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REVIEW, CONCEPT, AND SYNTHESIS

The food source of Sargasso Sea leptocephali

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Abstract

The mysterious food source of anguilliform leptocephali has been difficult to understand, so this review evaluates potential interrelationships among recent discoveries on this subject. There are typically few identifiable gut-content objects in leptocephalus intestines, which usually contain amorphous materials. Gut content observation studies and stable isotope research have suggested that marine snow detrital-type particles are a food source, but this was difficult to validate. Recent gut-content DNA-sequence analyses indicated that small 4–25 mm Sargasso Sea European eel larvae, *Anguilla anguilla*, frequently ingest calycophoran siphonophore tissues as well as other taxa not likely to be ingested individually. A high-magnification photographic study of Sargasso Sea leptocephalus gut contents recently detected possible hydrozoan tentacles and apparent fatty acid-rich single-celled, heterotrophic thraustochytrid protists (class Labyrinthulomycetes), which have been found in marine snow in previous studies, but are not amplified by some DNA primers. Calycophoran siphonophores are abundant in the Sargasso Sea and have extensive tentacle arrays and short-lived eudoxid reproductive stages that might be appropriate sizes to be eaten directly or contribute to marine snow aggregates. The two groups may be interrelated because thraustochytrids are ubiquitously present decomposers that colonize detrital materials in oceanic and coastal ecosystems, so both siphonophore tissues and thraustochytrids may be present in marine snow consumed by European eel and other leptocephali. Therefore, future research on what leptocephali consume as food should be approached from a size-scaling perspective using systematic direct gut-content observations in combination with appropriate primers for next-generation DNA sequencing.

Introduction

During larval surveys to find the spawning areas of anguillid eels that started with the Danish expeditions conducted by Johannes Schmidt and then continued in the North Atlantic and Indo-Pacific, many thousands of anguillid and other anguilliform larvae (leptocephali) were collected and examined microscopically (Schmidt 1935; Smith 1989; Miller 2009; Miller and Tsukamoto 2017). Remarkably, during examinations of all those collected leptocephali, no identifiable zooplankton were ever reported to be seen in the intestines of the larvae. This led to speculation about what these highly-transparent, laterally compressed larvae feed on (Pfeiler 1986; Westerberg 1990). Leptocephali are widely present at tropical to southern temperate latitudes, they have various unusual morphological and physiological characteristics, and they grow to large sizes (60 to > 300 mm) (Smith 1989; Miller 2009). Because fish larvae typically feed on zooplankton (Nunn et al. 2012) and considerable information has accumulated about their digestive physiology in recent decades (Rønnestad et al. 2013), it was a unique situation to not know what leptocephali feed on.

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While routine observations of oceanic eel larvae were conducted for identification, various amounts of indistinct amorphous gut content materials could often be seen. Eventually, non-living particulate organic matter (POM) objects such as discarded appendicularian houses and zooplankton fecal pellets that are classified as being contributors to marine snow were observed in leptocephalus gut content studies (Otake et al. 1993; Mochioka and Iwamizu 1996; Miller et al. 2011). Many types of detrital materials and bacterial and phytoplankton exudates can aggregate into marine snow, and particles can be colonized by a wide range of prokaryotic and eukaryotic species (Alldredge and Silver 1988; Shanks and Walters 1997; Kiørboe 2000). Important contributors to marine snow formation are transparent exopolymer particles (TEP) that act as glue to facilitate particle aggregation (Mari et al. 2017) and contain carbohydrates (Skoog et al. 2008). Some types of zooplankton feed on marine snow particles (Alldredge 1976; Dilling et al. 1998; Dilling and Brezenzinski 2004), so it has been hypothesized that leptocephali can ingest some types of marine snow as a food source and obtain nutrition from components of the material they ingest (Otake et al. 1993; Mochioka and Iwamizu 1996; Miller et al. 2011, 2013).

But the feeding behavior of leptocephali in the ocean has not been observed, and the exact source of the amorphous material that usually forms the majority of gut contents is not yet known. Preliminary stable isotope analysis of a few taxa of leptocephali (Otake et al. 1993), more detailed studies of leptocephali and a range of food-web species and POM (Feunteun et al. 2015), and the low trophic position of small Japanese eel leptocephali (Miller et al. 2013) were also consistent with the leptocephali obtaining nutrition from marine snow. But other than appendicularian houses and fecal pellets, what types of marine snow leptocephali feed on could not be determined.

DNA sequencing studies then found high proportions of sequences of hydrozoans (Cnidaria) in the amorphous gut contents of small European eel, *Anguilla anguilla*, leptocephali in the Sargasso Sea (Riemann et al. 2010; Ayala et al. 2018). Both studies also found a wide range of taxonomic groups in the gut content material in addition to either the comparatively high proportions of hydrozoan sequences or reads/amplicons (Riemann et al. 2010; Ayala et al. 2018). Relatively large Sargasso Sea marine snow particles were also used for DNA sequencing, which frequently contained high proportions of copepod sequences along with those of hydrozoan and other taxa (Ayala et al. 2018; Lundgreen et al. 2019). The most abundant type of hydrozoan sequences in both leptocephalus gut contents and marine snow particles were calycophoran siphonophores, which are abundant in the Sargasso Sea (Ayala et al. 2018; Lundgreen et al. 2019; Lüskow et al. 2019). Hydrozoan DNA sequences including those of calycophoran siphonophores were also confirmed to be present in the gut contents of larger leptocephali in the western North Pacific, but the external surfaces of the skin outside the intestines were suggested to be a source of DNA sequence contamination in the Sargasso Sea gut content studies (Chow et al. 2019).

Investigations of the gut contents also continued and higher magnification photographic examinations of the gut contents of leptocephali in the western North Pacific (Tomoda et al. 2018) and Sargasso Sea (Miller et al. 2019) found the presence of small spherical or ovoid objects. In the Sargasso Sea larval gut contents, distinctly round objects were present that appeared to be the single-celled fungus-like heterotrophic thraustochytrid protists (kingdom Straminipila, class Labyrinthulomycetes or Labyrinthulea) (Raghukumar 2002; Raghukumar and Damare 2011; Marchan et al. 2018), based on an apparent lack of visual similarity to other similarly shaped organisms of that size range. Thraustochytrids appear to colonize any type of detrital material (Raghukumar 2002) and they have been detected in marine snow aggregates (Li et al. 2013; Bochdansky et al. 2017), so their presence would not be unexpected if the larvae were feeding on marine snow particles (Miller et al. 2019).

It was also determined recently that artificially cultured Japanese eel leptocephali would consume phytoplankton and their TEPs and appendicularian houses (Tomoda et al. 2015) or particulate material collected in the ocean (Chow et al. 2017). These larvae had previously been feeding on an unnatural paste diet (e.g., Tanaka et al. 2001), but when offered those materials in the laboratory or oceanic particulate material on board a research cruise they would consume the POM materials. Little growth was observed in the oceanic trials, so it may not have been the type of POM that natural larvae typically consume (Chow et al. 2017).

While feeding trials have shown the ability of the larvae to consume POM, and some of the amorphous material and visible objects in leptocephalus gut contents can be considered to have originated from the ingestion of particles classified as marine snow, how eel larvae in the Sargasso Sea may ingest tissues of calycophoran siphonophores, which have complex tentacle systems and multi-stage life histories (Carré and Carré 1991; Bouillon et al. 2006; Mapstone 2014) is not known. The possible presence of thraustochytrids in marine snow that could be consumed by leptocephali also needs to be evaluated to help direct future research. Therefore, this review examines the diet of Sargasso Sea European eel and other species of leptocephali by briefly overviewing leptocephalus gut content studies, the basic life-cycle biology, morphology and size scaling of calycophoran and other siphonophores, and the newly realized possible presence of thraustochytrids in marine snow aggregates and leptocephalus gut contents. Combining these three types of information suggests that both groups of organisms may be components of marine snow aggregate formation in oligotrophic offshore

ocean areas such as the Sargasso Sea where temperate anguillid eel spawning areas are located, and leptocephali might also directly feed on some siphonophore life stages. These new types of information and knowledge gained from future research may be important for understanding the ecology of European eel larvae, which are one piece in the puzzle regarding what caused the drastic population decline of this species in recent decades, because larval survival could be a factor associated with the decline (Miller et al. 2016; Westerberg et al. 2018).

Visual observations of gut contents of leptocephali

Most collected leptocephali do not have much gut content material in their tubular intestines, probably because their bodies are soft and flexible and a lot of the material is evacuated while they are being agitated in the codend of nets after they are captured. All studies that have examined leptocephalus gut contents have observed various amounts of amorphous material in the intestines. The gut contents of leptocephali collected near coastal Japan included amorphous materials, but also zooplankton fecal pellets (Otake et al. 1993) and frequently contained discarded appendicularian houses (Mochioka and Iwamizu 1996). Govoni (2010) found amorphous or particulate material and small protists such as ciliates, dinoflagellates, and unidentified ovoid cells in the intestines of leptocephali from the continental shelf of the northern Gulf of Mexico. Photographs of the gut contents of leptocephali from the offshore western North Pacific and the Indonesian Seas also documented amorphous materials, appendicularian houses, fecal pellets and small round objects in the intestines (Miller et al. 2011; Tomoda et al. 2018). The most recent photographic study of gut contents was conducted on leptocephali in the Sargasso Sea, which included small European eel larvae 5–23 mm (Fig. 1) and other species of leptocephali whose intestines contained the same types of objects and amorphous materials (Miller et al. 2019) that were observed in Indo-Pacific species. Visible objects such as appendicularian houses and their associated fecal pellets and possible hydrozoan objects were seen in some of the leptocephali (Figs. 2, 3; Miller et al. 2019).

