2016

Endohelminth parasites of some midwater and benthopelagic stomiiform fishes from the northern Gulf of Mexico

Michael J. Andres
University of Southern Mississippi, michael.andres@usm.edu

Mark S. Peterson
University of Southern Mississippi, mark.peterson@usm.edu

Robin M. Overstreet
University of Southern Mississippi, robin.overstreet@usm.edu

Recommended Citation

GULF AND CARIBBEAN

R E S E A R C H

ENDOHELMINTH PARASITES OF SOME MIDWATER AND BENTHOPELAGIC STOMIIFORM FISHES FROM THE NORTHERN GULF OF MEXICO

Michael J. Andres*, Mark S. Peterson, and Robin M. Overstreet
The University of Southern Mississippi, Department of Coastal Sciences, 703 East Beach Drive, Ocean Springs, MS 39564; *Corresponding author, email: michael.andres@usm.edu

ABSTRACT: Mesopelagic fishes represent significant ecological links between mesozooplankton and the larger pelagic squids, fishes, and marine mammals. As such, these fishes also play a significant role as intermediate or paratenic hosts for parasites that require a crustacean intermediate host and mature in marine mammals or pelagic fishes. We examined a total of 208 individuals representing 5 species of Sternoptychidae and 88 individuals representing 2 species of Phosichthyidae from 20 locations in the northern Gulf of Mexico (nGOM). Six of the 7 species we examined are mesopelagic and one species was benthopelagic. We found the larval stages of *Anisakis brevispiculata, Anisakis typica, Hysterothylacium fortalezae* (all Nematoda: Ascaridoidea); *Bolbosoma* sp. (Acanthocephala: Polymorphidae); and Tetraphyllidea (Cestoda) plus an immature specimen of *Brachyphallus* sp. (Digenea). Molecular sequencing was used to identify the ascaridoids and *Bolbosoma* sp. and to confirm the identification of 3 host sternoptychid species. The mesopelagic fishes hosted *Anisakis brevispiculata* (that matures in pygmy and dwarf sperm whales) and *Hysterothylacium fortalezae* (that matures in pelagic fishes, primarily mackerels), whereas the benthopelagic species was parasitized by *Anisakis typica* (that matures in dolphins). We suggest this pattern of infection indicates a pelagic life—cycle for *Anisakis brevispiculata* and *Hysterothylacium fortalezae* and a demersal life—cycle for *Anisakis typica*. Our study represents the first published sequences from the nGOM for the fishes *Argyropelecus aculeatus, Maurolicus weitzmani*, and *Polyipnus clarus* and the first molecular identification of larval ascaridoids from mesopelagic fishes in the nGOM.

KEYWORDS: *Anisakis, Hysterothylacium,* marine mammals, Phosichthyidae, Sternoptychidae

INTRODUCTION

Mesopelagic fishes represent significant ecological links between mesozooplankton and the larger pelagic squids, fishes, and marine mammals (e.g., Pauly et al. 1998, Choy et al. 2013, Young et al. 2015). Members of the orders Stomiiformes and Myctophiformes account for the vast majority of such fishes (Bernal et al. 2015). Most parasitological studies of midwater fishes have revealed larval or juvenile stages of helminths that mature in large pelagic fishes and marine mammals rather than adult helminths (e.g., Noble and Collard 1970, Gartner and Zwerner 1989, Mateu et al. 2015). Typically, larval or juvenile stages of trematodes (that mature in fishes), cestodes (that mature in elasmobranchs), acanthocephalans (that mature in fishes and marine mammals), and nematodes, especially ascaridoids, have been reported (e.g., Noble and Collard 1970, Gartner and Zwerner 1989, Klimpel et al. 2006, 2010, Mateu et al. 2015).

The life—cycle of marine ascaridoids involves a crustacean first intermediate host and a vertebrate final host. Typically, members of the Raphidascarididae mature in teleosts, whereas those of Anisakidae mature in marine mammals. The crustacean first intermediate host is generally a copepod, but euphasiids serve as important intermediate hosts for species of *Anisakis* (see Smith and Wooten 1978). Larval ascaridoids commonly infect fishes and invertebrates that serve as paratenic, or transfer hosts (hosts that are not required for the development of the parasite). The use of these paratenic hosts acts as an adaptation to overcome the challenges of reaching appropriate final hosts in oceanic environments (Marcogliese 1995), and has led to the utility of ascaridoids as biological tags for fishes (Mattiucci et al. 2008) and trophic interactions (Palm and Klimpel 2008). The use of paratenic hosts also has public health implications for certain fisheries products because of the zoonotic (can be passed from animal to human) potential of some ascaridoids, especially those that mature in marine mammals (species of *Anisakis* and *Pseudoterranova*; see Sakanari and McKerrow 1989), but also some that mature in fishes (Overstreet and Meyer 1981, Overstreet 2012).

Despite the potential human health risk associated with some ascaridoids, the Gulf of Mexico (GOM) is poorly characterized with respect to the intermediate and paratenic host use patterns of those nematodes. Kuhn et al. (2011) modeled the geographic range of 9 species of *Anisakis* but did not include data for the GOM presumably because species of *Anisakis* were reported under junior synonyms, incomplete larval names, misidentifications, or they lacked molecular data (e.g., Gunter and Overstreet 1974). Cavallero et al. (2011) surveyed stranded cetacean hosts from the eastern GOM (eGOM), southeastern U.S. Atlantic coast, and Caribbean Sea and reported the presence of 7 species of *Anisakis, Pseudoterranova ceticola*, and larvae of *Contracaecum multipapillatum* (exclusively from inshore populations of the common bottlenose dolphin, *Tursiops truncatus*). However, the highly migratory nature of most marine cetaceans and the varied geographic stranding localities examined by Cavallero et al. (2011) do not provide a clear picture of the richness of *Anisakis* species in the northern GOM (nGOM).

