Metazoa and Related Diseases

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Abstract
Cephalopods and their metazoan parasites have coevolved in wild fisheries for many years. In fact, helminth larvae and parasitic copepods have been recorded in cephalopods worldwide. This is not surprising considering the important role cephalopods play in the transfer of energy and contaminants in marine food webs. Nerito-oceanic ommastrephid squids are by far the most noticeable trophic bridge for helminth parasites in the marine realm, coastal octopus, and cuttlefish serving as primary host for crustaceans. Although it is highly likely that parasitic infections occurred, relatively little is known about the pathogenic potential of metazoan parasites in naturally infected cephalopods. It is stated that heavy parasitic infections may probably cause host morbidity or poor condition but signs of disease are singularly rare with very few specimens exhibiting disease conditions. Unfortunately, neither robust scientific evidence nor available material is available to support this statement. It is more likely that metazoans may deplete energy stores of infected cephalopods, which are directed toward tissue repair and the host’s defense mechanisms. Parasitic infection may thus be considered an environmental stressor and as such a source of uncertainty in the evaluation of the potential productivity of cephalopod populations.

Keywords
Metazoan parasites • Pathogens • Trematodes • Cestodes • Nematodes • Crustaceans • Seafood security

12.1 Introduction
Metazoan parasites comprise a polyphyletic group made up of six parasitic taxa: flatworms (Platyhelminthes), tapeworms (cestodes), trematodes (flukes), roundworms (nematodes), acanthocephalans, and crustaceans. They exhibit complex life cycles and reproductive strategies, with a remarkable high diversity and prevalence in marine ecosystems. Ecto- and endoparasitic metazoans including monoxenous/heteroxenous and specialist/generalist species have been largely recorded in the different components (zooplankton, fish, large fish, marine mammals, and seabirds) of the trophic cascades characteristically defined in the marine realm (Rohde 2002).
Recently, spatially explicit modeling revealed that European cephalopod distributions match contrasting trophic pathways (Puerta et al. 2015), and therefore it is expected that cephalopods are common hosts for metazoan parasites. Such statement is not surprising considering that cephalopods are key element in the food web, its foraging behavior and diet facilitates endoparasite transmission. Furthermore, cephalopods inhabit in a wide array of biotopes (shallow to deeper waters/ecosystems (benthic to pelagic), and its varied social structure and behavior capabilities (solitary, scholar) may also enable an ectoparasitic recruitment into major cephalopod stocks. The above both arguments provide us a broad perspective to understand the great availability of ubiquitous microhabitats offered by cephalopods to colonization by metazoan parasites. In fact, the reported species composition of the metazoan fauna-infecting cephalopods being characterized by a relatively uniform and limited composition remains far from saturated, with empty micrones to be colonized.

The general qualitative character of the metazoan fauna of European Atlantic populations of octopus/cuttlefish/squid (coastal-slope species) and short-finned squid (slope-shelf and nerito-oceanic species) is almost the same as that of Mediterranean populations; only a few differences in species composition were observed, which clearly reflects that infection by metazoans (mostly at larval stage) are nonspecific. Overall, the community structure of parasitic metazoans of European cephalopods is similar among ecologically and taxonomically close species. On the basis of 2000 individuals comprising 10 cephalopod species collected at a microgeographic area (Galician waters, NW Spain), González et al. (2003) found some associations between parasite relative species diversity and cephalopod life cycle characteristics. Results showed that those species with similar risk of becoming infected with a given parasite fauna belong to one of three ecological groupings (coastal, intermediate, or nerito-oceanic). It was suggested that the ecological niche of a cephalopod species is more important in determining its risk of parasitic infection than is phylogeny.

Mostly, the narrow range of metazoan parasites found in European cephalopod populations is thus characterized by wide host specificity. In the life cycles of the reported parasitic helminths, cephalopods may be considered second intermediate or transport/paratenic hosts serving as trophic bridges for parasite flow to top predators (final hosts) (Pascual et al. 1996a; Abollos et al. 1998).

