Introduction

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Abstract

Cephalopods are valuable seafood for human consumption, and some of them are good candidates for aquaculture. In addition, they have evolved many characteristic features that make them interesting models for research. The recent inclusion of cephalopods in the Directive 2010/EU regulates the use of animals for scientific purposes and obliges cephalopod researchers to promote the best health and welfare practices during aquarium maintenance or aquaculture procedures. The identification of diseases of cephalopods, and the pathogens that cause them, is consequently of major interest to improve cephalopod welfare and husbandry. This work has been designed as a short, easy to follow 'handbook,' with the aim of presenting fundamental aspects of the anatomical and histological structures as well as the identification of different pathogens, the resulting histopathology, and the diagnosis of diseases in cephalopods. We hope it will provide a useful contribution that will also encourage marine pathologists, parasitologists, veterinarians and those involved in fishery sanitary assessment, aquarium maintenance, and aquaculture practice to increase our knowledge about the pathology of cephalopods further.

Keywords

Cephalopods • Pathology • Parasites • Infectious diseases • Fisheries • Aquaculture • Seafood

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Knowledge of pathologies of cephalopod mollusks in the wild is very limited. The information available is mainly based on *postmortem* examination of animals after capture, which limits the identification of the etiological agent responsible for the disease. Most recently, pathologies of cephalopods have also been identified in laboratory and small-scale culture conditions; it is predicted that the increasing interest in industrial cephalopod aquaculture will increase the risks of their occurrence (Sykes and Gestal 2014).

Identifying pathogens and the resulting diseases, and the potential risks to animals' health due to mechanical damage or injuries from capture or in the laboratory are considered some of the main requisites for improving welfare and husbandry for these animals, as required in 'assessment of health and welfare' of the Directive 2010/63/EU.

Cephalopods (i.e., nautilus, cuttlefish, squid, and octopus) are members of the phylum mollusca. The taxon currently numbers about 800 species, representing a large diversity of

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forms and adaptations. These are exclusively marine invertebrates distributed in all areas of the world, from the intertidal areas to deep sea.

The interest in cephalopods has increased considerably over the last few decades, mainly because they (i) represent a very important target for fisheries with high market value; (ii) constitute an important resource of seafood for human consumption, with a high protein and polyunsaturated fatty acid content; (iii) are characterized by features of their biology and physiology which are novel in design and evolutionary adaptation (Albertin et al. 2015; Shigeno et al. 2018); (iv) are the sole invertebrates included in the list of regulated species by the Directive 2010/63/EU (Fiorito et al. 2015; Di Cristina et al. 2015).

Coleoid cephalopods have been used for millennia as seafood by humans across the world and across different food cultures (Mouritsen and Styrbæk 2018). Cephalopod landings reached about 4 million tons in 2016 (FAO 2017), although a fall of approximately one-quarter from that total was reported for 2017 (G. Pierce, pers. communication). The continuously increasing demand from the market, the decline in fishing overall, and the search for a more sustainable food resource have all contributed to promote a great interest in cephalopod aquaculture over the last decade, with an important, associated research effort in the field (Iglesias et al. 2014).

Considered classically as 'marine guinea pigs' (Grimpe 1928), cephalopods have been studied for more than one century for the uniqueness of their biology (Grimpe 1928; Packard 1972; Marini et al. 2017). They have evolved many characteristic features that make them 'organisms of interest' for the study of the evolution of neural and behavioral complexity. Despite their typical molluscan design and body plan, cephalopods possess a highly differentiated multi-lobular brain, a camera eye resembling that of vertebrates, a 'closed' circulatory system, a sophisticated set of sensory organs and fast jet-propelled locomotion. Cephalopods, and squid in particular, are also the animals that donated to neuroscience the giant axon, the classic preparation that allowed the discovery of how neuronal action potentials and nerve propagation worked, together with the ionic mechanism of action potentials.

The identification and management of diseases are some of the major hurdles in the development of the aquaculture industry. The accurate identification of the different organs at histological level and the knowledge and management of infectious and non-infectious diseases that may affect cultured species are a priority for both the aquarium maintenance and aquaculture of cephalopods.

A range of diseases has been described in cephalopods, caused by a wide variety of pathogens, belonging to many phyla, including fungi, viruses, bacteria, and protozoan and metazoan parasites. Bacterial infections have been identified in wild cephalopods, while the collection, transfer, aquarium maintenance and weakening of animals under stress may facilitate and increase the development of the diseases (Hanlon and Forsythe 1990; Hochberg 1990). Wild cephalopods are also intermediate, paratenic, or definitive hosts of a range of parasites with different life cycle strategies. They occupy an ecological niche that makes them vulnerable to infection by specific groups of parasites, which are transmitted to the definitive host, namely fish, marine mammals, or birds. An association between relative species diversity of parasites and cephalopod life cycle characteristics has been observed in Atlantic waters, suggesting that the ecological niche of a cephalopod species is more important in determining its risk of parasitic infection than its phylogeny (González et al. 2003).