The purpose of this study was to document larval ascaridoids as well as other endohelminths in stomiiform fishes

that were incidentally caught in bottom trawl surveys along the nGOM outer continental shelf and continental slope. We examined 5 mesopelagic species (the Atlantic Sliver Hatchetfish, *Argyropelecus aculeatus*; the Silvery Hatchetfish, *Argyropelecus sladeni*; the Atlantic Pearlside, *Maurolicus weitzmani*; the Slope Hatchetfish, *Polyipnus clarus* (all Sternoptychidae); and the Stareye Lightfish, *Pollichthys mauli* (Phosichthyidae)) and one benthopelagic species (the Rendezvous Fish, *Polymetme corythaeola* (Phosichthyidae)) for helminths. Morphological identification of larval ascaridoids to species level is generally problematic (e.g., Zhu et al. 1998, Mattiucci and Nascetti 2006, Mattiucci et al. 2014); therefore, we molecularly genotyped the 28S and internal transcribed spacer region (ITS; = ITS1, 5.8S, and ITS2) ribosomal DNA (rDNA) regions to confirm identifications.

Materials and Methods

Sample collection

Representative stomiiform fishes were collected from the nGOM by bottom trawl as a part of the NOAA–Fisheries Southeast Small Pelagic Survey in 2011–2012. Trawls were towed for 30 min and depths (m) at the end of trawl were recorded. Following capture of fishes, we identified and froze them at −20°C while on ship. We examined 208 individuals representing 5 species of Sternoptychidae and 88 individuals representing 2 species of Phosichthyidae from 20 locations (Figure 1A). In the laboratory, hosts were defrosted at 1–3°C, identifications confirmed using the keys developed by McEachran and Fechhelm (1998), measured (nearest mm SL), weighed (nearest 0.01 g), and sexed. The visceral organs and gastrointestinal tract of all

fish were placed in a small amount of physiological saline and examined for helminths under a stereomicroscope. An initial subsample of 20 individuals each of *Maurolicus weitzmani*, *Pollichthys mauli*, *Polypinus clarus*, and *Polymetme corythaeola*, and all specimens of *Argyropelecus aculeatus* and *Argyropelecus sladeni*, were examined for larval helminths in the musculature by candling. Candling was conducted by putting fillets between two glass slides and examining them by stereomicroscope; however, no larvae were found in the musculature of any of the fishes examined, and the process was discontinued. Parasite specimens were fixed in 70% ethanol for morphological and molecular analyses. Nematodes were cleared in 95 parts 70% ethanol + 5 parts gylcerol for morphological examination. A single acanthocephalan and a single digenean were stained, cleared, and mounted according to the procedures in Andres and Overstreet (2013). Voucher specimens were deposited in the Gulf Coast Research Laboratory Museum (GCRLM), Ocean Springs, Mississippi (see Table 1 for accession numbers).

Ecological parasitology terms follow those defined by Bush et al. (1997): prevalence as the percentage of fish infected and mean intensity of infection as the mean number of parasites per infected fish. The 95% confidence interval for prevalence was calculated using the Clopper–Pearson exact CI (exactci) package found in the PropCIs library of R v. 2.15.2 (R Core Team, 2012).

Molecular analyses

Genomic DNA was extracted from a middle portion of 36 individual ascaridoids, a section of the posterior portion from the acanthocephalan, and a piece of muscle from a single individual of each sternoptychid species (with the exception of *Argyropelecus sladeni*; n = 4) using Qiagen DNAeasy tissue kit (Qiagen, Inc., Valencia, CA, USA) following the instructions provided. For ascaridoids, DNA fragments of ca. 1,020 base pairs (bp) long for the 28S ribosomal DNA (rDNA) gene and 800 bp long comprising the ITS rDNA gene were amplified from the extracted DNA by polymerase chain reaction (PCR) following the procedures of Nadler et al. (2000) and Zhu et al. (1998), respectively. For the acanthocephalan, a 668 bp partial portion of the mitochondrial cytochrome c oxidase 1 (*cox1*) was amplified following García–Varela and Nadler (2006) and for sternoptychids, ca. 640 bp partial portion of the *cox1* was amplified following Ward et al. (2005). See Table 2 for the list of primers used and their corresponding references. The resulting PCR products were excised from PCR gel using QIAquick Gel Extraction Kit (Qiagen, Inc., Valencia,

TABLE 1. *Gulf Coast Research Laboratory museum (GCRLM) and GenBank accession numbers for vouchers treated in this study.*

