of the product in order to kill parasites other than trematodes. This treatment must be applied for fishery products derived from finfish and cephalopod molluscs, which are consumed raw or almost raw. Additionally, cold-smoked fish (less than 60 °C) and all marinated and/or salted fishery products must be frozen, if the processing is insufficient to kill nematode larvae. Since nematodes live in stomachs of adult mammals, they are relatively robust against acid. Thus, for pickling solutions and marinades of fish, the duration until larvae are killed depends on the salt concentration. Additional rules are set up, e.g. tests on the viability of nematode larvae must be conducted after artificial digestion of salted Atlantic herring and salted sprat. Additionally, post-mortem migration of nematode larvae from the viscera into the musculature has been observed in a variety of fish host species. Therefore, immediate gutting after capture is mandatory for most marine fish species to avoid an increased number of nematodes in the fish musculature.

Post-mortem migration was demonstrated for, e.g. herring, smelt, anchovy and capelin. In contrast, there was no evidence of post-mortem migration in saithe (*Pollachius virens*), haddock (*Melanogrammus aeglefinus*), ocean perch

(e.g. *Sebastes marinus*) and Alaska salmon (*Oncorhynchus keta*, *O. gorbuscha*, *O. nerka*). Surprisingly, in the latter, over 90% of nematode larvae were found located in the musculature immediately after catch, which was associated with the anadromous behaviour and salinity changes. In herring, the largest proportion of flesh with residing *A. simplex* (s.s.) larvae was found in the belly flaps, without any differences between left and right side. In smelt, more than half of the nematodes from the musculature were located in the epaxial part, 18.4% in the hypaxial and 26.0% in the tail musculature compartments. Proportions of nematode larvae in the viscera and musculature can vary between fish host species. For example, in a study by Strømnes and Andersen (1998), 0.4% of nematodes found in saithe occurred in the belly flaps and 99.6% in the viscera, whereas in cod 3.2% occurred in the belly flaps and 96.8% in the viscera. Approximately 12% were present in the belly flaps and 88% in the viscera in golden redfish. It is still not clear which conditions and species trigger post-mortem migration of anisakid nematodes (in particular *A. simplex*). However, if the occurrence of post-mortem migration and high intensities in certain parts of the musculature in fish from specific harvest areas is known, this information could be used to set up processing steps and trimming guidelines, which would enable the fish processing industry to at least reduce the risk of nematode larvae in the final product.

Heating or cooking is much faster than freezing: at a core temperature of $>60\ ^{\circ}C$, and a duration of at least 1 min is recommended. Treatments of freezing or heating do not have to be conducted for fishery products from wild catches if epidemiological data are available showing that the fishes in the respective fishing grounds are not infected with hazardous parasites and if a competent authority has no objections and so authorises. In accordance with the scientific opinion of the EFSA, farmed fish exclusively reared in floating onshore tanks, cultured from embryos and exclusively fed with parasite-free food is also exempted from freezing requirements. In the case of both aforementioned exceptions, food business operators must ensure the origin from a parasite-free fishing ground or fish farm.

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Appendix

Roundfish Dissection: Checklist

1) ☐	Measurements	Measure total length (TL), standard length (SL) and optionally pre-anal length (PAL) to the nearest 0.1 cm
2) ☐	Weight	Take the total weight (TW) of the fish to the nearest 0.01 g
3) ☐	External examination	Macroscopically examine body surface and openings for ectoparasites (skin, eyes, nostrils, mouth, gills, fins, anus)
4) ☐	Tissue sample	If needed, cut tissue samples from the dorsal muscle sections and freeze at −20 °C or store in 100% EtOH for molecular examination. Include in carcass weight (step 10)!
5) ☐	Gill arches	Remove the operculi and gill arches, cover with saline solution and examine under a stereo microscope. Rinse the gill cavity and examine the fluid. Include the operculi and gill arches in CW (step 10)!
6) ☐	Enucleation	Isolate the eyeballs and check the eye fluid for the presence of parasite metacercariae
7) ☐	Dissection	Open the body cavity along the ventral mid-line. Carefully cut around the anus to prevent damaging the intestine. Remove the belly flap on one side, creating a 'window' to the internal organs
8) ☐	Internal organs	Remove the organs by cutting the oesophagus at the ventral end. Separate the organs and keep liver and pylorus for crushed preparations. Cover the other organs with saline solution
9) ☐	Organ weight	Weigh liver and gonads to the nearest 0.001 g
10) ☐	Carcass weight	Take the carcass weight (CW), adding the weight of tissue samples and including the operculi and gill arches
11) ☐	Stomach	Take the weight of the filled and empty stomach. Identify, count and weigh each food component (to the nearest 0.001 g). Check the stomach contents for parasites. Examine in portions covered with saline solution
12) ☐	Body cavity	Rinse the body cavity and examine for parasites
13) ☐	Crushed preparations	After checking the organ surface, crush the liver, pylorus, spleen, gall bladder and kidney between the lid and bottom of a petri dish. Examine via transillumination. Divide into portions if necessary
14) ☐	Intestines	Check the outside, then divide and check the contents for parasite stages.
15) ☐	Parasites	Process the parasites

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https://doi.org/10.1007/978-3-030-16220-7

Host dissection protocol

№_____

Fish species:_____

Researcher:_____

Dissection Date:_____ Sampling Date:_____

Sampling ID:_____ Haul №:_____

Sampling source:_____

Total length:_____ Standard length:_____

Total weight:_____ Carcass weight:_____

Gonad weight:_____ Liver weight:_____

Stomach weight (full/empty):_____/_____

Tissue sample ☐, Otolith ☐, Sex: ♂ ☐, ♀ ☐, Juv ☐, Unk. ☐

Food items/notes:

Parasite Species	Surface	Mouth/ Nostrils	Gills	Eyes	Body cavity	Liver	Stomach	Pyloric caeca	Gut	Gonad	Kidney	Muscle

Host dissection protocol №_____

Parasite Species	Surface	Mouth/ Nostrils	Gills	Eyes	Body cavity	Liver	Stomach	Pyloric caeca	Gut	Gonad	Kidney	Muscle

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