Tracing European eel in the diet of mesopelagic fishes from the Sargasso Sea using DNA from fish stomachs

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Abstract

Trophic interactions in complex mesopelagic ecosystems are poorly understood, for which it is important to trace diet remains of predators. In many cases, however, the prey remains obtained from predator stomachs are unidentifiable by visual inspection, and identification depends on new molecular techniques. Here we search for predators on larvae of the critically endangered European eel (*Anguilla anguilla*), a species which has shown a dramatic decline in recruitment to the stock. We sampled for predators among mesopelagic fishes using pelagic trawling in the known area of spawning and larval distribution of *A. anguilla* in the Sargasso Sea. We aimed at developing a species-specific quantitative PCR (qPCR) system, targeting the *nd4*-region of mitochondrial DNA (mtDNA), to search for eel remains in the stomachs of 17 mesopelagic fish taxa (62 specimens). Remains of *A. anguilla* was confirmed in 9.7 % of all fish stomachs investigated, representing six species (*Lampanyctys cuprarius*, *L. photonotus*, *Myctophum selenops*, *Notoscopelus caudispinosus*, *Melamphaes typhlops*, and *Chauliodus danae*). Thus, our study documents that mesopelagic fishes in the Sargasso Sea to some extent predate the *A. anguilla* larvae, motivating further studies on upper-level trophic interactions in this oceanic ecosystem.

1. Introduction

Assessment of trophic linkages in the ocean often depends on accurate identification of remains of species in stomachs of predators. Fish larvae are especially difficult to visually identify in stomach remains, and Schooley et al. (2008) estimated that the maximum time limit for proper visual identification of the smallest larvae consumed by a fish would be in the order of half an hour. Therefore, molecular methods for determining stomach contents of predatory fishes are needed in the case of fragile, easily digestible fish larvae (Teletchea 2009). By amplification of DNA in gut contents, the detection time of consumed prey can be prolonged to 12–24 hours after predation (Rosel and Kocher 2002; Albaina et al. 2010; Carreon-Martinez et al. 2011), and quantitative PCR (qPCR) based techniques are now widely used in assessing patterns of fish predation in aquatic systems (e.g. Hunter et al. 2012; Taguchi et al. 2014; Brandl et al. 2015).

DNA analyses of complex samples such as fecal (e.g. Valentini et al. 2009; Pompanon et al. 2012) and environmental samples (Taberlet et al. 2012) are increasingly used in ecological studies of contemporary biodiversity (Bohmann et al. 2014; Thomsen and Willerslev 2015), and the molecular characterization of stomach contents has been successfully used to elucidate diets of a wide range of taxa including piscivorous species of fish (Berry et al. 2015; Schreier et al. 2016), mammals (Berry et al. 2017), and birds (Thalinger 2016). These sample types share several characteristics such as being a complex mixture of DNA from multiple and often unknown target organisms and bacteria, having a fast degradation of DNA and that there is a lack of other useful techniques (i.e. visual inspection) to verify the biological content of the samples.

Trophic interactions in mesopelagic ecosystems are poorly understood, and detailed studies on this matter are scarce. We here present a study tracing the critically endangered European eel (*Anguilla anguilla*) (IUCN 2017) in stomach contents of a range of mesopelagic fishes of the Sargasso Sea. The European eel is comprised of a single panmictic population, whose spawning grounds were traced to the Sargasso Sea, drifting and swimming across the Atlantic to the nursery grounds on the European coasts (Schmidt 1923). There has been a massive decline in the recruitment to the stock of *A. anguilla*,

and consequently a need to improve our insight into the mortality risk during the early stages of this economically important species (Dufour et al. 2003; Åström and Dekker 2007).