| Taxon | GCRLM No. | GenBank Accession No. | | |
		ITS	28S	cox1
Platyhelminthes				
Brachyphallus sp.	06551	–	–	–
Acanthocephala				
Bolbosoma sp.	06552	–	–	KX098556
Nematoda				
Anisakis brevispiculata	06553–5	KX098557–9	KX098560	–
Anisakis typica	06556	KX098561	KX098562	–
Hysterothylacium fortalezae	06557	KX098563	KX098564	–
Chordata				
Argyropelecus aculeatus	36551	–	–	KX098553
Maurolicus weitzmani	36552	–	–	KX098554
Polyipnus clarus	36553	–	–	KX098555
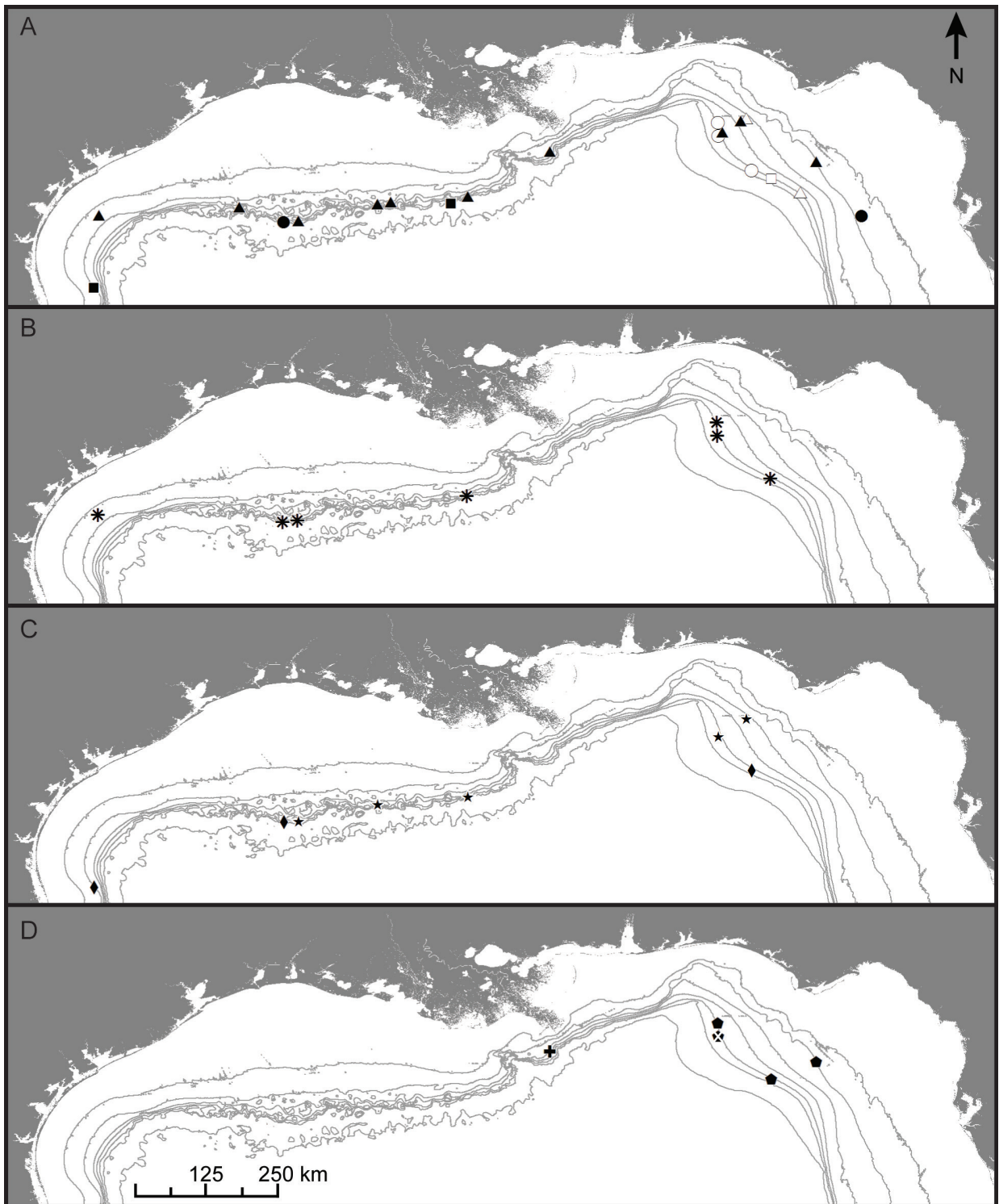
FIGURE 1. *Sampling localities in the northern Gulf of Mexico. A. Localities of sternoptychids (triangles), phosichthyids (squares), or both (circles) from 2011 (filled symbols) and (2012) (open symbols). B. Locations from which* Anisakis brevispiculata *were collected. C. Locations from which* Anisakis typica *(diamonds) and* Hysterothylacium fortalezae *(stars) were collected. D. Locations from which tetraphyllideans (pentagons),* Bolbosoma *sp. (+), and* Brachyphallus *sp. (white "X" overlaid a pentagon) were collected. Bathymetric contour lines represent 50 m, 100 m, 200 m, 300 m, 500 m, and 1,000 m.*

CA, USA) following kit instructions. They then were cycle—sequenced using ABI BigDye™ chemistry (Applied Biosystems, Inc., Carlsbad, CA, USA), ethanol—precipitated, and processed on an ABI 3130 Genetic Analyzer™.

Sequences used for pairwise comparison were aligned using MAFFT version 6.611b (Katoh et al. 2005) with 1,000 cycles of iterative refinement and the *genafpair* algorithm.

TABLE 2. *PCR primers used for the various taxonomic groups and regions in this study.*

Group	Region	Primer	Direction	Annealing Temp.	Reference
Acanthocephala	cox1	5′–AGTTCTAATCATAARGATATYGG–3′	Forward	40°C	Folmer et al. 1994
		5′–TAAACTTCAGGGTGACCAAAAAATCA–3′	Reverse		
Ascaridoidea	28S	391: 5′–AGCGGAGGAAAAGAAACTAA–3′	Forward	55°C	Nadler et al. (2000)
		501: 5′–TCGGAAGGAACCAGCTA-CTA–3′	Reverse		
	ITS	NC5: 5′–GTAGGTGAACCTGCGGAAGGATCATT–3′	Forward	60°C	Zhu et al. (1998)
		NC2: 5′–TTAGTTTCTTTTCCTCCGCT–3′	Reverse		
Sternoptychidae	cox1	FishF1: 5′–TCAACCAACCACAAAGACATTGGCAC–3′	Forward	54°C	Ward et al. (2005)
		FishF2: 5′–ACTTCAGGGTGACCGAAGAATCAGAA–3′	Reverse		

RESULTS

Host data

Of the 5 midwater species collected, *Polyipnus clarus* was the most common (n = 156 from 11 stations) followed by the phosichthyid *Pollichthys mauli* (n = 35 from 3 stations) whereas 53 individuals of the benthopelagic *Polymetme corythaeola* were collected from 4 stations. Individuals of *Argyropelecus aculeatus* and *Polymetme corythaeola* were collected from the deepest depths as well as the largest depth range (see Table 3). Individuals of both phosichthyid species tended to be longer and heavier than the sternoptychid species (Table 3). The *cox1* sequence of *Argyropelecus aculeatus* and *Maurolicus weitzmani* was identical to that of the unpublished sequence of *Argyropelecus aculeatus* (KF929623) collected from the slope water off Cape Hatteras and the sequence of *Maurolicus weitzmani* (KJ190037) from the Mid–Atlantic Bight (Davis et al. 2014), respectively. The *cox1* sequence of *Polyipnus clarus* differed from that of the *Polyipnus clarus* sequence FJ918932 collected from the slope water of southern New England (DeVaney 2008) by a single purine transition.

Genetic identification

Three ascaridoid species were found; *Anisakis brevispicu-* *lata* (Anisakidae), *Anisakis typica*, and *Hysterothylacium fortalezae* (Raphidascarididae). Of the 20 individuals of *Anisakis brevispiculata* sequenced, one 28S genotype and 3 ITS genotypes were found. All differences in those genotypes occurred at position 177 in the ITS2, with 15 individuals having a guanine; 4 individuals having an adenine, and one individual having an ambiguous purine. The most common ITS genotype (KX098557) was identical to the sequence of *Anisakis brevispiculata* AY826719 of Cavallero et al. (2011). No intraspecific variation occurred in either gene region of the 6 individuals of *Anisakis typica*. The ITS sequences we generated for *Anisakis typica* were identical to EU327688 (Iñiguez et al. 2009) and JQ912690 (Mattiucci et al. 2014) for *Anisakis typica* , but they differed from sequence AY826724 by Cavallero et al. (2011) that had 3 single bp indels in the ITS1 at positions 125, 131, and 161. No intraspecific variation occurred in either gene region of the 11 individuals of *Hysterothylacium fortalezae*. The 28S sequences we generated for *Hysterothylacium fortalezae* differed from the GenBank sequence U94760 (provided by RMO to Nadler and Hudspeth 1998) by a single bp indel at position 436.

