Parasites, pathogens, and other symbionts of copepods

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Abstract

There is a large diversity of eukaryotic symbionts of copepods, dominated by epizootic protists such as ciliates, and metazoan parasites. Eukaryotic endoparasites, copepod-associated bacteria, and viruses are less well known, partly due to technical limitations. However, new molecular techniques, combined with a range of other approaches, provide a complementary toolkit for understanding the complete symbiome of copepods and how the symbiome relates to their ecological roles, relationships with other biota, and responses to environmental change. In this review we provide the most complete overview of the copepod symbiome to date, including microeukaryotes, metazoan parasites, bacteria, and viruses, and provide extensive literature databases to inform future studies.

Copepods in ecosystems

Copepods are the most ubiquitous and abundant zooplankton forms in the oceans and they exist in almost all aquatic systems at all latitudes and depths, including deep-sea vents. Most of the >11 300 known species are marine – planktonic, benthic, and host-associated [1,2] – but they also occur in freshwater and damp terrestrial habitats. As primary consumers, copepods are at the base of most aquatic food webs with a clear link to higher trophic levels [3], and make important contributions to biogeochemical processing [4]. They are important contributors to carbon sequestration by virtue of their vast numbers, lipid content, production of sinking fecal pellets, moults, and carcasses and their ability to vertically migrate to the deep ocean [4,5]. Copepods exhibit a wide diversity of lifestyles and morphologies, and are important **parasites** (see Glossary) in their own right, having established symbiotic relationships with a diverse spectrum of metazoan hosts (**Figure 1**).

Long-term studies over multiple decades have shown that key abundant copepod taxa have altered their distribution and size in response to climate change and anthropogenic factors [6,7]. Anthropogenic changes to the marine environment include increasing temperature, decreasing pH, and the addition of chemical and microplastic pollutants, all of which have been associated with changes in copepod communities [8]. Increased aquaculture activity and intensity have provided copepods locally with radically modified, physicochemical, biotic, and epidemiological environments (e.g., [9]). However, the mechanisms behind the changes in copepod communities, distribution, and morphology remain unclear. Fully understanding the dynamics of these dominant zooplankton taxa will require an understanding of the

organisms with which they interact most closely: their **symbionts** (including parasites and pathogens; **Box 1**).

The Tara Oceans global marine biodiversity survey showed that symbiont–host interactions are highly abundant in a taxonomic interactome network assessment [10]. However, knowledge of the diversity and roles of copepod symbionts is patchy. We present here an integrated overview of these associations, intended to provide a basis for interpretation of data and experimental design in diverse fields: ecology, microbiology, pathology, and epidemiology. **Tables S1–S4**, in the supplemental information online, list the metazoan, microeukaryotic, bacterial, and viral symbionts of copepods, respectively, including copepod host taxa and relevant references, as retrieved via the search criteria explained in Box S1. Since the eukaryotic data in particular are too extensive to be included in the main text, they are summarized in **Figure 2**.

Metazoan parasites of copepods

Copepods have long been known as essential intermediate hosts for several major parasitic, metazoan groups, including Digenea, Cestoda, Nematoda, Acanthocephala, and even Crustacea, in both marine and freshwater habitats (e.g., [11], **Table S1**), but in most cases the impact of the parasite on the copepod host is as yet unknown. Species of the freshwater genera *Eucyclops*, *Macrocyclops*, and *Metacyclops* have successfully been propagated and used in the laboratory for infection experiments with cestodes and nematodes [12,13]. Most metazoan parasites infest the coelom of the copepod host. To ensure completion of the life cycle, parasites often induce behavioral change, facilitating infestation and transmission to another intermediate, transport, or final host [14]. The cestode

Schistocephalus solidus for example, not only manipulates the freshwater copepod host (*Macrocyclops albidus*) in its behavior to avoid predation prior to infection but also increases its activity upon reaching the infective stage to increase transmission [15]. If a copepod host is infected by multiple parasite species that manipulate its behavior in different ways, conflicts over host manipulations arise [16].

Freshwater copepods are intermediate hosts to one of the most successful invasive parasitic species, the Eastern Asian swimbladder nematode *Anguillicoloides crassus* (formerly *Anguillicola crassus*), which spreads throughout the population of the European eel (e.g., [17]). While many life-cycle studies focus on the experimental infection of the intermediate host and transmission of the parasite to its final host, very little is known about the effect on the copepod other than that its mortality rises with an increase in nematode dose [18].