Information on the potential predators of eel larvae is very limited. Miller et al. (2015) highlight the low occurrence rates of the ubiquitously present anguilliform leptocephalus larvae in stomach contents, and discuss factors that prevent them from being preyed on, such as active swimming and shape-changing behavior when curling up, which make them resemble gelatinous zooplankton. In a study of stomach contents of predatory fishes from the Sargasso Sea, Appelbaum (1982) was unable to detect leptocephali of *A. anguilla*, and so far, no studies have reported findings of leptocephalus larvae of *A. anguilla* in stomach content analyses, except for a single study in the Mediterranean (Grassi 1896).

In order to identify predators of the critically endangered *A. anguilla*, we here implement a molecular diet analysis to assess the range of predators that are potentially foraging on *A. anguilla* larvae, by screening the guts of mesopelagic fishes collected at the *A. anguilla* larvae abundance maximum, during the Danish Eel Expedition (Sargasso-eel) to the Sargasso Sea in 2014 (Fig. 1). Through the use of a newly developed qPCR system for *A. anguilla* and subsequent verification by amplicon sequencing, we here test the hypothesis that mesopelagic fishes are preying on European eel larvae.

2. Materials and Methods

2.1 Fish collection and identification

This study was carried out as part of the Danish Eel Expedition 2014 (Sargasso-eel), which used the research vessel R/V Dana and was led by the Danish Technical University, Aqua (DTU Aqua). During the period of March 30th to March 31st, three hauls were carried out with a large small-meshed pelagic trawl (FOTØ) with a 16-mm mesh size. The gear was in each case towed at various depths between 0-300 m for approximately two hours (Table 1). This was done as an attempt to catch ripe or spawning adult European eel, but it also provided bycatch of mesopelagic fishes. From the catches we

picked 62 specimens identified to 17 taxonomic groups either to species or genus level, representing the families Melamphaidae, Myctophidae, Scombrolabracidae and Stomiidae (Table 2). These taxa were regularly encountered in all the hauls carried out, and specimens were chosen based on abundance and in order to cover a broad range of predators.

2.2 Identification of predators

Morphology based identification of specimens was based on the identification keys provided by Morrow (1964), Ebeling and Weed (1973), Nafpaktitis et al. (1977), Hulley (1984), and Maul (1986). The 62 specimens ranged from 4.5–11.9 cm in Total Length (TL), and no fishes from the catches exceeded 15 cm TL. All specimens were preserved in 96% ethanol and stored at the Zoological Museum, University of Copenhagen (ZMUC). Institutional abbreviations follow Fricke and Eschmeyer (2017).

In order to get a molecular identification of the possible predator species, along with the morphological identification, gill arch tissue from 62 specimens (Table 2) was removed and stored in 96% ethanol. DNA was extracted from the gill arch tissue using the Qiagen DNeasy Blood and Tissue kit (spin column protocol) following the provided protocol. Extracted DNA was amplified in PCR (Text A, Supplementary Information), using both 16S primers 16sar-L (5'-CGCCTGTTTATCAAAAACAT-3') and 16sbr-H (5'-CTCCGGTTTGAACTCAGATC-3') (Palumbi et al. 1991), as well as the COI-targeting FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and FishR1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') (Ward et al. 2005). Resulting PCR products were subsequently commercially Sanger-sequenced by Macrogen Europe (www.macrogen.eu) (Sanger and Coulson 1975; Sanger et al. 1977) in both forward and reverse direction using the same primers as in the initial PCR setup. Forward- and reverse-reads obtained were assembled using Geneious v. 7.1.7 (Kearse et al. 2012), and visually inspected for erroneous or low chromatograph reads, before being prepared as consensus sequences. Identity of the individual

sequences obtained from vouchered tissue samples were then inferred using the nucleotide BLAST search engine provided by National Center for Biotechnology Information (NCBI), and also matched with sequences deposited at the Barcode of life Database (BOLD) systems database. To check for invalid BLAST matches, sequence matches were only compared with species known to be present in the North Atlantic Ocean according to Morrow (1964), Ebeling and Weed (1973), Nafpaktitis et al. (1977), Hulley (1984), Maul (1986), and Fahay (2007), as well as FishBase (Froese and Pauly 2017).