The *cox1* sequence generated from the acanthocephalan was 88% similar to sequence JQ040303 for *Bolbosoma balaenae* (Gregori et al. 2012); 66% similar to sequence JX442190 for *Bolbosoma* sp. (García–Varela et al. 2013); and 65% similar to sequence JX442189 for *Bolbosoma turbinella* (García–Varela et al. 2013). The *cox1* sequences of the latter 2 species were 84% similar, suggesting that the acanthocephalan we collected was a species of *Bolbosoma* (Polymorphidae). Morphologically, the proboscis of our specimen was not everted; however, it did poses a funnel–shaped bulb in the foretrunk.

Parasitological data

The prevalence of helminths was relatively low (Table 4). No individual of *Argyropelecus sladeni* was infected with any helminth. Individuals of *Anisakis brevispiculata* were identified from all other midwater fish with the

TABLE 3. *Host information for the species examined in this study. SL = Standard length in mm; g = grams; m = depth; Stations = number of stations that hosts were obtained from; + = number of stations that had parasitized fish.*

Taxonomic group	n	SL range	g	m	Stations n	+
Phosichthyidae						
Pollichthys mauli	35	61–154	1.00–17.79	396–419	3	3
Polymetme corythaeola	53	91–184	3.08–36.87	76–468	5	3
Sternoptychidae						
Argyropelecus aculeatus	16	45–66	1.18–9.13	76–468	4	2
Argyropelecus sladeni	6	25–43	0.51–2.83	336	1	0
Maurolicus weitzmani	30	30–47	0.36–1.21	102–396	5	4
Polyipnus clarus	156	21–57	0.33–3.85	66–430	11	4

exception of *Maurolicus weitzmani*. *Anisakis typica* was the only helminth found to infect *Polymetme corythaeola*, and was found to parasitize that host only. *Maurolicus weitzmani* had the highest prevalence of *Hysterothylacium fortalezae* because a single location in the western GOM (wGOM) had 5 infected individuals. The site of infection for individuals of *Anisakis brevispiculata* was in the mesenteric lining associated with the gonads, with the exception of 2 individuals in a single *Polyipnus clarus*, where one individual occurred near the gonads and the other near the liver. Likewise, all individuals of *Anisakis typica* occurred in the mesenteric lining around the gonads, even in the single fish with an intensity of 2. All individuals of *Hysterothylacium fortalezae* were found in the mesenteric lining of the liver. None of the fish examined from the 2011 eGOM locations (east of Mobile Bay, AL; see Figure 1A) were infected with ascaridoids, whereas all of the 2012 sampling locations occurred in the eGOM, and only the southernmost location exhibited uninfected fish. All three ascaridoid species were found across the nGOM (Figure 1B–C). The depth range of fish infected with ascaridoids was 66–468 m for *Anisakis brevispiculata*, 98–468 m for *A. typica*, and 212–419 m for *Hysterothylacium fortalezae*, and all but the shallowest two locations (both of which occurred over the Texas shelf) occurred in > 200 m of depth.

The mean intensity (Table 4) for all helminths, with the exception of larval tetraphyllideans (Cestoda) from *Polyipinus clarus*, was between 1.0–1.2. Tetraphyllideans were not identified past order, but were found in only mesopelagic fishes from the eGOM (Figure 1D) ranging from a depth of 102–419 m. The single specimen of *Bolbosoma* sp. was found in the proximal portion of the intestinal tract of *Maurolicus weitzmani* from the wGOM (Figure 1D) at a depth of 186 m. A single immature (based on possessing adult characters but not being mature) specimen of *Brachyphallus* sp. (Digenea: Hemiuridae) infected the stomach of *Polyipinus clarus* from the eGOM (Figure 1D) at a depth of 419 m.

Discussion

We provide the first molecular identification of anisakids and a species of *Bolbosoma* from fish in the nGOM, the first parasitological survey of 4 sternoptychid species and 2 phosichthyid species in the nGOM, and provide the first *cox1* sequences for sternoptychids from the GOM. We found the larval stages of 5 helminth taxa (*Anisakis brevispiculata*, *Anisakis typica*, *Hysterothylacium fortalezae*, *Bolbosoma* sp., and Tetraphyllidea) and an immature species of *Brachyphallus*. The presence of generalist parasite taxa in the hosts that we examined is similar to the findings of previous authors who examined midwater fishes (e.g., Noble and Collard 1970, Gartner and Zwerner 1989, Klimpel et al. 2007, 2008, 2010, Mateu et al. 2015). We found an overall low prevalence and mean intensity of all parasitic taxa across all host species examined. *Polyipnus clarus* had the largest number of recovered helminth taxa (4), but it also had the highest sample size (156). *Argyropelecus sladeni* was not infected with any helminths, but only 6 individuals were examined, and, consequently, we do not include it in the following discussion.

All midwater species were parasitized by *Hysterothylacium fortalezae*, with *Maurolicus weitzmani* having the highest prevalence of infection (20%) for any host–parasite pair. *Hysterothylacium fortalezae* primarily parasitizes pelagic, piscivorous fishes (e.g., species of *Scomberomorous* as well as the Leatherjacket, *Oligoplites saurus*; Deardorff and Overstreet 1980), but those authors also reported adults from the Black Grouper, *Mycteroperca bonaci*. Our finding of *Hysterothylacium fortalezae* parasitizing midwater fishes rather than the benthopelagic *Polymetme corythaeola* is likely indicative of those fishes acquiring an infection while feeding on epipelagic crustaceans. Deardorff and Overstreet (1981) described larvae that they attributed to *Hysterothylacium fortalezae* from the viscera of coastal and offshore pelagic fishes (3 species of *Anchoa*, 2 species of *Peprilus*, and the Atlantic Cutlassfish, *Trichiurus lepturus*) but also from a penaeid shrimp and the structure–associated Black Sea Bass, *Centropristis striata* (as *C. melana*). However, the demersal species they found to be infected with *Hysterothylacium fortalezae* larvae have pelagic life–history stages that could have allowed those species to acquire their infection before settling. Most of the locations Overstreet and Deardorff (1981) sampled occurred in shal-