Metazoan parasites that infect copepods as ectosymbionts include the Cycliophora, Hydrozoa, and Monogenea, all of which are commensal, and two ectoparasitic lineages in the Crustacea (Isopoda, Tantulocarida) (Table S1). Epicaridean isopods have a **heteroxenous** life cycle, involving two crustacean hosts. In all known life cycles they infect pelagic calanoid copepods as intermediate hosts, feeding on their blood. While these isopods can potentially castrate their final hosts (often decapods), virtually nothing is known about their effects on the copepod intermediate host [19].

Micro-eukaryotic symbionts of copepods

The majority of microeukaryotes (i.e., solitary and colonial unicellular eukaryotes, and microscopic fungi) known to be associated with copepods are members of Alveolata [20], which includes the ciliates (Ciliophora), a major group of free-living and epibiotic

protists, the diverse and abundant dinoflagellates (Dinoflagellata), and Apicomplexa. Ciliates (particularly peritrichs and suctorians) are the most speciose group (**Figure 2, Table S2**); however, their apparent predominance may be the result of observational bias. The epibiotic nature and relatively large size of most ciliates likely enhances their detection and characterization, compared to smaller, more cryptic or internally localized symbionts. Ciliate–copepod host relationships exhibit a range of specificity, including preferential associations with particular copepod species, sex, or attachment site. Apostome and suctorian ciliates are particularly diverse in association with calanoid and cyclopoid copepods [21,22]. For most ciliate associations, the impact on the copepod hosts is unknown, but some of the surface hitchhikers (e.g., *Epistylis daphniae*) seem to affect longevity negatively [23].

Dinoflagellates from two major lineages, Dinophyceae and Syndiniales, are relatively well known as parasites of copepods. Within the former, the parasitic castrator *Blastodinium* includes a group of gut parasites of calanoid and cyclopoid copepods [24]. Figure 3 shows *Blastodinium* as polyphyletic within Dinophyceae, with some species related to other parasites of copepods, such as representatives of *Chytriodinium*, *Dissodinium*, and *Syltodinium* [25].

Within Syndiniales, some *Syndinium* species (MALV-IV) are parasitoids of copepods, capable of population-level control [26,27]. More recent eukaryotic metabarcoding data provide evidence of interactions between a larger diversity of syndinians and copepods [10,28], which likely reflect both parasitic and trophic relationships. Interestingly, although the marine crustacean-infecting *Hematodinium* (also MALV-IV) is strongly associated with some copepod taxa [22,28,29], there is no direct (microscopy-based) evidence so far of a parasitic relationship [30]. Instead,

copepods are likely to feed on *Hematodinium* dinospores, and therefore may act as vectors between hosts susceptible to their infection.

Ellobiopsis chattoni, branching as part of MALV-I in **Figure 3**, is another parasitic castrator. Other *Ellobiopsis* species (and related genera) have been described, but not sequenced, and their taxonomic affinities should not be assumed [31]. The related *Thalassomyces* has not yet been reported from copepods, but utilizes rather larger crustaceans. Another enigmatic (and phylogenetically long-branched) member of MALV-I is a Red Plasmodial Parasite (RPP) discovered infecting *Clausocalanus* [32]. The genetically divergent 18S sequences of these parasites may lead to an underestimation of their occurrence and diversity by environmental sequencing studies [33]; nonetheless RPP and closely related sequences (within 5% difference) were represented in environmental studies on NCBI GenBank by 48 entries (as of January 2021), albeit mostly from a single study [34], and very similar to each other. There were 14 (putative) ellobiopsid sequences representing three or four distinct 18S types, but only one *Thalassomyces*-related clone. Given their high phylogenetic diversity (**Figure 3**) MALV-I may harbor many other, as yet unknown, copepod symbionts.