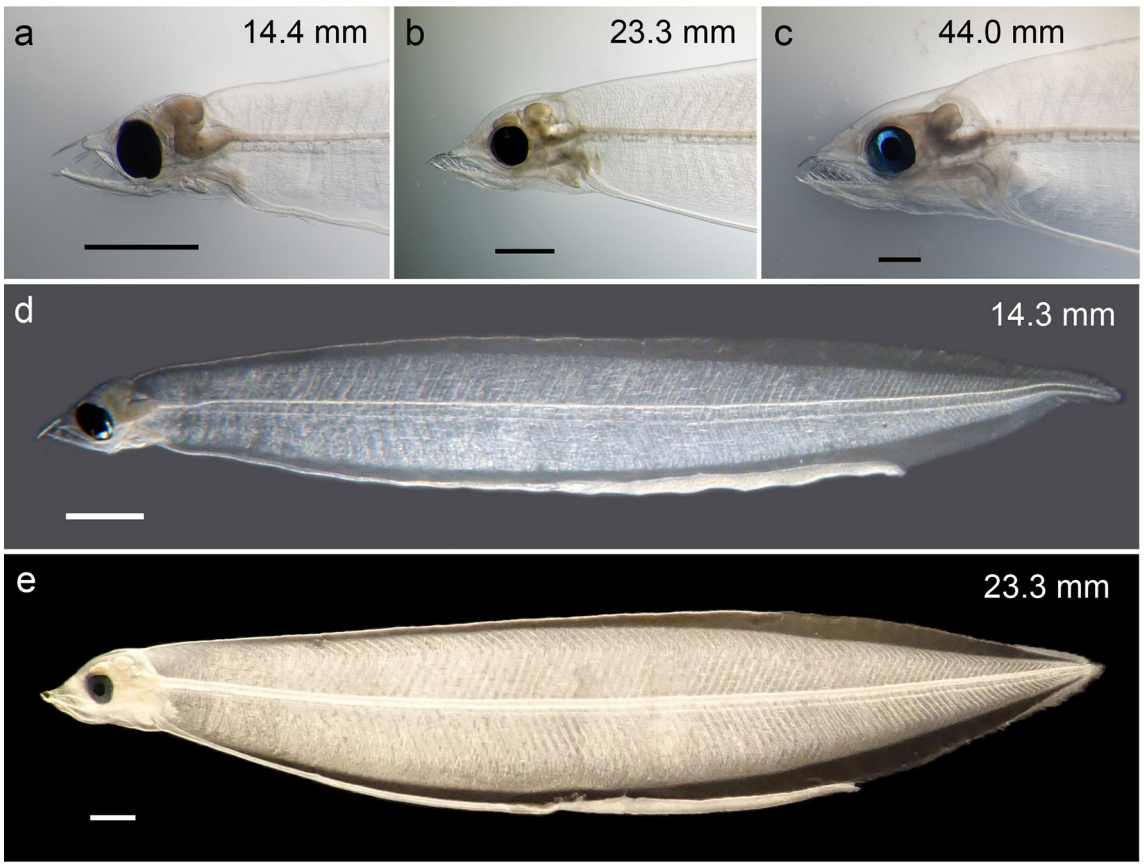

Fig. 1 Photographs of the head regions of **a** 14.4 mm *Anguilla anguilla*, **b** 23.3 mm *Anguilla rostrata* whose whole body is shown in **e**, 44.0 mm *A. anguilla* leptocephali modified from Miller et al. (2019), and **d** the whole body of a 14.3 mm *A. anguilla*. The different sizes of larvae show fewer proportionally longer teeth in the small larvae compared to the larger ones. Scale bars: 1 mm

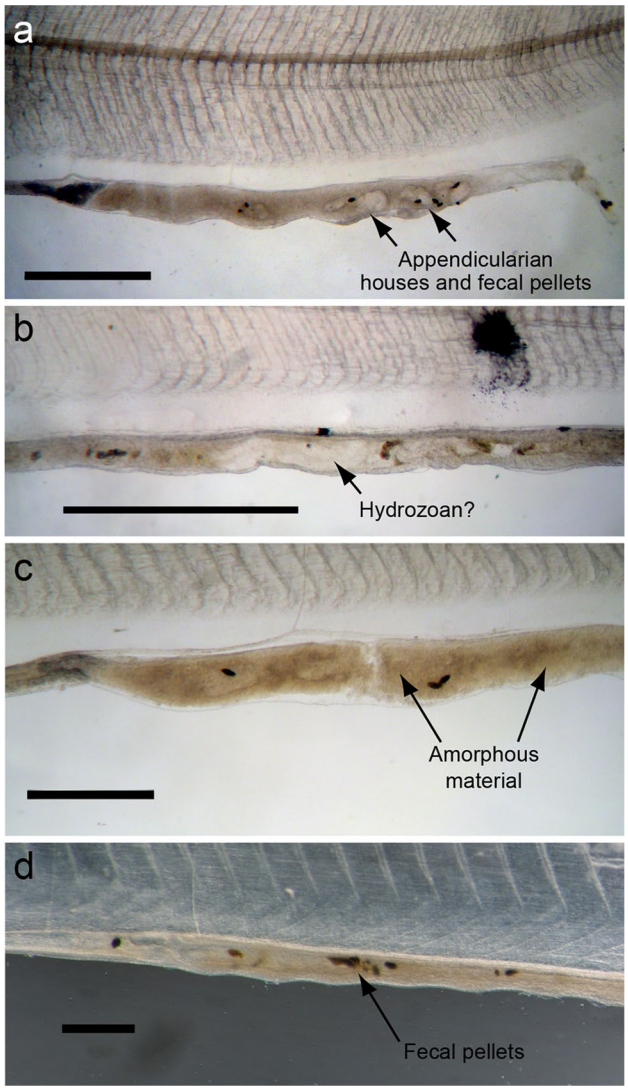

Fig. 2 Images of the intestines of **a** a 13.0 mm *Anguilla anguilla* that contain amorphous material, apppendicularian houses and fecal pellets, **b** a 20.1 mm *Avocettina infans* that contains translucent and pigmented objects that appear different than typical apppendicularian houses and could be hydrozoan tissues, amorphous materials, appendicularian houses, and fecal pellets inside the intestines of leptocephali of **c** a 14.0 mm *Anguilla rostrata*, and **d** a 43 mm *Anarchias similis* (Muraenidae) modified from Miller et al. (2019). Scale bars 1 mm

There was a lack of information about what was present in the amorphous materials in the gut contents though, until high-magnification oil-immersion microscopy imagery showed some of the types of materials in the intestines of several types of leptocephali in the Sargasso Sea (Miller et al. 2019). Those images showed that a wide range of shapes and sizes of mostly round or oval objects and amorphous material comprised of smaller objects were present (e.g., Fig. 3b), which might include many types of organisms, including fungi-related taxa that can be in marine aggregates (Richards et al. 2015; Bochdansky et al. 2017; Leonard et al. 2018) or other organisms. The most distinctive objects present in the highly magnified gut contents images were round ≤ 40 µm objects with many small particles inside them (Fig. 3a–d). Intensive searches of imagery and evaluations of sizes of marine nanoplankton (2–20 µm) and microplankton (20–200 µm) revealed that these objects appear identical to thraustochytrid life stages or species with round body forms (Fig. 3f, g; Miller et al. 2019). The apparent thraustochytrids in the leptocephalus gut contents seem to most resemble *Thraustochytrium striatum* (Boro et al. 2018) and *T. gaertnerium* (Bongiorni et al. 2005a) and are also similar to some *Parietichytrium* (Ou et al. 2016) and *Aurantiochytrium* or *Schizochytrium* species (Fig. 3f; Manikan et al. 2015; Fig. 3g; Boro et al. 2018).

Interestingly, a video of the gut contents flowing out of the intestine of a 19.0 mm European eel larva that are seen in photographs in Fig. 4 shows that many round objects were present within the amorphous materials (Supplementary video), which could be thraustochytrid cells. A piece of apparent exoskeletal material of unknown origin can also be seen. Many small round or oval objects were also visible in the gut contents flowing out of the intestine of a 33 mm Synaphobranchidae leptocephalus collected in Tomini Bay in Indonesia (Fig. 2 of Miller et al. 2011).

DNA sequences in leptocephali gut contents

The two studies on the DNA sequence content of European eel larvae collected within their Sargasso Sea spawning area provided important new information about what types of organisms or tissue-material might be contained in the whole-intestine contents of those leptocephali. The first study used 18S rRNA gene barcoding and found that hydrozoan sequences were present in 55% of the 42 European eel larvae intestines that contained DNA sequences (4.5–14.5 mm) (Riemann et al. 2010). Although no types of DNA were detected in 19 of the 61 larvae tested, sequences of a wide range of marine organisms of 17 eukaryotic taxonomic lineages, such as radiolarians (40% of larvae with sequences), copepods, chaetognaths, and dinoflagellates were also detected in the other larvae. Hydrozoans had 14 OTUs (unique sequences), and the remaining 61 OTUs were of other taxa, which included 18 radiolarian OTU sequences (Riemann et al. 2010).

