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INSIGHT INTO THE DIET COMPOSITION AND GASTROINTESTINAL PARASITE COMMUNITY OF THE COMMON SMOOTH-HOUND, *MUSTELUS MUSTELUS* (CARCHARHINIFORMES: TRIAKIDAE), IN THE NORTHERN ADRIATIC SEA

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We examined gastrointestinal tracts from 15 sharks, common smooth-hound (*Mustelus mustelus*), sampled between December 2005 and April 2007 by bottom trawls operating in the northern Adriatic Sea, and analysed diet composition and infestation levels of gastrointestinal helminth parasites. Decapod malacostracans were the dominant prey group (Index of Relative Importance, %IRI = 84), with *Liocarcinus corrugatus, L. depurator* and *Pilumnus* sp. as the most frequent prey (Frequency of occurrence, %F = 20 - 33). The second highest ranked prey group were ray-finned fish (%IRI = 9.5), represented mostly by *Engraulis encrasicolus* (%F = 13.3), while the third prey group were cephalopods (%IRI = 6.0), consisting mainly of *Sepia elegans*. Parasitological examination revealed 377 parasite specimens in 13 infected sharks (Prevalence, P = 86.7%), with a mean intensity (I) of 29 helminths per host. Nematode *Cucullanus micropapillatus* was the most prevalent (P = 60.0%) and dominant parasite (I = 26.9), recorded with 242 specimens. Trematode *Ptychogonimus megastomum* was recorded in 3 sharks (P = 20.0%) and numbered 89 individuals, while cestodes were represented by genus *Eutetrarhynchus* and genus *Phyllobothrium*, although in low prevalence (both P = 13.3%) and with only few individuals (I = 0.3 and 1.1, respectively).

Key words: elasmobranch, sharks, feeding, intermediate hosts, Mediterranean Sea

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Analizirali smo sastav probavila 15 morskih pasa mekuša, *Mustelus mustelus*, prikupljenih u razdoblju od prosinca 2005. do travnja 2007. godine pridnenim koćama u sjevernom Jadranu, te istražili sastav prehrane i stupanj invadiranosti probavnim nametnicima. Dominantna skupina plijena bili su deseto-

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nožni rakovi (Decapoda – Malacostraca, indeks relativne važnosti, %IRI = 84,0), među kojima su najčešći plijen bili *Liocarcinus corrugatus*, *L. depurator* i *Pilumnus* sp. (postotak učestalosti, %F = 20 - 33). Ribe koštunjače bile su po važnosti druga skupina plijena (%IRI = 9,5) najčešće zastupljene vrstom *Engraulis encrasicolus* (%F = 13,3), dok su treća skupina plijena bili glavonošci (%IRI = 6,0), većinom predstavljeni vrstom *Sepia elegans*. Parazitološkom analizom ukupno je zabilježeno 377 jedinki nametnika u 13 inficiranih morskih pasa (prevalencija, P = 86,7%), s prosječnim intenzitetom infekcije (I) od 29 jedinki po domaćinu. Oblić *Cucullanus micropapillatus* bio je najprevalentniji (P = 60,0%) i najbrojniji nametnik (I = 26,9), zabilježen s ukupno 242 primjerka. Metilj *Ptychogonimus megastomum* je pronađen u 3 morska psa (P = 20,0%) s ukupno 89 jedinki, dok su trakavice bile predstavljene s rodovima *Eutetrarhynchus* i *Phyllobothrium*, ali s niskom stopom prevalencije (oba P =13,3%) i u malom broju jedinki (I = 0,3 i 1,1).

Ključne riječi: hrskavičnjače, morski psi, prehrana, međudomaćini, Sredozemno more

INTRODUCTION

Sharks as top predators play an important role in shaping marine communities and regulating the dynamics of prey populations. As dietary opportunists sharks feed upon a wide range of available prey, determined by their body size and the composition of the local macrofauna. Coastal, medium-sized mesopredatory sharks from the genus *Mustelus* (Carcharhiniformes: Triakidae) have a diet based upon crustaceans, molluscs and small fish (Lipej *et al*., 2004), which is enabled by characteristic jaw musculature and tightly packed low-cusped teeth specialized for crushing hard-shelled prey (Compagno, 1984; Cortés, 1999; Motta, 2004; Gerry *et al*., 2008).