TABLE 4. *Percent prevalence (P) with 95% confidence interval and mean intensity of infection (I; ± SE) for the parasitic taxa of the 4 mesopelagic (M) hosts and one benthopelagic (B) host.*

Host	Zone	Tetraphyllidea		Anisakis brevispiculata		Anisakis typica		Hysterothylacium fortalezae	
		P	I	P	I	P	I	P	I
Pollichthys mauli	M	6 (0.7–19.2)	1	14 (4.8–30.3)	1	0	–	3 (0.1–14.9)	1
Polymetme corythaeola	B	0	–	0	–	9 (3.1–20.7)	1.2 ± 0.2	0	–
Argyropelecus aculeatus	M	0	–	13 (1.6–38.3)	1	0	–	6 (0.2–30.2)	1
Maurolicus weitzmani	M	5 (0.1–24.9)	1	0	–	0	–	20 (7.8–38.6)	1.2 ± 0.2
Polyipnus clarus	M	2 (0.4–5.2)	7 ± 1.9	8 (4.0–13.1)	1.1 ± 0.1	0	–	1 (0.2–4.6)	1

low water where trophic interactions between demersal and pelagic organisms are more likely to have occurred.

Anisakis brevispiculata parasitized all midwater fish species with the exception of *Mycteroperca weitzmani* and was the most numerous of all examined nematodes. One individual each of *Argyropelecus aculeatus* and *Polypinus clarus* had a co—infection of *Hysterothylacium fortalezae* and *Anisakis brevispiculata*. Pygmy and dwarf sperm whales (Kogiidae) serve as the final hosts for *Anisakis brevispiculata* (Mattiucci et al. 2001, Cavallero et al. 2011) and feed primarily on squids and to a lesser degree on mesopelagic fishes (Pauly et al. 1998). However, Mattiucci et al. (2001) reported that the demersal *Merluccius merluccius* (Macrouridae) served as a paratenic host in the central—eastern Atlantic Ocean; demersal fishes have been known to feed on midwater fishes, especially along continental slopes and seamounts (Pusch et al. 2004, Gartner et al. 2008), and this is reflected in their parasite fauna (e.g., Klimpel et al. 2008, Palm and Klimpel 2008). Therefore, we believe that *Anisakis brevispiculata* has a pelagic life—cycle in the nGOM but that additional midwater fish (especially myctophids) and squid species from the GOM should be studied to confirm this.

Anisakis typica parasitized *Polymetme corythaeola*, the only benthopelagic species examined. Palm et al. (2008) suggested a pelagic life—cycle for *Anisakis typica* off Indonesia based on their finding of larvae in scombrid and carangid fishes, but our findings suggest a demersal cycle in the nGOM. Cavallero et al. (2011) found that *Anisakis typica* was the dominant anisakid in offshore delphinids that had stranded along the coasts of the GOM, southeastern U.S., and Caribbean Sea. Our finding of *Anisakis typica* in the benthopelagic species only was surprising, considering that the diets of most oceanic delphinids consist of squid and epipelagic and mesopelagic fishes (e.g., Fiedler et al. 1998, Pauly et al. 1998, Davis et al. 2002). Therefore, additional paratenic hosts are likely necessary to transfer *Anisakis typica* to its final hosts. Additional benthopleagic and demersal fishes along the outer continental shelf and slope should be examined to further establish the life—cycle patterns of *Anisakis typica* in the nGOM.

The current underrepresentation of parasitological data for species of *Anisakis* in the nGOM is likely an artifact of the bathymetry of the GOM. The continental shelf is shallow and wide across most of the commercial and recreational fishing grounds, and the majority of ichthyoparasitological examinations in the region for ascaridoids (e.g., Overstreet 1978, Deardorff and Overstreet 1980, 1981) have focused on host species that occur in coastal waters or over the continental shelf. Likely reasons why anisakids are not commonly encountered in fisheries products from the GOM are the lack of surveys using molecular tools, the importance that oceanic euphasiids may play in the life—cycles of species of *Anisakis* (Smith and Wooten 1978), and the diversity of

marine mammals associated with the bathymetric features (e.g., salt domes and diapirs) of the nGOM continental slope (Davis et al. 2002).

In the GOM, the only species of *Bolbosoma* reported is *Bolbosoma vasculosum* from Blainville's beaked whale, *Mesoplodon densirostris*, (Salgado—Maldonado and Amin 2009) However, in the Caribbean Sea, *Bolbosoma vasculosum* has been reported from both the pygmy killer whale, *Feresa attenuata*, and the Atlantic spotted dolphin, *Stenella frontalis*; another species, *Bolbosoma capitatum*, has been reported from the short—finned pilot whale, *Globicephala macrorhynchus*, and an unidentified species of *Bolbosoma* infected the pygmy sperm whale, *Kogia breviceps* (see Mignucci—Giannoni et al. 1998). Unfortunately, the freezing and subsequent thawing of the single specimen that we attribute to *Bolbosoma* sp. was based on molecular sequencing of the *cox1* gene and did not allow for morphological identification to species level. Species of *Bolbosoma* are one of the two acanthocephalan genera known to parasitize marine mammals (Raga et al. 2009). Euphasiids and copepods act as first intermediate hosts (Hoberg et al. 1993, Gregori et al. 2012), and fishes act as paratenic or transport hosts (Raga et al. 2009).

Likewise, tetraphyllideans use crustacean and fish intermediate hosts, mature in elasmobranchs, and the larvae are commonly found in marine fishes (e.g., Jensen and Bullard 2009). We attempted to amplify the 28S from the extracted DNA of 3 individual larvae but were unsuccessful. Jensen and Bullard (2009) provided a key to 15 larval types that they identified but our specimens could not clearly be assigned to any of them, most likely because of the freezing and thawing of hosts. The only species of *Brachyphallus* reported from the GOM by Overstreet et al. (2009) is *Brachyphallus parvum* (as *Lecithochirium parvum*). *Brachyphallus parvum* has been reported from 27 teleost species in the GOM, 5 of which are pelagic. The life—cycle of the congener *Brachyphallus crenatus* involves an opisthobranch snail, calanoid copepods, matures in teleosts, and can include chaetognaths and ctenophores as paratenic hosts (Køie 1992); therefore, the finding of an immature species in *Polypinus clarus* is not necessarily unexpected.