The second study used next-generation 18S rRNA gene sequencing (NGS) and found a similar diversity of taxa, but hydrozoan DNA sequences were detected in the whole-intestine contents of all of the 75 European eel leptocephali (9.2–24.7 mm, 15.2 ± 2.6 mm) that were examined after being collected in March and April of 2014 (Ayala et al. 2018). The gut contents of the larvae were

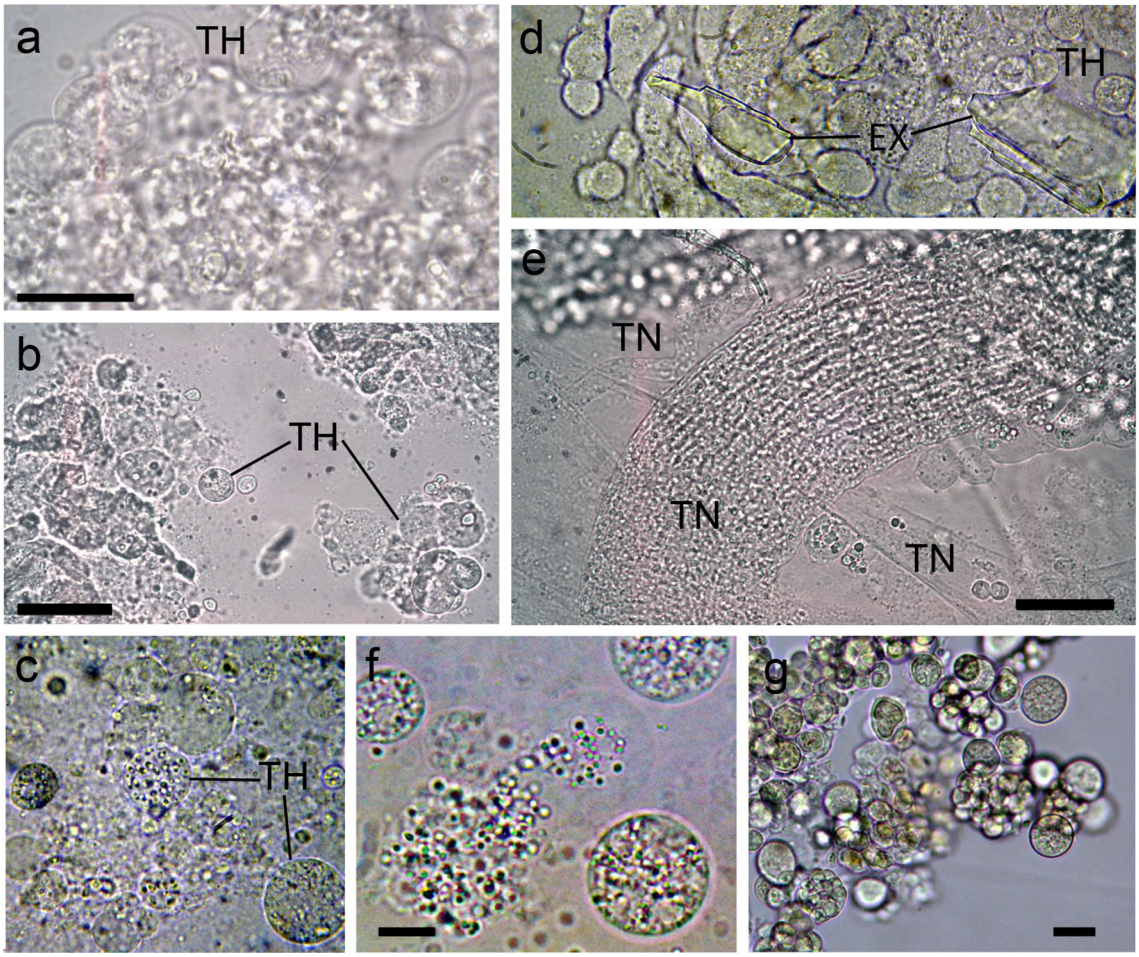

Fig. 3 High-magnification images of leptocephalus gut contents of **a** a 13.0 mm *Anguilla anguilla*, **b**, **c** a 27 mm *Eurypharynx pelecanoides* (Eurypharyngidae) that contain possible single-celled thraustochystrid protists (TH), **d** exoskeletal-like (EX) materials and **e** possible hydrozoan tentacles (TN), and **f** intact cells of the thraustrochytrid *Aurantiochytrium* and small zoospores released from another cell (small round objects) (modified from Manikan et al. 2015), and **g** aggregates of the thraustochystrid *Schizochytrium aggregatum* in the laboratory (modified from Boro et al. 2018). Scale bars **a**, **b**, **d**, **e** 50 µm, **f** 10 µm, **g** 20 µm

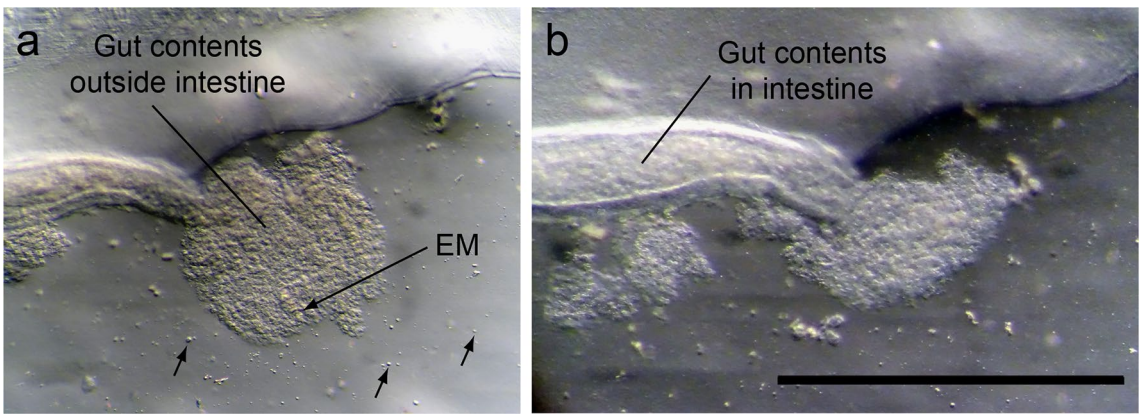

Fig. 4 Photographs of amorphous materials as they flowed out of the intestine of a 19.0 mm *Anguilla anguilla*. The gut content material is suspended in seawater added to the dish holding the leptocephalus. Arrows point to individual round objects that might be intact thraustrochytrid cells, and to a piece of apparent exoskeletal material (EM). Many round objects are present in the amorphous materials as can be seen more clearly in the Supplementary video. Scale bar **b**: 1 mm

visually examined with a dissecting microscope prior to the sequencing analyses (68% of guts were full) and were not found to contain distinguishable objects (*i.e.*, only amorphous material; Ayala et al. 2018). Among the sequences of 16 eukaryotic taxonomic lineages that were detected in the amorphous gut content material, 76% of the reads belonged to cnidarians (98% hydrozoans, 2% anthozoans) (Fig. 5a). The percentages of hydrozoan reads compared to other taxa was high in many of the leptocephali (Fig. 5b), but some larvae had gut contents that were not dominated by hydrozoan reads and had substantial read percentages of other taxa.

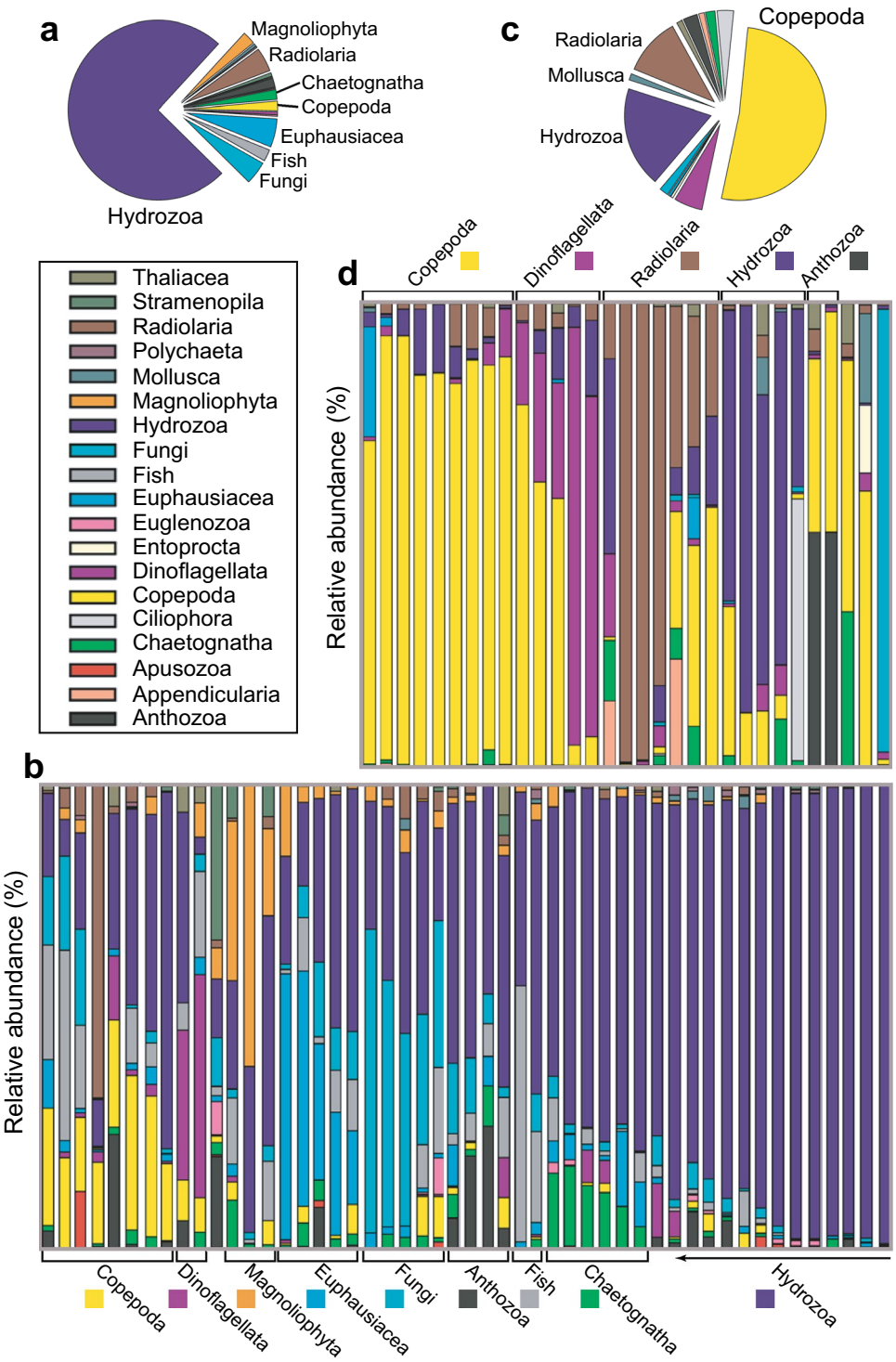

Fig. 5 Percentages of 18S rRNA gene sequence reads of various eukaryotic taxa that were detected in **a** the intestines of 61 European eel leptocephali collected in the Sargasso Sea in March and April 2014 in the study of Ayala et al. (2018), **b** proportions of reads of the taxa of 50 of the 75 individual leptocephali (one bar is one larva), **c** proportions of reads of the taxa detected in all 31 of the 1–10 mm size marine snow particles that were analyzed (Ayala et al. 2018; Lundgreen et al. 2019) and **d** the proportions of taxa in each of the marine snow particles. Plots are based on the 50 most abundant types of sequences that accounted for 92% of the reads in the gut contents and 82% in the marine snow. Selected taxa that are abundant in some individual larval gut contents or marine snow particles are labeled. Redrawn from Ayala et al. (2018)