Sharks are exposed to a variety of gastrointestinal parasites, acquired by the ingestion of infected prey. Gastrointestinal helminth parasites are endoparasites with complex life cycles which include phases of dispersal, host colonization and development through a number of intermediate, paratenic and final hosts (Poulin, 1998; Bush *et al*., 2001). Sharks typically serve as the final (definitive) hosts for the most helminth parasites, hosting a variety of platyhelminthes, nematodes and acanthocephalans (Caira & Healy, 2004). Since gastrointestinal helminths are trophically transmitted they can provide a valuable insight into both host and parasite biology, and food web structure (Marcogliese & Cone, 1997; Marcogliese, 2004). For commercially exploited and potentially threatened sharks health is an important issue, since parasitic disease can affect the abundance and population trends. Host–parasite systems have shown that parasites may reduce food intake and induce nutritional stress of the host (Petkevičius, 2007) which consequently influences growth, survival and reproduction of entire population (Anderson & May, 1978).

The common smooth-hound *Mustelus mustelus* (Linnaeus, 1758) is distributed throughout the Eastern Atlantic and Mediterranean Sea, where it is commercially fished for consumption. Increased fishing pressure has led to decreased abundance in a majority of populations, so the species is globally listed as Vulnerable (Serena *et al*., 2009), whereas in the Adriatic Sea it is categorised as Near Threatened (Jardas *et al*., 2008). The species is described as an opportunistic predator in the Adriatic Sea, with diet based upon benthic crustaceans, molluscs, and pelagic and benthopelagic teleost fish (Jardas *et al*., 2007a; Constantini *et al*., 2000), but no information exists to our knowledge on the gastrointestinal parasitofauna of the Adriatic smooth-hound populations. With this study we therefore describe the diet composition of the common smooth-hound in the Croatian northern Adriatic and link its feeding habits to the structure of the gastrointestinal parasite community, providing new insight into the life cycles of the identified parasites.

MATERIALS AND METHODS

We sampled 15 common smooth-hounds sharks (9 males and 6 females) on board of commercial bottom trawlers operating in the northern Adriatic Sea at depths between 45 and 84 m, from December 2005 to April 2007 (Fig. 1). Sharks were identified according to taxonomic keys (Jardas, 1996; Lipej *et al*., 2004; Serena, 2005), sexed by external examination, weighed (body weight, BW) and measured (total length, TL). We isolated the digestive tracts, stored them in labelled plastic bags and transported them on ice to the laboratory for examination. Gastrointestinal tracts were subsequently dissected; contents were rinsed in tap water through a 1-mm mesh sieve and preserved in 75% ethanol.

Prey items were examined under a stereomicroscope and identified to the lowest possible taxon, according to taxonomic keys (Riedl, 1970; Ševčić, 1990; Milišić, 1994; Jardas, 1996; Zavodnik & Šimunović, 1997). We wet weighed the identified prey taxa using a digital scale with precision of ± 0.01g and counted the total number of items. We quantified the diet composition by frequency of occurrence (%F), percentage number (%N), percentage weight (%W) and the index of relative importance (%IRI) (Pinkas *et al*., 1971; Cortés, 1997).

Trematodes and cestodes were isolated from food remains using a stereomicroscope, fixed in 10% formaldehyde and transferred to 75% ethanol, while nematodes were preserved in glycerine alcohol. For detailed parasitological examination, trematodes were processed using Semichon's acetocarmine staining technique; nematodes were prepared by the glycerin jelly technique, while cestodes were stained with Mayer's Haematoxylin and Eosin Y and mounted in Canada balsam (Lasee & True, 2004). Slides were examined under a light microscopy 20x and 100x magnifications and parasites were identified following taxonomic keys (Petter & Radujković, 1989; Khalil *et al*., 1994; Gibson *et al*., 2002; Jones *et al*., 2005; Bray *et al*., 2008). We used Quantitative Parasitology 3.0 software

Fig. 1. Map of the Adriatic Sea, with bathymetry and direction of major sea currents. Study area is marked with a dotted rectangle.

(Reiczigel & Rózsa, 2005) to calculate prevalence, mean abundance, mean intensity of infection (Bush *et al.*, 1997) and a variance-to-mean ratio as a measure of overdispersion.