Few other studies on the parasites of midwater fishes have included sternoptychids or phosichthyids as hosts. Gartner and Zwerner (1989) examined *Argyropelecus aculeatus* and *Sternoptyx diaphana* from the western North Atlantic; Klimpel et al. (2004, 2007) examined *Maurolicus muelleri* from the Norwegian Deep and Mid—Atlantic Ridge; and Bray and Gaevskaya (1993) provided a description of a monorchiid (Digenea) from *Polymetme corythaeola* from the eastern mid—Atlantic Ocean. Gartner and Zwerner (1989) found only cestode larvae in the 2 sternoptychids they examined. Klimpel et al. (2004, 2007) found parasite communities similar to ours, but they found 2 digenean species and the ascaridoid species *Anisakis simplex* (sensu stricto) and *Hystero-*

thylacium aduncum. We did not find *Anisakis simplex* (sensu stricto) in any of the fish we examined, but Cavallero et al. (2011) did find individuals in stranded delphinids, *Kogia breviceps*, and *Mesoplodon europaeus*. Cavallero et al. (2011) also found five additional species of *Anisakis* plus *Pseudoterranova ceticola*; therefore, some of the additional species of *Anisakis* they identified but were not collected by us likely occur in the nGOM.

Our results must also be accompanied with a few caveats. The first of which is that the midwater fishes treated in our study were collected incidentally with a bottom trawl. We do not believe that all of these fishes were collected near the bottom, but Gartner et al. (2008) reported large numbers of *Maurolicus weitzmani* and *Polyipnus clarus* associated with the benthic boundary layer. This may explain why we were able to attain a sample size greater than 100 for *Polyipnus clarus*. Our sample sizes were also relatively small for most species examined (only *Polyipnus clarus* and *Polymetme corythaeola* had n > 50); therefore, we elected to pool parasitological data for species across the nGOM rather than comparing infections by locations. Ross et al. (2010) found a homogenous mesopelagic fish assemblage across the nGOM that would support our consideration. However, based on diet data for some sternoptychid species in the eGOM, the species composition of available prey at lower taxonomic levels spatially varies slightly (e.g., Hopkins and Baird 1985). The stomachs of most of the fishes we examined were either inverted, empty, or contained unrecognizable (digested) prey, and could not be used to reliably compare diets. To generate a more concise picture of the parasite communities of sternoptychids and phosicthyids and to generate a better understanding of the life–cycles of such parasites, fishes collected by midwater trawls should be examined.

Literature Cited

Andres, M.J. and R.M. Overstreet. 2013. A new species of *Podocotyloides* (Digenea: Opecoelidae) from the grey conger eel, *Conger esculentus*, in the Caribbean Sea. Journal of Parasitology 99:619–623. doi: 10.1645/12–155.1

Bernal, A., M.P. Olivar, F. Maynou, and M.L. Fernández de Puelles. 2015. Diet and feeding strategies of mesopelagic fishes in the western Mediterranean. Progress in Oceanography 135:1–17. doi: 10.1016/j.pocean.2015.03.005

Bray, R.A. and A.V. Gaevskaya. 1993. *Bathymonorchis polyipni* (Reimer, 1985) n. g., n. comb. (Digenea: Monorchiidae) from bathypelagic fishes of the eastern mid–Atlantic Ocean. Systematic Parasitology 26:91–95. doi: 10.1007/BF00009216

Bush, A.O., K.D. Lafferty, J.M. Lotz, and A.W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revised. Journal of Parasitology 83:575-583. doi: 10.2307/3284227

Cavallero, S., S.A. Nadler, L. Paggi, N.B. Barros, and S. D'Amelio. 2011. Molecular characterization and phylogeny of anisakid nematodes from cetaceans from southeastern Atlantic coasts of USA, Gulf of Mexico, and Caribbean Sea. Parasitology Research 108:781–792. doi: 10.1007/s00436–010–2226–y

Choy, C.A., E. Portner, M. Iwane, and J.C. Drazen. 2013. Diets of five important predatory mesopelagic fishes of the central North Pacific. Marine Ecology Progress Series 492:169–184. doi: 10.3354/meps10518

Davis, M.P., N.I. Holcroft, E.O. Wiley, J.S. Sparks, and W.L. Smith. 2014. Species specific bioluminescence facilitates speciation in the deep sea. Marine Biology 161:1139–1148. doi: 10.1007/s00227–014–2406–x

Davis, R.W., J.G. Ortega–Ortiz, C.A. Ribic, W.E. Evans, D.C. Biggs, P.H. Ressler, R.B. Cady, R.R. Leben, K.D. Mullin, and B. Würsig. 2002. Cetacean habitat in the northern oceanic Gulf of Mexico. Deep–Sea Research I 49:121–142. doi: 10.1016/S0967–0637(01)00035–8

Deardorff, T.L. and R.M. Overstreet. 1980. Review of *Hysterothylacium* and *Iheringascaris* (both previously =*Thynnascaris*) (Nematoda: Anisakidae) from the northern Gulf of Mexico. Proceedings of the Biological Society of Washington 93:1035–1079.

Deardorff, T.L. and R.M. Overstreet. 1981. Larval *Hysterothylacium* (=*Thynnascaris*) (Nematoda: Anisakidae) from fishes and invertebrates in the Gulf of Mexico. Proceedings of the Helminthological Society of Washington 48:113-126.

DeVaney, S.C. 2008. The interrelationships of fishes of the Order Stomiiformes. Ph.D. thesis. University of Kansas, Lawrence, KS, USA. 233 p.

Fiedler, P.C., J. Barlow, and T. Gerrodette. 1998. Dolphin prey abundance determined from acoustic backscatter data in eastern Pacific surveys. Fishery Bulletin 96:237-247.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for the amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3:294-299.