2.3 *Extraction from fish stomachs and initial detection of DNA from* A. anguilla

Stomach sacs from the 62 specimens (Table 2) were cut open using sterile surgical blades, and the contents scraped into a 1.5 mL Eppendorf tube using scissors and tweezers. Between the treatment of each stomach sample, scissors and tweezers were thoroughly cleaned by first washing with 0.05 % bleach solution, and then dipping into 96 % alcohol before flaming. We extracted DNA from each stomach sample using Qiagen DNeasy Blood and Tissue kit (spin column protocol) following the manufacturer's protocol. To begin with, the resulting DNA extractions were screened in PCR reactions using primers AngangCBL 5'-TTCTTCGCATTCCACTTCCT-3' and AngangCBR 5'-CCGGATTGTTTGATCCTGTT-3' already tested positive for genus-specificity towards mitochondrial (mtDNA) cytochrome b (*cytb*) from *Anguilla* spp. These primers were developed previously as part of a qPCR system in relation to other work on eDNA from eels and are published here for the first time. Further details on PCR setup and the reagent mix are included in Text B (Supplementary Information). Extracted DNA from stomachs that returned positive amplification with the AngangCB-primer set in this initial PCR were assumed to contain DNA from *Anguilla* spp. and were marked as positive for further analysis using real-time PCR. Separate laboratories were used for pre- and post-PCR procedures, and rigorous controls were used, including extraction blanks and PCR blanks, in order to thoroughly monitor and ensure contamination-free samples.

Since the AngangCBL and AngangCBR primers turned out to be genus-specific, we designed a new primer- and probe set aimed at being species-specific towards *A. anguilla*. The primers were developed to target a short region of mtDNA, by inspecting alignments of sequences of mtDNA NADH dehydrogenase 4 (*nd4*) from Anguilliformes using the software Geneious v. R7.1.7 (Kearse et al. 2012). We then identified gene regions *in silico* with high levels of variation between the target species and other Atlantic non-target species (see e.g. Agersnap et al., 2017). Using Primer3 v.0.4.0 (Koressaar and Remm 2007; Untergasser et al. 2012) we checked whether potential primers and probes would match the target region, before finally matching the primers against the NCBI database using Primer-BLAST (Ye et al. 2012), to verify by database matching whether the primers matched the sequences known from the targeted species (i.e. matched sequences from *A. anguilla*). NCBI BLAST matches with non-marine species and species not occurring in the North Atlantic Ocean were disregarded. This resulted in a combination of primers: Angang_F10571 (5'-ATCTAGCAACGGACCCCTTA-3') and Angang_R10676b (5'-TTGGTTGGTTCTAGCCGCA-3') along with black hole quencher (BHQ1) probe (Angang_P10595) equipped with a FAM-dye at the 5'-end (5'-FAM-ACACCACTACTAGTTTTATCTTGCTGACT-BHQ1-3'), targeting a 106 base pair long sequence in the *nd4*-region of the mitochondrial genome (collectively referred to as AngangDN4_02). These primers, together with the probe, were then tested in qPCR on DNA extracted from tissue from vouchered specimens of Atlantic anguilliform representatives (Table 3), in order to test whether false positive amplification would arise from mtDNA from other anguilliform species. The qPCR setup (Text C) and details on optimization of primer- and probe concentrations (Text D) are included in the Supplementary Information.

2.4 *Standard dilution series to quantify the level of DNA in stomach extractions.*

To be able to quantify the number of target *Anguilla* sp. DNA copies per qPCR reaction in the extracted stomach samples, we made a standard dilution series for the AngangDN4_02 system. This

standard series was prepared by obtaining target amplicons generated in PCR with DNA extracted from tissue from *A. anguilla* (ZMUC P313229) using the primers Angang_F10571 and Angang_R10676b. Details on creating a standard dilution series and purifying the dsPCR product can be found in Text E (Supplementary Information). Using the Qubit ®2.0 Fluorometer and the QubitTM dsDNA high sensitive assay, we measured the concentration of the resulting dsPCR amplicon. With the aid of the Oligo-Calc engine (Kibbe 2007) we estimated the molecular weight of the dsPCR amplicon (65364.7 Da), and used the Qubit Fluorometer measured concentration of the dsPCR-product, and the Avogadro constant to calculate the number of target-copies per µL in the cleaned dsPCR product (Agersnap et al. 2017). We then diluted the dsPCR product with ddH_2O to obtain a working solution of 10^8 copies/µL, and stored this at -20 °C until we prepared a standard dilution series an hour prior to a subsequent real-time PCR on extractions from stomach samples (Agersnap et al. 2017).