A more recent next-generation 18S rDNA sequencing study of various larger sizes of 40 anguillid (*Anguilla japonica* and *A. marmorata*; 36.7–52.3 mm) and marine eel (6 families; 18.1–214.0 mm) leptocephalus gut contents verified that cnidarian (Anthozoa, Hydrozoa) sequences were present in the gut contents of some of the larvae (Chow et al. 2019). However, it was also determined that greater numbers of Cnidarian sequence reads including those of calycophoran siphonophores were detected in samples obtained from the body surfaces of the leptocephali. Cnidarian sequences were found in 18 of the 35 gut content samples and 13 of the 16 body surface samples (Chow et al. 2019). The studies of Ayala et al. (2018) and Riemann et al. (2010) both used whole intestines for analyzing the gut contents, which would have also included the external body surfaces. This suggests that some of the hydrozoan reads found by Ayala et al. (2018) and Riemann et al. (2010) could have come from the external skin surfaces associated with the intestines. All of the leptocephali from both the Sargasso Sea and Pacific studies were collected by plankton nets, so when the collected organisms of many taxonomic groups are concentrated in the codends of the nets, the DNA of many organisms likely becomes embedded in the mucus of the external surface of the body of each leptocephalus. This suggests that while hydrozoan sequences were likely present in the gut contents of the European eel larvae in the Sargasso Sea, some of the hydrozoan and other taxa sequence reads could have originated from the external body surface and not from the gut contents.

The primers used by Ayala et al. (2018), Riemann et al. (2010) and probably Chow et al. (2019) are not among the primers that are able to amplify thraustochytrid DNA (see below), so if those protists were present or not as suggested by the gut content imagery is unclear. Prokaryotic cyanobacteria (*Trichodesmium*, *Prochlorococcus*, *Synechococcus*) and bacteria sequence reads were found in the leptocephalus gut contents in the Sargasso Sea NGS study, but the proportions of taxonomic groups were different than those detected in the marine snow particles that were collected and analyzed using the same NGS methods in that study (Ayala et al. 2018).

Ayala et al. (2018) also found hydrozoan DNA sequences in more than 20 of the 31 marine snow particles they examined, and cnidarians comprised 21% of the reads in the particles (88% hydrozoan and 12% anthozoan reads) that included sequences of 13 eukaryotic taxonomic lineages (Fig. 5c, d). These lineages were mostly the same as those found in the leptocephalus gut contents, but the compositions and proportions in each marine snow particle were different than those of most individual larval gut contents (Ayala et al. 2018). Lundgreen et al. (2019) reported in greater detail on the 18S rRNA gene sequence content of the same marine snow particles used by Ayala et al. (2018) to compare to the leptocephalus gut contents. Many of those large (~ 1–10 mm) marine snow particles had high percentages of copepod reads, some had high radiolarian or dinoflagellate read proportions, but cnidarians were the second most abundant taxa overall (Lundgreen et al. 2019) as seen in Fig. 5c. Most significantly, the analysis of the marine snow particles clearly demonstrated that hydrozoan and anthozoan (corals, sea anemones etc.) tissues can aggregate into marine snow particles in that region of the Sargasso Sea. Four of the marine snow particles had more than 50% of their detected reads belonging to hydrozoans (Fig. 5d).

The comparison between the sequence composition of marine snow particles and gut contents did not find any clear evidence that the leptocephali had been feeding on the types of particles that were analyzed, so it seems useful to consider other factors that might contribute to why the 18S rDNA NGS study of Ayala et al. (2018) found such a high proportion of siphonophore sequence content in the leptocephalus intestinal materials even though no hydrozoans could be seen in the amorphous materials contained in the gut contents. One possibility suggested by Ayala et al. (2018) is that siphonophores are consumed, but they digest very quickly. Contamination from the external surface of the gut is also a possible factor, but Chow et al. (2019) also found that cnidarian sequences were the most abundant taxonomic group in the leptocephalus gut contents, just not to the same extent as in the Ayala et al. (2018) study.

Another possibility is that smaller quantities of siphonophore tissues are ingested along with other materials as suggested by the diversity of sequences found, but their DNA amplifies well compared to some other taxa. For example, a 2% amplification efficiency difference between two taxa can cause a 30% difference in sequence abundance after 35 PCR cycles (Pompanon et al. 2012). The gut content of the European eel larvae seen in the Supplementary video for example, does not seem to be filled with material from digested siphonophore bodies, but they do contain a few pieces of material that could be from siphonophores.

A potentially even more important factor is that there are significant differences in rRNA gene copy numbers per cell among marine species or strains of organisms (Herrera et al. 2009; Galluzzi et al. 2010; Schirrmeister et al. 2012; Perisin et al. 2016). This is an increasingly recognized problem in 18S rDNA NGS surveys of taxonomic abundances based on counts of the number of amplicons of each taxa after PCR (Kembel et al. 2012; Louca et al. 2018). For example, rRNA gene copy numbers were found to vary widely in photosynthetic picoeucaryotes (Chlorophyta), which greatly influenced the quantitative PCR results among species (Zhu et al. 2005). The rRNA gene copy number appears to increase with genome size in both plants and animals (Prokopowich et al. 2003), which is likely related to the critical role of that gene in protein synthesis.

Considering the ability for rapid tissue growth and regeneration in hydrozoans such as of tentacles (Siebert et al. 2015; Leclère and Röttinger 2017) and the critical role of muscle cells and proteins in tentacle function (retraction, extension) (see next section), it is possible that tentacle and other hydrozoan tissues (excluding gelatinous parts) are enriched in rRNA gene copy numbers to facilitate rapid transcription of mRNA for protein production. Ribosomal DNA gene copy number can be coupled with gene expression (Gibbons et al. 2014) and rapid growth. Siebert et al. (2011) used NGS methods to show that mRNA expression was higher in the gastrozoids (for prey capture) than the transparent nectophores (for swimming) of a siphonophore, as was found for another hydrozoan (Sanders et al. 2014). These types of tissue areas show rapid cell growth for new tissue formation (Siebert et al. 2015). Similarly, short development-duration copepod species (faster growth) have higher copy numbers of 18S rRNA genes than slower development species (White and McLaren 2000). A cnidarian ctenophore species was found to have very different characteristics of numbers of transcripts of rRNA compared to other types of animals (Francis et al. 2013). The 18S rRNA genes copy numbers do not seem to have been studied yet in hydrozoans, but if they have higher copy numbers than most of the other marine taxa detected in the leptocephalus gut contents by Ayala et al. (2018) and Chow et al. (2019), this would have made their relative abundance (reads per cell) appear larger.

Siphonophore life history and presence in gut contents

Understanding how parts of structurally complex siphonophores may enter the food materials of leptocephali requires an examination of their morphology and the biology of their different life history stages. Hydrozoans are cnidarians with multi-stage life histories that can include planula larva, polyp, and medusa stages, and complex morphological structures for swimming, prey capture, digestion, or defense (Bouillon et al. 2006; Leclère et al. 2016; Siebert and Juliano 2017). Siphonophores are pelagic colonial hydrozoans that include about 175 species in 16 families and 65 genera (Mapstone 2014). They lack the polyp and medusa stages of other hydrozoans, but still have multi-stage life histories (Fig. 6c) that include nectophores for swimming propulsion and gastrozoids for feeding (Dunn 2005). They vary in size from millimeters to several meters in length when their tentacles are included. Smaller species tend to live in the epipelagic layer (0–300 m) and larger more fragile species live in the mesopelagic zone (Mackie et al. 1987; Mapstone 2014).