RESULTS

Sampled sharks ranged from 50.5 to 152.5 cm TL, with a mean of 101.1 ± 27.3 cm. Out of 15 collected digestive tracts, 14 contained prey remains while one was empty. We identified four major prey groups (Tab. 1), along with decomposed prey remains which were classified as unidentified organic material. Malacostracans were the dominant prey with 86.7% of occurrence and accounting for 84.0 of total %IRI. The most frequently recorded malacostracans belonged to the genera *Liocarcinus* (*L. corrugatus* Pennant, 1777 and *L. depurator* Linnaeus, 1758) and *Pilumnus* (Tab. 2).

Tab. 1. Major prey groups in the diet of the common smooth-hound in the northern Adriatic Sea, by percentage number (%N), percentage weight (%W), frequency of occurrence (%F) and index of relative importance (%IRI).

Prey groups	%N	%W	%F	%IRI
Cephalopoda	8.3	14.5	40.0	6.0
Malacostraca	70.8	77.7	86.7	84.0
Actynopterigii	14.6	7.3	66.7	9.5
Plantae and Chromista	6.3	0.5	13.3	0.6

Tab. 2. Identified prey taxa in the diet of the common smooth-hound in the northern Adriatic Sea.

Prey taxa/species	Frequency of occurrence (%)
Cephalopoda	
Sepia elegans de Blainville, 1827	6.7
Malacostraca	
Brachyura indet.	13.3
Carcinus aestuarii Nardo, 1847	13.3
Liocarcinus corrugatus (Pennant, 1777)	33.3
Liocarcinus depurator (Linnaeus, 1758)	20.0
Meiosquilla desmaresti (Risso, 1816)	6.7
Pilumnus sp.	20.0
Portunidae indet.	6.7
Upogebia pusilla (Petagna, 1792)	6.7
Actinopterygii	
Engraulis encrasicolus (Linnaeus, 1758)	13.3
Gadidae indet.	6.7
Liliopsida	
Posidonia oceanica (Linnaeus) Delile, 1813	13.3

Tab. 3. Mean intensity with bootstrap 95% confidence limits (CI), mean abundance with bootstrap 95% confidence limits (CI), mean prevalence (%) with Stern's exact 95% confidence limits (CI) and variance-to-mean ratio of parasites isolated from the common smooth-hound in the northern Adriatic Sea.

Species / taxa	No. of parasites detected	No. of infected sharks	Mean intensity (95% CI)	Mean abundance (95% CI)	Prevalence (%) (95% CI)	Variance-to-mean ratio
NEMATODA						
Chromadorea						
Cucullanus micropapillatus	242	9	26.9 (13.4-51.8)	16.1 (7.3-35.5)	60.0 (33.2-80.9)	41.71
Ascaridida (larval stage)	36	5	5.1 (2.8-14.4)	2.4 (0.7-7.1)	33.3 (14.2-60.3)	11.71
PLATYHELMINTHES						
Cestoda						
Eutetrarhynchus sp.	2	2	0.3	0.1 (0.0-0.3)	13.3 (2.4-39.7)	0.93
Phyllobothrium sp.	8	2	1.1 (3.0-4.0)	0.5 (0.0-1.4)	13.3 (2.4-39.7)	3.98
Trematoda						
Ptychogonimus megastomum	89	3	12.7 (1.0-56.0)	5.9 (0.1-26.7)	20.0 (5.6-46.6)	71.47

The second highest ranked prey group was ray-finned fish (Actynopterigii) which represented 66.7% of occurrence, but with a low index of relative importance (9.5% IRI) and a small contribution to total wet weight (7.3%). Among ray-finned fish we identified European anchovies, *Engraulis encrasicolus* (Linnaeus, 1758) and a few representatives of the family Gadidae. Cephalopods were recorded in 6 sharks, but the majority of findings were partially digested beaks, so species identification was only possible for *Sepia elegans* de Blainville, 1827. Small parts of plants and algae were represented with a percentage weight <1% and were probably taken incidentally while feeding on other prey.