2.5 *Detection and quantification of DNA from* Anguilla *spp. in stomach contents of mesopelagic fishes.*

Extractions from stomach contents were tested in a qPCR setup with eight or sixteen technical qPCR replicates of each extraction from a stomach, together with a minimum of three Non-Template Controls (NTC) per setup, with inclusion of duplicate positive controls based on 10- to 100-fold ddH_2O diluted DNA extractions from tissue from *A. anguilla* (ZMUC P313229), *A. rostrata* (ZMUC uncat.) and a hybrid between the two (ZMUC uncat.), as well as duplicate negative controls of other anguilliform species, including slender snipe eel (*Nemichthys scolopaceus*, ZMUC P313229), European conger (*Conger conger*, ZMUC P313592), and stout sawpalata (*Serivomer beanii*, ZMUC P313606) (Table 3). Besides extractions from stomach samples, positive and negative controls, we also added the prepared ten-fold standard dilution series ranging from 10^8 copies/µL down to 0.1 copies/µL, with three replicates per ten-fold dilution level, for each assay (Agersnap et al. 2017). A qPCR amplification plot was deemed positive when a sigmoidal amplification curve was detected in at least one of eight replicates (Ficetola et al. 2014).

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187 **2.6** *Validation of obtained DNA from* Anguilla *spp. in stomach contents.*

188 Validation of qPCR amplicons amplified with *Anguilla*-specific primers was based on DNA from
189 stomach contents amplified in an ordinary PCR setup. This approach was used as initial cloning
190 attempts continuously failed, most likely because the TaqMan Environmental Master Mix comprises
191 uracil instead of thymin. The One Shot® Mach1™-T1R competent cells supplied with the TOPO TA
192 one-shot kit (Life Technologies) are genotype '+ung', and will most likely break down all plasmids
193 containing uracil (Howe 2007a, b), resulting in transformed clones that loose the plasmid together with
194 insert and resistance used for screening positive clones. To circumvent this, as well as to avoid relying
195 on amplification plots not within the limit of quantification (LOQ) (Bustin et al. 2009), we prepared an
196 ordinary PCR setup using the qPCR-reactions as template. The resulting dsPCR product was then
197 subject to a 3' A-overhang addition prior to cloning. Further details on the PCR and subsequent
198 cloning can be found in Text F (Supplementary Information). Positive amplification of the cloning
199 product was ensured by visualization on a 2% agarose gel, stained with GelRed, and amplicons were
200 Sanger-sequenced in both forward- and reverse direction using the sequencing service provided by
201 Macrogen Europe. Resulting sequence chromatographs were manually inspected for failed and
202 erroneous reads using Geneious v. R7.

203 **3. Results**

204 The 62 fish specimens examined for predation on European eel were found to belong to 17
205 different taxa, based on morphological evidence as well as COI and 16S primers (Table 2). Each of the
206 individual 62 stomach contents weighed between 0.01–0.05 g, and were all partly digested (pink fluid)
207 and hence visual identification was impossible. The stomach contents of eight of the 62 fishes returned
208 positive amplification in the initial PCR setup with the AngangCBL and AngangCBR primers,
209 indicating presence of *Anguilla* sp. An additional four specimens were also included in the further
210 analysis as DNA content in the stock was high (Table 3), giving a total of twelve specimens deemed