There are 3 main groups of siphonophores, which are the suborders Cystonectae, Physonectae and Calycophorae (Mapstone 2014), and the diversity of shapes and structures of hydrozoans have been shown for many species (Bouillon et al. 2006). The complexity of tentacle

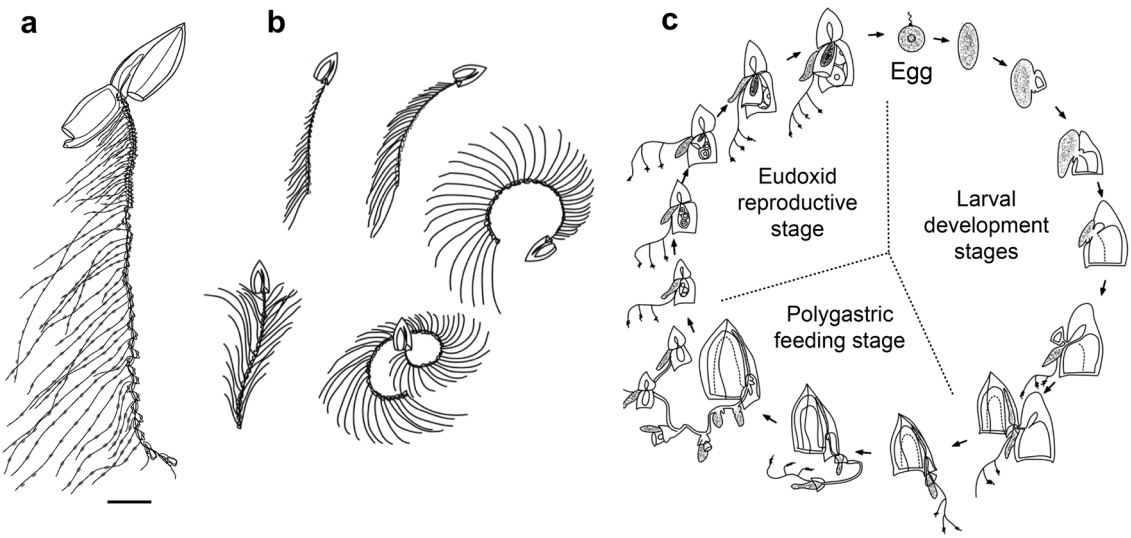

Fig. 6 Diagrams of calycophoran siphonophores showing *Lensia conoidea* with its tentacle array fully extended (**a**; modified from Mapstone, 2014), a sequential depiction of the movements of *Muggiaea atlantica* to deploy its tentacle array for feeding on small copepods (**b**; modified from Blackett 2015-originally drawn by Mackie and Boag, 1963), and the life-cycle stages of *Muggiaea kochi* that were reared in the laboratory (**c**; modified from Blackett 2015-originally drawn by Carré and Carré 1991). At 24 °C, the larval development period took 7 days, and after 1–2 days of feeding during the polygastric stage the eudoxid stages were released that matured during the next 5 days and released their eggs and then died. The dead eudoxid bodies would then contribute to POM in the water column and are a likely candidate for being consumed by leptocephali in the same way as discarded appendicularian houses

structures of siphonophores has also been shown clearly in recent studies or reviews (Haddock et al. 2005; Dunn and Wagner 2006; Mapstone 2014; Siebert et al. 2015). The main tentacle of siphonophores has many gastrozooids that each have one tentacle, which can have side branches with a nematocyst battery (Purcell 1984; Mackie et al. 1987). Using their tentacle systems and nematocysts, siphonophores feed on small invertebrates and fish, and various types of larvae (Biggs 1977; Mackie et al. 1987; Colin and Costello 2007; Mapstone 2014).

The small hydrozoans that predominate in the upper 300 m of the open ocean are mostly the widely present calycophoran siphonophores that consist of 106 species (Mapstone 2014). Calycophoran siphonophores were recently found to be the most abundant gelatinous zooplankton in the southern Sargasso Sea (Ayala et al. 2018; Lüskow et al. 2019), and they are the main type of siphonophore sequences found in both the leptocephalus gut contents and marine snow particles (Ayala et al. 2018; Lundgreen et al. 2019). Calycophoran siphonophores have complex tentacle systems (Fig. 6a) with batteries of nematocysts that mostly capture small copepods. Their feeding tactics were described by Mackie et al. (1987) as swimming in short bursts to spread out their tentacle arrays and waiting for prey to contact them (Fig. 6b). When prey contact a part of the tentacle array, they can become entangled in the tentacles and nematocysts that adhere to the surface of the prey before they are pulled up to the gastrozooids where they are digested (Purcell 1984; Mackie et al. 1987).

Damage to these extensive tentacle arrays is one obvious way that siphonophore tissue could be released into the ocean and become POM or marine snow. Calycophoran tentacle systems have numerous small structures associated with their batteries of nematocysts that are < 30 µm (Purcell 1984; Mackie et al. 1987). Some of these structures could theoretically break off while a prey animal is struggling to escape from entanglement, or while a larger animal accidentally swims through the tentacle array. Discharged or undischarged nematocysts of some hydrozoans are about ≤ 10 µm in size and form oval shapes, and the tubules of discharged nematocysts are much thinner (Colin and Costello 2007). The detailed morphology of *Abylopsis* (Fig. 6a) and other siphonophore tentacle filaments and nematocyst structures have been shown to be small enough (Kass-Simon and Scappaticci 2002) to be in marine snow aggregates that leptocephali could eat. For example, the nematocysts and tentacle diameters of the hydrozoan *Physalia utriculus* (Yanagihara et al. 2002) are small enough, and hydromedusa tentacles would appear large, similar to the tentacle-like objects seen in the *Eurypharynx* gut contents (Fig. 3e), but individual nematocysts would be < 20 µm (Corrales-Ugalde et al. 2017).

Another way that siphonophores could contribute to marine snow or be eaten directly is through the release of their short-lived reproductive-stage eudoxid stages with gonophores (Carré and Carré 1991; Fig. 6c). The eudoxids detach from the siphonophores, they release their eggs, and then die. These temporary eudoxid bodies could then be colonized by other organisms or aggregate with other detrital materials. Leptocephali could feed on these aggregates or directly on appropriately sized eudoxids. The smallest of the eudoxid stages (including gonophores) range from about one to several millimeters (Grossman et al. 2014). One gelatinous object was observed in a leptocephalus intestine that does not seem similar to an appendicularian house (Fig. 2b), which could be some kind of siphonophore object, such as an eudoxid. A similar object was seen in a previous study (Fig. 10c, d of Miller et al. 2011). Calcophoran siphonophores are abundant in the northern Sargasso Sea near Bermuda and their feeding-stages and eudoxids have peaks during the winter and spring months (Fig. 7; Lo and Biggs 1996) when small anguillid and other leptocephali would be present in the European eel spawning area. Species such as *Ablyopsis eschscholtzii* (most abundant), *Ablyopsis tretragona*, *Eudoxoides spiralis*, and *Eudoxoides mira* were abundant within the European eel spawning area in March and April (Lüskow et al. 2019) and they have peaks in abundance and eudoxid production near Bermuda during the early part of the European eel spawning season (Fig. 7), which is in February and March (Miller et al. 2015). Calycophoran siphonophores of several species similar to *Ablyopsis eschscholtzii* were found to comprise 11% of the DNA sequence reads in the marine snow particles in the Sargasso Sea (Lundgreen et al. 2019).

Calycophoran siphonophores are usually most abundant in the upper 100–400 m, with some species showing diel vertical migrations (Andersen et al. 1992), so their depth distributions directly overlap with leptocephali (Castonguay and McCleave 1987). Therefore, the production of the eudoxid reproductive stages, which then become POM/marine snow after they die, appears to be a likely mechanism for how calycophoran DNA is entering small European eel leptocephalus gut contents.

However, siphonophores develop from fertilized eggs, so some of the earliest life history stages might be small enough to be eaten directly by leptocephali. Several stages of the calycophoran siphonophore *Abylopsis tetragona* were shown at sizes of about 1 mm or smaller (Carré 1967). Eggs of a hydrozoan were about 30 µm (Prudkovsky and Neretina 2016), but eggs of other species were 150–275 µm (Freeman 1983). Planula larvae of the siphonophore, *Narzornia bijuga*, are > 100 µm wide (Sherlock and Robison 2000), but it is unclear how many eggs or larval stages would be the appropriate size for leptocephali to possibly ingest. Therefore, it seems possible that sources of DNA from siphonophores

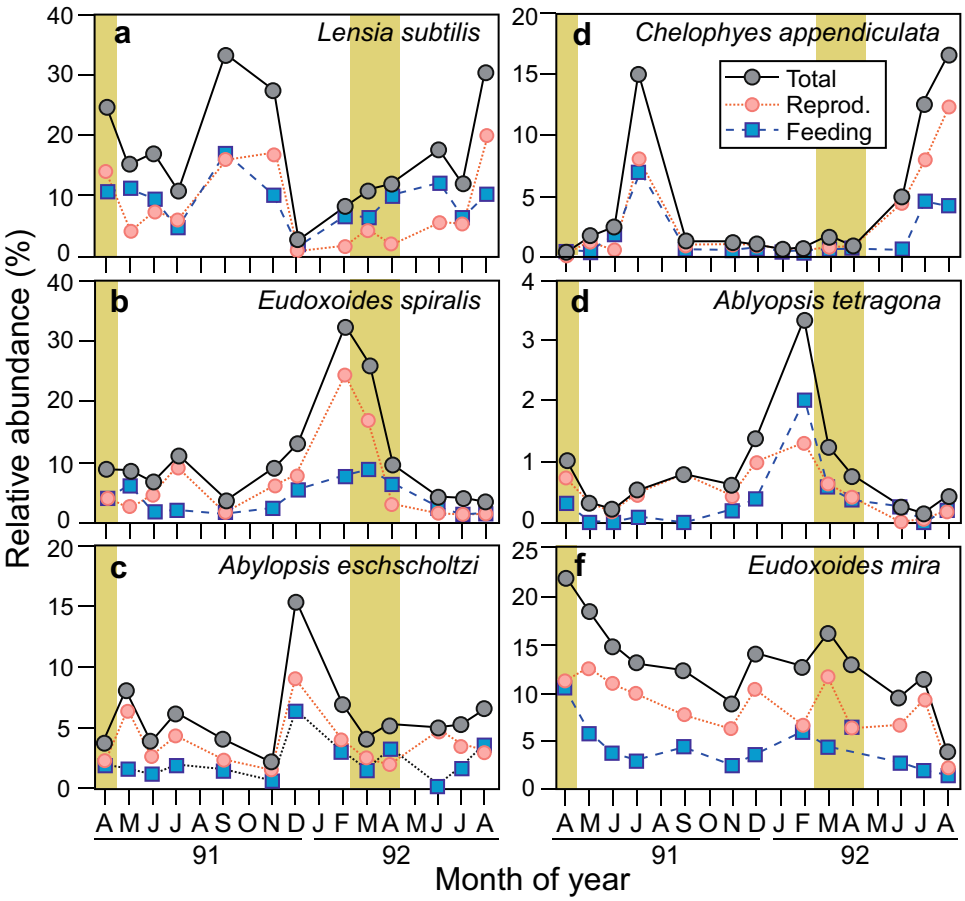

Fig. 7 Monthly relative abundance of the calycophoran siphonophore polygastric feeding stages (squares), the reproductive eudoxid stages (red circles), and the total of both stages (gray circles) during nighttime in the upper 100 m at the Bermuda Atlantic Time Series (BATS) station southeast of Bermuda in the northern Sargasso Sea. Vertical shading bars show the season when most anguillid leptocephali have been collected and studied for their gut contents. Redrawn from Lo and Biggs (1996)

in leptocephali gut contents could include eggs, larvae, or small-size individuals or eudoxid stages, as well as the contraction muscle fibers (Leclère and Röttinger 2017) or any other tentacle tissues that break off.