Other (putative) dinoflagellates have been described as copepod parasites (**Table S2**): *Schizochytriodinium*, thought to be related to *Chytriodinium*, *Dissodinium*, and *Syltodinium* (**Figure 3**), has not been recorded since its original description and lacks sequence data. *Schizodinium sparsum* has been synonymized with *Blastodinium mangini* (**Figure 3**), and both *Atelodinium* species with unidentified members of *Paradinium* [26]. Although initially thought to be dinoflagellates, molecular analysis has revealed *Paradinium* to be a member of Rhizaria (Endomyxa: Ascetosporea:

order Paradinida). Several *Paradinium* species are described from copepods, with some having been sequenced along with a few other unspecified copepod-infecting paradinid lineages [32,35]. However, PCR-amplified group-targeted environmental studies have unveiled a large diversity of other paradinids (ENDO-3) and related clades, at least some of which are also symbionts of copepods [35].

Microsporidians are endoparasites of a huge range of aquatic and terrestrial animals, particularly invertebrates. Copepods are acknowledged as important hosts of Microsporidia, with 40–50 microsporidian species previously associated with them [36,37]; we identify 64 copepod-associated microsporidian species in **Table S2**. However, many of these are either poorly defined and/or have been subject to confusing taxonomic revisions. Phylogenetic analyses are necessary to elucidate microsporidian taxonomy, and it is clear that most copepod-infecting Microsporidia remain unsequenced. However, currently available small subunit (SSU) rDNA data show that they branch in Clades 1, 4, and 5 (**Figure 4**). Clade 1 contains the majority of these, in two categories: those with aquatic insect (larval) hosts – mosquitoes and caddisfly larvae (some of which are also definitive hosts of copepod-infecting Microsporidia) – and those with no other known hosts. Although the latter have so far been observed only in copepods, they may also have alternative hosts. Their phylogenetic proximity to microsporidian sequences from soil/compost and mosquitoes suggests that phylogenetic patterns might not be a reliable indicator of host relationships/habitat, at least when so much diversity remains unknown. However, the *Amblyospora–Parathelohania* clade are all parasites of Culicidae (Insecta), and the ‘aquatic outgroup’ of Vossbrinck et al. [38] includes a clade of cladoceran parasites, in addition to the copepod and caddisfly parasites mentioned previously.

Even though some eukaryotic symbionts of copepods are relatively easy to observe, there is clearly much uncharacterized eukaryotic diversity associated with this host group. Examples are unsequenced taxa such as the diversity of euglenoids reported by Michajłow [39], a likely much higher diversity of copepod-associated oomycetes than currently recognized [40], and the yellow-hyphal parasite in *Calanus* spp., which is possibly *Ichthyosporidium* [41,42].

The diversity of parasites in general – and group-targeted environmental sequencing studies [10,34,35] – show that only the tip of the eukaryotic symbiont iceberg is currently known. High-throughput sequencing approaches (metabarcoding and metagenomics) have been applied to copepods only to a limited extent: to investigate gut content (eukaryotic and bacterial) [29,43], and zooplankton–Syndiniales interactions [28]. Savage (2020; Master's thesis [22]) pooled individuals of the most abundant copepod (and other zooplankton) taxa from the Strait of Georgia (BC, Canada) to investigate their symbiome and diet using 18S V4 primers biased against Metazoa. The copepod samples were dominated by ciliates and dinoflagellates (mostly Syndiniales), with lower numbers of Cercozoa and diatoms, concordant with the number of records of such interactions in the premetabarcoding literature. The syndinian diversity was predominantly from groups II and IV, and varied significantly between copepod species and seasonal sampling timepoints. Paradinids (mostly *Paradinium poucheti*) were particularly associated with cyclopoid copepods. Genetically divergent parasites (e.g., Microsporidia and nonparadinid Ascetosporea [35,44] were not detected, as broadly targeted primers do not effectively amplify them [33]; in these cases group-specific primers are required (e.g., [45,46]). Other groups,

such as euglenoids, have long insertions within the ribosomal genes often used in metabarcoding, requiring special consideration in bioinformatic pipelines.