211 eligible for further scrutiny and analysed using qPCR. The remaining 50 specimens that were negative
212 in the initial PCR with the AngangCBL and AngangCBR primers on stomach contents were not
213 analysed further. Eleven out of the twelve focal fishes showed positive signaling of anguilliform DNA
214 in the qPCR (Fig. 2, Table 3). The lowest frequency of detection occurred with *Lampanyctus*
215 *photonotus* (ZMUC P2394081), having 1/8 positive replicates, and the highest occurred with
216 *Myctophum selenops* (ZMUC P2394072), having 12/16 positive replicates (Table 3). Detection of
217 anguilliform DNA occurred at Cq-values between 38.38 and 43.73 on average, which translates into
218 DNA copy numbers between 0.135 and 1.43 per reaction (Fig. 2). DNA was successfully sequenced
219 from six identical haplotypes of the 11 samples initially positive in the qPCR runs, and all six showed
220 highest similarity to that of *A. anguilla*, when compared to all anguilliform sequences available in
221 NCBI Genbank (Fig. 2), when excluding the supposed misidentification of an *A. rostrata* (Accession
222 no. KJ564271) by Jacobsen et al. (2014). A quick Neighbour-Joining (NJ) tree with available *cytb*
223 sequences from anguilliform species in NCBI pairs KJ564271 with *A. anguilla*, and supports our
224 suspicion of a misidentified European eel. As hybrids of *A. rostrata* and *A. anguilla* are also occurring,
225 KJ564271 could also be a hybrid with the mtDNA of a European eel.

226 Six species (and six specimens in total), namely *M. selenops* (ZMUC P2394072), *L. photonotus*
227 (ZMUC P2394083), *L. cuprarius* (ZMUC P2394089), *Notoscopelus caudispinosus* (ZMUC
228 P2394046), *Melamphaes typhlops* (ZMUC P2394101), and *Chauliodus danae* (ZMUC P2394084)
229 were found to have been feeding on *A. anguilla* (Fig. 2). We found no correlation between the
230 amounts of positive replicates, the Cq-value at detection, and the success of cloned and sequenced
231 DNA (data not shown). The Atlantic non-target anguilliform species (*C. conger*, *N. scolopaceus*, and
232 *S. beanii*) showed amplification in some of the replicates (Table 3), with Cq-values of ~40, when tests
233 were performed on 10-fold dilutions of DNA extracted from tissue samples from these non-target
234 species (Table 3). The AngangDN4_02 assay is thus susceptible to amplifying sequences of unspecific
235 origin, when anguilliform DNA is present in high concentrations (i.e. from tissue samples). However,
236 the replicates of NTCs tested alongside in each qPCR setup did not amplify false positives, indicating

that cross-contamination is unlikely. If cross-contamination had occurred, it would have been detected through *A. anguilla* (template) DNA being present in NTC wells alongside wells containing template DNA.

Of the four stomachs that had not initially amplified for anguilliform DNA, two (*M. selenops*, ZMUC P2394072 and *N. caudispinosus*, ZMUC P2394046) were positive for *A. anguilla* DNA using the AngangDN4_02 assay and verified through subsequent cloning and sequencing, suggesting that the initial screening for presence of European eel using AngAngCBL and AngAngCBR *cytb* primers may not be as efficient as the AngangDN4_02 qPCR assay. Overall, 9.7 % of all fishes (6 out of 62) investigated were here verified to have been preying on *A. anguilla*, although it is likely that more have been preying on eel larvae, as the initial screening may have included false negatives, and due to the unsuccessful sequencing of the remaining five specimens, which were positive for anguilliform DNA in the qPCR assay.

4. Discussion

Using qPCR and DNA sequencing on stomach samples of mesopelagic fishes from the Sargasso Sea, we here identified six species of fish that feed on the larvae of European eel. The specimens found to feed on eel offspring belong to the dominant mesopelagic orders Myctophiformes, Stephanoberyciformes and Stomiiformes. The low concentrations of DNA detected in the stomachs are likely remnants from a very recent meal, as stomachs were stored in the freezer no later than 2 hours upon capture, i.e. well within the time limits proposed for detection by molecular methods (Rosel and Kocher 2002; Albaina et al. 2010). The sample sizes of each predator fish investigated here (n = 1-15, Table 2) are very low, warranting further genetic analyses of the diet of these mesopelagic fishes, in order to understand the levels of predation on the larval phase of the European eel. We especially highlight the need for further genetic diet studies on the six main predators identified here, but also an even broader study with a more comprehensive taxon sampling.