Helminth parasites were identified in 13 digestive tracts, with a total prevalence of 86.7%. We isolated 377 parasite specimens, with a mean intensity of 29 helminths per host. The largest number of parasites (N = 93) was isolated from one male shark with a TL of 99 cm, caught in December. Nematodes were the dominant parasites (73.7%), while remaining specimens were trematodes (23.6%) and cestodes (2.7%). Out of 13 infected sharks, 8 contained only nematodes in their digestive tract. In contrast, when cestodes or trematodes were recorded other helminth taxa were also present. Nematode *Cucullanus micropapillatus* Tornquist, 1931 was the dominant species with a total prevalence of 60.0% and a mean intensity of 26.9 parasites per host (Tab. 3). The majority of identified *C. micropapillatus* were in larval form (81.8%), while 44 specimens were adult. Since taxonomic identification was based on morphological characte-

ristics, some larval stage nematodes, belonging to families Anisakidae and Cucullanidae, could only be identified as Ascaridida. The only trematode species was *Ptychogonimus megastomum* (Rudolphi, 1819), with 89 individuals recorded, but with a small prevalence (20.0%). Cestodes were represented with the genus *Phyllobothrium* and genus *Eutetrarhynchus,* although in low prevalence (both P = 13.3%) and with only a few individuals (Tab. 3). According to variance-to-mean ratio, individuals of *C. micropapillatus* and *P. megastomum* exhibited typical aggregated, right skewed distribution in the analysed sharks, with most host individuals harbouring low numbers of parasites, and only a few sharks with numerous parasites.

DISCUSSION

Cranial anatomy and diet composition studies have shown that smooth-hounds are specialised for foraging on hard-shelled crustaceans, which has been also confirmed for populations in the Adriatic (Jardas *et al.,* 2007a,b; Lipej *et al.,* 2011; Gračan *et al.,* 2013). Portunid crabs from the genus *Liocarcinus* have been frequently identified prey of *M. mustelus* (Jardas *et al.,* 2007a) and *M. punctulatus* in the Adriatic Sea (Jardas *et al.,* 2007b; Lipej *et al.,* 2011; Gračan *et al.,* 2013), and juvenile *M. mustelus* in the western Mediterranean Sea (Morte *et al.,* 1997). This finding may be linked with high fishing effort in the study area since portunid crabs are scavengers, frequently found feeding on damaged or dead animals along trawl tracks (Kaiser & Spencer, 1994; Šimunović, 1997). Small pelagic fishes like anchovies use the northern Adriatic as a spawning ground (Gamulin & Hure, 1983; Sinovčić, 2000) and therefore present an easy available and energetically valuable prey species for this shark genus (Constantini *et al.,* 2000; Jardas *et al.,* 2007a,b; Lipej *et al.,* 2011; Gračan *et al*., 2013). Cephalopods from genera *Illex* and *Loligo* were reported as prey of the *M. mustelus* in the central Adriatic (Jardas *et al.,* 2007a), in the Aegean Sea (Kabasakal, 2002) and in South African waters (Smale *et al.,* 2001). In the present study we identified only one cephalopod species (*S. elegans*), whilst the decomposed state of remaining cephalopods have prevented the identification of other species.

Elasmobranchs have characteristic intestines with a spiral valve inside the intestinal tube, which is the most parasitized internal organ in sharks (Caira & Healy, 2004). The present study demonstrates that majority of common smooth-hounds in the northern Adriatic (86.7%) are infected with helminth parasites, especially with generalist nematodes, while specialised trematode and cestode taxa are not as common. Until now, the nematode *C. micropapillatus* was recorded in the Adriatic in ray-finned fish from genera *Symphodus* and *Labrus* (Janiszewska, 1949; Sey, 1970). Similar species, *C. longicollis,* was identified only in *Mullus barbatus* in the south Adriatic (Petter *et al.,* 1984) and central Adriatic (Jardas & Hristovski, 1985). Hence, this paper presents the first finding of *C. micropapillatus* for elasmobranchs in the Adriatic Sea. Although specimens from this genus are usually transferred through polychaets, copepods and fish (Køie, 2000a,b), based on the prevalence and intensity of infection in the common smooth-hound from our study and scarce *C. micropapillatus* reports in the Adriatic ray-finned fish, it is possible that frequently consumed crustaceans from genera *Liocarcinus* and *Pilumnus* may also be involved in transmission of this nematode.