Thraustochytrid biology and marine snow

Although appropriate primers do not appear to have been used to validate if thraustochytrids can be present in the materials eaten by leptocephali, the images obtained of the gut contents of several species of Sargasso Sea leptocephali are strikingly similar to some thraustochytrid species as mentioned above. Thraustochytrids are part of the taxonomic group referred to as stramenopiles (or heterokonts), which include many groups such as diatoms, golden and brown algae, and organisms with flagella, as well as the Labyrinthulomycetes (Caron et al. 2012; Simpson et al. 2017). The thraustochytrids and the other labyrinthulean groups, are for convenience, mostly referred to here as thraustochytrids; but see below.

Until recently, thraustochytrid sequences have frequently been absent in NGS studies on oceanic plankton communities simply because specific PCR primers are needed to amplify most labyrinthulean 18S sequences (Singh et al. 2014). Therefore, the universal primers listed as being used in the leptocephalus gut content studies or in research on particle flux in the North Atlantic including near Bermuda (Amacher et al. 2009, 2013) did not have the ability to detect thraustochytrids. The biological oceanography of the northern Sargasso Sea is actually one of the most intensively studied regions of the world due to the Bermuda Atlantic Time Series station (BATS) (Steinberg et al. 2001), and various recent studies have been conducted to the south in the European eel spawning area (Andersen et al. 2011; Riemann et al. 2011; Richardson et al. 2014), but these studies do not mention thraustochytrids protists despite the fact that they must be present based on studies in other regions.

Although some earlier studies have detected thraustochytrids (López-García et al. 2001; Dawson and Pace 2002; Stoeck et al. 2003), there is little evidence of most past studies using universal primers detecting thraustochytrids; but previously unknown protist diversity including labyrinthuleans began to be found in microplankton or benthic communities (Moreira and López-García 2002). Now that specifically designed primers have been used in studies on thraustochytrids, they are being found to be widespread in

marine environments (Collado-Mercado et al. 2010; Nakai et al. 2013; Marchan et al. 2018).

The observations of what appear to possibly be thraustochytrids in the gut contents of leptocephali in the Sargasso Sea (Miller et al. 2019) is consistent with recent reports of their presence in marine snow in coastal waters (Lyons et al. 2005), the open ocean (Fig. 8b, Li et al. 2013), phytoplankton detritus (Fig. 8c, Raghukumar and Shaumann 1993), and the bathypelagic benthic zone (Bochdansky et al. 2017). In addition to being present in the open ocean (see below), thraustochytrids are among the heterotrophic labyrinthulomycete protists that colonize and break down the surfaces of detrital plant material in coastal waters, such as in mangrove

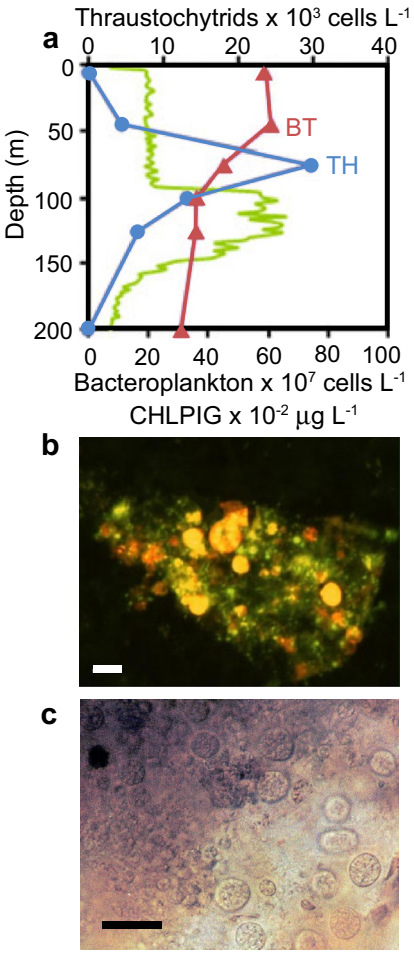

Fig. 8 Vertical distributions of thraustochytrid (TH) and bacterial (BT) abundance and chlorophyll concentration (line) at the Hawaii Ocean Time-series station ALOHA (22°45′ N, 158° W) in March 2010 (**a**), acriflavine-stained thraustochytrid cells (round yellow objects) in a marine snow particle from the station (**b**), that were modified from Li et al. (2013), and **c** a phytoplankton detritus sample from the North Sea viewed with contrast microscopy showing the presence of many round TH cells (modified from Raghukumar and Shaumann, 1993). Scale bar is 5 µm

areas (Bongiorni et al. 2005a; Ou et al. 2016; Boro et al. 2018).

Other groups of the Labyrinthulomycetes include species secreting ectoplasmic nets (aplanochytrids and labyrinthulids) that can extend across substrates (Leander et al. 2004; Ueda et al. 2015; Ou et al. 2016). *Aplanochytrium* cells have been recently observed in the laboratory to use these ectoplasmic nets to pull diatoms into their cell aggregations and systematically engulf their contents (Hamamoto and Honda 2019). Other species mostly just have individual round cells (thraustochytrids). Labyrinthulids are important in causing seagrass wasting disease (Sullivan et al. 2013). Some species are parasites on various types of marine organisms such as bivalves and cephalopods (Stokes et al. 2002; Raghukumar 2002; Schärer et al. 2007; Polglase 2019), and they are now being found to be abundant within aquaculture systems Boaventura et al. (2018). They have been enumerated in samples through direct counting, such as with pollen baiting (Raghukumar 2002; Gupta et al. 2012), epifluorescence (Raghukumar and Shaumann, 1993), and more recently using specific primers for 18S rDNA gene PCR amplification (see Marchan et al. 2018). There is presently a total of 40 species in 12 genera of thraustochytrids whose phylogeny has been examined (Honda et al., 1999; Yokoyama et al. 2007; Liu et al. 2014; Marchan et al. 2018).

Their actual abundance and widespread presence are only recently being recognized after research interest in their use in biotechnology or aquaculture stimulated a variety of studies because of their high omega-3 polyunsaturated fatty acid contents and production of extracellular polysaccharides that contain sugars and other compounds (Jain et al. 2005; Gupta et al. 2012; Liu et al. 2014; Singh et al. 2014; Chang et al. 2015; Marchan et al. 2018). They secrete a wide range of enzymes (Bongiorni et al. 2005b; Taoka et al. 2009; Liu et al. 2014) that break down detritus or likely the cell walls of other organisms during their heterotrophic feeding, apparently making them important competitors with bacteria to break down and remineralize organic materials in the ocean (Raghukumar and Damare 2011).

Thraustochytrid abundances have been studied in places such as the Seto Inland Sea of Japan (Naganuma et al. 1998; Kimura et al. 1999, 2001), the Arabian Sea (Raghukumar et al. 2001), the equatorial Indian Ocean (Damare and Raghukumar 2008), coastal waters and sandy shores of the Mediterranean Sea (Bongiorni et al. 2004), Greenland and Norwegian seas (Naganuma et al. 2006) and near Hawaii (Li et al. 2013). When direct enumeration methods or the appropriate PCR primers are used, thraustochytrids and other groups such as aplanochytrids have been found to be widely present in the oceanic water column (Hamamoto and Honda 2019), as was observed near Hawaii (Fig. 8a, Li et al. 2013) and other areas, with variations in abundance occurring with depth and season (Damare and Raghukumar 2006, 2008; Raghukumar

et al. 2001). Thraustochytrid peak abundance at a station near Hawaii shown in Fig. 8a occurred above the chlorophyll maximum layer, but their abundances varied with depth among stations/seasons (Li et al. 2013). Thraustochytrids can range from much lower to even higher than the bacterial volume/biomass at the same locations, with overall abundances being about 21–674 × 10^3 cells L^{-1} at the areas that have been studied through direct detection (see Raghukumar 2002; Raghukumar and Damare 2011; Singh et al. 2014) and maximum values being twice as high in some areas (see Marchan et al. 2018). Abundance estimates have also been made for surface waters around Japan using newly designed primers for quantitative PCR (Nakai et al. 2013).

The abundance of thraustochytrid cells may be linked to how much non-living POM is present, because they colonize detrital material. They can apparently also directly colonize TEP particles (Damare and Raghukumar 2012) and their numbers can be highest at the end of phytoplankton blooms when large amounts of TEP and POM are released (Raghukumar et al. 2001). Their numbers in or on particulate material may increase with time because they reproduce asexually through formation of zoosporangia and discharge of zoospores, which can result in one large individual splitting up into many small ones (see Fig. 3f and photographs in Bongiorni et al. 2005a; Boro et al. 2018). Thraustochytrids can feed on organisms such as bacteria (Raghukumar 1992) and diatoms (Hamamoto and Honda 2019), and ciliates, rotifers and zooplankton will eat thraustochytrids/aplanochytrids (Castillo et al. 2009; Damare et al. 2013).

Other interesting questions are related to the taxonomic composition of labyrinthuleans and how each group typically contributes to oceanic food webs. Genetic and other research has shown that the marine labyrinthuleans are mainly separated into 2 orders and 5 families (see Marchan et al. 2018). Hamamoto and Honda (2019) analyzed the labyrinthulean sequence read content from the Tara Oceans Project that sampled in many ocean basins (Pesant et al. 2015; de Vargas et al. 2015) and found they were widely present and were comprised of 45 aplanochytrid and 94 labyrinthulid (order Labyrinthulida), and 60 oblongichytrid and 131 thraustochytrid (order Thraustochytrida) OTUs. The widespread presence of these protists suggests the role of thraustochytrids in marine food webs have been underestimated or not yet clearly examined, and this might be particularly true regarding their possible presence in materials consumed by leptocephali.