Moreover, the composition of the parasitocoenoses of European cephalopods seems to remain stable over time. Another issue is the marked differences noted in the infection rate. The demographic infection parameters of a given cephalopod species within a particular ecological group may vary among ecoregions and even province of a particular European marine realm. As a rule, infections by metazoan parasites are significantly higher in northern European seas and Lusitanian provinces than in the Mediterranean basin. Furthermore, the size/weight/sexual maturity structure of a given cephalopod group is recognized as the key categorical predictor of parasite epidemiological values determining the intra- and interspecific variability of the metazoan fauna of European cephalopod stocks. Generally, no significant differences in the infection rate of males and females are observed, but as the size/weight/maturity increase the infection values substantially raise. Additionally, parasite recruitment may vary depending on the definitive host distribution (Kuhn et al. 2016) but especially, at the mesoscale, the recruitment in the mesozooplankton and hyperbenthos are affected by the oceanographic regime (Pascual and González 2007; Gregori et al. 2015). The latter authors gave evidence that in upwelling systems parasite faunas are impoverished, whereas downwelling relaxing conditions propitiate optimal conditions to successful. Similarly, ontogenic shifts in cephalopod diet from planktonic invertebrates and small fish planktophages on one side to largest fish preys on the other may contribute to age-related variations in the helminthofauna (from trematodes to cestodes/nematodes). These shifts may also enhance the accumulation process of parasites which largely favors the typical skewed binomial distribution of metazoans in cephalopod populations.

Fragmentary information on the metazoan fauna of European cephalopods based on opportunistic sampling plans within commercial fisheries or market surveys has been produced in 20 papers published in the last 30 years accounting from the latter revision provided by Hochberg (1990). This knowledge progress made on the biodiversity, pathology, and ecological relationships of metazoan parasites affecting cephalopods reflects a poor coverage for species/geographical areas. There can be no doubt of the lack of sufficient critical mass of European scientists in this field, but in a comparative analysis with other commercially important taxa, a historical negligible financial support for research on cephalopod diseases animal group was noted (Pascual and Guerra 2003). The rate of knowledge progress on cephalopod diseases becomes, therefore, a vexing, unbalanced question in fisheries research.

There is, nevertheless, some current regional and national surveillance plans for zoonotic parasites implicated in human allergy (following the scientific opinion on parasite risk in fishery products published by the European Food Safety Authority; EFSA 2010) which may serve as a promising platform for future biodiversity studies on metazoan parasites of cephalopods. The use of already certified biobanking tools in fish parasite research (González et al. 2018) can also aid to establish network opportunities for sampling and collection of traceable metazoan cephalopod parasites.

Against the overall background, the strategy defined in this chapter is not to make a comprehensive literature review of host–parasite systems, but illustrate the role of the most
prevalent and relevant metazoan parasites as pathogens and diseases-related agents in European cephalopods. Thereafter, scientific focus for the different taxa which is discussed under the new challenge perspectives.

### 12.2 Metazoan Parasites as Pathogens

#### 12.2.1 Trematodes

Several monogeneans have been described from European cephalopods (Hochberg 1990), but some forms were considered *incertae sedis* and the type material (holotype, paratype, and syntype) is not largely available. The single extent of evidence came by Llewellyn (1984), who reported gyrodactylids as epidermal browsers in the mantle cavity and on the gills of *Alloteuthis* squids from the North Sea and the English Channel off France and England. These monogeneans are thought to be recruited to the squids through direct contact of adults at the time of mating.