García–Varela, M. and S.A. Nadler. 2006. Phylogenetic relationships among Syndermata inferred from nuclear and mitochondrial gene sequences. Molecular Phylogenetics and Evolution 40:61-72. doi: 10.1016/j.ympev.2006.02.010

García–Varela, M., G. Pérez–Ponce de León, F.J. Aznar, and S.A. Nadler. 2013. Phylogenetic relationship among genera of Polymorphidae (Acanthocephala), inferred from nuclear and mitochondrial gene sequences. Molecular Phylogenetics and Evolution 68:176-184. doi: 10.1016/j.ympev.2013.03.029

Gartner, J.V., Jr. and D.E. Zwerner. 1989. The parasite faunas of meso– and bathypelagic fishes of Norfolk Submarine Canyon, western North Atlantic. Journal of Fish Biology 34:79-95. doi: 10.1111/j.1095–8649.1989.tb02959.x

Gartner, J.V. Jr., K.J. Sulak, S.W. Ross, and A.M. Necaise. 2008. Persistent near–bottom aggregations of mesopelagic animals along the North Carolina and Virginia continental slopes. Marine Biology 153:825-841. doi: 10.1007/s00227–007–0855–1

Gregori, M., F.J. Aznar, E. Abollo, Á. Roura, Á.F. González, and S. Pascual. 2012. *Nyctiphanes couchii* as intermediate host for the acanthocephalan *Bolbosoma balaenae* in temperate waters of the NE Atlantic. Diseases of Aquatic Organisms 99:37-47. doi: 10.3354/dao02457

Gunter, G. and R.M. Overstreet. 1974. Cetacean notes. I. Sei and Rorqual whales on the Mississippi coast, a correction. II. A dwarf sperm whale in Mississippi Sound and its helminth parasites. Gulf Research Reports 4:479-481. doi: 10.18785/grr.0403.07

Hoberg, E.P., P.Y. Daoust, and S. McBurney. 1993. *Bolbosoma capitatum* and *Bolbosoma* sp. (Acanthocephala) from sperm whales (*Physeter macrocephalus*) stranded on Prince Edward Island, Canada. Journal of the Helminthological Society of Washington 60:205-210.

Hopkins, T.L. and T.M. Lancraft. 1984. The composition and standing stock of mesopelagic micronekton at 27°N 86°W in the eastern Gulf of Mexico. Contributions in Marine Science 27:143-158.

Iñiguez, A.M., C.P. Santos, and A.C. Paulo Vicente. 2009. Genetic characterization of *Anisakis typica* and *Anisakis physeteris* from marine mammals and fish from the Atlantic Ocean off Brazil. Veterinary Parasitology 165:350-356. doi: 10.1016/j.vetpar.2009.07.012

Katoh, K., K.–I. Kuma, H. Toh, and T. Miyata. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Research 33:511-518. doi: 10.1093/nar/gki198

Klimpel, S., H.W. Palm, S. Rückert, and U. Piatkowski. 2004. The life cycle of *Anisakis simplex* in the Norwegian Deep (northern North Sea). Parasitology Research 94:1-9. doi: 10.1007/s00436–004–1154–0

Klimpel, S., H.P. Palm, M.W. Busch, E. Kellermanns, and S. Rückert. 2006. Fish parasites in the Arctic deep–sea: Poor diversity in pelagic fish species vs. heavy parasite load in a demersal fish. Deep–Sea Research I 53:1167-1181. doi: 10.1016/j.dsr.2006.05.009

Klimpel, S., E. Kellermanns, H.W. Palm, and F. Moravec. 2007. Zoogeography of fish parasites of the pearlside (*Maurolicus muelleri*), with genetic evidence of *Anisakis simplex* (*s.s.*) from the Mid–Atlantic Ridge. Marine Biology 152:725-732. doi: 10.1007/s00227–007–0727–8

Klimpel, S., E. Kellermanns, and H.W. Palm. 2008. The role of pelagic swarm fish (Myctophidae: Teleostei) in the oceanic life cycle of *Anisakis* sibling species at the Mid–Atlantic Ridge, Central Atlantic. Parasitology Research 104:43-53. doi: 10.1007/s00436–008–1157–3

Klimpel, S., M.W. Busch, T. Sutton, and H.W. Palm. 2010. Meso– and bathy–pelagic fish parasites at the Mid–Atlantic Ridge (MAR): Low host specificity and restricted parasite diversity. Deep–Sea Research I 57:596-603. doi: 10.1016/j.dsr.2010.01.002

Køie, M. 1992. Life cycle and structure of the fish digenean *Brachyphallus crenatus* (Hemiuridae). Journal of Parasitology 78:338-343. doi: 10.2307/3283485

Kuhn, T., J. García–Màrquez, and S. Klimpel. 2011. Adaptive radiation within marine anisakid nematodes: A zoogeographical modeling of cosmopolitan, zoonotic parasites. PLoS ONE 6(12):e28642. doi:10.1371/journal.pone.0028642

Jensen, K. and S.A. Bullard. 2009. Characterization of a diversity of tetraphyllidean and rhinebothriidean cestode larval types, with comments on host associations and life–cycles. International Journal of Parasitology 40:889-910. doi: 10.1016/j.ijpara.2009.11.015

Marcogliese, D.J. 1995. The role of zooplankton in the transmission of helminth parasites to fish. Reviews in Fish Biology and Fisheries 5:336-371. doi: 10.1007/BF00043006

Mateu, P., V. Nardi, N. Fraija–Fernández, S. Mattiucci, L. Gil de Sola, J.A. Raga, M. Fernández, and F.J. Aznar. 2015. The role of lantern fish (Myctophidae) in the life–cycle of cetacean parasites from western Mediterranean waters. Deep–Sea Research I 95:115-121. doi: 10.1016/j.dsr.2014.10.012

Mattiucci, S. and G. Nascetti. 2006. Molecular systematics, phylogeny and ecology of anisakid nematodes of the genus *Anisakis* Dujardin, 1845: An update. Parasite 13:99-113. doi: 2006132099

Mattiucci, S., L. Paggi, G. Nascetti, E. Abollo, S.C. Webb, S. Pascual, R. Cianchi, and L. Bullini. 2001. Genetic divergence and reproductive isolation between *Anisakis brevispiculata* and *Anisakis physeteris* (Nematoda: Anisakidae). International Journal of Parasitology 31: 9-14. doi: 10.1016/j.fishres.2007.09.032

Mattiucci, S., V. Farina, N. Campbell, K. MacKenzie, P. Ramos,

A.L. Ramos, A.L. Pinto, P. Abaunza, and G. Nascetti. 2008. *Anisakis* spp. larvae (Nematoda: Anisakidae) from Atlantic horse mackerel: Their genetic identification and use as biological tags for host stock characterization. Fisheries Research 89:146–151. doi: 10.1016/j.fishres.2007.09.032

Mattiucci, S., P. Cipriani, S.C. Webb, M. Paoletti, F. Marcer, B. Bellisario, D.I. Gibson, and G. Nascetti. 2014. Genetic and morphological approaches distinguish the three sibling species of the *Anisakis simplex* species complex, with a species designation as *Anisakis berlandi* n. sp. for *A. simplex* sp. C (Nematoda: Anisakidae). Journal of Parasitology 100:199–214. doi: 10.1645/12–120.1

McEachran, J.D. and J.D. Fechhelm. 1998. Fishes of the Gulf of Mexico Volume 1. University of Texas Press, Austin, TX, USA. 1112 p.