Siphonophores, marine snow, and thraustochytrids

While many components of the concepts overviewed here remain to be validated, some consistencies and possible interrelationships between different types of information appear to be emerging. Objects defined as marine snow (fecal pellets, discarded appendicularian houses, possible eudoxid siphonophore stages or tentacles) are ingested by leptocephali in the Atlantic and Pacific, a diversity of taxa including hydrozoans that were genetically detected in Sargasso Sea marine snow were also detected in European eel larval gut contents (Riemann et al. 2010; Ayala et al. 2018), and a variety of types of small round objects were seen in photographs of leptocephalus gut contents in the Sargasso Sea (Miller et al. 2019) and western Pacific (Miller et al. 2011; Tomoda et al. 2018). Leptocephali can consume TEPs or particulate materials if they are offered to them (Tomoda et al. 2015; Chow et al. 2017).

These observations are all consistent with leptocephali consuming marine snow aggregates even if the larvae also directly consume some life stages of siphonophores or other soft-bodied organisms. For example, if leptocephali did not ingest most of those materials in marine snow aggregates and were feeding directly on the live individuals of polygastric feeding-stage siphonophores (Fig. 6), copepods, euphausiids, chaetognaths, or fish that have been genetically detected in their intestines (Fig. 5; and by Chow et al. 2019), at least a few individuals of those plankton/nekton species would likely have been seen in their gut contents as whole or partially digested objects; but they have not. For taxa such as radiolarians, ciliates, dinoflagellates, and fungi that may have been present in the gut contents (Riemann et al. 2010; Terahara et al. 2011; Ayala et al. 2018; Chow et al. 2019), these organisms generally seem too small for leptocephali to directly ingest them individually, and small protists that have been detected in coastal leptocephalus intestines (Govoni 2010) could have been ingested along with particulate material. It is known that marine snow can be comprised of diverse taxa (Alldredge and Silver 1988; Shanks and Walters 1997; Kiørboe 2000; Lundgreen et al. 2019) including bacteria (see Thiele et al. 2015), which could include all of the taxa detected visually or genetically in leptocephalus gut content studies. The video of gut contents flowing out of the intestine of the European eel larva is remarkably uniform in texture (Supplementary video), with many small round objects and with some exoskeletal-type objects that could be from a siphonophore or other type of zooplankton. This seems consistent with having consumed a particular type of marine snow because it does not seem likely that small round objects would be produced from digesting zooplankton stages.

The other interesting factors described above are that until relatively recently, thraustochytrids seem to have rarely been considered as being potentially important components of oceanic marine snow. Because they are becoming increasingly realized to be present in detritus and marine snow particles (Raghukumar and Shaumann 1993; Lyons et al. 2005; Li et al. 2013; Bochdansky et al. 2017), they may be directly

related to the materials consumed by leptocephali. In addition, because calycophoran siphonophores are the most abundant cnidarians in the Sargasso Sea (Ayala et al. 2018; Lüskow et al. 2019) and are contributing materials to marine snow aggregates (Ayala et al. 2018; Lundgreen et al. 2019), they may also have a link with thraustochytrids. All types of detrital materials can be substrates for thraustochytrid colonization (Raghukumar 2002), so hydrozoan tissues and appendicularian houses may also be good surfaces for colonization by thraustochytrid zoospores, which would then grow and multiply and become part of marine snow aggregates. Interestingly, both aplanochytrid labyrinthulids and calycophoran siphonophore DNA sequence reads were relatively abundant in the gut contents of a common Japan Pacific coast copepod species, suggesting a possible association between the two groups if the copepods were grazing on marine snow (Hirai et al. 2018). Also interesting is that the DNA sequence contents study of large leptocephali in the Pacific (Chow et al. 2019) found sequences of intestinal conoid parasites (phylum Apicomplexa, class Conoidasida, subclass Coccidia and Gregarinasina) that are found in many types of terrestrial and aquatic animals including marine fishes (Molnár et al. 2012; Lovy and Friend 2015; Rosenthal et al. 2016). They have apparently not been reported in other fish larvae intestines, but some of their life history stages form round shapes (Sitjà-Bobadilla and Palenzuela 1996; Girard et al. 2016) that can appear somewhat similar to round thraustochytrid cells.

While considering this set of potentially interrelated types of information and how to approach future investigations, factors such as size-scaling of food objects and the jaws of the larvae should also be considered. For example, the DNA sequence analysis of gut contents and relatively large marine snow particles both found the presence of similar taxa, but they were present in different proportions (Ayala et al. 2018; Lundgreen et al. 2019). However, the size of the teeth and jaws of the 14 mm European eel larva relative to the 1 mm scale bar in Fig. 1a, suggests that the ~ 1–10 mm marine snow particles used for NGS DNA sequencing (Lundgreen et al. 2019) in comparison to leptocephalus gut contents (Ayala et al. 2018) are too large to be ingested by most of the leptocephali that were analyzed (38 of the 61 larvae were ≤ 14 mm; 15 mm mean size, with only 7 larvae > 17 mm). In the Pacific, particulate material collected with mesh sizes smaller than 0.35 mm was found to be more suitable food material for the survival of artificially cultured leptocephali (Chow et al. 2017), which may suggest the composition of smaller particles is more appropriate for leptocephali.

In addition to possible contamination from DNA on the external surface of the leptocephalus body (Chow et al. 2019), the size-scaling factor may explain part of the mismatch between most individual marine snow particles and individual leptocephali that were studied in the Sargasso Sea (Ayala et al. 2018), since most of the larvae probably consumed smaller particles than were analyzed. The abundance and taxonomic composition of marine snow particles was also found to vary geographically in relation to hydrographic features of the Sargasso Sea (Lundgreen et al. 2019).

Feeding on small marine snow particles also seems most likely, because Munk et al. (2018) found that small marine snow particles (0.05–0.53 mm; 15–23 particles L^{-1}) were much more abundant than larger particles (medium and large, 0.53–4.0 mm; < 0.23 particles L^{-1}) in observations using an Underwater Vision Profiler at a station in the European eel Sargasso Sea spawning area (Fig. 9a). The depths with the highest abundances of small marine snow particles corresponded with the depth of the highest daytime catches of European eel larvae (Munk et al. 2018; Fig. 9a, b), which were just below the chlorophyll maximum at the bottom of the thermocline (Fig. 9b). As a result of vertical migration by the leptocephali (Castonguay and McCleave 1987; Munk et al. 2018), they were most abundant at night above the thermocline near 40 m in the warmer mixed layer, but some were also within or below the thermocline, possibly because the amount of vertical migration increases with larval size (Castonguay and McCleave 1987). This is also consistent with the nighttime depths of anguillid and other leptocephali relative to the thermocline in other studies (Miller 2015; Onda et al. 2017), and POM can sometimes be most abundant at or just below the bottom of the surface mixed layer (MacIntyre et al. 1995; Pilskaln et al. 2005).

The correspondence between the daytime abundance-depths of the European eel larvae and the highly-abundant small marine snow particles at a station in the Sargasso Sea (Munk et al. 2018) is interesting, and larger leptocephali may also be able to consume these smaller < 1 mm particles. The relative length and number of teeth in anguillid (Fig. 1a–c) and other leptocephali increase with growth (Schmidt 1916; Castle 1963, 1965, 1970), while the actual between-teeth distance may remain similar (Miller et al. 2019). This suggests that the long widely spaced teeth of small leptocephali (4 teeth on each jaw side in smallest larvae) are able to feed on relatively large particles compared to their head size, which could also be eaten by larger leptocephali (17–20 teeth on each side for *A. anguilla* and *A. rostrata*) due to their more numerous and narrowly spaced teeth relative to the length of the jaw (Fig. 1; Schmidt 1916; Miller et al. 2019). The forward pointing teeth of anguillid and other leptocephali (Fig. 1) do not appear designed for cutting into objects that are too large to ingest directly, but appear more functional for grasping soft whole objects (Westerberg 1990; Miller 2009; Miller et al. 2019).

While direct feeding on small soft organisms is possible for young leptocephali based on laboratory observations of rotifers being consumed by first-feeding larvae (Tanaka et al.