Cephalopods are also parasitized by digenean trematodes, at larval stages (metacercaria) or adults, acting as second intermediate, paratenic, or final host, but never as first intermediate host (Hochberg 1990). Metacercariae of didymozoid are the most important group of digeneans which infects oceanic squids, and some of them have been described in *Illex coindetii* (Fig. 12.1a) or *Todaropsis eblanae* in European waters (Hochberg 1990). Other groups of digenean trematodes were reported in 10 species of squids, cuttlefish, and octopus from the French and Italian coasts (Mediterranean) and from the English Channel off France and England (Hochberg 1990). Again, the several morphs recognized present taxonomic uncertainty. *Derogegenes varicus* were reported in *Sepia officinalis* in the coast of Plymouth, England, where more than 80% of the specimens over 10 cm mantle length appeared to be infected. *Lecithochirium* sp. was reported in *Octopus vulgaris* in the North East Atlantic, NW Spain (Fig. 12.1b–d). This hemiurid shows a fusiform body, with a sub-terminal oral sucker, a small pharynx, big acetabulum, or ventral sucker, with conspicuous acetabular cleft, and excretory vesicle with a characteristic Y-shape.

With some exceptions, most reports of trematodes in cephalopods show a low prevalence of infection, acting as paratenic hosts. Metacercaria and adult digeneans infected the digestive system (especially, the stomach and caecum) and despite they can form oval cysts with thin, transparent envelopes no significant host tissue reaction was noted.

#### 12.2.2 Cestodes

Larval and post-larval stages of cestodes repeatedly have been described from European cephalopods (Hochberg 1990). The prevalence and intensity of infection are high in theuthoid squids, showing a wide range of sizes and shapes in relation to the squid host (Fig. 12.2a, b). Plerocercoid larvae belonging to the orders Tetraphyllidea and Trypanorhynchidea dominate among cestodes (Pascual et al. 1996b), which become sexually mature in the digestive tracts of elasmobranch fishes, sharks, and rays. The scolex of Tetraphyllidean larvae has characteristically four large

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**Fig. 12.1** Digenean trematodes from European cephalopods. Whole specimens (ventral view) of didymozoid from from *Illex coindetii* (a), and adult *Lecithochirium* sp. (b, c, e) from *Octopus vulgaris* showing oral and ventral sucker, pharynx, acetabular cleft, and excretory vesicle. View at light microscopy (a, b), and (c, d) scanning electron microscopy (SEM). Scale bars: A: 200 μm; B: 200 μm; C: 200 μm; D 100 μm
Fig. 12.2 Larval cestodes tetraphyllidean taken from a variety of cephalopod species of Europe. Note the biomass of plerocercoids (a) and the range of sizes and shapes (b) in relation to the squid host. Light microscopy of two typical body architecture of tetraphyllideans (c, d), showing the phenotypic plasticity of their scolices: with wavy-edged bothridia (e–g), smooth-oval bothridia (i–k), and scalloped-edged bothridia (l). Tegment (h) and apical and bothridial sucker in apical (m, n) view of scolex. Characteristically, in some cases, the scolices are invaginated as seen in scanning electron microscopy (o) and histological section (p). (e–h) Alcoholic Gill’s Haematoxylin. (p) H&E. Scale bars: C: 1 mm; D: 5 mm; E: 100 µm; F: 100 µm; G: 100 µm; H: 500 µm; I: 200 µm; J: 100 µm; K: 100 µm; L: 100 µm; M: 200 µm; N: 500 µm; O: 500 µm; P: 250 µm.
leaf-like bothridia. The genus *Phyllobothrium* is the most common and widely dispersed in decapod and octopod cephalopods from European waters (Fig. 12.2c, d). Phyllobothrid larvae are divided into three groups by the form of their bothridia: with wavy-edged bothridia (Fig. 12.2e, g), smooth-oval bothridia (Fig. 12.2i, k), and scalloped-edged bothridia (Fig. 12.2l). An apical sucker is also observed (M, N). They show one accessory sucker by bothridia and one apical sucker. The tegument of the body is covered by microvilli or microtrics (Fig. 12.2h). Characteristically, in some cases, the scolices are invaginated (Fig. 12.2o, p).

Plerocercoids are mainly lying free in the lumen or attached to the organs of the digestive tract (stomach, caecum, and rectum) (Fig. 12.2p), although sometimes found free in the liver, mantle cavity, and even leaving the host. Plerocercoids move freely between these organs, especially when changes in temperature and other physico-chemical factors occurred in postmortem condition. This movement of large forms has been suggested to impair the marketability of the infected specimens considerably due to the unaesthetic appearance of the fish product.