The nematode genus *Anisakis* Dujardin, 1845 consists of generalist species which use various invertebrates like crustacean euphausiids as intermediate hosts, occur in fish and cephalopods, and parasitize digestive tract of cetaceans and pinnipeds (Mattiucci & Nascetti, 2006, 2008). In the Adriatic, larval anisakids were recorded in commercially

important fish like *Trachurus* sp., *Merluccius* sp., *Scomber* sp. and *E. encrasicolus* (Janiszewska, 1949; Sey, 1970; Petter *et al.*, 1984; Jardas & Hristovski, 1985; Mladineo *et al.*, 2012), cephalopods (Petrić *et al.*, 2011) and loggerhead sea turtles (Gračan *et al.*, 2012). Since small pelagic fish, like the European anchovy, and small cephalopods, like short-finned squid *Illex coindetii,* are found with high infection rates of *Anisakis* larvae in the Adriatic Sea (Petrić *et al.*, 2011; Mladineo *et al.*, 2012) it is possible that they represent one of its vectors to *M. mustelus*.

The trematode *P. megastomum* was recorded in genera *Galeorhinus, Galeus, Scyliorhinus, Squalus* and *Mustelus* in the Adriatic by Dollfus (1937) and Gibson & Bray (1977). However, there are no data on other hosts for this parasite in the Adriatic Sea. Since the suggested intermediate hosts in the Mediterranean region are scaphopod molluscs and crustaceans from genera *Portunus* and *Pilumnus* (Yamaguti, 1975), it is possible that these crustaceans, identified in the diet of the common smooth-hound in the Adriatic, may present an infectious link for *P. megastomum* in the Adriatic food web.

The identified helminth parasitofauna in our study was dominated by nematodes and trematodes, similar to previous reports of helminth communities in the Adriatic Sea (Sey, 1968, 1970a,b; Jardas & Hristovski, 1985; Petter & Radujković, 1986; Mladineo *et al*., 2012). As parasites are recognized as an important part of a food-web structure (Lafferty *et al.*, 2006), similar further investigations will contribute to the knowledge on predator-prey interactions in this region. Moreover, the high prevalence values of helminth parasites emphasize the need for future parasitological studies to improve our understanding of the factors that influence host populations, which could have demographic effects on commercially exploited species such as the smooth-hound shark.

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SAŽETAK

Uvid u sastav prehrane i zajednice probavnih nametnika psa mekuša, *Mustelus mustelus* (Carcharhiniformes: Triakidae), u sjevernom Jadranu

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Analiziran je sastav prehrane i stupanj invadiranosti probavnim nametnicima na uzorku od 15 jedinki morskih pasa mekuša prikupljenih na komercijalnim pridnenim koćama u razdoblju od prosinca 2005. do travnja 2007. godine u sjevernom Jadranu. Ukupna dužina tijela analiziranih životinja bila je između 50,5 i 152,5 cm, pri čemu je ustanovljeno 9 mužjaka i 5 ženki. Analizom sadržaja probavila zabilježene su sljedeće taksonomske kategorije plijena: desetonožni rakovi, ribe koštunjače, glavonošci te biljke i alge. Desetonožni rakovi su bili najzastupljeniji plijen (indeks relativne važnosti, %IRI = 84,0), najčešće predstavljeni vrstama *Liocarcinus corrugatus*, *L. depurator*, *Carcinus aestuarii* i jedinkama roda *Pilumnus.* Ribe koštunjače su bile druga najzastupljenija skupina plijena (%IRI = 9,5) među kojima je zabilježena vrsta *Engraulis encrasicolus* i predstavnici porodice Gadidae, dok je treća po važnosti bila skupina glavonožaca (%IRI = 6,0), zabilježena s učestalošću od 40,0% i predstavljena vrstom *Sepia elegans.*

Analizom sadržaja probavila utvrđeno je da je nametnicima invadirano 13 morskih pasa (prevalencija, P = 86,7%), pri čemu su oblići utvrđeni u 12 životinja (P = 80,0%), a metilji i trakavice u 3 životinje (P = 20,0%). Od spomenutih nametnika najveću prevalenciju (60,0%) je imala vrsta *Cucullanus micropapillatus,* za koju je ukupno zabilježeno 198 ličinačkih primjeraka i 44 odrasle jedinke. Metilj *Ptychogonimus megastomum* je zabilježen s učestalošću od 20,0% i 89 primjeraka, dok su trakavice iz rodova *Eutetrarhynchus* i *Phyllobothrium* bile manje prisutne u uzorcima (P = 13,3%). S obzirom na intenzitet i učestalu zaraženost morskih pasa vrstama *C. micropapillatus* i *P. megastomum* te dominantnost rakova u prehrani psa mekuša, moguće je da rakovi služe kao međudomaćini za ove nametnike.