Some comparisons of symbionts associated with other planktonic Crustacea have been detailed previously. The differences recognized so far between microeukaryotic symbionts of copepods, ostracods, euphausiids, and amphipods range from closely related microeukaryotic taxa being associated with different hosts (e.g., the copepod and *Daphnia*-infecting Microsporidia in clade 1 (**Figure 4**), and ellobiopsids in copepods and euphausiids [47]), to much larger-scale differences. It is striking that Microsporidia have yet to be conclusively identified in euphausiids, and conversely gregarines, otherwise diverse and ubiquitous gut symbionts of many invertebrates, are apparently scarce in copepods. Reasons for both of these observations could be uneven sampling and/or biological factors such as host size and habitat (e.g., the small size of copepods relative to krill, or perhaps other symbionts such as *Blastodinium* inhibiting gregarine invasion of copepod guts).

Bacterial symbionts of copepods

Copepods harbor epibiotic and endobiotic bacterial communities distinct in composition from each other and the surrounding water column [48,49] (**Table S3**), although generally bacterial genera reported from copepods are also free-living and/or associated with other hosts. The abundance of bacteria associated with zooplankton can be orders of magnitude higher than in the ambient water, and as such copepods (and their fecal pellets) represent 'microbial hotspots' in the water column [50., 51., 52.] where bacteria often exhibit increased growth rates and production relative to free-living counterparts [53,54]. Associations between copepods and bacteria may be permanent or transient, and are influenced by host

life-history stage, moulting, and physiological activity [55] as well as environmental conditions such as prey availability and water depth [56,57]. There is active microbial exchange between the water column and zooplankton: the latter are known to facilitate the 'hitchhiking' of bacteria through the water column during vertical migration, increasing exchange and interaction between spatially separated communities [50,51,57].

Colonization of the copepod surface appears to be site-specific, with bacteria preferentially attaching around the antennules, mandibles and egg sacs [53,58]. The exact nature of many of these associations remains unknown. Colonization by nonpathogenic bacteria may prevent secondary colonization by harmful algae or pathogenic bacteria, and surface-colonizing bacteria may also consume detritus produced by host feeding, in return reducing the build-up of debris on the copepod's surface which may otherwise hinder its swimming ability [59].

Culture-dependent studies show that epibiotic communities are dominated by marine *Vibrio* spp. in estuarine and coastal environments [60], including the human pathogens *V. parahaemolyticus*, *V. alginolyticus*, *V. vulnificus*, and *V. cholerae* [61,62]. The adhesion of V. cholerae to live copepods has been demonstrated to extend its survival and culturability in both marine and fresh water when compared to adhesion to dead copepods, or free-living cells [63], suggesting that copepods may function as environmental reservoirs for the pathogen [64]. The pathogenic impact of associations with *Vibrio* spp. on copepods is unknown, but a diversity of other functional roles of copepod-associated *Vibrio* spp. are being revealed [53,65., 66., 67.].

Culture-independent microbiome studies often show copepods to be dominated by a small number of operational taxonomic units (OTUs), often Flavobacteriaceae (Bacteroidetes), Rhodobacteraceae (Alphaproteobacteria), and Oceanospirillaceae and Pseudoalteromonadaceae (both Gammaproteobacteria) [50,51,55,56]. Despite their abundance in cultivation-based studies, *Vibrio* spp. may often be present at only very low frequencies [55,56] or absent from high-throughput sequencing datasets [50,51,68], although they can be more highly represented in generally Gammaproteobacteria-dominated metatranscriptomic copepod gut-derived data [67]. Datta et al. [55] analyzed microbiomes of 200 *Calanus finmarchicus* individuals, showing a small 'core' microbiome of 34 OTUs, with community differences related to host physiology and microbial interactions.

However, most functional aspects of copepod–microbiome interactions remain largely unknown. Environmental and ecological factors are likely to be important here, for example habitat (e.g., deep- vs shallow-dwelling) and feeding history [55], physicochemical variables like pH [68], and the impact of seasonal changes on the environment [54,56]. Influences of human activities also have far-reaching consequences – for example, there is evidence of negative physical and physiological impacts of plastic contamination in copepods [69,70]; however, it is unknown (but very likely) that this will impact their symbionts, with possible feedback effects on the host.