Other Tetraphyllidean larvae have been reported from European cephalopods within the *Scolex spp.* species complex. Despite the yet unresolved uncertainty of their taxonomic affinities, these forms are well recognized by their smaller sizes and bothridia with a characteristic number of suckers.

Larval Tetrarhynchidean metacestodes characteristically with four hook-armed tentacles are well represented by *Nybelinia* plerocerci in European cephalopods. *Nybelinia* is commonly encountered found in a variety of cephalopods in the NE Atlantic and Mediterranean cephalopods (Pascual et al. 1996a). *N. lingualis* (Cuvier 1817) localizes in intestinal mesenteries, on the ovary, on the linings of the coelomic cavity, and on the external covering of the stomach (Fig. 12.3a). The scolex is composed of four sessile bothridia showing four tentacles or proboscids armed by small helicoidally placed hooks (Fig. 12.3b). They can be observed evaginated (Fig. 12.3a, b, d), or invaginated into the body (Fig. 12.3c). Despite the intensity of infection can be significantly high in larger squids and the tentacles are used to anchor the host’s tissues, no noticeable report on the pathological effect of these cestodes was found in the literature, with the exception of specific light infiltration of hemocytes in the infected area (Fig. 12.3e).

Most reports of cestodes in European cephalopods simply document the presence of cestodes, site of infection, sample...
locality, and some demographic infection parameter. Despite singular effort has been concentrated on the ecological relationships of cephalopod–cestode systems (e.g., Gaevskaya and Nigmatulli 1978; Pascual et al. 1996b) as a whole neither pathological nor disease symptoms have been mentioned or figured associated to the cestode infections.

12.2.3 Nematodes

In the last years, larval ascaridoid nematodes are by far the most commonly reported parasitic agent in European cephalopods. Survey of anisakids with zoonotic and human allergic potential in cephalopod food products has been a target of some national surveillance plans. In fact, despite in the older literature specific identification for nematode larval types in cephalopods has been suggested as a main taxonomic concern, from the Hochberg’s revision (1990) molecular methods have been largely applied to specifically identify the anisakids and their distribution in the organs and tissues of the host (Abollo et al. 2001; Melani et al. 2014; Serracca et al. 2013; Pico-Duran et al. 2016; Cost et al. 2016). Larval stages of Anisakis simplex sensu stricto and A. pegreffii have been identified parasitizing Octopus vulgaris, Eledone cirrhosa, Sepia officinalis, and other sepideae species, and with higher prevalence have been identified in loliginids and ommastrephids in European waters (Abollo et al. 1998, 2001). As a rule, anisakids are found covering the outer and inner membranes of internal organs, especially the gonads (ovary and testes), nidamental glands and on the wall of stomach. Sometimes they were found on the coelomic membrane of the mantle wall (Fig. 12.4a, b). In cephalopods at fresh postmortem condition, the viable anisakid larvae can be also found actively moving within the mantle cavity. This provokes commercial rejection by consumers due to the unaesthetic appearance of squid products.

General morphological diagnostic characteristics of larval stages of Anisakis, such as anterior end showing a boring

**Fig. 12.4** Anisakid nematodes from various cephalopod species in Europe. Third-larval stages are easily recognized macroscopically (a, b) coiled and encysted in different organs (arrows). Light microscopy images of the anterior (c) and posterior (d) extremities with the characteristic ventriculus (e) of Anisakis simplex showing some morphological structures (striated cuticle, boring tooth, excretory pore, lips, esophagus, intestine, anal gland, anus, and mucron) in lateral view. Cystidicola sp. nematode from Octopus vulgaris (f, g). Anterior (f) and posterior (g) extremities showing the characteristic pseudolabia and mucron, respectively. Scale bars: C: 50 µm; D: 30 µm; E: 150 µm; F: 20 µm; G: 20 µm
tooth, mouth, lips with papilla and excretory pore, ventriculo- 
ous with specific length and shape, and tail end showing the 
anus and mucron terminal, are shown in Fig. 12.4c, e.