Although the qPCR reveals presence of anguilliform DNA, the attempt to quantify the amounts of DNA present seems very imperfect at such low quantities. A DNA copy number does not provide useful information on the extent of predation on *A. anguilla* leptocephali. Stomach content volume is, in our study, unknown, but we could point to the range of potential predators, which appeared to be quite substantial. The method used here may aid in unraveling the predation pressure on the larval stage of *A. anguilla*, although we would recommend that future studies implement a DNA metabarcoding approach (e.g. Hibert et al. 2013; De Barba et al. 2014), as it allows for simultaneous identification of several fish species, the relative importance of European eel in the diet, as well as prevalence of other sources of diet in future studies of fish predation. In order to address and test consequential questions in ecology, a larger sample size would be required. Predicting whether the predation from these mesopelagic fishes may affect the dynamics of the European eel larvae would also require a complete diet analysis of the predators in question (Piñol et al. 2014).

The method used here is a sensitive and cost-effective method to screen a large number of potential predatory species for the presence of specific taxon of conservation concern. That being said, the primer and probe set developed in this study may not be completely species-specific when running 50 cycles of qPCR, as it also detects low amounts of DNA in otherwise very copy-abundant samples of the control species (*A. rostrata*, *C. conger*, *N. scolopaceus*, and *S. beanii*). Consequently, we propose that the initial PCR and qPCR could be indicative as a screening tool for *A. anguilla*, but in order to safely determine the presence/absence of *A. anguilla*, especially when present in low concentrations, the cloning and subsequent sequencing should also be performed.

Related to diet analyses are the analyses of DNA from actual environmental samples (environmental DNA – eDNA) such as freshwater (e.g. Ficetola et al. 2008; Thomsen et al. 2012a; Sigsgaard et al. 2015), seawater (e.g. Thomsen et al. 2012b; Thomsen et al. 2016; Sigsgaard et al. 2017), and soil (e.g. Andersen et al. 2012; Yoccoz et al. 2012; Drummond et al. 2015), which have been used to describe community compositions in various different ecosystems, and can even be used

to make indirect inferences on diet (Sigsgaard et al. 2016). Hence, future studies of the trophic relationships of the European eel could be supplemented with eDNA analyses of water samples.

Diurnal vertical migration in the water column plays an important role in feeding habits of mesopelagic fishes, especially among Myctophiformes (Dypvik et al. 2012), *Melamphaes* sp. (Sutton et al. 2010), and *C. danae* (Merrett and Roe 1974; Roe and Badcock 1984). As larvae of *A. anguilla* also perform diurnal vertical migration (Castonguay and McCleave 1987), the respective migratory behaviors have to be considered for predation to occur. Many mesopelagic fishes are known to migrate to the upper 200 m during night, and are present in these water layers during dusk and dawn. *Anguilla anguilla* leptocephali migrate from the 100-200 m water layer during daytime to more shallow layers above 100 meter during night (Castonguay and McCleave 1987, Munk et al. 2018), and thus the distribution of mesopelagic fishes overlaps that of *A. anguilla*, during the darker – but not fully dark – parts of the 24 hour period. Accordingly, catches of the mesopelagic fishes and the *A. anguilla* leptocephali were all from the 0-300 m depths and caught during the dark hours (Table 1).