The most well-studied endobiotic copepod bacterial communities are those in the gut, which include transient members associated with host feeding, and longer-term associations [29]. The copepod gut offers a suboxic, low pH environment compared to the ambient water and copepod surface, with a continuous supply of organic

substrates from host feeding [71]. As such, it may provide suitable conditions for the growth of bacterial groups unable to survive in the oxygenated water column, in exchange for the metabolic benefits provided by bacterial symbionts [54]. A range of bacteria associated with the copepod gut microenvironment have been shown to be involved in a number of important biogeochemical processes, including methanogenesis, methylmercury production, iron remineralization, and the degradation of high-molecular-weight organic compounds [51,52,71]. Copepod-associated gammaproteobacteria carry out nitrogen fixation [65], nitrate reduction to nitrite [66], and alkaline phosphatase activity in response to phosphorus limitation (primarily by *Vibrio* spp. [67]).

Copepods also appear to be able to influence bacterial communities in their immediate surroundings, termed the 'copepod zoosphere' [72]. In addition to the increased concentration of organic carbon and other nutrients, certain bacterial groups – particularly Vibrionaceae, Rhodobacteraceae, and Oceanospirillales – may selectively benefit over other bacterial groups present from inclusion within the zoosphere [72].

Very few copepod bacterial endoparasites are known. Bacteria have been observed to invade copepod body cavities and form large masses. Afflicted copepods may appear opaque and exhibit impaired swimming behavior [58,73]. *Wolbachia* species are well known as reproductive parasites of insects, nematodes, and arthropods [74,75] and have been shown to affect the composition of the microbial communities of affected hosts [74]. Wolbachial infections of the freshwater *Mesocyclops thermocyclopoides* observed in Thailand had a potentially feminizing effect on the host [76].

Copepods and viruses

Despite the fact that viruses are extremely abundant and diverse in planktonic samples [77,78], very little is known about viruses associated with copepods, as vectors, reservoirs, or pathogens. Most virus–copepod studies relate to viruses of parasitic copepods of economic concern (salmon lice), and the potential role of the virus in copepod–finfish host relationships (**Table S4**).

Two rhabdoviruses, LSRV-No9 and LSRV-No127 [negative-sense single strand (–ss) RNA], were characterized from the salmon louse *Lepeophtheirus salmonis* from salmon farming sites in Norway, in developmental stages and glandular tissues of the louse [79]. LSRV-No9 was also very closely related to a rhabdovirus N protein gene from *L. salmonis* from the Canadian Pacific Ocean. Virus-infected lice appear to elicit a reduced inflammatory response from their host compared to uninfected lice [80]. A further rhabdovirus, *Caligus rogercresseyi* rhabdovirus (CrRV-Ch01), which branches as sister to LSRV-No9 and LSRV-No127, was characterized from another sea louse *Caligus rogercresseyi* from Atlantic salmon in Chile [81]. These three together are referred to as *'Caligrhavirus'*, pending ICTV ratification [82]. More recently a fourth –ssRNA virus, *Lepeophtheirus salmonis* negative-stranded RNA virus (LsNSRV-1), was described from *L. salmonis*, branching within the newly defined family Artoviridae, genus *Hexartovirus* [82].

A different route to viral discovery, metagenomics on hand-picked copepod samples, identified two ‘circo-like’ (ssDNA) viruses in the calanoid copepods *Acartia tonsa* and *Labidocera aestiva* from Tampa Bay, FL, USA. *Labidocera aestiva* copepod circo-like virus (LaCopCV) showed up to 100% prevalence in some samples, with high viral loads, while *Acartia tonsa* copepod circo-like virus (AtCopCV) was detected

sporadically year-round [83]. However, we were unable to find any other reports of relationships between viruses and free-living copepods, other than those in which the copepod acts as a vector or/and reservoir of viruses.

Several studies have looked into the potential of copepods as reservoirs/vectors of viruses that can then be transferred to often economically important fish or crustacean hosts. Frada et al. [84] showed that >80% of copepods sampled during a North Atlantic cruise carried viable virions of the coccolithophore *Emiliania huxleyi* virus (EhV). *Lepeophtheirus salmonis* can act as a mechanical vector of IHNV (infectious haematopoietic necrosis virus), an important rhabdovirus of fish [85], and as vector of a salmonid alphavirus (SAV3) (**Table S4**). The sea louse *Caligus rogercresseyi* vectors the orthomyxovirus (–ssRNA) ISAV (infectious salmon anemia virus) to the salmon host, while more indirect effects also operate; for example, infection of Atlantic salmon by *L. salmonis* (and other parasitic sea lice) has been shown to increase their susceptibility to ISAV [86]. Viruses of penaeid shrimps are of research interest because of their importance in shrimp aquaculture [87., 88., 89.]. Copepods have been shown to amplify and vector the dsDNA white spot syndrome virus (WSSV) [87,88], and Taura syndrome virus (TSV) replicates in the copepod *Ergasilus manicatus*, while yellow head virus (YHV) may also be mechanically vectored to shrimp [89].