Mignucci–Giannoni, A.A., E.P. Hoberg, D. Siegel–Causey, and E.H. Williams, Jr. 1998. Metazoan parasites and other symbionts of cetaceans in the Caribbean. Journal of Parasitology 84:939–946. doi: 10.2307/3284625

Nadler, S.A. and D.S.S. Hudspeth. 1998. Ribosomal DNA and phylogeny of the Ascaridoidea (Nemata: Secernentea): Implications for morphological evolution and classification. Molecular Phylogenetics and Evolution 10:221–236. doi: 10.1006/mpev.1998.0514

Nadler, S.A., S. D'Amelio, H.P. Fagerholm, B. Berland, and L. Paggi. 2000. Phylogenetic relationships among species of *Contracaecum* Railliet and Henry 1912 and *Phocascaris* Høst, 1932 (Nematoda: Ascaridoidea) based on nuclear rDNA sequence data. Parasitology 121:455–463. doi: 0.1017/s0031182099006423

Noble, E.R. and S.B. Collard. 1970. The parasites of midwater fishes. In: S.F. Snieszko, ed. A Symposium on Diseases of Fishes and Shellfishes. American Fisheries Society Special Publication 5, Washington, D.C., USA. p. 57–68.

Overstreet, R.M. 1978. Marine Maladies? Worms, Germs, and Other Symbionts from the Northern Gulf of Mexico. Mississippi–Alabama Sea Grant Consortium, Ocean Springs, MS, USA. 140 p.

Overstreet, R.M. 2012. Waterborne parasitic diseases in the ocean. In: R.A. Meyers, ed. Encyclopedia of Sustainability Science and Technology, Volume 17. Springer, New York, USA. p. 12018–12062. doi: 10.1007/978–1–4419–0851–3

Overstreet, R.M., J.C. Cook, and R.W. Heard. 2009. Trematoda (Platyhelminthes) of the Gulf of Mexico. In: D.L. Felder and D.K. Camp, eds. Gulf of Mexico–Origins, Waters, and Biota. Biodiversity. Texas A&M University Press, College Station, TX, USA. p. 419–486.

Overstreet, R.M. and G.W. Meyer. 1981. Hemorrhagic lesions in stomach of *Rhesus* monkey caused by a piscine ascaridoid nematode. Journal of Parasitology 67:226–235. doi: 10.2307/3280642

Palm, H.W. and S. Klimpel. 2008. Metazoan fish parasites of *Macrourus berglax* Lacepède, 1801 and other macrourids of the North Atlantic: Invasion of the deep sea from the continental shelf. Deep–Sea Research II 55:236–242. doi:10.1016/j.dsr2.2007.09.010

Palm, H.W., I.M. Damriyasa, Linda, and I.B.M. Oka. 2008. Molecular genotyping of *Anisakis* Dujardin, 1845 (Nematoda: Ascaridoidea: Anisakidae) larvae from marine fish of Balinese and Javanese waters, Indonesia. Helminthologia 45:3–12. doi: 10.2478/s11687–008–0001–8

Pauly, D., A.W. Trites, E. Capuli, and V. Christensen. 1998. Diet composition and trophic levels of marine mammals. ICES Journal of Marine Science 55:467–481. doi: 10.1006/jmsc.1997.0280

Pusch, C., A. Beckmann, F.M. Porteiro, and H. von Westernhagen. 2004. The influence of seamounts on mesopelagic fish communities. Archive of Fishery and Marine Research 51:165–186. doi: 0944–1921/2004/51/1–3–165

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3–900051–07–0, URL http://www.R–project.org/.

Raga, J.A., M. Fernández, J.A. Balbuena, and F.J. Aznar. 2009. Parasites. In: W.F. Perrin, B. Würsing, and H.G.M. Thewissen, eds. Encyclopedia of Marine Mammals, 2nd ed. Academic Press/Elsevier, Amsterdam, Netherlands. p. 821–829.

Ross, S.W., A.M. Quattrini, A.Y. Roa–Varón, and J.P. McClain. 2010. Species composition and distributions of mesopelagic fishes over the slope of the north–central Gulf of Mexico. Deep–Sea Research II 57:1926–1956. doi: 10.1016/j.dsr2.2010.05.008

Sakanari, J.A. and J.H. McKerrow. 1989. Anisakiasis. Clinical Microbiology Reviews 2:278–284. doi: 10.1128/CMR.2.3.278

Salgado–Maldonado, G. and O.M. Amin. 2009. Acanthocephala of the Gulf of Mexico. In: D.L. Felder and D.K. Camp, eds. Gulf of Mexico–Origins, Waters, and Biota. Biodiversity. Texas A&M University Press, College Station, TX, USA. p. 539–552.

Smith, J.W. and R. Wootten. 1978. *Anisakis* and anisakiasis. Advances in Parasitology 16:93–163.

Ward R.D., T.S. Zemlak, B.H. Innes, P.R. Last, and P.D.N. Hebert. 2005. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences 360:1847–1857. doi: 10.1098/rstb.2005.1716

Young, J.W., B.P.V. Hunt, T.R. Cook, J.K. Llopiz, E.L. Hazen, H.R. Pethybridge, D. Ceccarelli, A. Lorrain, R.J. Olson, V. Allain, C. Menkes, T. Patterson, S. Nicol, P. Lehodey, R.J. Kloser, H. Arrizabalaga, and C.A. Choy. 2015. The trophodynamics of marine top predators: Current knowledge, recent advances and challenges. Deep–Sea Research II 113:170–187. doi: 10.1016/j.dsr2.2014.05.015

Zhu, X.Q., R.B. Gasser, M. Podolska, and N.B. Chilton. 1998. Characterisation of anisakid nematodes with zoonotic potential by nuclear ribosomal DNA sequences. International Journal of Parasitology 28:1911–1921. doi: 10.1016/S0020–7519(98)00150–7