Some nematodes other than ascaridoids occasionally have 
been observed in European cephalopods. As an example, 
spirurida larval nematodes of a cystidicolid were found 
encapsulated in the external and internal walls of the crop, and 
on the connective tissue sheath surrounding the digestive 
gland and intestine of the common octopus O. vulgaris caught 
at NW Spain (Gestal et al. 1999a). Larval of Cystidicolidae 
shows mouth dorsoventrally elongated with a pair of pseudo- 
dolabia with conical protuberances, two lateral cephalic alae. 
The esophagus shows an anterior short muscular part and 
posterior part longer and glandular. Post-anal tail short, with 
weakly nodulose truncated mucron (Fig. 12.4f, g).

Most recently, some efforts have been made to show the 
histopathological effect of nematode infections (Pascual et al. 
1996b; Gestal et al. 1999a). Early infections consisted of 
necrotic tissue displaying a light inflammatory reaction fol- 
lowed by haemocyte infiltration. In more advanced case of 
infection, the response of cephalopod tissues to invading 
nematodes was a typical cell-mediated immune response (Ford 
1992), with parasite encapsulation as an immune strategy to 
avoid parasite migration and destruction of host tissues 
(Fig. 12.5a–h). Most infected organs displayed evidence of 
mechanical compression and displacement of host tissue ele- 
ments at sites close top or in direct contact with parasitic larvae, 
with varying degrees of cellular infiltration. The spaces sur- 
rounding worms were usually coated with tissue fragments, cell 
debris, and extensive secretion of mucus. Anisakids were thus 
postulated as responsible for parasitic castration in those 
heavily infected mature squids as a consequence of partial 
destruction and alteration of gonad tissues (Abollo et al. 1998).

12.2.4 Crustaceans

Few published reports dealt with the crustaceans infecting 
European cephalopods. Branchiurans and cymothoid isopods 
have been accidentally found on the skin and in the mantle 
cavity of cuttlefish (Sepia and Sepiola species). The majority of 
crustacean reports refer to copepods: harpacticoids of Cholydia 
intermedia from the mantle cavity and gills of a cirroteuthid cephalopod 
collected in the Faroe-Shetlands Channel; lichomolgids on the gills of Sepia officinalis 
and T. sagittatus from NW Mediterranean (Rosas and Banyuls) 
and the Adriatic (Trieste) (Hochberg 1990; Costanzo et al. 
1994), and from the gills of Illex coindetii off the Atlantic 
coast of the Iberian Peninsula (Lopez-González and Pascual 
1996); females and males of a cyclopoid copepod parasite in Octopus vulgaris 
from Banyuls (Hochberg 1990).

Larval stages of Lernaeoceridae assigned to Pennella 
varians have been largely recorded at the Mediterranean on 
the gills of teuthoids (L. vulgaris, T. eblanae), cuttlefish 
(Sepia officinalis, S. elegans, S. orbignyana, Rossia caroli, 
Sepietta oweniana) and octopus (Octopus vulgaris, E. 
moschata, Bathypolypus sponsalis) (Gestal et al. 1999b) 
(Fig. 12.6a–e).

Heavy infestation by the postembryonic stages of the 
siphonostomid copepod Pennella sp. has also been com- 
monly reported in the gills of several commercially impor-
tant cephalopod species from temperate waters of the NE 
Atlantic (Pascual et al. 1996a). The spatiotemporal distri-
bution of this mesoparasitic copepod revealed a marked 
aggregated and seasonal pattern of parasites that fits well 
with their mating behavior in the gills of cephalopods 
(Pascual et al. 2001). Adults of the cyclopoid copepod of the 
genus Octopicola, Octopicola superbus have been identified 
in European octopuses at the English Channel, Mediter-
anean, and Atlantic at NW Spain (Fig. 12.6i–l).