A further perspective on copepod–virus interactions can be gained from the study of endogenous viral elements (EVEs): viral genomic fragments integrated into the genome of their eukaryotic hosts, some of which results from recent endogenization of currently circulating viruses. Metegnier et al. [90] found 13 EVEs in the *L. salmonis* genome, from *Circoviridae* and *Parvoviridae* (both ssDNA), and *Bunyaviridae* and

Mononegavirales (both –ssRNA). In the free-living copepod Eurytemora affinis they found ten *Mononegavirales* EVEs, branching with rhabdoviruses, underlining the directly observed relationships described previously between this group of viruses and copepods. Sequencing of copepod genomes and copepod-enriched metagenomes will enable much deeper investigations into past and current copepod–virus interactions.

Recent advances in understanding the diversity and nature of crustacean-infecting viruses indicate that significantly more copepod-associated viral diversity likely awaits discovery. There are at least 100 viral pathogens across multiple viral families (DNA and RNA) described within shrimp, crabs, crayfish, and lobsters. Currently, high-throughput sequencing virome studies are revealing a plethora of previously unknown viruses in a range of crustacean hosts [91., 92., 93., 94.], often highlighting the similarity of crustacean-derived viral sequences to avian, insect, plankton, and bacteriophage-like viruses.

Functional diversity of copepod symbionts

Host-symbiont relationships occupy one or more positions on a ‘symbiotic continuum’ [33]; **Box 1**, ranging from commensal ectosymbionts, to endosymbionts with limited negative effects on the host, symbionts that manipulate the host’s behavior, to parasitoids that kill the host in order to complete their life cycle and thereby influencing copepod community structure and population size (**Figure 5**). We emphasize that the impact of most organisms (and viruses) associated with copepod hosts is unknown, and in many cases may be context-dependent. For example, little is known about bacterial and viral pathogenicity in copepods, and those pathogenic in other (related) organisms cannot be assumed to also be so in copepods. On the

other hand, the association between bacteria and copepods is proving to be particularly interesting, with copepods providing distinct microenvironments for bacteria and acting as vehicles for amplification of their effects. For all copepod symbionts, particularly for noneukaryotes, specificity and residence times of associations (**Box 1**) are largely unknown, as are triggers for pathogenesis and other physiological shifts.

Hypersymbiosis (including **hyperparasitism**) between host-associated copepods and their symbionts (including parasites) was comprehensively reviewed by van As (2019) [95]. In addition to being directly affected by pathogens, copepods act as mechanical and biological vectors, reservoirs, and alternative hosts for a wide range of pathogens, many of commercial relevance. Parasitic copepods, especially of the genera *Caligus* and *Lepeophtheirus* (Caligidae) and *Ergasilus* (Ergasilidae), have been shown to act or have high potential to act as vectors for bacteria and viruses between fishes [96]. Some cases are relatively well known: salmon lice acting in different vectoring capacities for viruses (and influencing susceptibility to viruses; [86]) and pathogenic bacteria, for example, *Aeromonas salmonicida*, *Tenacibaculum maritimum*, *Pseudomonas*, and *Vibrio* [97,98]. *Caligus elongatus* is a potential vector for *Photobacterium damsela* in seabass [99]. *Ergasilus rotundicorpus* is suspected to act as vector for the lymphocystis virus in marine and freshwater fishes [96]. The fish-pathogenic protist (*Neo*)*paramoeba perurans* may also be transferred via *L. salmonis* as vector [100]. The oyster *Ostrea edulis* parasite *Marteilia refringens* can be transmitted to the bivalve host by *Paracartia grani* and potentially *P. latisetosa* [101]. From a different perspective, parasites of parasitic copepods could be considered as biological control agents (e.g., microsporidians; [102]). However, endosymbionts of parasitic copepods are much less well known than those of free-living taxa.