Despite the increased interest in cephalopods as seafood and the recommendations of Food Safety Authorities on parasite risk in fishery products, currently only fragmentary information on pathogens and diseases in cephalopods exists. This information has been mainly gathered from opportunistic sampling plans within commercial fisheries or market surveys, and it is small in comparison with information available for other commercially important taxa (Pascual and Guerra 2003; González and Pascual 2018). At the present, there is no available information on the risk that cephalopod parasites pose to human consumers. In order to carry out good Regulatory Science, (which is described as the scientific and technical foundations upon which regulations are based) knowing what risks cephalopod disease pose to consumers will be a key point. Future research should be addressed to this, together with building the knowledge base overall, which is also a critical point in this research area. Although human consumption of cephalopods worldwide is much lower than that of fish, potential risk should be managed appropriately. As an example, González and Pascual (2018) pointed out that 'risk management should configure and consistently implement policies to ensure that scientific evidence is translated into action, while also considering aspects such as the key general principles established in EU food law (necessity, proportionality, minimum effect on competence, and guarantee of level playing field) that guarantee and protect the functioning of markets.' The use of certified biobanking in fish (González et al. 2018) can aid the establishment of a similar network for sampling and collection of traceable cephalopod parasites.

Knowledge of the most important pathogenic agents identified in cephalopods has been reviewed in volume III (1990) of the seminal serial work 'Diseases of Marine Animals,' edited by Otto Kinne. A general overview of each group of pathogens, together with a compilation of information on microorganisms and parasite species identified per cephalopod host species, is included in the original work (Hochberg 1990; Hanlon and Forsythe 1990). In more recent years, a review by Castellanos-Martínez and Gestal (2013),

and some additional papers on specific pathogens or parasites added additional data on the knowledge of cephalopod parasitology and diseases.

However, to the best of our knowledge, no guide to histological identification has yet been published; this book aims to contribute to fill this gap. It originates as one of the outcomes of the activities of the COST Action FA1301, Cephs *In* Action, which established an interdisciplinary network for improvement of cephalopod welfare and husbandry in research, aquaculture, and fisheries.

The first part of the book offers tools that advise one on how to make an accurate pathological analysis. Several chapters provide a review of sampling methodology (including necropsy and postmortem examination), organ anatomy, as well as a detailed description of the histology of larval stages and adults for three species of cephalopods (*Sepia officinalis, Loligo vulgaris,* and *Octopus vulgaris*). We consider these species as valuable 'morphotype' models of the taxonomic groups Sepioidea, Myopsida, and Octopoda, which include most of the species with highest culture potential (Iglesias et al. 2014).

Additionally, knowledge of organ architecture and tissue structure at histological level is a key factor to identify and analyze pathological conditions. The histological identification of organs of the selected species of cephalopods is discussed for both larval stages and adults.

In the second part of this book, methods for assessment of parasites and pathogens in cephalopods are thoroughly described. Diseases conditions are diverse in the wild- and aquarium-maintained cephalopods, depending on the combination of physiological and immunological host factors, as well as the virulence of the pathogens. Current techniques involving molecular tools are being used to support the diagnosis of different pathologies. However, conventional diagnostic tools, including gross pathology, histopathology, and identification of signs of diseases, remain not only useful but also very valuable techniques. The combination of both approaches, i.e., diagnosis taxonomy and molecular biology, is needed for the accurate identification of pathogens. Aquarium maintenance and conditions (e.g., seawater quality, tank materials, density of individuals per tank) provoke stress that increases the susceptibility of cephalopods to suffer diseases. Consequently, knowledge of these disorders is a bottleneck for the assessment and improvement of the health status and welfare in cephalopods, as required by the European Directive 2010/EU (EU 2010; see also Fiorito et al. 2015).

The material selected for this compendium represents a comprehensive overview of the pathologies observed in wild- and aquarium-maintained cephalopods, in the form of a short, easy to follow handbook. We aim to present fundamental aspects of the anatomical and histological structures, as well as the identification of different pathogens, the resulting histopathologies and diagnosis of diseases in cephalopods.

We hope this will provide a useful contribution that will also encourage marine pathologists, parasitologists, veterinarians and those involved in fishery sanitary assessment, aquarium maintenance, and aquaculture practice, to increase our knowledge regarding the pathology of cephalopods further.

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