Myctophiformes are important in the mesopelagic food webs worldwide (Cherel et al. 2010; Dypvik et al. 2012), but previous studies have found that these species mainly feed on crustaceans such as copepods, euphausiids, and amphipods (Tyler and Pearcy 1975; Sameoto 1988; Sameoto 1989). The same is the case for species of Stephanoberyciformes, e.g. *Scopelogadus beanii* (Gartner and Musick 1989), although they may mainly feed on gelatinous zooplankton such as tunicates (Bartow and Sutton 2008). Stomiiform fishes such as *C. danae*, however, have often been found to be feeding on fish (Mauchline and Gordon 1983; Butler et al. 2001). The four species of Myctophiformes feeding on *A. anguilla* represents several lineages, indicating that most myctophids can feed on leptocephali, and since Myctophiformes can comprise up to 50 % of the deep water fish biomass (Gjøsaeter and Kawaguchi 1980), these may be the main predators on *A. anguilla* leptocephali in the Sargasso Sea.

311 In conclusion, DNA-based methods enable broad analyses of the diets of large sample sizes of
312 predators, and also targeted screening for the presence of specific taxa of conservation concern. In this
313 study, we genetically identified previously unknown trophic interactions involving predation of the
314 critically endangered European eel larvae by six mesopelagic fish predators. These findings contribute
315 with much needed and otherwise lacking information on important trophic interactions in poorly
316 understood mesopelagic ecosystems.

317

318 **Compliance with ethical standards**

319 All applicable international and national guidelines for the care and use of animals were followed. All
320 procedures performed in this study were in accordance with the ethical standards of the institution at
321 which the study was conducted.

322

323 **Conflict of interests**

324 The authors declare no financial or other conflict of interests.

325

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559

560 **Table legends**

561 **Table 1** An overview of the stations where mesopelagic fishes were sampled in connection with the
562 occurrence of *A. anguilla* larvae

563

564 **Table 2** List of the 17 taxa (62 specimens), which were initially run in PCR and subsequently screened
565 on a gel for *Anguilla* sp. content in their stomach contents. Specimens were determined to genus or

566 species level by using morphological characters and mitochondrial 16S- and COI target regions to
567 compare with sequences in the NCBI Genbank and BOLD databases. Asterisks indicate that not all
568 specimens have a DNA barcode deposited in the NCBI Genbank
569
570 **Table 3** List of the 12 fishes analysed together with control species and their respective qPCR
571 detection values. Sample types are either SC (stomach content) or TS (tissue sample). The ddH_2O
572 dilution factor is listed for TS, whereas SC samples were not diluted. ZMUC-museum vouchers are
573 listed with catalogue numbers, institutional abbreviations follow Fricke and Eschmeyer (2017).
574 Average Cq-values and target DNA copies per reaction inferred from the standard curve of the qPCR,
575 are listed together with the ratio of positive replicates. Sequences obtained by cloning of stomach
576 samples were all 5'-
577 TGTGGTTTTGGCTTGCTAAAATTATTAATGGTRGAAGTCAGCAAGATAAAACTAGTAGTG
578 GTGTTGA-3', and match *A. anguilla* in NCBI BLAST searches, here presented with accession
579 numbers
580
581 **Figure legends**
582 **Fig. 1** Position of the study in the southern Sargasso Sea. Sea surface temperature is illustrated by
583 isotherms for the Atlantic, based on data from the Operational Sea Surface Temperature and Sea Ice
584 Analysis project (OSTIA, http://ghrsst-pp.metoffice.com). Filled circle indicates sampling position of
585 mesopelagic fish. Extension of the distribution area of larval European eel is indicated by ellipse, as
586 from Schmidt (1923)
587
588 **Fig. 2** Boxplot showing DNA target copies per qPCR reaction. Median values are marked by the bold
589 line in the middle, upper and lower hinges display the first and third quartiles, the extreme upper and
590 extreme lower whisker display highest and lowest value recorded, and outliers (circles) are shown
591 where deemed pertinent. Each boxplot pertains to the stomach of a single individual. Positively cloned

592 and sequenced qPCR stomach samples with confirmed sequence similarity to *A*. *anguilla* are marked

593 "Y". All drawings were prepared by S.W. Knudsen. Drawings are not scaled