Concluding remarks

In conclusion this review has, for the first time, brought together literature on the huge number and diversity of symbionts that interact with copepods, revealing an enormous diversity of organisms, from viruses to metazoans, many of which display parasitic/pathogenic relations in certain contexts. Most interactions between copepods, their parasites, and the pathobiome are uncharacterized physiologically and ecologically. There is a clear need to improve basic knowledge and understand biological consequences of taxa within the copepod pathobiome. More targeted investigations are required into the nature of copepod–symbiont associations, their functionality, and consequences. Sequence-based taxonomic studies of copepod **holobionts** and communities are valuable for indicating host-symbiont associations, and how these vary according to space, time, and ecology. However, investigations into the genetics and physiology of symbionts – both independently of, and interactively with their host – will reveal many new aspects of their combined responses to their environment, and their contributions to aquatic ecology and ecosystem services.

A critically neglected aspect of pelagic climate-change ecology is how host–parasite dynamics are changing in a warming world. Future climatic shifts [103] are likely to cause major reorganization of food web structure [104] as thermal adaptation limits are exceeded for hosts and parasites, either releasing hosts from their parasites or creating new host niches for parasites. The extent and diversity of copepod–symbiont interactions are likely to have been highly underestimated by direct inspection/analyses of individual copepods. In many cases the relationship between host and microbe is unknown or can change depending on the environmental conditions. Further, pathogenic effects of symbionts should not be considered at the

level of individual symbiotic taxa but rather in the context of the pathobiome ([105]; **Box 1**).

The recent diversification and tractability of high-throughput ‘omics technologies is already facilitating these new lines of research. However, the importance of integrating more traditional techniques, such as histopathology and in situ hybridization techniques, for visualization and functional interpretation of the **copepod symbiome** should not be overlooked. Conceptually parallel to the importance of considering interactions between host, environment, and symbionts to understand pathogenesis and disease manifestation, a synthetic understanding of the same three components is required to understand the variable states and functionality of the copepod holobiont, which, in their multitudes, constitute a mostly unexplored diversity of ecological, physiological, and genetic forces in aquatic ecosystems (see **Outstanding questions**).

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Declaration of Interests

The authors declare no competing interests.

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Glossary

Castration (parasitic)

complete or partial blocking of host reproduction, to the benefit of the parasite.

Copepod symbiome

all organisms, including eukaryotes, bacteria, and viruses, associated with copepod hosts, encompassing negative, neutral, and positive interactions. The nature and duration of the association is not specified by this term; in many cases both are unknown.

Heteroxenous

refers to a parasite life-cycle involving more than one host organism.

Holobiont

a unit of biological organization comprising a host and its associated bacteria, Archaea, viruses, and eukaryotes.

Hypersymbiosis (including **hyperparasitism)**

the symbiotic (or parasitic) habit of one species upon another symbiotic (or parasitic) species.

Parasite

a host-associated organism that feeds on, or derives resources from its host.

Pathogen

a host-associated organism that causes disease in its host.

Symbiont

a member of the symbiome.

Figure legends

Figure 1. Phylogenetic hypothesis of interordinal relationships within the Copepoda

Based on small subunit (SSU) rDNA after Huys et al. [2], the hypothesis summarizes convergent evolution of different modes of life and host utilization; the position of Gelyelloida (no molecular data available) is intercalated, based on morphological data. The color shading in the phylogram (red to blue) indicates relative numbers of species in these copepod clades that are either free-living (white), endosymbiotic (red), or ectosymbiotic (blue). The circles on top of the graph indicate if copepods parasitize invertebrate (purple) or fish (yellow) hosts.

Figure 2. Summary of the diversity of eukaryotic symbionts of copepods.

Bars to the left indicate the number of species known for each eukaryotic symbiont taxon. Bars to the right indicate the number of copepod species known to have symbiotic associations with the indicated non-copepod taxon. Organisms exclusively from marine habitats are indicated in light gray shading, those from freshwater habitats are indicated in black shading, while brackish habitats or both marine and freshwater are indicated by mid-grey shading.

Figure 3. Maximum Likelihood phylogeny based on partial 18S rRNA gene sequences from parasitic dinoflagellates associated with copepods and other taxa.