Although in the older literature the cephalopod-copepod 
relationships have been categorized as highly host specific, 
most of the species were considered commensals. They 
moved on the skin (of head, arm, or mantle), mantle cavity, 
and gills, and feed on mucus (Fig. 12.6f–h, m–o), thereby 
they were considered not true parasites when affecting wild 
cephalopod populations. In fact, the infected cephalopods did 
not appear stressed and no damage to the tissues was reported. 
However, pennellid larvae deserve a special mention. Cor- 
relation between heavy gill infections and poorer squid con-
dition at the infrapopulation level has also been demonstrated 
(Pascual et al. 2005). This work provides strong evidence that 
mechanical lysing of large areas of functional tissues pro-
duced by pennellids contributes to the variability in squid 
growth, being one of the multiple categorical predictors of 
size-at-age data in several infested cephalopod species com-
mercially exploited in European waters.

12.3 New Coming Challenges

Modern conception of fisheries management under the 
Common Fishery Policy and the H2020 Research and 
Innovation Framework is based on two driven pillars: the 
ecosystem-based concept and the accommodation of fish 
production systems to the new seafood system challenge. 
The final goal is to conquer a better understanding of the 
natural and anthropogenic impacts on fish resources at the 
ecosystem level to render productive ecosystems and healthy 
seafood products.

12.3.1 Seafood Security

Parasitic diseases have been largely recognized as a bottleneck 
that hampers fish production systems (Shinn et al. 2014).
However, as it was summarized in this chapter, apart from histopathological evidences the role of metazoans as etiological agents of pathologies in cephalopod species, stocks, or individuals still remains unexplored. Of particular interest is to estimate the impact of aggregated metazoan infrapopulations on the condition and productivity of cephalopod populations, especially for those species that have fast-growing potential in open-caged systems. Similarly, considering that multiple
infections are common in nature, it would be desirable to analyze the synergistic/antagonistic effect of the different parasitic agents in the well-being of cephalopod populations.

12.3.2 Seafood Safety

Problems in the rational utilization of commercial fisheries include the management of biohazards along fish production value chains. The high pathogenicity for man of the anisakid larvae and their high prevalence and abundance in various commercially exploited fish and cephalopod species draws particular attention to this biohazard (EFSA 2010). Parasites have zoonotic potential (i.e., cause anisakidosis in humans) but also they affect the marketability of seafood products. Exposure to the anisakidosis pathogens at high rate in the mantle cavity of European squids should be properly evaluated as a causative vector in risk analysis of this emerging zoonotic and allergic disease in southern European countries (EFSA 2010).

To achieve the above challenges, systematic study on the biodiversity of the metazoan parasitic fauna of European cephalopods should clearly be a hot spot. Considering the larval stage for most recorded metazoans and in many cases the absence of type specimens, biobanking, and genetic markers are relevant tools to assure the
identification of the etiological agent, the characterization of the hazard and the implementation of critical control points.

12.4 Concluding Remarks

Relatively little is known about the pathogenic potential of metazoan parasites in naturally infected cephalopods. It is stated that heavy parasitic infections may probably cause morbidity or poor condition of cephalopods but signs of disease are singularly rare with very few specimens exhibiting disease conditions. Gaevskaya and Nigmatullin (1981) suggested that the greatest injury to the cephalopod Sthenoteuthis pteropus may be done by Tentacularia larvae, large Porrocaecum (which destroy the integrity of the mantle musculature), Phyllobothrium (which assimilate part of the nutrients of the squid’s food as they undergo growth and development), and anisakid larvae (which apparently destroy oocytes). Unfortunately, neither robust scientific evidence nor available material is available to support this statement.

As a whole, judging by the magnitude of the extent/intensity of infection, the site of infection, and the fact they can undergo growth, it seems that metazoans may deplete energy stores of infected cephalopods, which are directed toward tissue repair and the host’s defense mechanisms. Parasitic infection may thus be considered an environmental stressor and as such a source of uncertainty in the evaluation of the potential productivity of cephalopod populations (Pascual et al. 2007a, b, c).

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