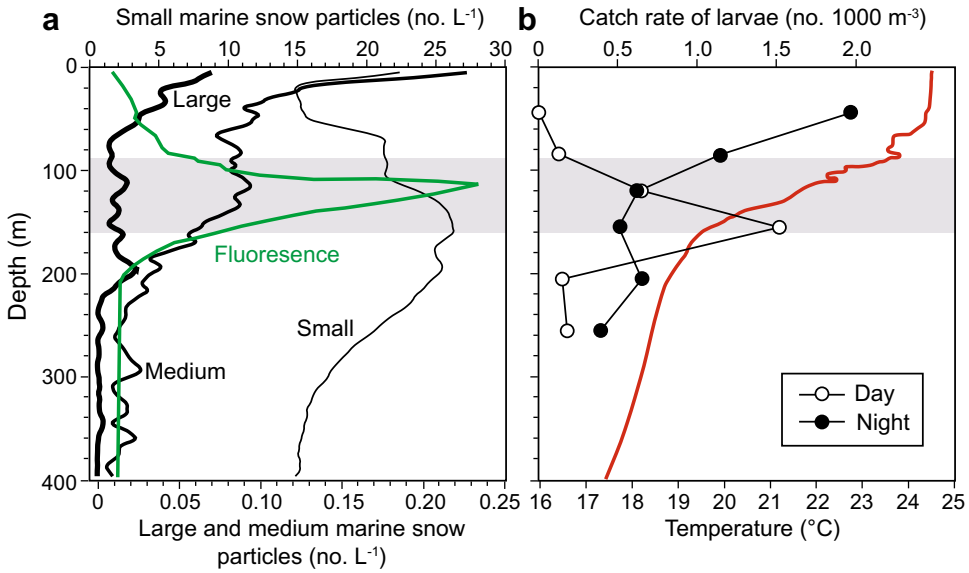

Fig. 9 Plots of observations made at a station in the spawning area of the European eel (25°38′ N, 62°48′ W) sampled from 31 March to 1 April 2014 by Munk et al. (2018) that show **a** the abundance of 3 sizes of marine snow particles in the upper 400 m (small: 0.05–0.53, medium: 0.53–1.06, large: 1.06–4.0 mm) that were recorded by an underwater video profiler in relation to the chlorophyll maximum layer (fluorescence scale not shown), and **b** the catch rates of European eel larvae at 6 depths that were sampled with a horizontally towed 3.5 m diameter mouth opening ring net deployed during the day (12:00–18:00) and night (00:00–06:00) in relation to the temperature structure. The approximate depths of the thermocline are shown with shading. Redrawn from Munk et al. (2018)

1995), the most frequently observed oval objects in the gut contents are consistent with being discarded appendicularian houses, because they usually have oval fecal pellets with them (Taguchi 1982), or appendicularian house filters have been visually confirmed (Mochioka and Iwamizu 1996; Miller et al. 2019). Some objects look different though (e.g., Fig. 2b) and could be hydrozoans. While the high siphonophore sequence read composition found in many of the Sargasso Sea or Pacific larval gut contents (Ayala et al. 2018; Chow et al. 2019) suggests some objects or materials that have been observed are from hydrozoans, it does not answer the question of what kind of tissues are typically ingested and if those tissues are nutritionally valuable. For example, discarded appendicularian houses aggregate together with other materials and are colonized by organisms such as ciliates (Hansen et al. 1996), so they could be targeted by leptocephali as much for the associated aggregates than for the houses themselves; and the houses are typically surrounded by amorphous material in the gut contents (Fig. 2a, c). This could also apply to the discarded post-reproduction eudoxid stages of calycophoran siphonophores (Fig. 6c).

The stable isotopic signatures of leptocephali have not yet been studied in the Sargasso Sea to compare their trophic position to other food web components such as siphonophores, but in the western Indian Ocean cnidarians were found to have at least slightly higher trophic levels (∂^{15}N) than leptocephali and had more enriched ∂^{13}C levels (Fig. 10). This suggests that the leptocephali were not

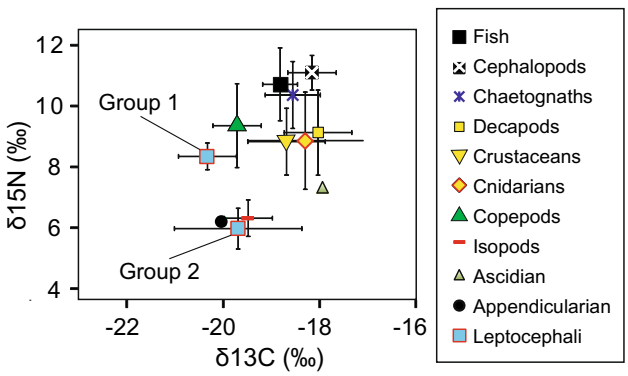

Fig. 10 Plots of average ∂^{15}N and ∂^{13}C stable isotope values (bars are standard deviations) of the 2 groups of leptocephali (blue squares) and other groups of planktonic/nektonic animals (other symbols) in the western Indian Ocean (redrawn from Feunteun et al. 2015). The taxa of leptocephali included in Group 1 (*Anguilla bicolor bicolor*, Congridae, Muraenidae, Chlopsidae, Ophichthidae, Serrivomeridae) do not grow to large maximum sizes compared to those of Group 2 (*Ariosoma* of the Congridae, *Nemichthys* and *Avocettina* of the Nemichthyidae)

obtaining a large proportion of their nutrition from cnidarians or other zooplankton (Feunteun et al. 2015), which was also indicated by amino acid isotope analysis of young Japanese eel larvae, *Anguilla japonica*, in comparison to other marine organisms (Miller et al. 2013). Interestingly though, all stable isotope studies conducted up to now have found evidence of the existence of two groups of taxa of

leptocephali that have different isotopic signatures, even though their values vary geographically among studies or with latitude etc., and long-larval duration larvae can mix among areas (Miyazaki et al. 2011; Feunteun et al. 2015; Liénart et al. 2016; Quattrini et al. 2019; Ghinter et al. 2020). The larvae in Group 1 (higher ∂^{15}N) consist of most families/taxa and their maximum larval sizes are smaller than those of Group 2 (lower ∂^{15}N). Comparisons of isotopic signatures of leptocephali to POM from each sampling area in the Indian and Pacific oceans were also conducted (Miyazaki et al. 2011; Feunteun et al. 2015; Liénart et al. 2016; Ghinter et al. 2020), which were generally consistent with feeding on marine snow, but direct fractionation correspondences with filtered bulk POM (marine snow and free-living microorganisms > 0.7 µm filtered from water samples) were not often observed. The ∂^{15}N signatures of Group 1 leptocephali were not much lower than cnidarians however in the Feunteun et al. (2015) study (Fig. 10). The 5 siphonophores that were among those cnidarians had ∂^{15}N signatures of 8.7 ± 0.8 and 2 *Anguilla bicolor bicolor* larvae were 8.6 ± 1.2 (Feunteun et al. 2015), which does not exclude the possibility of siphonophores contributing to some proportion their diets. The average signatures of Group 2 were much lower. These isotopic studies have found that POM signatures vary with depth in the water column (Miyazaki et al. 2011; Feunteun et al. 2015; Ghinter et al. 2020), so many factors may influence the signatures, such as feeding at different depths.

Another factor to consider is that the type of marine snow particles that leptocephali may ingest could contain other more nutritious/easily assimilated materials than hydrozoan tissues or discarded appendicularian houses. Marine snow formation is facilitated by transparent exopolymer particles (TEP), which contain carbohydrates (Skoog et al 2008; Mari et al. 2017) that could be easily assimilated by leptocephali if they are present in ingested food materials. The high fatty acid content of thraustochytrids would also likely provide excellent nutrition for leptocephali if they can be digested, and leptocephali have high concentrations of omega-3 polyunsaturated fatty acids in their bodies (Deibel et al. 2012; Liénart et al. 2016). Therefore, marine snow has the potential to contain carbohydrates and fatty acids in addition to other organismal materials that may or may not be digested (i.e., fecal pellets may not be digested).

The amount of TEP produced by eukaryotic phytoplankton is usually related to productivity levels and phytoplankton blooms (Mari et al. 2017), which may affect the amount and quality of marine snow available to first-feeding Sargasso Sea anguillid larvae. This food availability and competition with the first-feeding leptocephali of mesopelagic eels that are born during the same season has been proposed to affect levels of early larval survival and to be a possible contributor to European eel recruitment fluctuations (Miller et al. 2016). These recruitment fluctuations have shown correlations with ocean–atmosphere factors such as the North Atlantic Oscillation (NAO) that can affect ocean productivity (e.g., Friedland et al. 2007; Bonhommeau et al. 2008), so understanding the feeding ecology of leptocephali in the Sargasso Sea and worldwide is a key component of evaluating the causes of the declines or interannual recruitment fluctuations of northern hemisphere anguillid eels, which are under a high level of concern for their conservation (Jacoby et al. 2015).

Summary and conclusions

This review overviews and synthesizes existing information about observations of amorphous and marine snow-related materials in leptocephalus intestines in the Sargasso Sea and elsewhere, the genetic detection of hydrozoans in small European eel and larger Pacific leptocephalus gut contents, and the biology and life histories of the ubiquitously present calycophoran siphonophores and thraustochytrid protists. The findings of 18S rRNA gene sequence analysis of leptocephalus gut contents indicate hydrozoan tissues are frequently ingested, and the diversity of other taxa present is consistent with marine snow having been consumed; but primers needed to detect thraustochytrids were not used. The tentacle systems of the calycophoran siphonophores contain components that are small enough to break off and aggregate into marine snow and be ingested by leptocephali before or after degradation by heterotrophic organisms. The smallest life stages of calycophorans could be directly ingested, and their deceased reproductive stages seem especially likely to contribute to marine snow aggregation and be consumed by leptocephali in the same way that occurs for discarded appendicularian houses. While siphonophore and other cnidarian DNA has been found to be consistently present in small European eel and other species of leptocephali gut contents, those species as well as a similar diversity of eukaryotic taxa were also found in large marine snow particles from the Sargasso Sea. Selective PCR primer amplification and rRNA gene copy number differences should be evaluated to help understand the contribution of hydrozoan tissues to leptocephalus diets, and specific primers should be used to test for thraustochytrid protists in the larval gut contents. The possible presence of intestinal parasites in leptocephali also needs further examination. Detailed isotopic studies of marine snow, gut content components and the bodies of the larvae may also be useful. Each part of this interesting mystery that is not yet fully understood requires carefully designed and interpreted future research that combines direct observations of gut contents that are also analyzed using specific primers. Leptocephali are consistent components of marine food webs throughout the tropical and subtropical latitudes of the world's oceans, so comparative

research should be done on the food sources of different sizes of leptocephali and on what they consume in different types of marine environments, especially for species with long larval migrations such as anguillid eels. These studies will be essential for understanding the feeding ecology of eel larvae to help determine the possible causes of the decadal population declines of species such as the European eel, as well as for understanding the role of leptocephali in the ocean carbon cycle.

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Author contributions MJM developed the review concept and conducted the literature research, RH and KT led research cruises that provided information about the gut contents of leptocephali, EF led research efforts on the stable isotopic compositions of leptocephali, other food web components and POM, and authors critically revised the review.

Compliance with ethical standards

Conflict of interests The authors declare they have no conflict of interests.

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