All dinoflagellate and related parasites of copepods for which sequence data were available at the time of writing are indicated by bold text. Representative sequences of parasites of other groups of organisms are indicated as shown in the key to the

figure. The tree with the highest log likelihood (–15461.04) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A General Time Reversible (GTR) model was used for analysis. A discrete Gamma distribution was used to model evolutionary rate differences among sites (five categories (+G, parameter = 0.5227)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 17.83% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Maximum likelihood bootstrap support was calculated for 100 replicates, and values >50% are shown next to the nodes. A Bayesian phylogeny was also constructed and thick branches represent Bayesian posterior probabilities >0.95. The scale bar corresponds to a 7% sequence divergence. The alignment contained 116 nucleotide sequences with 690 positions in the final dataset.

Figure 4. Bayesian phylogeny based on partial 18S rRNA gene sequences from microsporidians infecting copepods and other taxa.

Bayesian posterior probabilities are shown for major clades and the backbone of the tree. Black circles represent probabilities of 0.95 or higher. The Bayesian analysis ran for 5 million generations each with one cold and three heated chains; 1.25 M generations were discarded as burnin. The evolutionary model applied a GTR substitution matrix, with a 4-category autocorrelated gamma correction. All parameters were estimated from the data. The standard microsporidian clade numbering system is labelled from the left of the tree. All microsporidian parasites of copepods for which sequence data were available at the time of writing are indicated by black stars. Representative Microsporidia from other small aquatic crustacean

hosts (Cladocera, Ostracoda, Orthoptera, and culicid larvae in the 'aquatic outgroup' clade [38]) and proximally branching sequences are indicated by bold text labels and/or labeled brackets. Representative environmental sequences are included only when closely related to copepod-associated Microsporidia. These show that the 'aquatic outgroup' clade includes soil-derived lineages of unknown biology very closely related to copepod parasites, and the diverse aquatic cluster in Clade 4 from [46] does not (yet) include any copepod-derived sequences; however, other environmental sequences from [46] do group in the ‘parasites of Daphnia’ clade (data not shown). Sequence types A, B, and C from Jones et al. (2012) [106] are specifically labelled to clarify their identity, which includes the first description of *Facilispora margolisi* infecting *Lepeophtheirus cuneifer* and *L. parviventris*.

Figure 5. A subset of symbiont types found in copepods.
Symbionts affect a range of tissues (as indicated by the position of the small circles on the copepod) and have a variety of impacts on their hosts (severity of impact indicated by the shade of the larger circles surrounding the symbionts), ranging from lethal (black circles) to little or unknown impact (light-grey circles). In many cases the nature of the host-symbiont interaction is unknown.

Box 1. Pathogens, parasites, symbionts, and pathobiomes

In this review we use the term ‘symbiont’ (collectively ‘symbiome’) to represent any member of the set of host-associated organisms (including viruses), encompassing negative, neutral, and positive interactions. ‘Symbiont’ is an appropriate term per se, but also because it does not assume a particular type of interaction, for example, parasitic (feeding on host) or pathogenic (causing disease), when the nature of the interaction is unknown or context-dependent. It also does not assume an extended residence time; the duration of association between host and symbiont is very often unknown. The terms ‘parasite’ and ‘pathogen’ are used when the relationship is known to negatively impact the host. In any case there is of course significant overlap between these two categories of interaction with the host.

It is worth noting that ‘parasite’ and ‘pathogen’ are often used inconsistently. Further, an organism that is pathogenic in one host may or may not be in others, and its virulence may vary widely even in a single host. The reasons underlying this variability are many but they center on interactions between, and within, the multiplicity of symbionts associated with most organisms, the host organism, and the environment. The ‘symbiotic continuum’ [33] illustrates how different symbionts represent a range of host association types that are context-dependent, and thus variable across space and time.

The related pathobiome concept [105] recognizes that host health is potentially influenced by all symbionts (bacteria, viruses, and eukaryotes), their own interactions, and those with the host and surrounding environment. Consequently, the definition of ‘symbiont’ must be expanded to include all host associations, however transient, and whether or not the nature of the association is currently

known [105]. This is a useful basis for understanding the roles of symbionts of copepods as more of their diversity is revealed and functional interpretations of multiagent, holobiotic systems are investigated.